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The effects of sr2w-1 supplementation on high-intensity cycling performance and lactate metabolism

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THE EFFECTS OF SR2W-1 SUPPLEMENTATION ON
HIGH-INTENSITY CYCLING PERFORMANCE AND
LACTATE METABOLISM

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A thesis submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

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ABSTRACT

Purpose: The purpose of this investigation was to examine the effects of SR2W-1 herbal supplementation on cycling performance, muscle and blood lactate, and various physiological parameters including blood glucose, heart rate (HR), rating of perceived exertion (RPE), oxygen consumption (VO_2), expired ventilation (VE), respiratory exchange ratio (RER), and femoral artery blood flow. **Methods:** Seven recreational cyclists (Age: 26.7 ± 9.8 yrs, Height: 172.5 ± 13.3 cm, Weight: 67.1 ± 10.7 kg, and $\text{VO}_{2\text{max}}$: 59.5 ± 11 mL/kg/min) performed 20-min of steady-state cycling ($\sim 85\%$ $\text{VO}_{2\text{max}}$, 212.1 ± 25.0 W) followed by three 1-min high intensity intervals at $\text{VO}_{2\text{max}}$ workload (272.9 ± 26.9) with 30-sec active recovery periods at 100 watts. Following intervals, a 15-min passive recovery period preceded a ride to fatigue at $\text{VO}_{2\text{max}}$ workload. Subjects completed trials on four occasions; preceding and following 21 days of 1000mg/d SR2W-1 (EXP) or 1000mg/d placebo (PLA) assigned in random order. The Wilcoxon Signed-Rank Test was used to compare change-scores from pre- to post- PLA and EXP conditions. **Results:** No differences reported for any dependent variable and performance times were not different between PLA (pre-PLA: 180 ± 48 s; post-PLA: 198 ± 56 s) and EXP (pre-EXP: 170 ± 57 s; post-EXP: 191 ± 50 s). **Conclusion:** Notwithstanding the small sample size, 3 weeks of SR2W-1 supplementation does not appear to aid cycling performance, attenuate skeletal muscle fatigue, or modify general physiological responses to exercise.

CHAPTER ONE

INTRODUCTION

Following the pioneering work of A.V. Hill and others, the Harvard Fatigue Laboratory opened in the fall of 1927 in Cambridge, Massachusetts with the purpose of examining fatigue in man under various environmental and working conditions. Although the lab is no longer in existence, the complex nature of fatigue has necessitated continued work in this area, especially as it relates to fatigue from exercise. Of the many physiological parameters implicated in exercise-induced fatigue, hydrogen concentration (pH) and lactate are two of the most widely studied.

High levels of blood lactate and associated reductions in pH are strongly associated with the onset of fatigue. In 1907, Fletcher and Hopkins examined lactic acid levels in working frog muscles and stated that “an acid reaction of the muscle is, as most agree, a constant mark of the fatigued condition and a constant condition of the state of rigor,” providing evidence that acidity has been associated with decreased function and fatigue for over a century (33). The relationship between exercise-induced blood acidity and intensity in man was elucidated by Margaria et al. in 1933 (63). It is now known that carbohydrate metabolism (glycolysis) increases with exercise intensity to rapidly re-synthesize ATP for contracting skeletal muscle. A high rate of glycolysis leads to pyruvate production that exceeds the speed at which pyruvate can be aerobically metabolized in the mitochondria, thus resulting in the ‘anaerobic’ formation of

lactic acid. Once lactic acid is formed it immediately dissociates into lactate and hydrogen which contributes to a decrease in muscle and blood pH (Figure 1.1).

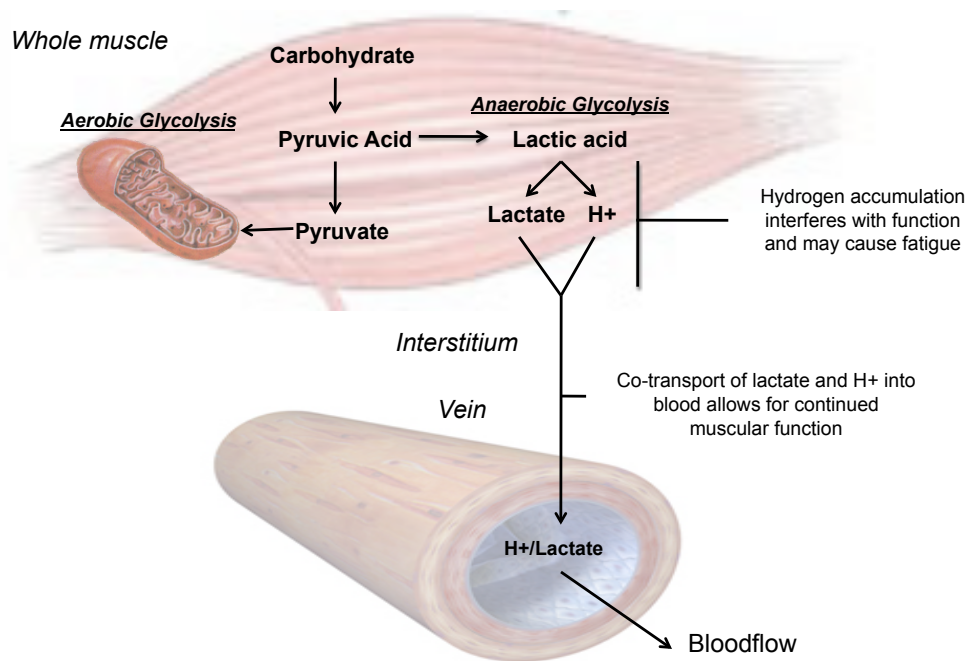


Figure 1.1 - Lactate Dynamics

Lactic acid's role in fatigue has been debated due to a lack of supporting evidence regarding lactic acid's proton contribution and the positive energetic properties of lactate. However, the deleterious effects of pH on skeletal muscle function are well-documented. In-vitro skeletal muscle experiments have shown that low intra-muscular pH can interfere with several reactions within the muscle cell, inhibiting function and causing fatigue (13, 30, 59). It has also been suggested that hydrogen ion accumulation may inhibit the release of calcium from the sarcoplasmic reticulum and interfere with the transmission of neural impulses which can also decrease function (45). Although there are other potential sources

of muscular fatigue which can hinder performance such as fatigue of the central nervous system, psychological factors, and inorganic phosphate accumulation in the muscle cell, acidosis is often regarded as the most prevalent source of fatigue during intense exercise.

Because intense exercise is associated with a drop in pH and subsequent fatigue, athletes can benefit from strategies that mitigate the acidifying effect of exercise to enhance their performance. In 1924, A.V. Hill acknowledged the importance of tissue and blood buffers and their effect on muscular effort (39). As early as the 1930's, researchers experimented with induced alkalosis and its effect on performance. They hypothesized that by increasing blood alkalinity, more lactic acid could be produced implying more energy from anaerobic glycolysis and achievement of higher intensities (64). After being largely ignored for nearly 30 years due to initial null findings, a resurgence of research in the 1970's began to indicate that manipulating extracellular buffering capacity can impact intracellular pH. Bicarbonate supplementation became the predominant mode of inducing blood alkalinity since the bicarbonate buffer system is a primary source of blood's buffering capacity. Research shows that increasing blood pH with bicarbonate increases the rate of lactate and hydrogen efflux from muscle cells despite the sarcolemma's relative impermeability to bicarbonate (18, 43, 48, 88, 92). This suggests that hydrogen ion efflux from the cell works down a concentration gradient. Conceptually, an increased concentration gradient facilitated by changes in extracellular lactate/H⁺ should allow for more lactate/H⁺

transport from the active skeletal muscle, thereby delaying fatigue. As a result, lactate/H⁺ supplement research has become prevalent due to its potential performance-enhancing benefits.

SR2W-1 is a commercially available herbal supplement designed to improve high-intensity exercise performance. Research supporting the efficacy of SR2W-1 is promising as two investigations have demonstrated that SR2W-1 prolongs exercise endurance and promotes lactate clearance after exhaustive exercise as well as improves overall performance (1, 57). However, the two existing SR2W-1 studies were not peer reviewed nor were they cross-over, placebo-controlled, double-blind designs examining multiple factors. The comprehensive nature of our studies' protocol is more likely to reveal a mechanism of action. If the ergogenic effects of SR2W-1 are facilitated by factors related to acid-base balance, the effects are likely mediated by one or both of the following physiological mechanisms: 1. Direct effect on lactate and hydrogen production in skeletal muscle or intracellular buffering, and 2. Indirect effect on skeletal muscle lactate and pH through modifications to blood lactate and/or pH. Although there has been a sparse amount of information gathered on the effects of herbal supplements on blood flow, the latter mechanism could be a result of blood flow distribution due to its role in lactate exchange (34, 96).

Aims and Hypotheses

Aim 1 - To determine if 21 days of SR2W-1 supplementation improves high-intensity cycling performance compared to a placebo.

Hypothesis 1 - SR2W-1 will improve cycling exercise performance compared to a placebo.

Aim 2 - To determine if 21 days of SR2W-1 supplementation alters blood lactate levels during cycling exercise compared to a placebo.

Hypothesis 2 - SR2W-1 will reduce blood lactate levels during exercise compared to a placebo.

Aim 3 - To determine if 21 days of SR2W-1 supplementation alters blood lactate levels during 15 minutes passive recovery compared to a placebo.

Hypothesis 3 - SR2W-1 will reduce blood lactate levels during high-intensity intervals when compared to a placebo.

Aim 4 - To determine if 21 days of SR2W-1 supplementation alters skeletal muscle lactate levels during 15 minutes of passive recovery compared to a placebo.

Hypothesis 4 - SR2W-1 will decrease skeletal muscle lactate levels and increase muscle pH compared to a placebo.

Aim 5 - To determine if SR2W-1 alters femoral artery blood flow during 15 minutes of passive recovery compared to a placebo.

Hypothesis 5 - SR2W-1 will increase femoral artery blood flow when compared to a placebo.

Significance of the Study

Although initial studies have indicated that SR2W-1 supplementation has positive effects on lactate levels and performance, the current study will be the first to implement a randomized, placebo-controlled, cross-over design. Thus, the finding will provide a strong scientific foundation for the relative efficacy of the supplement which will serve to better inform coaches and athletes. Further, the physiological effect of SR2W-1, combined with the performance outcome, will provide further insight in to the role of blood flow, blood lactate and pH, and skeletal muscle lactate and pH as factors in fatigue.

CHAPTER TWO

REVIEW OF THE LITERATURE

Objectives

The objectives of this chapter are to provide an overview of how: 1) lactate affects skeletal muscle fiber function in animals independent of pH, 2) pH affects skeletal muscle fiber function in animals, 3) lactate and pH affects cycling and leg exercise in man, 4) sodium bicarbonate/citrate supplementation affects cycling performance and, 5) herbal supplementation affects cycling performance.

Lactate and Skeletal Muscle Fiber Contractile Function in Animals

Lactate and hydrogen ions are by-products of anaerobic glycolysis, produced in excess when exercise intensity exceeds the aerobic system's ability to provide energy. Once lactate is produced in the cytoplasm of a muscle cell (fiber) it is: 1) released into the blood and used by the liver as a gluconeogenic precursor 2) shuttled to an adjacent muscle fiber and used for immediate energy provision or stored as glycogen, 3) released into the blood and taken up by other skeletal muscle fibers and the heart, or 4) excreted in urine or sweat. The production of hydrogen ions is generally associated with fatigue while lactate itself is considered a fuel. However, some research does indicate that lactate may inhibit muscle function independent of pH in animal fibers.

Research on the effects of lactate concentration on the contractile machinery, and consequently force production, is contentious. Two studies concluded that lactate has no effect on excitation-contraction coupling (22, 79) and may actually promote the maintenance of submaximal and maximal dynamic force and maximal isometric force (49). However, other studies have shown that lactate significantly decreases tetanic force (5, 87) and tension development of animal muscle fibers (28, 40).

On a mechanistic level, numerous studies have examined the influence of lactate concentration on the functionality of animal sarcoplasmic reticulum (SR), a muscle fiber organelle responsible for releasing the calcium necessary for contraction. Andrews et al. found that lactate exposure reduces calcium uptake of the SR which could theoretically decrease calcium availability for contraction for subsequent contractions (6). Lactate has also been shown to inhibit both caffeine-induced calcium release (22, 32) and calcium-induced calcium release by the sarcoplasmic reticulum (32), thus interfering with contractile function. In contrast, others have indicated that lactate either increases calcium-induced calcium release (6) or has no effect on the normal voltage-gated calcium release of the sarcoplasmic reticulum (22) since the physiological mechanism of calcium release is different than artificially induced mechanisms. More research is needed to determine the role of lactate on calcium dynamics of the SR.

Although mechanisms for how lactate may inhibit muscle function have not been identified and lactate's role as a source of fuel is widely accepted, there

is still some evidence suggesting that the lactate anion may contribute to the skeletal muscle fatigue process. However, research regarding the effect of high intracellular lactate on human muscle function *in vitro* is lacking and translating animal findings to human models is speculative, albeit not completely unrealistic.

Table 2.1: Lactate and Skeletal Muscle Fiber Contractile Function in Animals

Author/ Year	Species & Muscle	Design	Findings
Hogan et al. 1995 (40)	Dog Gastroc	<ul style="list-style-type: none"> • Infused with either [La⁺] or placebo <i>in situ</i> • Muscle stimulated via sciatic nerve for two 60-minute period with 45-minute rest in a step-wise fashion 	<ul style="list-style-type: none"> • ↑ [La⁺] ↓ tension development in proportion with a change in muscle oxygen uptake
Andrews et al. 1996 (5)	Rabbit Psoas and Soleus	<ul style="list-style-type: none"> • Chemically skinned SMF bathed in varying [La⁺] concentrations within physiological and supraphysiological range for rabbits and humans 	<ul style="list-style-type: none"> • ↑ [La⁺] ↓ Ca²⁺ activated force in fast-twitch psoas and slow-twitch soleus • In supraphysiological [La⁺] conditions (30-50 mM), force returned to control levels • ↑ [La⁺] ↓ muscle function and force of contractile apparatus, responsible for up to 1/3 of force depression
Favero et al. 1997 (32)	Rabbit Hindleg and Back	<ul style="list-style-type: none"> • SR vesicles extracted • Quantified Ca²⁺ efflux, ryanodine binding, and single Ca²⁺ channel dynamics in varying [La⁺] environments 	<ul style="list-style-type: none"> • ↑ [La⁺] ↓ Ca²⁺ and caffeine induced Ca²⁺ release • ↑ [La⁺] ↓ Ca²⁺-dependent binding of ryanodine to its receptor • ↑ [La⁺] ↓ single-channel caffeine-activated Ca²⁺ release
Andrews and Nosek 1998 (6)	Rat Extensor Digitorum Longus	<ul style="list-style-type: none"> • SMF bathed in varying [La⁺] concentrations • SR loaded and depleted under different [La⁺] conditions 	<ul style="list-style-type: none"> • SR Ca²⁺ uptake ↓ most with 10 mM [La⁺] and less with 20 and 30 mM up to 60 seconds exposure • Ca²⁺ induced Ca²⁺ release ↑ as [La⁺] ↑ at pCa 5 but not pCa > 8.5

Table 2.1: Lactate and Skeletal Muscle Fiber Contractile Function in Animals (continued)

Author/ Year	Species & Muscle	Design	Findings
Spangenburg et al. 1998 (87)	Mouse Extensor Digitorum Longus	<ul style="list-style-type: none"> • Muscle incubated in 10, 20, 30, and 50 mM [La⁺] at 21 and 37 °C • Force output monitored 	<ul style="list-style-type: none"> • ↓ tetanic force at ↑ extracellular [La⁺] levels especially at higher temperature • ↑ [La⁺] ↓ rate of Ca²⁺ release
Dutka and Lamb 2000 (22)	Rat Extensor Digitorum Longus and Toad Iliofibularis	<ul style="list-style-type: none"> • Skinned SMF bathed in varying [La⁺] concentrations • Contractile apparatus, caffeine-induced Ca²⁺ release from SR, and depolarization induced Ca²⁺ release evaluated 	<ul style="list-style-type: none"> • [La⁺] small ↓ on contractile apparatus • [La⁺] ↓ caffeine-induced Ca²⁺ release • [La⁺] ↔ Ca²⁺ release from normal voltage gate mechanism • No real inhibitory effect on excitation-contraction coupling
Posterino and Fryer 2000 (80)	Rat Extensor Digitorum Longus and Soleus	<ul style="list-style-type: none"> • Skinned SMF bathed in varying [La⁺] concentrations • Quantified effect of [La⁺] on contractile function, voltage dependent Ca²⁺ release, and Ca²⁺ leak from SR 	<ul style="list-style-type: none"> • [La⁺] ↓ maximum Ca²⁺ activated force, Ca²⁺ sensitivity • [La⁺] ↑ Ca²⁺ leak from SR • Inhibitors of [La⁺] transport did not alter effect of [La⁺] on excitation-contraction coupling • [La⁺] has small effect on Ca²⁺ release, Ca²⁺ handling, and contractile process
Posterino et al. 2001 (79)	Rat Extensor Digitorum Longus	<ul style="list-style-type: none"> • Skinned SMF bathed in varying [La⁺] concentrations • Measured Ca²⁺ uptake, contractile apparatus function, twitch and tetanic force in presence of [La⁺] 	<ul style="list-style-type: none"> • [La⁺] ↔ Ca²⁺ activation of contractile apparatus, rate of rise or peak twitch and tetanic response, Ca²⁺ release of action potential, and Ca²⁺ uptake of SR • [La⁺] ↔ excitation-contraction coupling

