EFFECT OF CAPSAICIN SUPPLEMENTATION ON PERFORMANCE OF AND PHYSIOLOGICAL RESPONSE TO REPEATED SPRINTING

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ABSTRACT

Aim: Fatigue during team sports requiring multiple sprints can result from the combined effects of metabolic, mechanical, neurological, and immune factors. The purpose of this study was to investigate the influence of capsaicin on performance of and the physiological response to an exercise test simulating the fitness demands of team sport game conditions. Methods: This study was a placebo-controlled, crossover design. Nineteen healthy male experienced athletes age 18-30 yr consumed either 3 g/d cayenne (25.8 mg/d capsaicin) or placebo for 1 wk. Directly following the supplementation period, they completed a repeated sprint test consisting of 15 30 m maximal effort sprints on 35 s intervals. Sprint times were recorded via electronic dual-beam timing system. Fasted blood draws for interleukin-6 (IL-6) were taken at baseline prior to supplementation, 45-min pretest, and immediately post test. Heart rate (HR), blood pressure (BP), rate of perceived exertion (RPE), muscle soreness (MS), and gastrointestinal distress (GD) were measured 1-min pretest, during, posttest, and 1-min posttest. MS was also measured for 3 d posttest. Results: Relative to the placebo, capsaicin significantly reduced maximum HR by 9.3%, total average HR by 8.5%, and sprinting average HR by 6.0% ($P<0.05$). Capsaicin caused GD of at least 2/5 in 24.5% of subjects. There was no difference between treatments in fastest or mean sprint time, fatigue, percent change or difference in IL-6, BP, RPE, sprint or posttest MS. Conclusion: Capsaicin did not influence repeated sprint performance or the inflammatory response, but reduced HR during intense activity and causes substantial GD.

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Table of Contents

LIST OF TABLES

Chapter 2 Tables

Manuscript Tables

Appendix Tables

LIST OF FIGURES

Manuscript Figures

Chapter 1: INTRODUCTION

Introduction

The combination of aerobic endurance and anaerobic speed and power requirements in sports involving multiple sprints can make it difficult to simulate the conditions experienced during a game and assess performance. Repeated sprint testing (RST) is considered to be a valid, reliable, and sensitive assessment of these fitness components in such sports (122, 42). The impairment of performance as exercise progresses during RST can be attributed to the complex interaction of factors (40, 38, 4, 10, 99).

High intensity exercise can induce muscle damage and hinder performance (134). The cytokine IL-6 is important in inflammation and is produced in response to exercise in greater amounts than any other (108). While inflammation is important in the recovery process, a prolonged or exaggerated IL-6 response can be detrimental, delaying recovery and leading to muscle atrophy (47). Additionally, reactive oxygen species (ROS) can cause cellular damage and oxidative stress if unbalanced by antioxidants. ROS production is increased during intense exercise and contributes to the pronounced inflammatory response (28, 71). Non-steroidal anti-inflammatory drugs are commonly used to reduce the pain associated with exercise-induced muscle damage and inflammation. However, there can be side effects in taking these drugs (8). If the initial exercise-induced inflammation can be reduced, it may be to the athletes' benefit in facilitating recovery, and thereby improve subsequent performance.

The factors that may contribute to fatigue arise from different biological systems. Intracellular calcium uptake and release by the sarcoplasmic reticulum (SR) is imperative in muscular contraction. Inhibition of this flux due to metabolite accumulation can result in impaired muscle function and lead to fatigue (4, 40, 50, 102). Additionally, ROS have been linked to the onset of fatigue (85, 83) and impaired muscle function for a variety of cellular

mechanisms including calcium regulation (36, 114). Providing an additional mechanism for calcium flux in active muscle might delay the onset of fatigue. Vasodilation may also help through enhanced blood flow; accelerating recovery and metabolite clearance (48, 118). Another ergogenic strategy for repeated sprints could be through central nervous system (CNS) stimulation (14, 128). This neurological component of fatigue regulated by catecholamines is thought to affect variables such as motivation, drive, mood, alertness, reaction time, and rating of perceived exertion (RPE) (27, 86). Additionally, voluntary muscle contraction is controlled via input from the CNS, and reduced neuromuscular activity is correlated with impaired performance in repeated bouts of intense exercise (111, 88). It may therefore be of benefit to ensure optimal CNS activity through increased catecholamine levels to delay the onset of fatigue.

Ephedrine and caffeine are popular drugs used as ergonenic aids in endurance and intense exercise, including repeated sprints. Both ephedrine, caffeine, and their combination have been shown to reduce RPE during exercise, which is likely a consequence of their effect on catecholamines (13, 23). Of concern regarding the use of caffeine and ephedrine are the cardio-stimulatory effects (increased blood pressure and heart rate) that accompany ingestion of these compounds (39, 72). These concerns are part of the reason that ephedrine has been banned by the FDA and should be taken into consideration when recommending a supplement for sports performance (109).

Capsaicin (CAP) is the spicy component of the chili pepper that gives it the characteristic pungent flavor. Its physiological effects are similar to that of ephedrine and caffeine in that it induces catecholamine secretion and stimulates the CNS (78, 141), though the mechanism is through activation of TRPV1 receptors (24). It does not have the cardiac effects of the aforementioned stimulants, and may have a variety of other physiological effects that may prove

beneficial to intense exercise. It has been shown to activate ion channels in the SR of muscle cells, encouraging intercellular calcium flux and improving ischemic threshold through calcitonin gene-related peptide (CGRP) mediated vasodilation (37, 136, 58). It can also function as an anti-inflammatory; activating PPAR-γ, suppressing macrophage infiltration, and reducing cytokine production (2, 21, 123, 63, 104). Additionally, it is an antioxidant, inhibiting LDL oxidation and formation of ROS (80, 113, 59, 97). However, this research has been *in vitro* or utilizing rodent models. Additionally, the limited research involving CAP in humans has focused on its ability to decrease respiratory quotients and shift fuel utilization from carbohydrate to fat, aiding in weight loss and endurance exercise (77, 78). Nevertheless, considering the evidence reviewed above, it is within reason to expect that it may be beneficial for brief intense exercise as well.

Statement of Problem

Fatigue during repeated sprinting is the result of the complicated interaction of a number of mechanisms. A nutritional supplement designed to reduce fatigue should address multiple causes. Few nutritional interventions have been effective in improving repeated sprint performance. Although caffeine and ephedrine have been shown in some studies to be of benefit, they have undesirable side effects. CAP has effects on metabolism and physiology that may translate to improved repeated sprint performance with fewer cardiovascular side effects.

Purpose and Significance

The purpose of this study is to investigate whether chronic supplementation of CAP benefits the performance of and physiological response to repeated sprinting in a healthy population. There is very limited research pertaining to CAP in exercise, particularly in humans. Of those available, all studies utilize aerobic exercise, and no research to date has investigated

the application of CAP towards brief high intensity exercise. This study is the first to examine the effect of CAP supplementation on repeated sprint performance and the physiological and inflammatory response. If effective, this study may lay the groundwork for future research evaluating the mechanisms underlying the action of CAP during exercise.

Hypotheses

Following seven d of CAP supplementation:

1. Sprint performance during repeated sprints will be improved as compared to the placebo (PCB) group as indicated by:
    a. Reduced mean sprint time
    b. Reduced percent fatigue decrement
2. The inflammatory response to repeated sprinting will be improved as compared to the PCB as indicated by:
    a. Reduced concentration of serum IL-6
3. The cardiac response to repeated sprinting will not be different than the PCB group as indicated by:
    a. No difference in heart rate (HR) before, during, and immediately after sprinting
    b. No difference in blood pressure (BP) before, during, and immediately following sprinting
4. Muscle soreness (MS) in response to repeated sprinting will be improved as compared to the PCB group as indicated by:
    a. Reduced rating of MS during sprinting across four leg regions
    b. Reduced rating of MS over three days following sprinting across four leg regions

5. Perception of exertion during sprinting will be improved as compared to the PCB group as indicated by:
    a. Reduced rate of perceived exertion (RPE)