Table 2.1: Lactate and Skeletal Muscle Fiber Contractile Function in Animals (continued)

Author/ Year	Species and Muscle	Design	Findings
Erdogan et al. 2002 (28)	Rat Diaphragm	<ul style="list-style-type: none"> • Muscle strips bathed in 20 mM [La⁺] or placebo for 20 minutes • Muscle stimulated via phrenic nerve • Evaluated resting membrane potential and amplitude of sarcolemma action potential 	<ul style="list-style-type: none"> • [La⁺] ↓ mean tension development • [La⁺] ↔ resting membrane potential or amplitude of sarcolemma action potential
Karelis et al. 2004 (49)	Rat Plantaris	Whole muscle bathed in saline or [La ⁺] solution and stimulated for 60 minutes	<ul style="list-style-type: none"> • [La⁺] infusion preserved submaximal dynamic force • Maximum dynamic and isometric force higher at end of stimulation period with [La⁺] • [La⁺] ↔ muscle glycogen utilization or neuromuscular fatigue

SMF = Single Muscle Fiber, SR = Sarcoplasmic Reticulum, [La⁺] = Lactate Concentration, Ca²⁺ = Calcium, mM = Millimole, ↑ = Increase, ↓ = Decrease, ↔ = No Change

pH and Skeletal Muscle Fiber Contractile Function in Animals

pH is the negative logarithm of hydrogen ions in solution. In other words, pH describes how acidic or basic a solution is. At rest, human skeletal muscle operates optimally at approximately 7.0 pH units. Heavy muscular work (i.e. exercise) disrupts homeostasis by reducing pH. Numerous studies have evaluated whether or not decrements in skeletal muscle pH impact function of various animals *in vivo* and *in vitro*. In 1906, Fletcher and Hopkins were amongst the first to identify that “lactic acid,” or an acidified condition of the muscle, affected the muscular function of amphibians. Since then, many studies have been conducted with various animals, most of which finding that muscle acidification diminishes both muscle contractile velocity (16, 19, 24, 78, 98, 100) and peak isometric force (21, 30, 78, 90). Additionally, the rate of relaxation and force recovery also appears to be impaired in fatigued and acidified skeletal muscle (12, 82, 98). Although acidosis may contribute to fatigue, Nielsen et al. speculated that it might be a protective strategy due to its beneficial contributions to force recovery in the presence of elevated potassium (75). This hypothesis is attractive, but Krisensen et al. found that acidosis is not protective under normal physiologic conditions (55).

A primary weakness of the existing *in vitro* animal data is that experiments are typically performed at non-physiological temperatures which makes interpreting the generalizability of findings difficult. For instance, both Westerblad et al. and Pate et al. found that acidification of animal muscle below

physiological temperatures significantly reduced maximum shortening velocity while at normal temperature shortening velocity is actually slightly increased (78, 98). There is also evidence that induced acidosis during experimentation does not mimic acidification caused by muscle fatigue from stimulation since there is a smaller decrease in stiffness of muscle when acidification is artificially created (24). Thus, methodological limitations make it difficult to apply these findings to *in vivo* human skeletal muscle function.

From a mechanistic standpoint there are many explanations for how lowered pH could induce muscle fatigue in animals. Calcium release from sarcoplasmic reticulum (SR) vesicles has been shown to decrease during acidosis and high-lactate conditions through the Ryanodine calcium channel, a very specific channel in which binding is governed by compounds that stimulate calcium release and inhibition (31, 61). However, other research using whole muscle fibers has shown that pH does not decrease calcium release from the SR, may leak more calcium in an acidotic state, and increase the amount of tetanic calcium available (14, 58). Regardless of calcium availability, lowered pH reduces calcium re-uptake time from the myoplasm creating a longer duration of depolarization-induced response (58) and greatly reduces binding of calcium to troponin C, which inhibits the contractile machinery (14, 21, 30). Finally, an acidified intramuscular condition may affect certain reactions in the glycolytic cascade, which could inhibit function. In mouse and frog muscle, phosphofructokinase (PFK) is very sensitive to pH changes and acidosis reduces

the affinity of PFK for fructose-6-phosphate (4, 93). An abundance of research implicates pH as a cause of fatigue but the exact role of pH is unknown.

Table 2.2: pH and Skeletal Muscle Fiber Contractile Function in Animals

Author/ Year	Subjects	Design	Findings
Fletcher and Hopkins, 1906 (33)	Amphibian Limb	<ul style="list-style-type: none"> Frog limb muscles stimulated electrically in different anaerobic environments (hydrogen and nitrogen) at varying temperatures “Lactic acid” measures and muscle weights taken at different time points 	<ul style="list-style-type: none"> ↑ “lactic acid” levels ↓ muscle function Muscle function ceased at certain pH levels and temperatures
Trivedi and Danforth 1966 (93)	Mouse Gastrocnemius	<ul style="list-style-type: none"> PFK extracted and placed in solution pH altered within physiological range 	<ul style="list-style-type: none"> ↓ pH, ↓ affinity of PFK for F-6-P PFK is very sensitive to pH changes
Donaldson 1977 (21)	Rabbit Soleus and Adductor Magnus	Skinned SMF stimulated in Ca^{2+} buffer solutions of consistent chemical composition at pH 6.5 or 7.0 and 1 mM or 7 mM of Mg^{2+}	<ul style="list-style-type: none"> ↔ Maximum tension at the same Mg^{2+} concentration but ↓ at lower pH Greater ↓ of submaximal force at 1 mM Mg^{2+} in acidotic conditions
Fabiato 1978 (30)	Frog Semitendinosus (skinned)	Skinned SMF stimulated in solutions of varying free Ca^{2+} and pH	<ul style="list-style-type: none"> Small acidosis ↑ Ca^{2+} content of SR and large acidosis ↓ Ca^{2+} content Acidosis ↓ maximum tension production H^+ accumulation affected force production despite Ca^{2+} saturation
Renaud and Mainwood 1985 (82)	Frog Sartorius	Muscles stimulated at different rates (0.2 - 2 trains/second) in various extracellularly buffered solutions	<ul style="list-style-type: none"> ↓ extracellular pH ↓ the rate of force recovery ↔ rate of change from a normal to fatigued state
Spriet 1985 (90)	Rat Gastrocnemius-Plantaris-Soleus Complex (Hindquarter)	<ul style="list-style-type: none"> Whole muscle stimulated in control condition (C), metabolic acidosis (MA), or respiratory acidosis (RA) Bx pre and post perfusion Analyzed for PPO_2, PPCO_2, pH, O_2 saturation of Hb, Hb concentration, plasma HCO_3^-, arterial Hct, ATP, ADP, AMP, CP, and $[\text{La}^+]$ 	<ul style="list-style-type: none"> $[\text{La}^+]$ release greatest in C Muscle Gly utilization and $[\text{La}^+]$ accumulation ↓ in MA and RA ↔ Muscle CP and ATP Acidosis ↓ isometric tension, aerobic, and anaerobic metabolism

Table 2.2: pH and Skeletal Muscle Fiber Contractile Function in Animals (continued)

Author/ Year	Subjects	Design	Findings
Cooke 1987 (16)	Rabbit Psoas	<ul style="list-style-type: none"> • SMF stimulated in buffers of differing pH, temperature, and chemical composition to determine MgATP, NADH, and ATPase activity 	<ul style="list-style-type: none"> • ↓ pH at 3 mM PO₄ ↓ PO₄ by 45%, ↓ maximum velocity of contraction, ↓ ATPase activity by 25-30% • Curve of the force-velocity relationship ↔ with PO₄ or pH changes
Metzger and Fitts 1987 (71)	Rat Diaphragm	<ul style="list-style-type: none"> • Muscle strips bathed in Ringer solution in temperature-controlled chamber • Stimulated to fatigue at high and low frequencies and time intervals • Intracellular pH measured 	<ul style="list-style-type: none"> • ↔ high low frequency groups and recovery of pH • Recovery of pH highly correlated to the recovery of peak tetanic tension • Fatigue from high and low frequency partially due to effects of intracellular acidosis on excitation-contraction coupling
Curtin 1988 (19)	Frog Anterior Tibialis	<ul style="list-style-type: none"> • SMF perfused in Ringer solution of varying pH (6.00 - 7.99) and constant HCO₃⁻ • Intracellular pH and temperature measured 	<ul style="list-style-type: none"> ↑ fiber acidity, ↓ force and heat
Edman 1990 (24)	Frog Anterior Tibialis	<ul style="list-style-type: none"> • SMF placed in standard or pH lowering solution at constant temperatures • Stiffness measured throughout isometric tetanus • SMF fatigued via continual stimulation or chemical solution • Caffeine introduced to determine whether failure of activation of contractile system caused fatigue 	<ul style="list-style-type: none"> • Chemical and mechanical fatigue did not duplicate contractile behavior of fatigued muscle • Smaller stiffness ↓ from artificially lowered pH versus fatigue • Induced acidification ↑ rate of rise of stiffness with ↓ rise of force • Force-producing capability of attached cross bridges ↓ in fatigued condition despite ↔ in activation

Table 2.2: pH and Skeletal Muscle Fiber Contractile Function in Animals (continued)

Author/ Year	Subjects	Design	Findings
Amorena 1990 (4)	Frog Semitendinosus	<ul style="list-style-type: none"> Fiber bundles superfused in acid or control solutions at 22°C Electrodes monitored pH SMF analyzed for ATP, CP, G-6-P, and F-6-P 	Acidosis slowed glycolysis by ↓ conversion of F-6-P to F-1,6-DP and inhibiting PFK enzyme
Lamb 1992 (58)	Skinned Toad Iliofibularis	<ul style="list-style-type: none"> SMF placed in Ca²⁺, Na, K⁺ solutions and stimulated chemically in activating solutions to elicit depolarization-induced response Ca²⁺ uptake and leakage from the SR at different pH evaluated by bathing SMF in different pH solutions and depolarizing at normal pH 	<ul style="list-style-type: none"> ↓ pH, SR can not uptake Ca²⁺ as rapidly from myoplasm creating longer duration of depolarization-induced response Acidosis ↑ Ca²⁺ leak from SR and releases less calcium with depolarization ↓ pH has little effect on depolarization-induced Ca²⁺ release
Favero 1995 (31)	Rabbit Hindleg and Back White Skeletal	<ul style="list-style-type: none"> Extracted SR vesicles placed in buffer solution at pH 7.1-6.5 Ca²⁺ efflux measured via effluxing agent and dual-wavelength spectrophotometer To measure [H] Ryanodine binding, SR membranes incubated in chemical medium, introduced to various channel-modifiers, placed in scintillation cocktail, shaken overnight, and analyzed 	<ul style="list-style-type: none"> Decreasing pH ↓ [H] Ryanodine binding regardless of other experimental conditions [La³⁺] is strong inhibitor of [H]Ryanodine binding
Pate 1995 (78)	Rabbit Psoas	<ul style="list-style-type: none"> Skinned SMF buffered to pH of 7.0 or 6.2 SMF stimulated at either 30°C (physiological condition) or 10°C 	<ul style="list-style-type: none"> At 10°C in acidified condition, maximum velocity of shortening and isometric tension significantly ↓ At 30°C, maximum velocity of shortening slightly ↑ while isometric tension still ↓ while acidified

Table 2.2: pH and Skeletal Muscle Fiber Contractile Function in Animals (continued)

Author/ Year	Subjects	Design	Findings
Wiseman 1996 (100)	Mouse Hindlimb and Rabbit Psoas	<ul style="list-style-type: none"> • Whole mouse extensor digitorum longus used to measure pH changes during stimulation and rabbit psoas SMF used to control proton concentration and manipulate temperature • Whole muscle and SMF bathed in normocapnic or hypercapnic solution, stimulated electrically at 13 to 25°C 	<ul style="list-style-type: none"> • Hypercapnia ↓ pH at 15 and 25°C but force inhibition greater at lower temperatures in mouse muscle • ↔ Temperature-dependent inhibition of force in individual rabbit fibers at maximum Ca²⁺ activation • Temperature affects inhibitory action of pH on muscle function
Westerblad 1997 (98)	Mouse Flexor Brevis	<ul style="list-style-type: none"> • SMF perfused in Tyrode control, acidifying, or alkalinizing solution • Muscles stimulated at either 12, 22, or 32°C (all below physiological temperature) • Fluorescent pH indicator SNARF-1 used to measure pH 	<ul style="list-style-type: none"> • In acidified fibers, force ↓ more at lower temperatures than at 32°C • Acidification slowed relaxation but mitigated by ↑ temperature • Shortening velocity ↓ with acidification and low temperature but ↔ at 32°C
Bruton 1998 (12)	Rat Flexor Brevis	<ul style="list-style-type: none"> • SMF perfused in 30% CO₂ environment at 28°C (acidified condition) • SMF stimulated until force declined to 40% of control's force production • pH analyzed via fluorescent probe 	<ul style="list-style-type: none"> • Intracellular acidification ↔ tetanic force production or number of stimulations required to induce fatigue • Low pH ↓ relaxation rate of muscle
Chin 1998 (14)	Mouse Flexor Brevis	<ul style="list-style-type: none"> • SMF perfused in solution designed to determine intensity dependence of pH and pH dependent mechanisms of fatigue • Muscle stimulated at different intensities to induce fatigue 	<ul style="list-style-type: none"> • Ca²⁺ release by SR ↑ with ↑ acidosis and fatigue • Ca²⁺ sensitivity of contractile proteins ↓ with large acidosis • Ca²⁺ release failure does not require nor is augmented by acidosis

Table 2.2: pH and Skeletal Muscle Fiber Contractile Function in Animals (continued)

Author/ Year	Subjects	Design	Findings
Laver 2000 (61)	Rabbit Skeletal	<ul style="list-style-type: none"> • Extracted SR vesicles incorporated into lipid bilayer membrane to study Ryanodine (RyR) receptor Ca^{2+} release channels • pH altered and cytoplasmic and luminal pH studied in the pH range of 5-8 	Acidic cytoplasmic pH ↓ RyR activity while luminal pH inhibited at much lower pH values (5-5.5)
Nielsen 2001 (75)	Rat Soleus	<ul style="list-style-type: none"> • Whole muscle incubated in K^+ solution and affixed to force transducer • Muscles stimulated through motor nerve and analyzed for membrane potential, Ca^{2+} influx, Ca^{2+} content, Na^+/K^+ pump activity, and intracellular pH • Lactic acid added to isolated muscle whose force had been depressed by elevated K^+ 	<ul style="list-style-type: none"> • Addition of lactic acid recovered force more quickly and prevented reduction in force when introduced with K^+ • Acidification may be protective during high-intensity exercise when extracellular K^+ accumulates to potentially inhibitory levels
Kristensen 2005 (55)	Rat Soleus	<ul style="list-style-type: none"> • Isolated muscle placed in lactate, high K^+, or bicarbonate-free medium and stimulated electrically • Role of pH on K^+ uptake rate observed 	<ul style="list-style-type: none"> • Study performed in response to Nielsen et al. • Lactate and lactic acid incubation lead to force recovery in K^+ depressed <i>in vitro</i> muscle • <i>In vivo</i> mechanism is not as such and lactate/lactic acid is not protective

PFK = Phosphofruktokinase, F-6-P = Fructose-6-Phosphate, G-6-P = Glucose 6 Phosphate, Mg^{2+} = Magnesium, mM = Millimole, Ca^{2+} = Calcium, Na^+ = Sodium, K^+ = Potassium, SR = Sarcoplasmic Reticulum, H⁺ = Hydrogen, Bx = Biopsy, PPO₂ = Partial Pressure of Oxygen, PPCO₂ = Partial Pressure of Carbon Dioxide, O₂ = Oxygen, Hb = Hemoglobin, HCO₃³⁻ = Bicarbonate, Hct = Hematocrit, ATP = Adenosine Triphosphate, ADP = Adenosine Monophosphate, AMP = Adenosine Monophosphate, Lac = Lactate, CP = Creatine Phosphate, Gly = Glycogen, NADH = Nicotinamide Adenine Dinucleotide, PO₄ = Phosphate, SMF = Single Muscle Fiber, F-1,6-DP = Fructose-1,6-Diphosphate, ↑ = Increase, ↓ = Decrease, ↔ = No Change

The Impact of pH and Lactate on Cycling, Leg Kicking, and Isometric Exercise in Man

As early as 1933 it was observed that a fatigued state coincided with lactic acid accumulation from muscular work in man (63). Since then, the relationship between intensity and blood and skeletal muscle pH during high-intensity exercise, specifically cycling exercise, has been well documented (36, 38, 48, 53, 83, 85). When pH declines and lactate increases in working skeletal muscle and blood during heavy exercise, performance starts declining and fatigue ultimately forces retirement.