Limitations

- Subjects were free living.
- Results of the study can only be applied to a population similar to the subject sample.
- Blood draw time points were limited to baseline, 45 minutes pretest, and immediately posttest.
- No biochemical assessments of nutritional status or muscle biopsies were performed on subjects.
- All subjects received a standard dose of CAP, regardless of body mass.
- Subjects were able to infer which treatment they were on despite blinding protocol.
- Subjects performed testing under non-realistic "game day" conditions, i.e. fasted with treatment.
- Only a single measure of inflammatory response was measured
- HR and BP were not taken rested pretest but warmed up.
- HR and BP measurements may have varied slightly timing from subject to subject due to equipment operation
- The washout time between treatments varied between subjects.
- Subjects were not restricted to a standardized diet, but required to maintain their usual.
- Subjects were given a dose of treatment prior to testing, therefore the difference in acute versus chronic treatment cannot be separated.
- No mechanisms beyond inflammation were assessed in this study.

- Post-test activity was not regulated.
- Treatment was not pure CAP, but a cayenne supplement containing CAP.

Delimitations

- Subjects were males, ages 18 – 30 years, healthy non-smokers.
- Subjects were free of injury, illness, or disease that would affect performance or measurements, or present a risk to the subjects or researchers.
- Subjects were tolerant of spicy foods.
- Subjects were trained in sports requiring repeated high intensity sprint efforts and/or runners training with intervals and/or speed work.
- The order in which the subjects received their supplements was randomly selected.
- The exercise test was a repeated sprint test consisting of 15 30 m sprints on 35 s intervals.
- The independent variable for the CAP group was consumption of three g of cayenne supplements per d for seven d and one g on testing day.
- The independent variable for the PCB group was consumption of three g of toasted wheat flour per d for seven d and one g on testing day.
- The dependent variables were sprint time, fatigue, serum IL-6, HR, BP, RPE, MS, and gastrointestinal distress (GD).

Basic Assumptions

- Subjects adhered to their dietary restrictions.
- Subjects consumed all required supplements.
- Subjects were honest in completing their questionnaires and surveys.
- Subjects did not exercise in the 24 hours or compete in the 72 hours preceding the test.

- Subjects did not consume alcohol in the 24 hours or caffeine in the 12 hours preceding the test.
- Subjects arrived under 12 hour fasted conditions for testing.
- Subjects wore the same non-compression fitness clothing and footwear for each test.
- Subjects were healthy and gave their maximal efforts during testing.
- HR monitor, BP monitor, timing system, ELISA kit and reader were all working properly.

Abbreviations and definitions of terms

- ATP – adenosine triphosphate: high energy molecule that is the energy source for most cellular processes.
- BMI – body mass index: an anthropometric using an individual's weight and height (kg/m^2).
- BP – blood pressure: the pressure exerted on blood vessel walls via circulating blood, systolic (sys) when blood is pumped, diastolic (dia) when heart is at rest (mmHg).
- CAP – capsaicin: 8-methyl-N-vanillyl-6-nonenamide, a phytochemical exclusive to the genus Capsicum, the "spicy" component of peppers.
- Capsinoid: substances found in peppers that are similar in structure to CAP, but lack the characteristic pungency and, largely, physiological implications.
- CGRP – calcitonin gene related peptide: a peptide released from motor and sensory neurons which has a number of biological effects, including vasodilation.
- CNS – central nervous system
- CRP – C-reactive protein: cytokine found in blood, elevated levels serving as an indicator of inflammation