Although the majority of mechanistic research regarding pH and muscle function has been performed with animals, a few studies have evaluated the effects of lowered pH on human muscle fibers *in vitro*. For example, a reduction in pH is detrimental to glycolysis during exercise (46, 53), possibly by inhibiting adenylylase and phosphorylase *b* kinase (13) or glycogen phosphorylase (89), necessary enzymes in the glycolytic cascade. Messonnier et al. showed that higher concentrations of MCT1 and MCT4, hydrogen transport proteins in skeletal muscle responsible for maintaining acid-base homeostasis, improves cycling performance, thus demonstrating a clear link between pH regulation and fatigue (70). However, Sahlin et al. reported that force recovers much more quickly than pH after exhausting leg exercise, implying that pH is not the sole source of anaerobic muscle fatigue (84).

As previously detailed, lactate is created as a by-product of anaerobic glycolysis during intense exercise. Even though lactate itself is a fuel and is not

considered a direct cause of muscle fatigue, measuring blood lactate serves as a good indicator of the myocellular environment during exercise. Hydrogen ions are shuttled to the blood via the same carrier as lactate (MCT4) which makes lactate an indirect measure of pH. Further, various studies collectively indicate that lactate is associated with fatigue during high-intensity exercise (2, 9, 41, 50, 97). However, McCartney et al. documented a smaller lactate accumulation following exercise with induced acidosis (65). Although it should be emphasized that associations do not equal causality, the general relationship between muscle and blood lactate and pH and fatigue suggests that changes in blood and muscle pH are likely two factors of many that contribute to fatigue during high-intensity exercise.

Table 2.3: The Impact of pH and Lactate on Cycling, Leg Kicking, and Isometric Exercise in Man

Author/ Year	Subjects	Design	Analyses	Findings
Margarita et al. 1933 (63)	1 healthy male subject	<ul style="list-style-type: none"> • Subjects ran at different intensities on different days • Exercise BS and EG 	BS analyzed for “lactic acid” accumulation	<ul style="list-style-type: none"> ↑ exercise intensity, ↑ “lactic acid” in blood until fatigue
Hermansen and Osnes 1972 (38)	2 female and 11 male subjects	<ul style="list-style-type: none"> • On bicycle ergometer or treadmill, 40-60 sec x 5 bouts, 4 minutes REC between • BS and quadriceps Bx post-exercise 	BS and Bx analyzed for pH	Blood and muscle pH ↓ during continuous and intermittent exercise
Klausen 1972 (53)	4 healthy males	<ul style="list-style-type: none"> • Supramaximal VO₂ test on a cycle ergometer (T1) • On separate day, subjects performed test again preceded by 5 minutes of heavy arm exercise to elevate blood [La⁺] (T2) • Exercise BS and EG 	<ul style="list-style-type: none"> • Blood lactate and VO_{2max} evaluated • Endurance time was the performance criterion 	<ul style="list-style-type: none"> • ↓ blood pH and ↑ peak lactate with T2 • ↔ Maximal aerobic power but tendency towards ↓ endurance time • ↓ Anaerobic glycolysis in T2
Karlsson 1975 (50)	3 male subjects	<ul style="list-style-type: none"> • 2 trials separated by 5-7 days: Exhaustive leg cycling followed by exhaustive arm cycling and vice versa • Deltoid and VL Bx immediately and 5 min after exercise • BS pre, during, and post exercise • Exercise EG 	<ul style="list-style-type: none"> • Bx analyzed for ATP, CP, pyruvate, and Lac • TTE was performance criterion 	• Performance ↓ in following bout of exercise after rest

Table 2.3: The Impact of pH and Lactate on Cycling, Leg Kicking, and Isometric Exercise in Man (continued)

Author/ Year	Subjects	Design	Analyses	Findings
Sahlin 1976 (85)	16 males and 3 women aged 20-29 years	<ul style="list-style-type: none"> • Subjects exercised until exhaustion or at a % of VO_{2max} • VL Bx of 12 subjects post-exercise • Experiment conducted twice, one week apart • Exercise BS 	<ul style="list-style-type: none"> • BS analyzed for $[La^+]$ and pH • Bx analyzed for pH 	<ul style="list-style-type: none"> • Muscle pH ↓ from 7.08 to 6.60 post-exercise and linearly related to muscle $[La^+]$ and pyruvate content • Muscle $[La^+]$ ↑ post-exercise and ↓ exponentially during recovery while pyruvate ↑
Harris 1977 (37)	53 healthy males between 18-33 years	<ul style="list-style-type: none"> • Subjects performed isometric quadriceps exercise or 6 minutes cycling • Medial quadriceps Bx of both groups at various time points 	<ul style="list-style-type: none"> • Bx analyzed for ATP, ADP, AMP, CP, pyruvate, and $[La^+]$ 	<ul style="list-style-type: none"> • ATP:ADP ratio ↓ linearly with $[La^+]$ • CP showed nonlinear relationship to $[La^+]$
Jones 1977 (48)	5 healthy males	<ul style="list-style-type: none"> • 3 trials: 0.3 g/kg placebo, NH_4Cl (acidosis), and $NaHCO_3$ • Subjects supplemented, rested 3 hours, and cycled at 33 and 66% VO_{2max} for 20 minutes and rode to exhaustion at 95% VO_{2max} • BS pre, during, and post exercise • EG measured during exercise 	<ul style="list-style-type: none"> • BS analyzed for $[La^+]$, glucose, glycerol, FFA, pH, PPO_2, $PPCO_2$, and 2,3-DPG • Endurance time was performance criterion 	<ul style="list-style-type: none"> • $[La^+]$ and endurance time ↑ with $NaHCO_3$ and ↓ with NH_4Cl

Table 2.3: The Impact of pH and Lactate on Cycling, Leg Kicking, and Isometric Exercise in Man (continued)

Author/ Year	Subjects	Design	Analyses	Findings
Sahlin 1978 (83)	8 males aged 23-31 years	<ul style="list-style-type: none"> • Cycling at pace selected to cause fatigue within 6 minutes • BS pre, during, and post exercise • Medial quadriceps Bx post exercise 	<ul style="list-style-type: none"> • BS analyzed for [La⁺], pyruvate, protein, electrolytes, and acid-base parameters • Bx analyzed for water, electrolytes, [La⁺], and acid-labile CO₂ 	<ul style="list-style-type: none"> • pH ↓ from 7.00 to 6.4 post exercise • pH almost fully recovered after 20 minutes rest and HCO₃⁻ still significantly ↓
Weltman 1979 (97)	9 males	<ul style="list-style-type: none"> • 4 trials, 5 minutes maximal cycling at variable work rate (T1) • Passive recovery, active recovery below anaerobic threshold, active recovery above anaerobic threshold, or active recovery above anaerobic threshold while breathing 100% oxygen • 5 minutes maximal cycling (T2) • Recovery BS 	<ul style="list-style-type: none"> • BS analyzed for [La⁺] • Work output was performance criterion 	Type of recovery affected [La ⁺] levels but [La ⁺] ↔ work output during subsequent exercise bouts
Adams 1980 (2)	6 males	<ul style="list-style-type: none"> • 3 trials: 17, 21, or 60% O₂ following equilibration period • 10 minutes cycling at 55% VO_{2max} and ride to exhaustion at 90% • BS pre, during, and post exercise. • EG pre and during exercise 	<ul style="list-style-type: none"> • BS analyzed for PPO₂, PPCO₂, [La⁺], and pH • TTE was performance criterion 	Hypoxia ↑ [La ⁺] and ↓ pH, PPCO ₂ , and HCO ₃ ⁻ , and TTE

Table 2.3: The Impact of pH and Lactate on Cycling, Leg Kicking, and Isometric Exercise in Man (continued)

Author/ Year	Subjects	Design	Analyses	Findings
Chasiotis 1983 (13)	3 healthy male subjects age 21-19 years	<ul style="list-style-type: none"> • Experiment A: subjects' leg was occluded for 50-60 s and Bx after adrenaline infusion • Experiment B: isometric contraction after leg occlusion and Bx after adrenaline infusion 	<ul style="list-style-type: none"> • Bx pH determined via homogenization and micro-electrode • Bx analyzed for metabolites, cyclic AMP, enzyme activity, phosphorylase, synthetase, G-6-P, G-1-P, F-6-P, and Lac 	<ul style="list-style-type: none"> • Cyclic AMP and phosphorylase both ↓ in acidotic conditions • Acidic inhibition of adenylcyclase and phosphorylase <i>b</i> kinase
Hogan 1984 (41)	6 healthy males	<ul style="list-style-type: none"> • 3 trials 1 week apart • 5 minutes at 95% VO_{2max}, 4 minute rest period, and ride to exhaustion at 90% VO_{2max}. • Trials differed by varying percent oxygen inhalation (16% O₂, 21% O₂, or 60% O₂) during 5 minutes exercise to elicit different blood [La⁺] levels • Exercise BS and EG 	<ul style="list-style-type: none"> • BS analyzed for [La⁺], pH and PCO₂ • Total power output during the 5 minutes at 95% and time to exhaustion were the performance criterion 	<ul style="list-style-type: none"> • Performance time significantly ↑ for hyperoxic condition versus hypoxic • H⁺ and [La⁺] ↔ at exhaustion • [La⁺] significantly ↑ after 5 minutes of exercise in hypoxic condition
Sahlin and Ren 1989 (84)	16 healthy males	<ul style="list-style-type: none"> • Isometric quadriceps contraction at 66% MVC until force declined • Force recovery measured by performing short contractions at different time intervals during REC • Endurance measured by timing initial contraction • Bx after initial contraction 	<ul style="list-style-type: none"> • Bx analyzed for NADH, IMP, ATP, ADP, AMP, CP, [La⁺], and G-6-P • Contraction endurance time was performance criterion 	<ul style="list-style-type: none"> • ↔ force with low pH but endurance ↓ • Force restored more quickly than endurance and pH • pH impaired capacity to re-phosphorylate ADP, inhibiting the ATP-generating process

Table 2.3: The Impact of pH and Lactate on Cycling, Leg Kicking, and Isometric Exercise in Man (continued)

Author/ Year	Subjects	Design	Analyses	Findings
Spriet 1989 (89)	7 men and 1 woman	<ul style="list-style-type: none"> • 3 30-second maximal isokinetic cycling bouts at 100 rpm separated by 4 minutes rest • VL Bx between bouts 2 and 3 	<ul style="list-style-type: none"> • Bx analyzed for glycogen, CP, ATP, AMP, Glucose, G-6-P, F-6-P, G-1-P, F-1,6-P2, Glyc-3-P, pyruvate, [La⁺], and pH • Maximal average power was performance criterion 	<ul style="list-style-type: none"> • Work in bout 3 ↓ to 82% of work from bout 2 while glycogenolysis in bout 3 was 32% of bout 2 • Glycogen phosphorylase possibly downregulated by H⁺ accumulation in bout 3
Bangsbo 1996 (9)	7 healthy college-aged male students	<ul style="list-style-type: none"> • One-legged quadriceps-specific supine ergometer exercise for 10 minutes at 10 W • 33 minutes rest (C) or intense arm exercise (HL) • Exhaustive knee-extensor exercise with experimental leg • Arm exercise used to elevate blood [La⁺] levels before exhaustive kicking exercise • Bx pre and post for each condition 	<ul style="list-style-type: none"> • Kick frequency > 55 rpm was performance criterion • Blood flow measured via thermodilution and analyzed for O₂ saturation, Hb concentration, Hct, and plasma K⁺ • Bx analyzed for [La⁺], glycogen, and CP 	<ul style="list-style-type: none"> • HL ↓ duration of exercise • ↔ Muscle [La⁺] concentration and rate of accumulation after exercise in both conditions • ↑ muscle H⁺ concentration after exercise in HL • Muscle fatigue occurred at ↓ pH but ↔ femoral blood K⁺ content in HL condition • ↓ pH ↔ rates of glycogen breakdown or [La⁺] production

Table 2.3: The Impact of pH and Lactate on Cycling, Leg Kicking, and Isometric Exercise in Man (continued)

Author/ Year	Subjects	Design	Analyses	Findings
Hargreaves 1998 (36)	6 male subjects	<ul style="list-style-type: none"> • 3 30-second maximal cycling bouts, 4 minute passive recovery between, 30 minutes exercise at 30-35% peak oxygen consumption and 60 minutes rest prior to bout 4 • Bx pre-exercise and after third and fourth bouts, BS throughout 	<ul style="list-style-type: none"> • BS analyzed for Hb concentration, Hct, plasma H⁺, [La⁺], and K⁺ • Bx analyzed for peak SR Ca²⁺ uptake, pH, and metabolites 	<p>Bout 3 and 4 performance ↓ not result of ↓ muscle glycogen but ↓ CP availability, ↑ H⁺ concentration, or impairment of SR function</p>
Messonnier 2007 (70)	3 healthy sedentary women and 5 men	<ul style="list-style-type: none"> • Work Capacity Event: 2 trials 3 days apart • Ingest sodium citrate (E) or lactose (C) and exercise at 120% W_{max} until exhaustion • BS post exercise • Standardized Event: Same as above except fixed-time so work rate and duration are controlled • VL Bx pre and post • BS post-exercise 	<ul style="list-style-type: none"> • BS analyzed for [La⁺] and pH • Bx analyzed for [La⁺], pH, fiber type, MCT1, MCT4, NHE1 isoform, and membrane-bound carbonic anhydrase isoforms • TTE was the performance criterion in work capacity event 	<ul style="list-style-type: none"> • Performance and work capacity closely related to concentrations of pH/[La⁺] regulating proteins (MCT1, MCT4, NHE1, and carbonic anhydrase) • Those who benefitted most in E condition were those with ↑ content of pH/[La⁺] regulating proteins

Table 2.3: The Impact of pH and Lactate on Cycling, Leg Kicking, and Isometric Exercise in Man (continued)

Author/Year	Subjects	Design	Analyses	Findings
Iaia 2010 (46)	7 healthy males	<ul style="list-style-type: none"> • Exhaustive cycle exercise preceded by a warm-up, very high (30 seconds), high (3 minutes), and low (2 hours) intensity cycling • Post 2 minutes recovery, subjects rode to exhaustion at 130% VO_{2max} • BS pre, during, and post exercise • VL Bx before and after both exhaustive bouts 	<ul style="list-style-type: none"> • BS analyzed for plasma ammonia, glucose, [La⁺], and FFA • Bx analyzed for lactate, CP, glycogen, and pH 	Muscle glycogen and pH do not control but affect the rate of glycogenolysis/ glycolysis and fatigue during high intensity exercise lasting less than 2 minutes

s = Seconds, REC = Recovery, BS = Blood Sample, Bx = Biopsy, EG = Expired Gasses, VL = Vastus Lateralis, [La⁺] = Lactate concentration, ATP = Adenosine Triphosphate, ADP = Adenosine Monophosphate, AMP = Adenosine Monophosphate, CP = Creatine Phosphate, HCO³⁻ = Bicarbonate, F-6-P = Fructose-6-Phosphate, G-6-P = Glucose-6-Phosphate, G-1-P = Glucose-6-Phosphate, H⁺ = Hydrogen, MVC = Maximal Voluntary Contraction, F-1,6-P₂ = Fructose-1,6-Biphosphate, Glyc-3-P = Glycerol-3-Phosphate, K⁺ = Potassium, HR = Heart Rate, MCT1 = Monocarboxylate Transporter 1, MCT4 = Monocarboxylate Transporter 4, NHE1 = Na/H⁺ Exchanger 1, FFA = Free Fatty Acid, 2,3-DPG = 2,3-Diphosphoglycerate, TTE = Time to Exhaustion, ↑ = Increase, ↓ = Decrease, ↔ = No Change

Sodium Bicarbonate/Citrate Supplementation and Cycling Performance

The independent effects of sodium bicarbonate and citrate supplementation on cycling exercise of various durations and intensities are well-documented. A number of studies have shown that both bicarbonate and citrate improve performance during high-intensity, short duration, interval type cycling (11, 18, 47, 60, 62, 66, 67, 69, 77), aerobic cycling (≥ 30 minutes) (68, 72, 92), and intermittent cycling (81). However, the literature is not unanimous as others have shown no benefits of supplementation during high-intensity (44, 51, 54, 94, 99) and long-duration cycling (86, 91).

Bicarbonate is a prominent extracellular buffer in the blood and ingesting either sodium bicarbonate or citrate elevates blood bicarbonate levels. Bishop et al. suggests that improved performance with bicarbonate supplementation is likely the result of increased extracellular buffering causing greater efflux of hydrogen from muscle to blood (11). Since intracellular hydrogen is suspected to play a role in muscle fatigue, this efflux of hydrogen would be beneficial during intense anaerobic exercise. McNaughton et al. also illustrated that sodium bicarbonate supplementation during intense aerobic cycling of one hour yielded a performance benefit, citing an alteration in extracellular buffering capacity similar to Bishop et al.'s postulation (68). Despite its impact on blood and muscle pH (18, 86, 91, 99), blood and muscle lactate (42, 92), as well as performance (11, 47, 60, 66, 67, 72, 77), bicarbonate is not generally used as an ergogenic aid because of gastric discomfort often resulting in urgent diarrhea (35, 91) or possible lightheadedness

(54). However, the fact that cycling performance is improved by altering blood and muscle pH via artificial augmentation of buffering capacity provides compelling evidence that pH has a role in muscular fatigue during heavy exercise.

Table 2.4: Bicarbonate/Citrate Supplementation and Cycling Performance

Author/ Year	Subjects	Design	Analyses/ Performance Criterion	Findings
Sutton 1981 (92)	5 males	<ul style="list-style-type: none"> • 3 trials: placebo, NH₄Cl (acidosis), or NaHCO₃ (alkalosis) • Cycle for 20 minutes at 33% VO_{2max}, 20 minutes at 66% VO_{2max}, then 95% VO_{2max} to exhaustion • Exercise BS and EG 	TTE was performance criterion	<ul style="list-style-type: none"> • Endurance longest in alkalotic condition and shortest in acidotic condition • [La⁺] ↑ most from rest to exhaustion with alkalosis and least with acidosis • Plasma [La⁺] least with acidosis implying inhibition of glycolysis and ↓ [La⁺] efflux
Inbar 1983 (47)	13 males	<ul style="list-style-type: none"> • 2 Trials: 10 grams NaHCO₃ or placebo • 6 minutes cycling, 4-6 second sprints at 400 watts every 2 minutes, 30 seconds all-out at 4.41 J/pedal revolution/kg • BS pre and post exercise • VL Bx pre-exercise 	<ul style="list-style-type: none"> • BS analyzed for pH and [La⁺], fiber type • Mean and peak power output were the performance criterion 	<ul style="list-style-type: none"> • Mean power output significantly ↑ with NaHCO₃ • ↔ Peak power output • No relationship between fiber type distribution and NaHCO₃ sensitivity
Costill 1984 (18)	2 men and 1 woman	<ul style="list-style-type: none"> • NaHCO₃ (E) or NaCl (C) an hour before cycling • 4 1-minute bouts, ride to exhaustion at 100% VO_{2max} • BS and EG throughout exercise and recovery • BL Bx before ingestion, first bout, and final bout 	<ul style="list-style-type: none"> • BS analyzed for lactic acid, pH, PCO₂, and blood HCO₃ • Bx analyzed for lactic acid and pH • TTE in final bout was performance criterion 	<ul style="list-style-type: none"> • During and following exercise, blood pH and HCO₃ ↑ with E • NaHCO₃ ↑ Performance

Table 2.4: Bicarbonate/Citrate Supplementation and Cycling Performance (continued)

Author/Year	Subjects	Design	Analyses/ Performance Criterion	Findings
Katz 1984 (51)	8 males	<ul style="list-style-type: none"> • 3 trials: 0.2 g/kg NaHCO₃ or placebo • Ride to exhaustion at 125% VO_{2max} • BS pre, during, and post exercise • Recovery EG 	<ul style="list-style-type: none"> • BS analyzed for [La⁺], pH, and pCO₂ • TTE was the performance criterion 	<ul style="list-style-type: none"> • [La⁺] efflux ↑ with bicarbonate ingestion • NaHCO₃ ↔ performance but may benefit following repeated work bouts
Wijnen 1984 (99)	4 well-trained males	<ul style="list-style-type: none"> • 4 conditions: Injection of either 0.18, 0.36 g/kg NaHCO₃ or placebo • 4 1-minute bouts at 125% VO_{2max} separated by 1 minute rest and a fifth bout to exhaustion • BS pre, during, and post exercise • Exercise EG 	<ul style="list-style-type: none"> • BS analyzed for pH, base excess, HCO₃⁻, and [La⁺] • TTE was performance criterion 	<ul style="list-style-type: none"> • ↑ pH proportional to NaHCO₃ administration • Inconclusive whether NaHCO₃ improved performance
McKenzie 1986 (66)	6 males	<ul style="list-style-type: none"> • 3 Conditions: placebo, 0.15, or 0.30 g/kg of NaHCO₃ • 5 bouts of 60 seconds at 125% VO_{2max} and a sixth bout to exhaustion • BS pre exercise 	<ul style="list-style-type: none"> • BS analyzed for pH and HCO₃⁻ • Work production and TTE were performance criterion 	<ul style="list-style-type: none"> • Work in kJ and TTE significantly ↑ in both NaHCO₃ conditions • ↔ between bicarbonate conditions
Parry-Billings 1986 (77)	6 males	<ul style="list-style-type: none"> • 4 conditions: sodium bicarbonate, sodium bicarbonate and sodium citrate, sodium citrate, and placebo • 3 30 s Wingate tests separated by 6 min recovery • BS pre, during, post exercise 	<ul style="list-style-type: none"> • BS analyzed for pH, [La⁺], and PPCO₂ • Mean peak power output were the performance criterion 	<ul style="list-style-type: none"> • Bicarbonate ↑ total work performed and performance in subsequent bouts

Table 2.4: Bicarbonate/Citrate Supplementation and Cycling Performance (continued)

Author/Year	Subjects	Design	Analyses/ Performance Criterion	Findings
Horswill 1988 (44)	9 endurance trained male cyclists	<ul style="list-style-type: none"> • 4 Trials: placebo, 0.10 , 0.15, or 0.20 g/kg of NaHCO₃ • Cycle sprint for 2 minutes • BS pre and post ingestion, post exercise 	<ul style="list-style-type: none"> • BS analyzed for lactic acid, pH, PCO₂, and PO₂ • Total work output generated was performance criterion 	<ul style="list-style-type: none"> • ↑ in post blood bicarbonate related to dosing • This dosing scheme yielded ↔ performance during 2 minutes cycling
Lavender and Bird 1989 (60)	8 females and 15 males	<ul style="list-style-type: none"> • 6 trials: 3 placebo and 3 300 mg/kg NaHCO₃ • 10 10-second cycle sprints with 50 seconds rest between 	Peak power and average power output measured using light-sensitive monitor of the flywheel	<ul style="list-style-type: none"> • NaHCO₃ ↑ average power and peak power output • Difference in average power ↑ as sprint repetitions ↑
Mitchell 1990 (72)	8 lean men	<ul style="list-style-type: none"> • 2 Conditions: HCO₃ or placebo infusion prior to exercise • Ride to exhaustion at 80% VO_{2max} • Exercise BS and EG 	<ul style="list-style-type: none"> • BS analyzed for [La⁺], pH, FFA, glycerol, alanine, insulin, norepinephrine, and epinephrine • TTE was performance criterion 	<ul style="list-style-type: none"> • Endurance ↑ with HCO₃ • Plasma glucose ↑ at exhaustion in control and [La⁺] ↑ in HCO₃ condition • Performance ↑ not explained by acid-base change
McNaughton 1991 (67)	8 trained male cyclists	<ul style="list-style-type: none"> • 3 Trials: control, placebo, and CaHCO₃ • One minute all-out sprint on a bicycle ergometer • BS pre and post 	<ul style="list-style-type: none"> • BS analyzed for [La⁺], pH, HCO₃, PPO₂, and PPO₂ • Work performed in the minute and peak power were the performance criterion 	<ul style="list-style-type: none"> • CaHCO₃ ↑ total work and maximal power output • Post-exercise Lac ↑ with bicarbonate

Table 2.4: Bicarbonate/Citrate Supplementation and Cycling Performance (continued)

Author/ Year	Subjects	Design	Analyses/ Performance Criterion	Findings
McNaughton 1992 (69)	24 trained males separated evenly into 3 groups	<ul style="list-style-type: none"> • 3 trials: control, placebo, or NaHCO₃ • 10, 30, 120, or 240 seconds of all-out cycling • BS pre and post 	<ul style="list-style-type: none"> • BS analyzed for PPO₂, pH, HCO₃⁻, and [La⁺] • Total amount of work and peak power were the performance criterion 	<ul style="list-style-type: none"> • NaHCO₃ ↑ blood HCO₃ post exercise but ↔ performance at 10 or 30 s • At 120 and 240 s, performance ↑ compared to control/ placebo
Kozak- Collins 1994 (54)	7 competitive female cyclists	<ul style="list-style-type: none"> • 2 conditions: placebo or 300 mg/kg of NaHCO₃ • 1 minute intervals at 95% VO_{2max} until exhaustion • BS pre, during, and post exercise • Exercise EG 	<ul style="list-style-type: none"> • BS analyzed for PPO₂, PPCO₂, and pH • Intervals completed and total time during intervals were performance criterion 	<ul style="list-style-type: none"> • ↔ Intervals completed and total time during intervals • NaHCO₃ ↔ performance • NaHCO₃ caused lightheadedness in 4 subjects
Verbitsky 1997 (95)	6 healthy males	<ul style="list-style-type: none"> • 3 trials: 3 minutes of cycling at VO_{2max}, VO_{2max} + 17%, or VO_{2max} + 17% and ingestion of 400 mg/kg of NaHCO₃ • Functional electrical stimulation test pre and post exercise to determine quadriceps fatigue • Exercise EG 	Peak torque was performance criterion	Peak torque ↑ with NaHCO ₃ ingestion

Table 2.4: Bicarbonate/Citrate Supplementation and Cycling Performance (continued)

Author/ Year	Subjects	Design	Analyses/ Performance Criterion	Findings
Linossier 1997 (62)	3 moderately active college-aged women and 5 men	<ul style="list-style-type: none"> • 5 trials: VO₂peak test, 2 exhausting rides at 120% VO₂peak after sodium citrate or placebo ingestion, 2 trials at same relative intensity as previous trials minus 20 seconds • BS throughout all trials and VL Bx before 4th and 5th trials 	<ul style="list-style-type: none"> • BS analyzed for pH and [La⁺] • Bx analyzed for CP, ATP, G-6-P, [La⁺], and citrate • TTE was the performance criterion. 	Sodium citrate ingestion ↑ TTE during supramaximal exercise but is not associated with improved muscle metabolic state
McNaughton 1999 (68)	10 trained male cyclists	<ul style="list-style-type: none"> • 3 trials: control, placebo, or NaHCO₃ • 60 minutes cycling • BS pre, during and post exercise 	<ul style="list-style-type: none"> • BS analyzed for pH, HCO₃, PPO₂, PPCO₂, and [La⁺] • Amount of work, peak power, and power:mass ratio were the performance criterion 	<ul style="list-style-type: none"> • Amount of work ↑ in NaHCO₃ trial, but peak power and power:mass ratio ↔
Hollidge-Horvat 2000 (42)	8 healthy males	<ul style="list-style-type: none"> • 2 trials separated by 2-3 weeks: 0.3 g/kg of NaHCO₃ or placebo • 15 minutes at 30, 60, and 75% VO₂max • BS and EG throughout exercise and leg blood flow measured via thermodilution • 5 VL Bx at rest, post-ingestion, and 3 during exercise 	<p>Bx analyzed for pyruvate dehydrogenase, glycogen phosphorylase (Phos) activity, acetyl-CoA, free CoASH, total CoA, acetlycarnitine, free carnitine, total carnitine, ATP, pyruvate, [La⁺], CP, glucose, G-6-P, G-1-P, F-6-P, and Gly-3-P. BS analyzed for O₂, CO₂, [La⁺], glucose, FFA, and HCO₃</p>	<ul style="list-style-type: none"> • Induced alkalosis ↑ glycolysis at Phos level relative to maximal PDH activation, creating discrepancy between pyruvate production and oxidation • ↑ [La⁺] production

Table 2.4: Bicarbonate/Citrate Supplementation and Cycling Performance (continued)

Author/ Year	Subjects	Design	Analyses/ Performance Criterion	Findings
Schabert 2000 (86)	8 male cyclists	<ul style="list-style-type: none"> • 4 trials 3-7 days apart: 40 km time trials with interspersed sprinting • Placebo and 3 different sodium citrate doses (0.2 g/kg, 0.4 g/kg, 0.6 g/kg) • Exercise BS 	<ul style="list-style-type: none"> • BS analyzed for [La⁺], glucose, pH, and PPCO₂ • Time to completion and sprint performance were performance criterion 	Despite influencing blood pH, no dosage of sodium citrate improved time trial performance, sprint performance, or average power output
Stephens 2002 (91)	7 endurance trained men	<ul style="list-style-type: none"> • 2 trials: 0.3 g/kg NaHCO₃ or placebo • 30 minutes cycling at 77 +/- 1% VO_{2peak} followed by ~30 min at ~80% VO_{2peak} • BS pre, during, and post exercise • Bx pre and post 	<ul style="list-style-type: none"> • BS analyzed for pH, gas partial pressure, [La⁺], Na, K⁺, and Cl • Bx analyzed for glycogen, [La⁺], ATP, CP, and H⁺ • Performance time and average power output were performance criterion 	<ul style="list-style-type: none"> • NaHCO₃ caused small but significant ↓ in muscle pH maintained through exercise • Induced metabolic alkalosis ↔ prolonged cycling performance • NaHCO₃ did not detriment muscle glycogen utilization, muscle [La⁺] accumulation, or CP degradation • 2 subject defecated urgently as result of bicarbonate
Price 2003 (81)	8 healthy males	<ul style="list-style-type: none"> • 2 trials: NaHCO₃ or placebo • Intermittent cycling protocol of 3-minute blocks: 90 s at 40% VO_{2max}, 60 s at 60% VO_{2max}, 14 s maximal sprint, and 16 s rest for 30 minutes • BS pre, during, and post exercise 	<ul style="list-style-type: none"> • BS analyzed for pH and HCO₃ • Peak power output, minimum power output, mean power output, and fatigue index were performance criterion 	<ul style="list-style-type: none"> • ↔ Peak power output between trials, fatigue index significantly different between trials, and ↑ peak power output with NaHCO₃ • Blood HCO₃ concentration ↑ post exercise with NaHCO₃ and pH remained higher throughout exercise

Table 2.4: Bicarbonate/Citrate Supplementation and Cycling Performance (continued)

Author/ Year	Subjects	Design	Analyses/ Performance Criterion	Findings
Bishop 2004 (11)	10 active females	<ul style="list-style-type: none"> • 2 trials: NaHCO₃ or placebo • 5 x 6 s all-out sprints every 30 s • BS and EG throughout protocol • Quadriceps Bx pre and post 	<ul style="list-style-type: none"> • BS analyzed for [La⁺] and pH • Bx analyzed for [La⁺], pH, and buffering capacity • 95% of first 6 seconds of 10 second preliminary test sprint performance was performance criterion (kJ) 	<ul style="list-style-type: none"> • NaHCO₃ ↑ total work and ↑ work and power in sprints 3, 4, and 5 • ↔ post-test muscle pH but NaHCO₃ yielded significantly ↑ [La⁺] • ↑ Performance is likely result of ↑ extracellular buffering and hydrogen efflux from muscle to blood
Zoladz 2005 (102)	7 healthy men	<ul style="list-style-type: none"> • 2 trials: placebo or 3 mmol/kg of NaHCO₃ • Cycling at 40 or 87% VO_{2max} for 6 minutes separated by 20 minutes rest • BS pre, during, and post exercise • Exercise EG 	BS analyzed for [La ⁺], pH, HCO ₃ , blood gasses, Na, K ⁺ , CP, Hb, Hct, erythrocyte, and leukocyte count	Primary component of VO ₂ kinetics not altered by NaHCO ₃ at low intensity (40% VO _{2max}) but was at high intensity (87%)

Table 2.4: Bicarbonate/Citrate Supplementation and Cycling Performance (continued)