- GD – gastrointestinal distress: subjective gauge of sensation in gastrointestinal system due to treatment and/or exercise, measured on a 0-5 scale for nausea, intestinal cramping, diarrhea, flatulence, and stomach discomfort.
- HR – heart rate: the number of heart beats per minute (bpm).
- IL-6 – interleukin 6: a cytokine produced by immune cells and muscle cells, used as a marker for inflammation.
- MS – muscle soreness: subjective gauge of pain in of the legs as a result of sprinting, measured by % of a visual scale representing each of 4 leg regions: region 1; upper front (quadriceps), region 2; upper back (hamstrings), region 3; lower front (shins), region 4 lower back (calves).
- NF-κB – nuclear factor κ B: a transcription factor involved in cellular responses, including inflammation.
- PCB – placebo
- PCr – phosphocreatine: anaerobic fuel source for skeletal muscle during brief high intensity activity
- PPAR-γ – peroxisome proliferators activated receptor gamma: a transcription factor involving in cellular responses, including inflammation.
- PST – progressive shuttle-run test: a test used for research and training purposes, requiring repeated sprinting of a set distance on progressively shorter intervals until exhaustion. Used to estimate VO2max.
- ROS – reactive oxygen species: a variety of molecule containing one unpaired electron that is highly reactive and may be damaging on the cellular level if unbalanced by antioxidants

- RPE – rate of perceived exertion: subjective gauge of effort level exhibited while performing exercise, on based on a 6-20 scale.
- RST – repeated sprint test: a test used for research and training purposes to simulate game conditions in sports requiring multiple sprints, using a set number of sprints, distance, and time interval.
- SR – sarcoplasmic reticulum: an organelle found in smooth and striated muscle fibers that functions in calcium ion flux for excitation-contraction coupling.
- SHU – Scoville heat units: a subjective unit of measurement gauging the potency of a product containing CAP, 15 SHU is equivalent to about 1ppm CAP.
- TRPV1 – transient receptor potential vanilloid channel 1: termed the "capsaicin receptor", a cellular receptor that binds to noxious stimuli, functioning in nociception, and serving as a cation channel.
- TNF-α – tumor necrosis factor alpha: a pro-inflammatory cytokine
- $VO2_{max}$: a measure of fitness, an individual's maximum capacity to transport and utilize oxygen during exercise (ml/kg/min)

CHAPTER 2: REVIEW OF LITERATURE

Introduction

Repeated sprint tests (RST) are used to simulate the fitness requirements of team sports for training and research purposes. Performance can be influenced by fatigue, which is the result of a number of factors. Some nutritional interventions have been designed to combat fatigue and improve performance. Capsaicin (CAP), a potentially ergogenic compound, has yet to be investigated. This literature review will first cover RST, the causes of fatigue, factors influencing performance, and physiological consequences. It will then introduce CAP and address its effects which may benefit performance of RST

REPEATED SPRINT TESTING

Validity, Reliability, and Sensitivity

Because performance in team sports such as soccer, lacrosse, field hockey, or rugby is complex and involves many variables, it is a challenge to the researcher to simulate game conditions. Due to this variability of conditions experienced during a game and the nature of the equipment and measurements required, it is exceptionally difficult to measure physiological responses and quantify performance during team sport events. The researcher must therefore simulate game conditions as best as possible under a controlled testing protocol. A good test should be valid in terms of its relevance to team sports, reliable regarding its measurements, and should be sensitive enough to detect differences in performance (29).

RST, while substantially different from game conditions is the best method of assessing the physical responses regarding these criteria (40). Rampinini et al. evaluated the validity of different performance tests; an incremental run to exhaustion, vertical jump, and repeated sprint test of 6 x 40 m separated by 20 s, as measurements of performance in team sports. Physical performance of 18 soccer players was analyzed during games by video and computer image

recognition systems and match-analysis comparisons to performance during the tests conducted. The authors found that the repeated sprint and incremental run to exhaustion tests were valid in reproducing conditions of game play and assessing physical performance (112). Glaister et al. used 10 male subjects and compared the validity and reliability of eight different methods of analyzing fatigue in two repeated sprint protocols. They determined that in repeated sprint tests, percentage decrement calculation (% fatigue decrement = (100 x (total sprint time/ideal sprint time)) – 100) was the most reliable and valid method for gauging fatigue (41).

RST protocols can vary, but generally involve a set distance, number of sprints and a recovery period between sprints that is either as part of an interval or a set amount of time. For team sport simulations, the majority of tests involve a 5 to 6 s sprint of 15 to 40 m, with 5 to 15 repetitions (9, 34, 42, 41, 52, 112, 105). Spencer et al. speculated that shorter duration and fewer sprints may be more accurate in reproducing game conditions (121). However, a limitation to shorter tests is that they may not be sensitive enough to identify changes in performance.