Author/Year	Subjects	Design	Analyses/ Performance Criterion	Findings
Vanhatalo 2009 (94)	8 active males	<ul style="list-style-type: none"> • 2 trials: 0.3 g/kg of NaHCO₃ or placebo • 3-minutes all out cycling with fixed resistance halfway between gas exchange threshold and VO₂peak • Exercise EG • BS pre, during, and post exercise 	<ul style="list-style-type: none"> • BS analyzed for [La⁺] changes • Change in critical power, or the boundary between heavy and severe exercise associated with maximal [La⁺] steady state, and the curvature constant of power (<i>W'</i>), or anaerobic work capacity, were the performance criterion 	<ul style="list-style-type: none"> • NaHCO₃ ↔ CP, <i>W'</i>, or overall performance despite enhanced blood buffer capacity • No relationship between blood alkalosis and power-duration relationship for all-out exercise

BS = Blood Sample, EG = Expired Gasses, Bx = Muscle Biopsy, [La⁺] = Lactate
 NH₄CL = Ammonium Chloride, NaHCO₃ = Sodium Bicarbonate, HCO₃ = Bicarbonate, K⁺ = Potassium, Na = Sodium, CP = Creatine Phosphate, H⁺ = Hydrogen, CL = Chloride, ATP = Adenosine Triphosphate, Hb = Hemoglobin, Hct = Hematocrit, VL = Vastus Lateralis, TTE = Time to Exhaustion, CaCO₃ = Calcium Bicarbonate, PPO₂ = Partial Pressure of Oxygen, PPCO₂ = Partial Pressure of Carbon Dioxide, s = Seconds, ↑ = Increase, ↓ = Decrease, ↔ = No Change

Herbal Supplementation, Cycling Performance and Muscle Fatigue

Herbal supplementation to improve exercise performance has become a popular alternative to bicarbonate and citrate since there are generally less gastrointestinal ramifications. The supplement being tested in this study, SR2W-1, consists of a proprietary blend of the following herbal ingredients: enoki mushroom, eleuthero extract (*Eleutherococcus senticosus*), reishi mushroom, tangerine extract, cordyceps mushroom (*Cordyceps sinensis*), and asian ginseng (*Panax ginseng*). The efficacy of SR2W-1 was evaluated on one other occasion in cyclists and the results indicated that the supplement decreased lactic acid accumulation, increased pH, and increased blood bicarbonate during recovery from intense exercise. However, time to complete a 60,000 Joule time trial was not affected (57). No other supplement contains the combined ingredients in SR2W-1, but some of the individual constituents of the proprietary blend have been evaluated for potential ergogenic effects during cycling, namely *Eleutherococcus senticosus* (eleuthero extract), *Cordyceps sinensis* (cordyceps mushroom), and *Panax ginseng* (asian ginseng).

Two studies evaluating *Eleutherococcus senticosus* in cyclists have shown improved performance. Asano et al. found that supplementation with eleuthero extract improved VO_{2max} and time to exhaustion. However, glaring limitations in the experimental design make it difficult to interpret these findings, consequently limiting generalizability (7). Kuo et al. found similar results to Asano's study utilizing a cycling protocol at 75% VO_{2peak} . The authors also observed elevated

free fatty acid levels with supplementation (56). In contrast, Eschbach et al. found that eleuthero supplementation failed to confer any performance or metabolic advantages during endurance cycling (29). It is important to note that the loading period of Eschbach's study was only one week as compared to eight weeks in Kuo's study.

Cordyceps sinensis (cordyceps mushroom) is another herb that has garnered attention as an ergogenic aid for cyclists. The primary claim regarding *cordyceps sinensis* is that it improves muscle oxygenation in mice and potentially in humans. However, the three studies evaluating cordyceps usage in cyclists all failed to yield gains in performance or any metabolic parameters (15, 23, 76).

A third and most popularly tested constituent of SR2W-1 is asian (panax) ginseng. While studies involving other modes of exercise have yielded positive performance results with ginseng supplementation, asian ginseng does not appear to elicit similar results among cyclists (3, 25-27). Although research involving elements of SR2W-1 and cycling performance have not been convincing, existing SR2W-1 research reveals that the combined and synergistic effects of the various herbals could potentially improve performance.

Table 2.5 Herbal Supplementation, Cycling Performance and Muscle Fatigue

Author/ Year	Subjects	Design	Analyses/ Performance Criterion	Findings
Asano 1986 (7)	6 males	<ul style="list-style-type: none"> • 4 trials: 2 VO₂max tests on consecutive days was control • 8 days placebo supplementation and VO₂max test then 8 days Eleutherococcus senticosus (ES) supplementation and VO₂max test • EG during exercise 	TTE was performance criterion	<ul style="list-style-type: none"> • ES ↑ VO₂max compared to control but not placebo. ES ↑ TTE in all instances • Protocol is single-blind. Possible learning/training effect
Morris 1996 (73)	7 males and 1 female	<ul style="list-style-type: none"> • DB, XO • 8 mg/kg Panax Ginseng (PG) and placebo or 16 mg/kg and placebo for seven days • Ride to exhaustion at 75% VO₂max • BS pre, during, and post exercise • Exercise EG 	<ul style="list-style-type: none"> • BS analyzed for [La⁺], glucose, and FFA • TTE was performance criterion 	↔ TTE with PG supplementation
Engels 1997 (27)	36 healthy men	<ul style="list-style-type: none"> • R, DB, PC • Placebo, 200 mg/day, or 400 mg/day PG over 8 week period • Graded cycling test starting at 100 watts and increasing 50 watts every 3 minutes to exhaustion • Exercise EG • BS pre, during, and post exercise 	<ul style="list-style-type: none"> • BS analyzed for [La⁺] • VO₂, RER, VE, HR, and RPE were recorded 	↔ physiological or psychological parameters

Table 2.5 Herbal Supplementation, Cycling Performance and Muscle Fatigue (continued)

Author/ Year	Subjects	Design	Analyses/ Performance Criterion	Findings
Allen 1998 (3)	20 men and 8 women	<ul style="list-style-type: none"> • R • 200 mg PG or placebo for 3 weeks • Graded maximal cycling test to exhaustion • BS post-exercise • Exercise EG 	<ul style="list-style-type: none"> • BS analyzed for [La⁺] • Peak aerobic capacity and exercise TTE were performance criterion 	↔ Peak aerobic performance and TTE
Lahr 1999 (57)	12 subjects	<ul style="list-style-type: none"> • DB, between-groups design • Subjects paired based on [La⁺] threshold and assigned to supplement with 950 mg/day of herbal formulation (SR2W-1) or placebo for 5 weeks • Cycle at [La⁺] threshold for 20 minutes, rest for 12 minutes, then complete 60,000 J TT • BS pre and post exercise 	<ul style="list-style-type: none"> • BS analyzed for lactic acid • TT performance was performance criterion 	<ul style="list-style-type: none"> • SR2W-1 ↓ lactic acid accumulation, ↑ pH, and smaller drop in HCO₃ during recovery • TT not significantly different but strong effect size present
Eschbach 2000 (29)	9 trained males	<ul style="list-style-type: none"> • DB, R, XO • 1200 mg/day of ES or placebo for 7 days • 120 minutes at 60% VO₂max followed by 10 km TT • BS every 20 minutes during steady state exercise • Exercise EG 	<ul style="list-style-type: none"> • BS analyzed for [La⁺] and glucose • TT performance was performance criterion 	↔ metabolic parameters or performance with ES
Engels 2001 (26)	24 healthy women	<ul style="list-style-type: none"> • DB, R • 400 mg of PG or placebo for 8 weeks • 30-second Wingate cycling test 	Peak and mean anaerobic output was performance criterion	↔ performance

Table 2.5 Herbal Supplementation, Cycling Performance and Muscle Fatigue (continued)

Author/ Year	Subjects	Design	Analyses/ Performance Criterion	Findings
Kiew 2001 (52)	Nine male cyclists	<ul style="list-style-type: none"> • DB, XO • 2 trials: 3 ml/kg of placebo or Argomas herbal supplement • Cycle to exhaustion at 70% VO₂max for 90 minutes then 80% VO₂max to exhaustion • Exercise EG 	TTE was the performance criterion	↔ TTE or metabolic parameters
Earnest 2004 (23)	17 competitive male cyclists	<ul style="list-style-type: none"> • Pre and post 14 day loading period with Optygen (primarily cordyceps sinensis and rhodiola rosea) • 4 minute stages starting at 100 watts, increasing 25 watts until exhaustion • Exercise EG • BS pre, during, and post exercise 	<ul style="list-style-type: none"> • BS analyzed for [La⁺] • TTE and peak wattage were the performance criterion 	↔ performance or metabolic parameters
Parcell 2004 (76)	22 college-aged male cyclists	<ul style="list-style-type: none"> • 5 weeks of 3.15 g of CordyMax Cs-4 (cordyceps sinensis) or placebo • 1-hour TT at 75% max wattage calculated from pre-test • EG during and after exercise 	TT completion time was the performance criterion	↔ aerobic capacity or TT performance
Colson 2005 (15)	8 healthy males age 18-50 years	<ul style="list-style-type: none"> • DB, R, PC • 2000 and 1000 mg cordyceps sinensis and rhodiola rosea for 6 days and seven days respectively or placebo • Graded test to exhaustion • Exercise EG • Muscle tissue oxygenation and blood flow measured via NIRS 	TTE was performance criterion	↔ VO ₂ max, muscle oxygenation, or TTE

Table 2.5 Herbal Supplementation, Cycling Performance and Muscle Fatigue (continued)

Author/ Year	Subjects	Design	Analyses/ Performance Criterion	Findings
Kuo 2009 (56)	9 males	<ul style="list-style-type: none"> • DB, R, XO • 800 mg/day of ES and placebo for 8 weeks separated by four weeks wash-out • Cycling at 75% VO₂max until exhaustion • BS pre, during, and post exercise • Exercise EG 	<ul style="list-style-type: none"> • BS analyzed for [La⁺], FFA, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, blood urea nitrogen, and creatinine • TTE was performance criterion 	<ul style="list-style-type: none"> • ↑ VO₂peak, TTE, and FFA production
Dalbo 2010 (20)	15 active men	<ul style="list-style-type: none"> • 3 trials: water, rice flour (placebo), or 250 mg of a mineral antioxidant complex (MAC) supplement • VO₂peak test • BS pre, during, and post exercise • Total body water assessed pre and post exercise 	<ul style="list-style-type: none"> • BS analyzed for [La⁺] • VO₂peak test served as the performance criterion 	<ul style="list-style-type: none"> • ↔ aerobic exercise performance or [La⁺] response • MAC ↑ total body water and intracellular water

ES = Eleutherococcus Senticosus, PG = Panax Ginseng, BS = Blood Sample, EG = Expired Gasses, TTE = Time to Exhaustion, Lac = Lactate, FFA = Free Fatty Acid, RER = Respiratory Exchange Ration, VE = Ventilation, HR = Heart Rate, RPE = Rate of Perceived Exertion, DB = Double-Blind Design, R = Randomized Design, PC = Placebo-Controlled Design, XO = Cross-Over Design, HCO₃ = Bicarbonate, TT = Time Trial, CS = Cordyceps Sinensis

Summary

Much attention has been paid to the possible role that acidosis has in muscular fatigue during high-intensity exercise. Proposed mechanisms include inhibited calcium release and uptake by the sarcoplasmic reticulum, inhibitions in calcium binding to troponin C, and functional disruptions of enzymes found in the glycolytic pathway, all of which could inhibit skeletal muscle function. There is also evidence that lactate itself could influence calcium dynamics of the sarcoplasmic reticulum despite lactate being a potential source of fuel during exercise. At the very least, it is agreed that hydrogen ions and lactate accumulate during high-intensity exercise and that exercise intensity cannot be maintained for an extended duration under these conditions. Supplementing with sodium bicarbonate and citrate, compounds known to improve buffering capacity directly in the blood and indirectly in the muscle, improves performance and provides convincing evidence for the fact that pH management is important for preventing fatigue. Concentration of the pH/lactate management proteins MCT1 and MCT4 substantiates this since possessing higher levels correlates to improved high-intensity exercise performance. Since bicarbonate/citrate supplementation often causes gastrointestinal problems, herbal supplementation has become popular as an alternative for altering pH during intense exercise. The current project was therefore designed to address the efficacy of a multi-ingredient herbal supplement on high-intensity cycling performance.

CHAPTER THREE

METHODOLOGY

Subjects

Seven male and female recreational cyclists, aged 18-55 were recruited from James Madison University and the surrounding Harrisonburg area. Subject characteristics are summarized in Table 3.1. Subjects were provided with written and verbal information about the experimental procedures, including potential risks, prior to completing the informed consent (Appendix II). All procedures were approved by the James Madison University Institutional Review Board prior to testing.

Table 3.1: Subject Characteristics

	Age (years)	Weight (kg)	Height (cm)	VO_{2max} (mL/kg/min)	20-min Ride Wattage	Interval Wattage
Mean ±	26.7 ±	67.1 ±	172.5 ±	59.5 ±	212.1 ±	272.9 ±
SE	9.8	10.7	13.3	11	25.0	26.9

Testing Procedures

Cardiorespiratory Fitness (VO_{2max})

Subjects performed a graded exercise test to determine peak oxygen uptake (VO_{2max}) on a Racermate Velotron (Seattle, WA) cycle ergometer. Initial workload was subjectively determined during a self-selected 5-minute warm-up. Subjects selected an intensity maintainable for 90 minutes at an “easy to

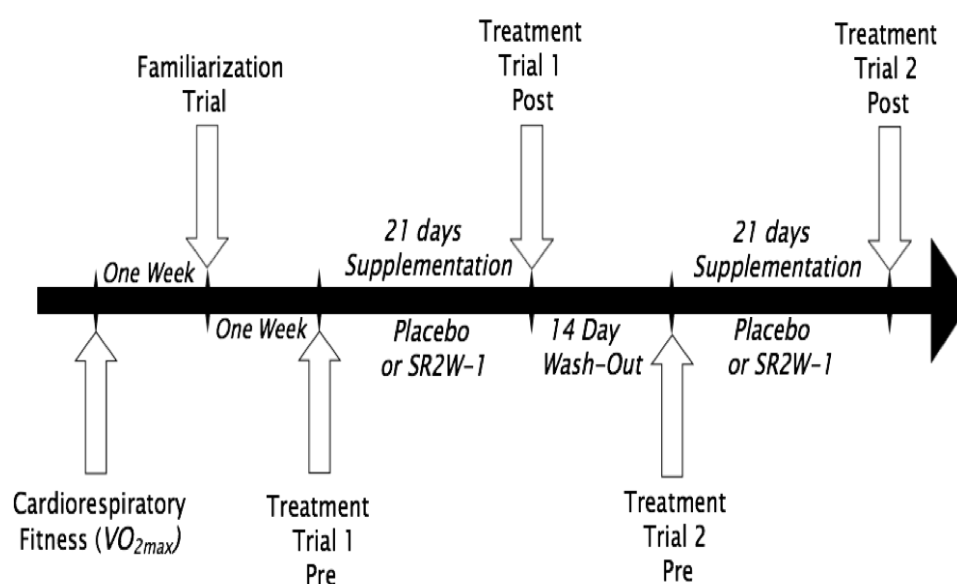
moderate intensity.” Following the warm-up, workload was incrementally increased by 20 W every 3 minutes until volitional fatigue or until subjects could no longer maintain a pedaling cadence ≥ 50 revolutions per minute. VO_2 , RER, and VE were continuously monitored with a Sensormedics Spectra metabolic cart (Yorba Linda, CA). Heart rate throughout each test was monitored using a Polar heart rate monitor (Lake Success, NY). Blood lactate levels were assessed via finger stick in the final minute of each exercise stage using a YSI 2300 STAT glucose/lactate analyzer (Yellow Springs, OH) to determine lactate threshold (≥ 1 mmol/L above resting lactate levels), after which blood sampling was discontinued. RPE was obtained in the final minute of each exercise stage and the workload (watts) at max was used to determine workloads during the familiarization and treatment trials.

Familiarization and Treatment Trials

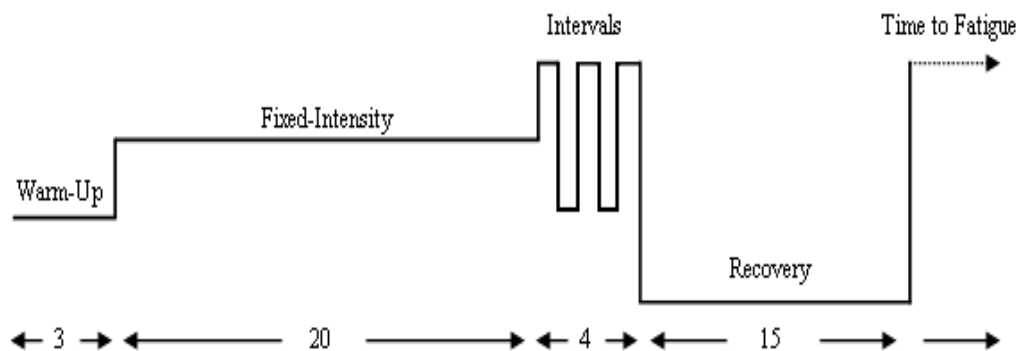
Each subject performed five trials over the course of nine weeks. The initial trial was included as a familiarization session with four subsequent trials serving as the treatment trials (TT). The general study design is outlined in Figure 3.1. The four treatment trials were conducted using a randomized, double-blind, cross-over design. The first two treatment trials occurred before and after three weeks of supplementation with either placebo or SR2W-1. Following a 14-day washout phase, subjects completed two more treatment trials conducted before and after three weeks of supplementation (placebo or SR2W-1, depending

on first phase). All trials were performed at ambient temperatures of 21-22°C. An Essential Home fan, set on ‘medium’ speed, was placed 2 meters from the handlebars of the cycle ergometer for cooling purposes during trials. Subjects were instructed to perform each trial as a competitive event.

Figure 3.1: Study Design



A visual representation of the exercise protocol is displayed in Figure 3.2. Subjects reported to the lab in the morning after a 10-12 hour overnight fast. To standardize for postural-related hemodynamics, subjects laid down for ten minutes prior to blood flow measurements. During that time, an indwelling catheter was inserted into the arm while their right leg was prepped for the muscle biopsy (Lidocaine, incision, and bandaging). Resting blood flow was then assessed by the same investigator using a Shenzhen Mindray DC-6 5-10 MHz duplex ultrasound machine (Shenzhen, Nanshan, China).

Figure 3.2: Treatment Trial Protocol

Following catheter insertion, biopsy preparation, and femoral ultrasound, subjects performed a 3-minute warm-up at 100 watts, after which the subjects rode for 20 minutes at a fixed-wattage that elicited 85% of the subject's VO_{2max} . Immediately following the 20-minute ride, subjects completed three one-minute intervals at a workload coinciding with VO_{2max} with 30-seconds of active recovery at 100 watts between each interval. Following the final interval, subjects stopped cycling and began a 15-minute passive recovery phase during which the subject underwent a muscle biopsy (1 minute into recovery) and femoral artery ultrasound (8 minutes into recovery). Following recovery, subjects cycled at a workload coinciding with VO_{2max} until fatigued (cadence ≥ 50 RPM).

Exercise Performance

Performance was measured by cycling duration (seconds) during the time to fatigue portion of the treatment trial.

Muscle Biopsy

Muscle biopsies were obtained from the vastus lateralis (VL) during the 15-minute recovery period of all treatment trials (10). The biopsy was taken within 1 minute of dismounting the ergometer in order to capture the metabolic state of muscle immediately after exercise. The muscle sample was quickly trimmed of excess adipose tissue and frozen in liquid nitrogen in order to halt metabolism. Each sample was then stored at -80°C for later analysis of muscle lactate. The subjects were re-bandaged in order to complete the ride to exhaustion.

Muscle Lactate

Muscle samples from each treatment trial were thawed to -20 to -15°C and cut into 15-30 mg pieces using a refrigerated scale. Samples were then refrozen at -80°C , transferred to a glass culture tube containing 0.5 ml 3 M perchloric acid (PCA) solution, homogenized, diluted with 1 M glycine/hydrazine stock (pH 9.8 - 10), and stirred with a pestle. NADH was then measured using a spectrophotometer at 340 nm. Muscle lactate values were calculated mathematically based on fluorescence results (17).

Blood Lactate, pH, and Glucose

Preceding each exercise trial, an indwelling antecubital venous catheter was inserted by a phlebotomy-trained investigator. During exercise, the catheter was flushed periodically with an injectible saline solution to prevent coagulation. Blood samples were obtained at the following time points: minutes 10 and 20 during the 20-minute fixed intensity ride, after the second high-intensity interval, and minutes 1.5, 3, 6, 9, 12, and 15 of recovery. Glucose and lactate levels were measured immediately from whole blood using a YSI 2300 STAT glucose/lactate analyzer (Yellow Springs, Ohio).

VO₂, RER, and Ventilation

VO₂, RER, and Ventilation were assessed at minutes 10 and 20 during the fixed-intensity ride and after the second high-intensity interval. A SensorMedics Spectra (Yorba Linda, CA) metabolic cart was used to monitor breath-by-breath gas exchange and average respiratory data over thirty seconds.

Heart Rate

Heart rate was recorded at minutes 10 and 20 during the fixed intensity ride, after the second high-intensity interval, and at minutes 3, 6, 9, and 12 during the recovery phase using a Polar heart rate monitor (Lake Success, NY).

Ratings of Perceived Exertion (RPE)

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

Blood Flow

Left femoral artery peak velocity and diameter were measured 5-7 mm below the inguinal crease using a Mindray DC-6 (Nanshan, Shenzhen, China) 5-10 MHz duplex ultrasound machine. Blood flow was calculated using the equation: πr^2 (mm) x peak velocity (cm/s) x 60 seconds. Pre-exercise peak velocity and diameter was assessed at rest and post-exercise measurements were obtained eight minutes following the completion of the high-intensity intervals.

Supplement

Subjects were randomly assigned to receive either 1000 mg/day of SR2W-1 or 1000 mg/day of placebo. Subjects ingested 2 x 500 mg capsules every morning for 21 days during the supplementation periods. To promote supplementation compliance, subjects were required to text, e-mail, or call a member of the investigative team immediately after supplementation. SR2W-1 is a proprietary blend of herbs and fungi (primary ingredients: Enoki mushroom, Eleuthero 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

Throughout the duration of the study, subjects were instructed to: 1. maintain consistent dietary habits for 72 hours prior to each trial, 2. complete a diet record (Appendix V) for the 24 hours preceding each trial, 3. avoid heavy exercise for 48 hours prior to each trial, 4. maintain consistent physical activity habits starting 72 hours 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 24 hours preceding each trial (Appendix VI). Subjects consumed their final 'self-selected' meal no less than 10 hours prior to the start of treatment trials (i.e. dinner on the evening prior to testing) and were allowed to consume only water *ad libitum* until the end of each treatment trial (total of ~10-12 hours of fasting with water intake).

Statistical Analyses

Due to the small sample size in this study, the Wilcoxon Signed Rank test was applied to compare change scores from pre- to post- supplementation (EXP vs. PLA for each dependent variable). More specifically, differences between pre- and post-supplementation cycling time to fatigue, muscle lactate, blood lactate, blood glucose, VO_2 , RER, VE, HR, and skeletal muscle blood flow were

compared between treatments at a given time point during exercise and recovery. Significance was set at $p < 0.05$. All blood lactate data during recovery was analyzed using differences in area underneath a curve. Results are reported as means \pm SE.

CHAPTER FOUR

MANUSCRIPT

THE EFFECTS OF SR2W-1 SUPPLEMENTATION ON HIGH-INTENSITY
CYCLING PERFORMANCE AND LACTATE METABOLISM

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Running Head: Herbal supplement, Blood and Muscle Lactate, and Cycling
Performance

ABSTRACT

Purpose: The purpose of this investigation was to examine the effects of SR2W-1 herbal supplementation on cycling performance, muscle and blood lactate, and various physiological parameters including blood glucose, heart rate (HR), rating of perceived exertion (RPE), oxygen consumption (VO_2), expired ventilation (VE), respiratory exchange ratio (RER), and femoral artery blood flow. **Methods:** Seven recreational cyclists (Age: 26.7 ± 9.8 yrs, Height: 172.5 ± 13.3 cm, Weight: 67.1 ± 10.7 kg, and $\text{VO}_{2\text{max}}$: 59.5 ± 11 mL/kg/min) performed 20-min of steady-state cycling ($\sim 85\% \text{VO}_{2\text{max}}$, 212.1 ± 25.0 W) followed by three 1-min high intensity intervals at $\text{VO}_{2\text{max}}$ workload (272.9 ± 26.9) with 30-sec active recovery periods at 100 watts. Following intervals, a 15-min passive recovery period preceded a ride to fatigue at $\text{VO}_{2\text{max}}$ workload. Subjects completed trials on four occasions; preceding and following 21 days of 1000mg/d SR2W-1 (EXP) and 1000mg/d placebo (PLA) assigned in random order. The Wilcoxon Signed-Rank Test was used to compare change-scores from pre- to post- PLA and EXP conditions. **Results:** No differences reported for any dependent variable and performance times were not different between PLA (pre-PLA: 180 ± 48 s; post-PLA: 198 ± 56 s) and EXP (pre-EXP: 170 ± 57 s; post-EXP: 191 ± 50 s). **Conclusion:** Notwithstanding the small sample size, 3 weeks of SR2W-1 supplementation does not appear to aid cycling performance, attenuate skeletal muscle fatigue, or modify general physiological responses to exercise.

INTRODUCTION

High levels of blood lactate and concomitant reductions in pH are strongly associated with the onset of fatigue. In 1907, Fletcher and Hopkins examined lactic acid levels in working frog muscles and stated that “an acid reaction of the muscle is, as most agree, a constant mark of the fatigued condition and a constant condition of the state of rigor,” providing evidence that acidity has been associated with decreased function and fatigue for over a century (33). The precise role that lactic acid plays in muscular fatigue has been debated, partially as a result of the lack of supporting evidence regarding lactic acid’s proton contribution and the positive energetic properties of lactate. However, the deleterious effects that hydrogen ions have on skeletal muscle function are well-documented. In-vitro skeletal muscle experiments have shown that low intramuscular pH can interfere with several reactions within the muscle cell, inhibiting function and causing fatigue (13, 30, 59). It has also been suggested that hydrogen ion accumulation may inhibit the release of calcium from the sarcoplasmic reticulum and interfere with the transmission of neural impulses which can also decrease function (45). Although there are other potential sources of muscular fatigue such as fatigue of the central nervous system, psychological factors, and inorganic phosphate accumulation in the muscle cell, acidosis is often regarded as the most prevalent source of fatigue during intense exercise.

Since intense exercise is associated with a drop in pH and inevitable fatigue, athletes can benefit from strategies that mitigate the acidifying effect of

exercise to enhance their performance. In 1924, A.V. Hill acknowledged the importance of tissue and blood buffers and their effect on muscular effort (39). As early as the 1930's, researchers experimented with induced alkalosis and its effect on performance. They hypothesized that by increasing blood alkalinity, more lactic acid could be produced implying more energy from anaerobic glycolysis and achievement of higher intensities (64). After being largely ignored for nearly 30 years due to initial null findings, a resurgence of research in the 1970's began to indicate that manipulating extracellular buffering capacity can impact intracellular pH. Bicarbonate supplementation became the predominant mode of inducing blood alkalinity since the bicarbonate buffer system is a primary source of blood's buffering capacity. Research shows that increasing blood pH with bicarbonate increases the rate of lactate and hydrogen efflux from muscle cells despite the relative impermeability of the sarcolemma to bicarbonate (18, 43, 48, 88, 92). This suggests that hydrogen ion efflux from the cell works down a concentration gradient. Conceptually, an increased concentration gradient facilitated by changes in extracellular lactate/H⁺ should allow for more lactate/H⁺ transport from the active skeletal muscle, thereby delaying fatigue. As a result, lactate/H⁺ supplement research has become prevalent due to its potential performance-enhancing benefits.

SR2W-1 is a commercially available herbal supplement designed to improve high-intensity exercise performance. Preliminary research on the efficacy of SR2W-1 is promising as two investigations have demonstrated that

SR2W-1 prolongs exercise endurance, promotes lactate clearance after exhaustive exercise, and improves overall performance (1, 57). Additionally, some research involving the constituents of SR2W-1 have yielded performance benefits during cycling (7, 56). However, the two existing SR2W-1 studies were not peer reviewed and possessed major methodological limitations. Thus, the primary aims of the current study were to evaluate the effect of the herbal supplement SR2W-1 on high-intensity cycling performance as well as blood and skeletal muscle lactate metabolism. Blood glucose, VO_2 , RER, VE, HR, RPE, and femoral artery blood flow were also observed to further elucidate the effects of the supplement. We hypothesized that SR2W-1 supplementation would improve cycling performance, reduce blood lactate levels during varying intensities of cycling exercise and during exercise recovery, reduce skeletal muscle lactate levels, and increase blood flow during recovery.

METHODS

Subjects

Seven male and female recreational cyclists, aged 18-55 were recruited from James Madison University and the surrounding Harrisonburg area. Subject characteristics are summarized in Table 3.1. Subjects were provided with written and verbal information about the experimental procedures, including potential risks, prior to completing the informed consent (Appendix II). All procedures

were approved by the James Madison University Institutional Review Board prior to testing.

Table 4.1: Subject Characteristics

	Age (years)	Weight (kg)	Height (cm)	VO_{2max} (mL/kg/min)	20-min Ride Wattage	Interval Wattage
Mean ±	26.7 ±	67.1 ±	172.5 ±	59.5 ±	212.1 ±	272.9 ±
SE	9.8	10.7	13.3	11	25.0	26.9

Testing Procedures

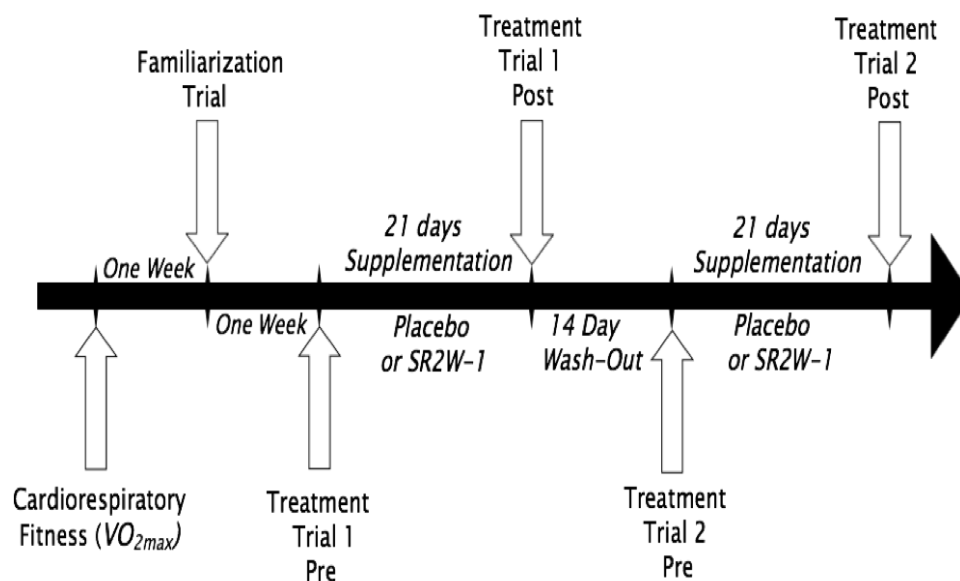
Cardiorespiratory Fitness (VO_{2max})

Subjects performed a graded exercise test to determine peak oxygen uptake (VO_{2max}) on a Velotron Racermate (Seattle, WA) cycle ergometer. Initial wattage was subjectively determined during a self-selected 5-minute warm-up. Subjects selected an intensity that could be maintained for 90 minutes at “easy to moderate intensity.” Following warm-up, workload was incrementally increased by 20 W every 3 minutes until volitional fatigue or until subjects could not longer maintain a pedaling cadence ≥ 50 revolutions per minute. VO₂, RER, and VE were continuously monitored with a Sensormedics Spectra (Yorba Linda, CA) metabolic cart. Heart rate was monitored throughout each test using a Polar heart rate monitor (Lake Success, NY). Blood lactate levels were assessed in the final minute of each exercise stage using a YSI 2300 STAT glucose/lactate analyzer

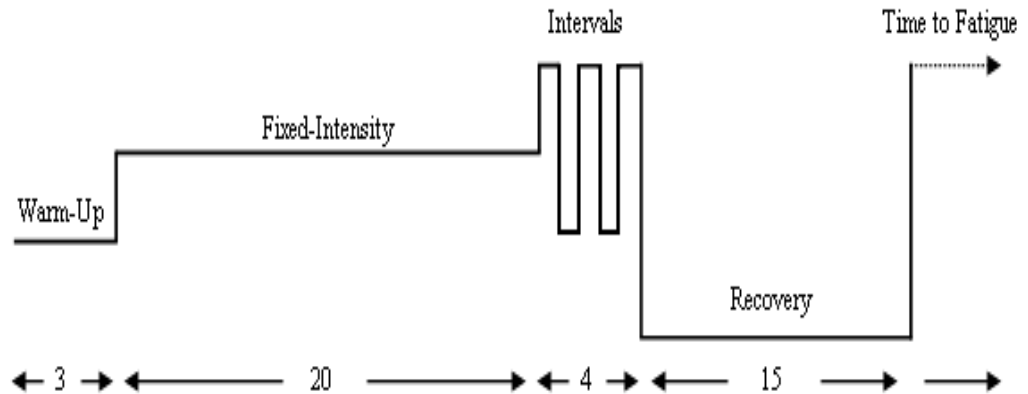
(Yellow Springs, OH) to determine lactate threshold, after which blood sampling was discontinued. RPE was obtained in the final minute of each exercise stage and wattage at $\text{VO}_{2\text{max}}$ was used to determine workloads during the familiarization and treatment trials.