RST can be reliable without prior subject familiarization to the protocol. Glaister et al. studied RST in 25 male team sport athletes, performing 12 x 30 m sprints on 35 s intervals. The protocol was found to be a dependable gauge for evaluating fitness in athletes participating in sports requiring multiple sprint efforts, without prior familiarization to the testing protocol (42). Subjects should be accustomed to sprinting however, to eliminate a potential training effect.

As with any simulation, it is important to be able to apply the results of an RST. Spencer et al. examined a 6 x 30 m RST in 10 highly trained male field hockey players. The typical error of measurements was 0.7% with the smallest worthwhile change calculated to be 0.15 s, or 0.6%. Based upon their results, they estimated that a 0.04 s improvement over a 30 m sprint,

corresponding to a "real and worthwhile" difference, would provide roughly a 26 to 28 cm advantage; enough to beat an opponent to a ball or intercept a pass (122).

Not surprisingly, there is a decrease in performance observed over an RST. Dupont et al. found a significant relationship between the relative decrement in speed and cumulated sprint time ($R=0.71$, $p<0.05$) in 11 male soccer players performing 15 x 40 m repeated sprints with 25 s active recovery in between, with the first sprint's time not being significantly different from the second, but significantly ($p<0.01$) shorter than the subsequent 13 (34). Our own lab group found that following an RST protocol using 12 x 30 m on 35 s intervals, sprint times were significantly longer (5.9 %, $P<0.05$) by the 9th sprint (unpublished lab data). In consideration of this decline in sprint performance during repeated sprinting and the potential consequences during a game that only a few tenths of a second may have, the importance of maintaining sprint ability is apparent.

Since beating an opponent to a ball in a sprint may be determined by the matter of centimeters or fractions of a second (122), the accuracy of the timing apparatus in measuring performance in RST is very important. In a comparison between hand held stop watches and electronic timing systems (Speedtrap II, Brower Timing Systems, Draper, UT) measuring sprint times, Hetzler et al. found that when high degrees of precision are necessary, an electronic system is superior (49). However, this study used a single beam laser system, which is susceptible to false triggering by the subject due to differences in stride length. Accordingly, Yeadon compared single beam to dual beam timing systems and found that the dual beam was capable of accurately measuring sprint times without having to adjust the sensors for varying subject characteristics, thereby eliminating additional unnecessary variability in the study (146).

Causes of Fatigue

Fatigue exhibited in repeated bouts of high intensity activity is the result of a complex combination of factors. Phosphocreatine (PCr) availability and the ability to resynthesize adenosine triphosphate (ATP) is one believed to be one potentially limiting factor. Anaerobic ATP production shifts during repeated sprints, from PCr contributing about 50 % in the first to upwards of 80 % in the tenth (38). Yquel et al. studied PCr resynthesis in nine healthy male subjects performing repeated bouts of maximal exercise following creatine supplementation. They found that the group ingesting creatine had a 5 % significantly ($p<0.05$) higher power output and increased PCr resynthesis than the control group (147).

The concentration of intracellular calcium ions is regulated by the sarcoplasmic reticulum (SR) and is important to muscular contraction. The ability of calcium ions to enter and exit the SR has major implications on intense exercise (4) and is considered to be one of the major causes of high-intensity muscular fatigue (40). Hill et al. studied the flux of SR calcium in intensely exercised muscle in nine healthy subjects. They found that there was a significant ($p<0.05$) correlation of depressed calcium release with a 33 % decrease in maximum voluntary torque following exercise (50). Ortenblad et al. examined the SR calcium flux in trained and untrained male subjects performing repeated sprints. The authors found that there was a significantly ($p<0.05$) increased rate of SR calcium release, 12 % increase in power, and reduced fatigue in trained compared to untrained subjects (102). These findings suggest that increased calcium availability through enhanced SR release is important to intense exercise, and may benefit repeated sprint performance. The flux of other ions, as regulated by sodium-potassium pump activity, may also contribute to muscular fatigue (40). Nielsen et al. studied potassium accumulation in trained versus untrained legs performing incremental exercise to exhaustion.