Familiarization and Treatment Trials

Each subject performed five trials over the course of nine weeks. The preliminary trial served as a familiarization trial while the remaining four trials were the treatment trials (TT). The general study design is outlined in Figure 3.1. The four treatment trials were conducted using a randomized, double-blind, cross-over design and the two three-week supplementation phases were separated by a 14-day washout period. The first two treatment trials occurred before and after three weeks of supplementation with either placebo or SR2W-1. Following the washout phase, subjects completed two more treatment trials conducted before and after three weeks of supplementation (placebo or SR2W-1, depending on first phase). All trials were performed at ambient temperatures of 21-22°C. An oscillating fan, set on 'medium' speed, was placed 2 meters from the handlebars of the cycle ergometer for cooling purposes during trials. Subjects were instructed to perform each trial as a competitive event.

Figure 4.1: Study Design

A visual representation of the exercise protocol is displayed in Figure 3.2. Subjects reported to the lab in the morning after a 10-12 hour overnight fast. To standardize for posture-related hemodynamics, subjects laid down for ten minutes prior to blood flow measurements. During that time, an indwelling catheter was inserted into the arm while their right leg was prepared for a muscle biopsy (Lidocaine, incision, and bandaging). Resting blood flow was then assessed by the same investigator using a Shenzhen Mindray DC-6 (Shenzhen, Nanshan, China) 5-10 MHz duplex ultrasound machine.

Figure 4.2: Treatment Trial Protocol

Following catheter insertion, biopsy preparation, and femoral ultrasound, subjects performed a 3-minute warm-up at 100 watts, after which the subjects rode for 20 minutes at a fixed-wattage that elicited 85% of the subject's VO_{2max} . Immediately following the 20-minute ride, subjects completed three one-minute intervals at 100% of wattage at VO_{2max} with 30-seconds of active recovery at 100 watts between each interval. Following the final interval, subjects stopped cycling and began a 15-minute recovery phase during which the subject underwent a muscle biopsy (1 minute into recovery) and femoral artery ultrasound (8 minutes into recovery). Following recovery, subjects cycled at 100% wattage at VO_{2max} until volitional fatigue (cadence ≥ 50 RPM).

Exercise Performance

Performance was measured by cycling duration (seconds) during the time to fatigue portion of the treatment trial.

Muscle Biopsy

Muscle biopsies were obtained from the VL during the 15-minute recovery period of all treatment trials (10). The biopsy was taken within 1 minute of dismounting the ergometer in order to capture the metabolic state of muscle immediately after exercise. The muscle sample was quickly trimmed of excess adipose tissue and frozen in liquid nitrogen in order to halt metabolism. Each sample was then stored at -80°C for later analysis of muscle lactate. The subject was re-banded in order to complete the ride to exhaustion.

Muscle Lactate

Muscle samples from each treatment trial were thawed to -20 to -15°C and cut into 15-30 mg pieces using a refrigerated scale. Samples were then frozen again to -80°C , transferred to a glass culture tube containing 0.5 ml 3 M perchloric acid (PCA) solution, homogenized, diluted with 1 M glycine/hydrazine stock (pH 9.8 - 10), and homogenized again. NADH was then measured using a spectrophotometer at 340 nm. Muscle lactate values were calculated mathematically based on fluorescence results (17).

Blood Lactate, pH, and Glucose

Preceding each exercise trial, an indwelling antecubital venous catheter was inserted by a phlebotomy-trained investigator. During exercise, the catheter was flushed periodically with an injectible saline solution to prevent coagulation.

Blood samples were obtained at the following time points: minutes 10 and 20 of the 20-minute fixed intensity ride, immediately following the second high-intensity interval, and minutes 1.5, 3, 6, 9, 12, and 15 of recovery. Glucose and lactate levels were measured immediately from whole blood using a YSI 2300 STAT glucose/lactate analyzer (Yellow Springs, Ohio).

VO₂, RER, and Ventilation

VO₂, RER, and Ventilation were assessed at minutes 10 and 20 during the fixed-intensity ride and after the second high-intensity interval. A SensorMedics Spectra (Yorba Linda, CA) metabolic cart was used to monitor breath-by-breath gas exchange and average respiratory data over thirty seconds.

Heart Rate

Heart rate was recorded at minutes 10 and 20 during the fixed intensity ride, after the second high-intensity interval, and at minutes 3, 6, 9, and 12 during the recovery phase using a Polar heart rate monitor (Lake Success, NY).

Ratings of Perceived Exertion (RPE)

Subjective ratings of exertion were obtained by having the subject point to the corresponding exertion level on a Borg RPE scale (numerically rated from 6-20). RPE was obtained at minutes 10 and 2 of the fixed intensity ride and after the second high-intensity interval.

Blood Flow

Left femoral artery peak velocity and diameter was measured 5-7 mm below the inguinal crease using a Mindray DC-6 (Nanshan, Shenzhen, China) 5-10 MHz duplex ultrasound machine. Blood flow was calculated using the equation: πr^2 (mm) x peak velocity (cm/s) x 60 seconds. Pre-exercise peak velocity and diameter was assessed at rest and post-exercise measurements were obtained eight minutes following the completion of the high-intensity intervals.

Supplement

Subjects received either 1000 mg/day of SR2W-1 and 1000 mg/day of placebo in randomly assigned order. Subjects ingested 2 x 500 mg capsules every morning for 21 days during the supplementation periods. To promote supplementation compliance, subjects were required to text, e-mail, or call a member of the investigative team immediately after supplementation. SR2W-1 is a proprietary blend of herbs and fungi (primary ingredients: Enoki mushroom, Eleuthero 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

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

Statistical Analyses

Due to the small sample size in this study, data was analyzed with Wilcoxon Signed Rank tests utilizing change scores for each dependent variable from pre- to post- supplementation under EXP and PLA conditions. Specifically, differences between pre- and post-supplementation cycling time to fatigue, muscle lactate, blood lactate, blood glucose, VO_2 , RER, VE, HR, and skeletal muscle blood flow were compared between treatments at a given time point during exercise. Significance was set at $p < 0.05$. All blood lactate data

during recovery was analyzed using differences in area underneath the curve. Results are reported as means \pm SE.

RESULTS

Exercise Performance

Time to fatigue was not differentially impacted by PLA and EXP supplementation. Individual performance times are displayed in Figure 4.3 (pre-PLA: 180 ± 48 sec; post-PLA: 198 ± 56 ; pre-EXP: 170 ± 57 ; post-EXP: 191 ± 50).

Skeletal Muscle Lactate

PLA and EXP supplementation did not differentially affect skeletal muscle lactate levels during recovery. Muscle lactate levels are displayed in Figure 4.4 (pre-PLA: 13.8 ± 2.4 mmol/kg wet wt; post-PLA: 13.0 ± 2.1 ; pre-EXP: 14.3 ± 2.0 ; post-EXP: 12.8 ± 2.2).

Blood Lactate and Glucose

Blood lactate and glucose levels were not differentially impacted by PLA and EXP supplementation. Blood lactate and glucose levels are displayed in Table 4.2.

Figure 4.3 Exercise Performance

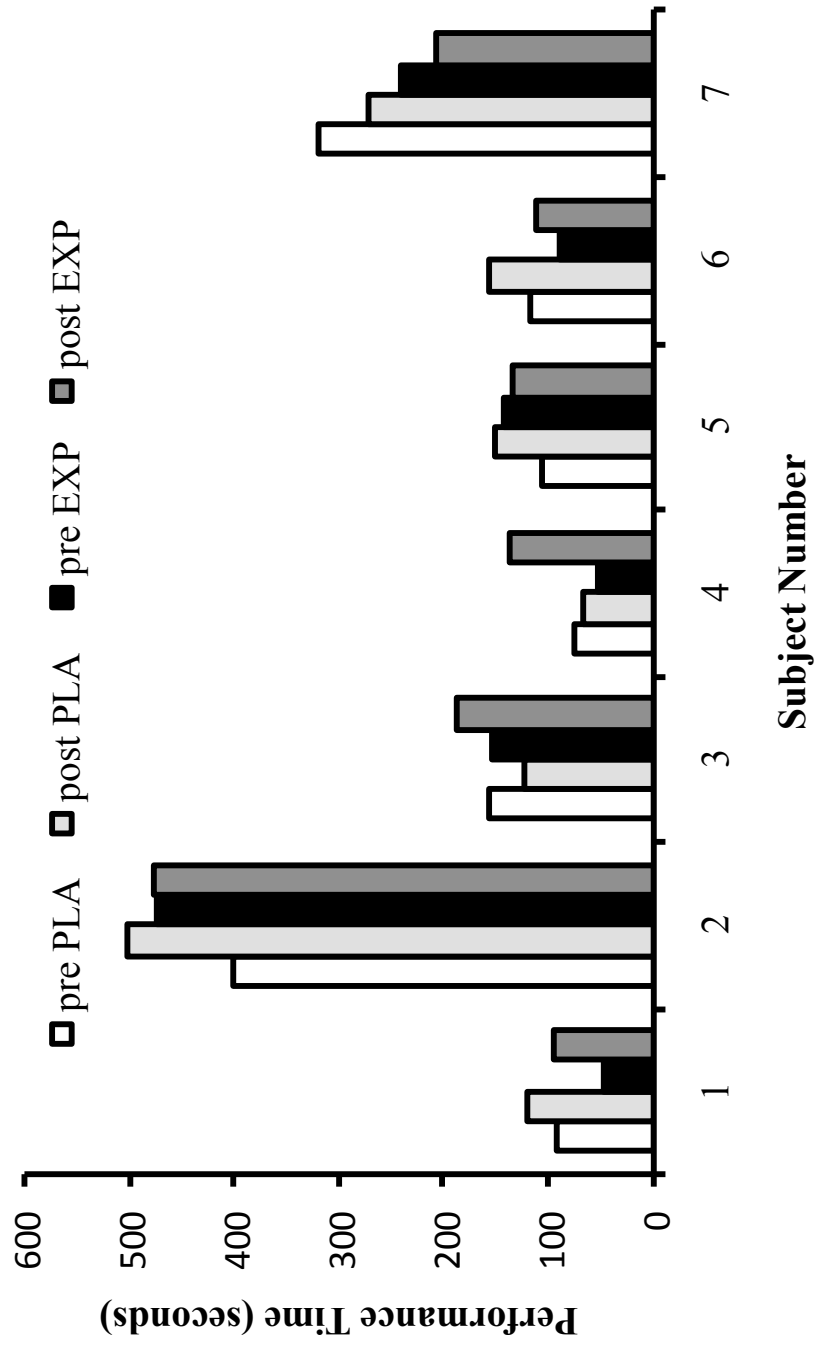


Figure 4.4 Skeletal Muscle Lactate

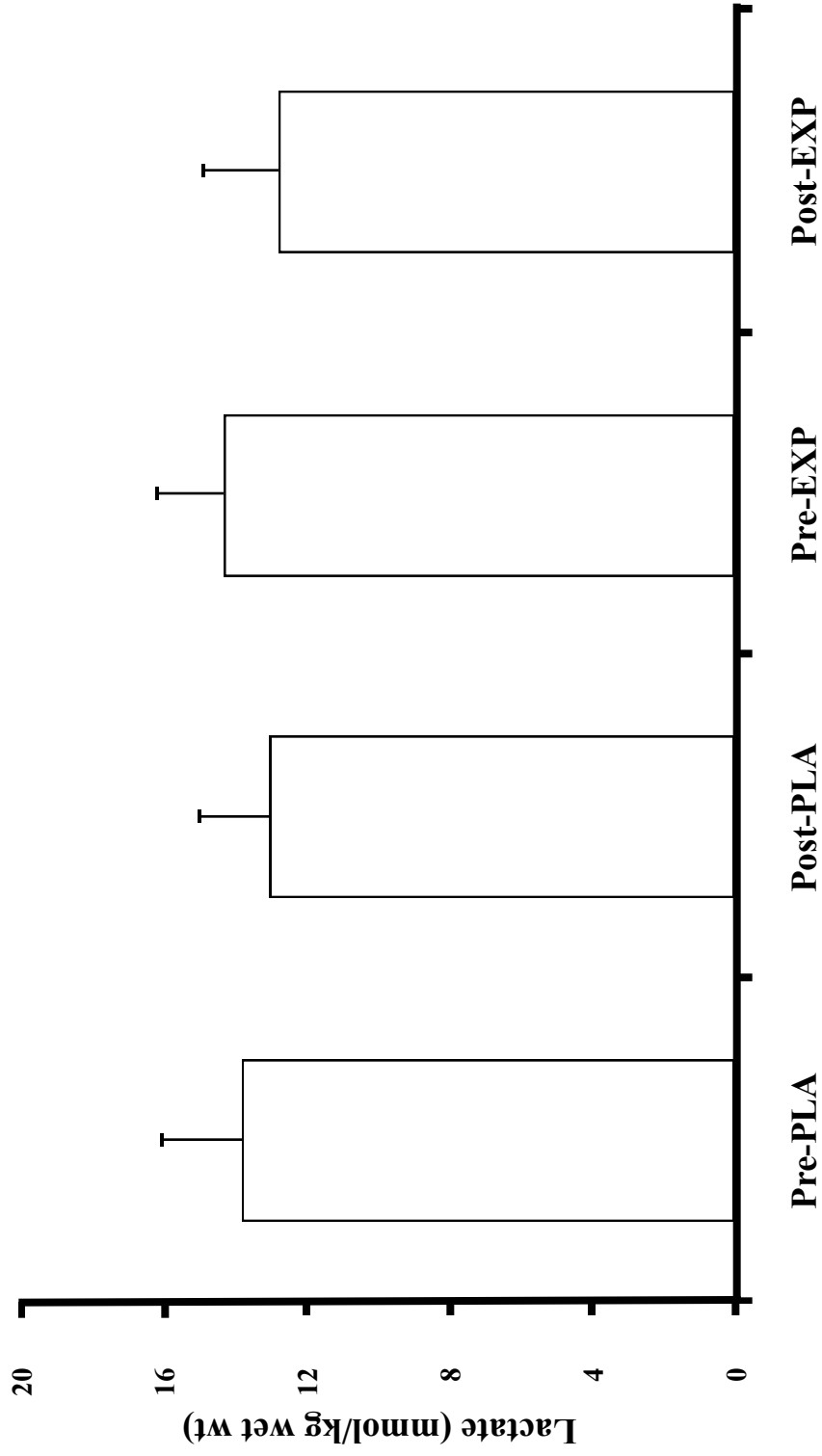


Table 4.2 Blood Glucose and Lactate Levels Before and After Supplementation

Time (min)	Variable	Placebo		Experimental	
		Pre	Post	Pre	Post
10	Glucose (mg/dL)	75.9 ± 4.0	72.0 ± 3.2	76.7 ± 3.3	71.5 ± 3.1
	Lactate (mmol/L)	3.0 ± 0.5	2.7 ± 0.3	3.2 ± 0.3	3.1 ± 0.5
20	Glucose (mg/dL)	80.1 ± 4.1	75.2 ± 3.2	82.2 ± 3.2	76.9 ± 2.4
	Lactate (mmol/L)	3.5 ± 0.6	3.2 ± 0.5	3.7 ± 0.3	3.6 ± 0.5
22:30	Glucose (mg/dL)	83.0 ± 4.1	77.5 ± 2.5	86.0 ± 3.2	80.9 ± 2.6
	Lactate (mmol/L)	4.7 ± 0.7	4.3 ± 0.6	5.0 ± 0.4	4.6 ± 0.6
15-min recovery AUC	Lactate (mmol/L)	56.7 ± 12.6	55.1 ± 11.3	60.5 ± 8.2	57.0 ± 12.7

AUC = Area Under the Curve

VO₂, RER, and Ventilation

PLA and EXP supplementation did not differentially impact VO₂ (ml/min and mL/kg/min), RER, or VE (L/min). VO₂, RER, and VE are displayed in Table 4.3.