The authors found a significantly ($p<0.05$) 28 % longer time to exhaustion in the trained leg versus the untrained leg and attributed this to the significantly higher concentration of potassium in the untrained leg during exercise (99).

Muscle oxygen availability is not likely to be a cause of fatigue in repeated sprints. Following exercise at VO_{2max}, the content of oxygen bound to myoglobin is reduced to 50 % of resting values within 20 s, but fully restored within 20 s of recover (91, 115). Accordingly, during repeated sprints with a sufficient recovery period, muscle oxygen should not be a limiting factor. However, blood flow and oxygen delivery may influence recovery and thereby affect fatigue in a subsequent sprint. Norman et al. studied blood flow in response to sprint exercise in healthy and myoadenylate deaminase (mAMPD) deficit individuals. mAMPD affects the accumulation of adenosine following exercise. Adenosine is a potent vasodilator, and its presence results in increased blood flow. The authors found that following intense sprinting, individuals deficit in mAMPD had a 33 % greater peak blood flow ($p<0.05$) and faster decrease in heart rate (HR) ($p<0.01$) post-exercise, and twice as fast return to normal blood flow during recovery than in normal individuals ($p<0.001$) (100). These results suggest that vasodilation enhances blood flow which may aid recovery following sprint activity.

Another possible cause of fatigue in repeated sprints regards the central fatigue hypothesis. Voluntary muscle activation requires neurological input, e.g. a stimulating signal from the CNS, to induce contraction. As exercise continues, the demand for this central drive increases in order to maintain intensity. Failure to deliver this stimulation can result in fatigue and reduced performance (61). Due to the nature of running and the equipment involved, it is very difficult to study neuromuscular activity in repeated running sprints. However, Mendez-Villanueva et al. studied neuromuscular factors of fatigue using surface electromyogram (EMG)

recordings in eight cyclists performing 10 x 6 s repeated sprints on a cycle ergometer with 30 s rest in between. They found that from first sprint to the tenth, there was a significant ($p<0.05$) decrease in peak power output (24.6 %), mean power output (28.3 %), a 14.6 % decrease in neuromuscular activity (as indicated by EMG RMS), and a strong linear relationship ($R^2=0.97$, $p<0.05$) between the neuromuscular activity and mean power output decline (88). Similarly, Racinais et al. examined nine cyclists performing the same cycling protocol and measuring neuromuscular activity. They found that there was a significant ($p<0.05$) decrease in maximal power in the last five sprints, and following the repeated sprints a 16.5 % decrease in maximum voluntary contraction from prior to sprinting, associated with an 11.4 % decrease in neuromuscular efficiency (force/RMS activity) (111). These results suggest that the reduced power exhibited during the progression of repeated sprints may, in part, be due to reduced neuromuscular drive.

Muscle Damage and Soreness

Intense activity, particularly eccentric exercise, can induce muscle damage and lead to soreness that may impair subsequent activity. Twist et al. investigated exercise- induced muscle damage and repeated sprint performance in 10 team sport university level athletes. Following a muscle damage inducing protocol of 10 sets of 10 maximal vertical jumps with 1 min recovery between, subjects performed 10 x 10 m sprints with 12 s rest in between. The authors found that peak running time was significantly ($p<0.05$) higher than baseline times at 30 min, 24 and 48 hr following the muscle damaging protocol, approximately corresponding to a 3% increase in sprint time over 10 m (134). Howatson et al. had 20 healthy collegiate males perform an RST involving 15 30 m sprints with a 10 m deceleration area and 60 s rest between sprints in order to determine the magnitude of muscle damage. Measures of muscle damage, soreness, and function

(maximal voluntary contraction, creatine kinase, muscle soreness (MS), and lower limb girth) were gauged 1 hr pre and posttest, and 24, 48, and 72 hr posttest. The authors found that this protocol was sufficient in inducing significant muscle damage in all variables by 24 and 48 hr posttest ($P<0.05$). Compared to pretest values, force production was reduced by 28 % at 24 hr, a 3 % limb girth increase remained for up to 48 hr, and MS peaked at 48 hr with a ~ 110 % increase (52). This pain may result in impaired short term future performance.