Table 4.3 VO₂, RER, and VE Before and After Supplementation

Time (min)	Variable	Placebo		Experimental	
		Pre	Post	Pre	Post
10	VO ₂ (L/min)	3.4 ± 0.4	3.4 ± 0.3	3.5 ± 0.3	3.4 ± 0.3
	VO ₂ (ml/kg/min)	49.0 ± 3.5	50.7 ± 3.1	51.2 ± 3.8	50.6 ± 4.0
	RER	0.91 ± 0.01	0.92 ± 0.01	0.91 ± 0.01	0.93 ± 0.02
	VE (L/min)	85 ± 7	87 ± 6	91 ± 8	92 ± 8
20	VO ₂ (L/min)	3.4 ± 0.3	3.5 ± 0.3	3.5 ± 0.3	3.4 ± 0.3
	VO ₂ (ml/kg/min)	49.6 ± 3.3	51.1 ± 2.9	51.2 ± 3.4	50.5 ± 3.4
	RER	0.89 ± 0.01	0.89 ± 0.02	0.91 ± 0.01	0.89 ± 0.01
	VE (L/min)	89 ± 6	91 ± 6	99 ± 8	93 ± 6
22:30	VO ₂ (L/min)	3.5 ± 0.3	3.6 ± 0.3	3.4 ± 0.4	3.5 ± 0.3
	VO ₂ (ml/kg/min)	51.1 ± 2.1	53.0 ± 2.2	52.3 ± 2.8	52.2 ± 2.8
	RER	0.99 ± 0.02	0.97 ± 0.03	0.97 ± 0.02	0.97 ± 0.03
	VE (L/min)	107 ± 6	109 ± 7	117 ± 6	112 ± 6

Heart Rate and Ratings of Perceived Exertion

Heart rate and RPE numbers were not differentially impacted by PLA and EXP supplementation. HR and RPE are displayed in Table 4.4.

Table 4.4 Heart Rate and RPE Before and After Supplementation

Time (min)	Variable	Placebo		Experimental	
		Pre	Post	Pre	Post
10	HR (bpm)	171 ± 5	169 ± 4	173 ± 5	172 ± 6
	RPE	14.9 ± 0.3	14.1 ± 0.5	14.9 ± 0.5	14.8 ± 0.6
20	HR (bpm)	175 ± 4	173 ± 4	177 ± 5	177 ± 5
	RPE	16.7 ± 0.6	15.9 ± 0.5	16.4 ± 0.4	16.1 ± 0.7
22:30	HR (bpm)	184 ± 4	182 ± 4	185 ± 4	183 ± 5
	RPE	18.3 ± 0.5	17.7 ± 0.6	17.7 ± 0.7	17.9 ± 0.7
3R	HR (bpm)	100 ± 6	104 ± 6	107 ± 5	104 ± 5
6R	HR (bpm)	96 ± 7	97 ± 4	98 ± 5	98 ± 4
9R	HR (bpm)	95 ± 4	92 ± 4	95 ± 4	98 ± 6
12R	HR (bpm)	91 ± 6	89 ± 4	93 ± 5	91 ± 5

R = Recovery

Blood Flow

Blood flow (L/min) was not differentially impacted by PLA and EXP supplementation . Blood flow is displayed in Table 4.5.

Table 4.5 Blood Flow (L/min)

	PLA		EXP	
	Pre Exercise	8-minute Post Exercise	Pre Exercise	8-minute Post Exercise
Pre Supplementation	1.1 ± 0.1	1.6 ± 0.1	1.1 ± 0.1	1.5 ± 0.2
Post Supplementation	1.2 ± 0.1	1.5 ± 0.2	1.2 ± 0.1	1.7 ± 0.1

DISCUSSION

The primary aims of this study were to evaluate the effect of the herbal supplement SR2W-1 on high-intensity cycling performance as well as blood and skeletal muscle lactate metabolism. Blood glucose, VO_2 , RER, VE, HR, RPE, and femoral artery blood flow were also observed to further elucidate the effects of the supplement. We hypothesized that SR2W-1 supplementation would prolong cycling time to fatigue, reduce blood lactate levels during varying intensities of cycling exercise and during exercise recovery, and reduce skeletal muscle lactate levels and increase blood flow during recovery. Contrary to our hypotheses, the collective results indicate that SR2W-1 does not affect time to fatigue, blood or muscle lactate levels, or any of the aforementioned physiological parameters. Therefore, SR2W-1 supplementation does not appear to be an effective ergogenic strategy, at least in the context of high-intensity cycling.

Despite some evidence supporting the efficacy of herbal supplementation (7, 56, 57), the performance findings in this study are consistent with the larger body of literature. Only one study has evaluated the impact SR2W-1 supplementation on cycling performance, which reported that 60,000 Joule time trial performance was unchanged (57). It is worth noting that the authors did not implement a cross-over design and could not account for individual variations in performance. However, the study did implement a five-week supplementation phase (versus 21 days in the current study), which would theoretically maximize the treatment effect. At least ten other studies evaluated the efficacy of one of the

primary constituents of SR2W-1 (*Cordyceps sinensis*, *Eleutherococcus senticosus*, or *Panax Ginseng*), eight of which reported no change in cycling time to exhaustion, time trial performance, or anaerobic power output. Thus it is logical that SR2W-1, with all constituents combined, would not yield performance benefits.

The effects of one to eight weeks of *Panax Ginseng* (PG) supplementation at varying dosages was investigated by Morris (73), Allen (3), and Engels (25-27). All five studies reached the similar conclusion that PG supplementation does not benefit cycling performance. The different supplementation schemes and similar testing protocols (high-intensity rides to exhaustion or graded exercise tests) provide compelling evidence that PG is not ergogenic. Research regarding the efficacy of *Eleutherococcus senticosus* (ES) on cycling performance is scant and inconclusive. Specifically, Eschbach et al. reported that 10-km time trial performance was unaffected by 7 days of ES supplementation (1200 mg/day) when compared to a placebo (29). In contrast, a subsequent study implemented a much longer supplementation period (8 weeks) with lower dosing (800 mg/day) and observed improvements in cycling time to exhaustion with ES (56). Another relevant investigation, albeit with glaring methodological limitations (single blind, possible learning effect), reported that time-to-exhaustion was enhanced with ES (7). It is possible that ES may be ergogenic during high-intensity cycling but further research involving different supplementation periods and dosages is required before drawing firm conclusions.

The results of studies investigating Cordyceps senesis (CS) and cycling performance consistently show no ergogenic effect. Three CS supplementation studies implementing exhaustive protocols of less than one hour found no increase in time to exhaustion or time trial performance (15, 23, 76). Although findings are not unanimous, the preponderance of literature suggest the primary constituents of SR2W-1 do not benefit cycling performance of short duration (<60 min) which is substantiated by the results of the current study.

It is well-established that high-intensity cycling performance benefits from lower blood lactate levels, as illustrated by the efficacy of bicarbonate supplementation (11, 18, 47, 60, 62, 66, 67, 69, 77). However, to our knowledge, only one study has provided any evidence to suggest that blood lactate levels are diminished by herbal supplementation in the context of cycling. Specifically, Lahr and colleagues documented that SR2W-1 provision reduced lactate levels during recovery from intense cycling. The authors speculated that less stress was placed on the HCO_3^- buffering system with supplementation (57). However, it should be noted that Lahr's study did not use a cross-over design and can not completely account for individual variation in performance.

Beyond the cycling-specific literature, a few studies have reported altered lactate metabolism during high-intensity exercise with herbal supplementation. Morrissey et al tested a complex herbal formulation using a double-blind, randomized, placebo-controlled design and found that lactate was lower during recovery from intense treadmill running (74). Further, Wu et al tested the efficacy

of Ciwujia (ES) with high-intensity exercise and found lactate to be lower during recovery while potentially promoting glycogen sparing (101). However, it should be noted that Wu's study, much like Lahr's, did not employ a cross-over design. The majority of studies, however, indicate blood lactate is not affected by PG (3, 27, 73), CS (23), ES (29), or alternative herbal (20, 52) supplementation. Further, to our knowledge, our study was the first to examine skeletal muscle lactate levels after herbal supplementation. Considering the close association of blood to muscle lactate levels at comparable observed time points ($r = 0.67 - 0.91$) in our investigation, our novel findings confirm the validity of measuring blood lactate to determine the state of muscle during exercise and reinforces the reliability of our testing procedures. Provided the positive effect that lowered lactate has on performance, the lack of alteration in blood or muscle lactate levels are consistent with our performance findings as well as those found in the literature.

Of the studies that have evaluated similar physiological parameters (VO_2 , RER, VE, HR, RPE, blood glucose, and skeletal muscle blood flow), there has only been one report suggesting that herbal supplementation modifies high-intensity cycling physiology. Kuo et al. found that VO_{2peak} and highest heart rate attained increased while RPE and RER decreased during a 75% VO_{2peak} ride to exhaustion with ES supplementation (56). Other data suggests VO_2 (27, 29, 52, 73), RER (27, 52, 73), VE (15, 76), HR (3, 20, 23, 27, 29, 52), RPE (3, 20, 27, 29, 52), or blood glucose (29, 52, 73) remain unchanged with herbal supplementation. Although Colson et al evaluated the effects of *Cordyceps sinensis* on circulatory

dynamics during cycling and found no significant changes in tissue oxygenation (15) we are the first to examine femoral artery blood flow in response to herbal supplementation. Increased blood flow to working skeletal muscle could theoretically facilitate lactate clearance from skeletal muscle (8). However, our findings suggest that blood flow is not affected by SR2W-1 supplementation, which may or may not have contributed to our null performance and lactate findings.

Contrary to our hypotheses, the results of the current study are consistent with the existing literature, which collectively indicates that herbal supplementation is not effective at improving cycling performance or altering lactate levels. In addition, supplementing with SR2W-1 does not alter VO_2 , RER, VE, RPE, blood glucose, or arterial blood flow during/following high-intensity cycling. The small sample size, supplementation adherence, and training stipulations associated with the study present limitations to the generalizability of our findings. However, the double-blind, placebo-controlled, cross-over design of our study, in concert with the intensity of the cycling protocol and numerous physiological parameters investigated, provides a strong basis for our conclusion that herbal supplementation, SR2W-1 in particular, is of limited ergogenic value.

CHAPTER FIVE

SUMMARY

The primary aims of this study were to evaluate the effect of SR2W-1 supplementation on high-intensity cycling performance, blood and muscle lactate levels, and a host of other physiological parameters (i.e. blood glucose, VO_2 , RER, VE, HR, RPE, and skeletal muscle blood flow) utilizing a randomized, double-blinded, placebo-controlled crossover design. We hypothesized that SR2W-1 supplementation would: 1) improve time to fatigue compared to a placebo, 2) elicit lower blood lactate levels during a 20-minutes fixed-intensity ride, high-intensity intervals, and recovery compared to a placebo, 3) elicit lower skeletal muscle lactate levels during 15-minutes of passive recovery from high-intensity cycling compared to a placebo, 4) increase skeletal muscle blood flow compared to a placebo.

Contrary to our hypotheses, SR2W-1 did not improve high-intensity cycling performance or decrease blood or skeletal muscle lactate levels. Furthermore, there were no differences in blood glucose, VO_2 , RER, VE, HR, RPE, and skeletal muscle blood flow with SR2W-1 supplementation compared to placebo. Some possible explanations for a lack of efficacy include, but are not limited to, a short supplementation period of 21 days, short duration of the cycling protocol, difficulty adhering to the supplementation (pill ingestion every day) and training stipulations (maintenance of training intensity and duration throughout the study) required for participation, and a small sample size ($n = 7$).

Notwithstanding these limitations, our findings indicate that SR2W-1 supplementation is of little ergogenic value.

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 3x 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:

Pre-testing/Screening (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 ≥ 40 ml/kg/min if female or ≥ 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.

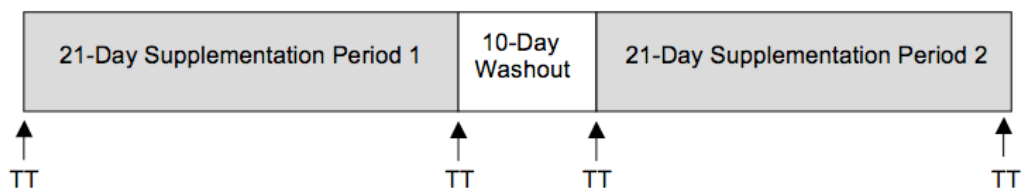
Familiarization Trial: (n = 1 trial)

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)

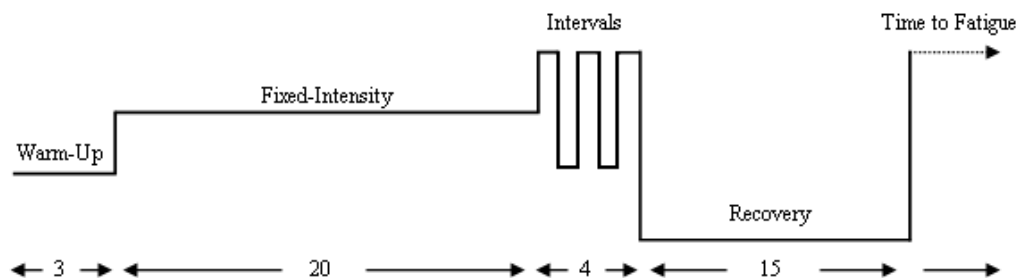
Within 5-14 days of the familiarization trial, you will be asked to perform the first of four treatment trials (TT). The treatment trials will take place before and after 21 days of sports formula supplementation and before and after 21 days of placebo supplementation. You will be randomly assigned to 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 muscle function - central fatigue test OR a thigh muscle biopsy, 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 doses 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 handling 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)

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***.

- _____ pacemaker/implantable cardiac
- _____ defibrillator/rhythm disturbance
- _____ heart valve disease
- _____ heart failure
- _____ heart transplantation
- _____ congenital heart disease

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)

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.

Cardiovascular risk factors

- _____ 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?

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 VII

Inventory of Supplies Necessary to Complete this Project

Pre-Screening Trials*Lactate Threshold Blood Analysis*

Supplies Needed	Brand and Item Number
Sterile Alcohol Prep Pads	Fisher Brand: 06-669-62
Mini-capillary Blood Collection Tubes	Ram Scientific: 07 6101
Unistik Lancets	Owen Mumford Ltd: AT 1013 CE053
Kendal Versalon All-Purpose Sponges, 2 x 2	Tyco Healthcare Group: 9022

Treatment Trials

Lactate Blood Analysis

Supplies Needed	Brand and Item Number
Excel Safelet Catheter Needles (20G x 1 1/4")	Superior Silver: EP85465
Baxter Standard Bore 3-Way Stopcock	Med Specialties Distr.: 2C5600
Bacteriostatic 0.9% Sodium Chloride	Hospira: NDC 0409-1966-07
Monoject 6 mL Luer-Lok Syringes	Tyco Healthcare: 1180600777
Kendal Versalon All-Purpose Sponges, 2 x 2	Tyco Healthcare Group: 9022

Treatment Trials

Muscle Biopsy

Supplies Needed	Brand and Item Number
Biopsy Needle	Stille: 119-29187-50
Crosstex self-sealing sterilization pouch	Fisher Brand: 01-312-51
Lidocaine HCl 0.1%	Hospira: NDL 0409-4276-02
BD 3 mL Syringe, Luer-Lok Tip	Becton Dickinson: 309585
23G TW Needles, Precision Glide	Becton Dickinson: 305193
Monoject Safety Needles, 20G x 1"	Tyco Healthcare: 8881850010
Safety Lock carbon steel surgical blades	Bard-Parker: 371151
1" Durapore Tape	3M: 1538-1
Kendal Curity Gauze Sponges 4 x 4	Tyco Healthcare: 2187
Kendal Versalon All-Purpose Sponges, 2 x 2	Tyco Healthcare Group: 9022
Betadine Swab Stick	Purdue Products: NDC 67618-153-01
Poly lined sterile field	Basse: 696
Elastikon Tape	Johnson and Johnson: 005171

Supplies Needed	Brand and Item Number
Coban	3M: NDC 8333-1582-01
Maxizyme	Henry Schein: 101-9031
Kenal 140 mL Luer-Lok Syringe	Tyco Healthcare: 8881114063
33" Tubing, latex free	Smiths Medical: 2009-12

Treatment Trials*Tissue Processing*

Supplies Needed	Brand and Item Number
Disposable Scalpel, #10	Feather: 2975
Cryo Tube Vials	Nunc: 375418
Petri Dishes for 47 mm cultures	Fisher Brand: 09-720-500
Kendal Curity gauze Sponges 4 x 4	Tyco Healthcare: 2187
Dulmont Medial Tweezers, 110mm, #5	Ted Pella, Inc.: 38125

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