Beyond testing of game condition simulating protocols, muscle damage and impaired performance has been documented following actual matches. Ascensão et al. studied the muscle damage in 16 male soccer players following a 2 45 min half soccer match. MS, muscle strength, sprint ability, and blood markers of muscle damage and oxidative stress were measured at 30 min and 24, 48, and 72 hr post match. The authors found that the soccer match caused significant MS, reduction in sprint ability by at least 5 % for up to 72 hr, reduction in peak hamstring and quadriceps torque by 10 % at 48 hr, and increase in blood markers of muscle damage for several days post test, as well as evidence for increased oxidative stress (6).

Inflammation and Oxidative Stress

Inflammation follows intense exercise, as indicated by circulating cytokines. Interleukin-6 (IL-6) is reported to be produced in greater amounts than any other cytokine as a result of exercise (108). This acute response is believed to be functional in adaptation to exercise and muscle recovery (107). It is the issue of prolonged inflammation that is of concern, as chronic elevated levels of IL-6 may result in muscle atrophy and inhibit muscle recovery (47). Attenuation of the inflammatory response may be desired in tournament style game situations where athletes are required to engage in matches on consecutive days.

Meckel et al. studied the anabolic and catabolic hormone and inflammatory markers resulting from RST. In 12 elite junior handball players performing 4 x 250 m sprints, there was a significant ($p<0.002$) ~ 2-fold increase in plasma IL-6 that remained elevated at 1 hr following cessation of the sprints (84). This inflammatory response has been observed to be more pronounced following repeated sprints than in a single bout. Meyer et al. found that in 12 active but not specifically trained male subjects that there was a significantly ($p=0.002$) greater increase in plasma IL-6 15 min following exercise in a repeated sprint protocol than following a single sprint test. This was about a 5.5-fold increase when compared to resting values and 3.75-fold compared to the single sprint test. C-reactive protein (another marker of inflammation) was also significantly ($p<0.05$) elevated for 24 hr above single sprint and resting values following RST (89). These inflammatory responses in RST parallel those observed in multiple sprint team sport games. Ispirlidis et al. found that in 24 elite male soccer players, following a soccer game there was a significant ($p<0.05$) increase in IL-6 by ~ 5.3-fold immediately post game that returned to baseline by 24 hr, and a ~ 2.5-fold increase in C-reactive protein that lasted 24 hr post game. There was also a significant impairment of vertical jumping ability, squatting, and 20 m sprint performance at 24, 48, and 72 hr post game (55). These results suggest an association between the inflammatory response to repeated sprinting and impaired physical performance up to several days afterwards.

It is important not to attribute the presence of IL-6 as the cause of MS and impaired performance. Elevated levels of IL-6 have been reported following exercise in the absence of muscle damage, and exercise-induced IL-6 is now thought to be the result of muscle contraction, (103) though its role in recovery is still a point of contention. However, the problem still remains of the potential detrimental effects of prolonged inflammation. Constant elevated

inflammation could be deleterious during excessive training and intense competition situations. Therefore, attenuation of the inflammatory response to exercise may be of benefit.

Reactive oxygen species (ROS) are metabolic byproducts that stimulate some important metabolic processes but can also cause cellular damage. Their production is increased during physical activity, and the inability to remove all ROS via antioxidants can result in oxidative stress. Cuevas et al. studied oxidative stress and NF-κB (a transcription factor activated by ROS and used as an indicator of inflammation) in eight professional cyclists performing single and multiple intense bouts of cycling sprints. The authors found that following the single and repeated sprints, there was a significant ($p<0.05$) activation in NF-κB and decrease in IκB (an NF-κB inhibitor), indicative of ROS production (28). Accordingly, exercise-induced activation of NF-κB is thought to be brought about by a number of mechanisms, including oxidative stress and ROS production. As NF-κB influences the expression of over 150 genes including those of acute-phase response proteins and pro-inflammatory cytokines, the activation of NF-κB has important implications, particularly in the inflammatory response (71). A nutritional intervention to increase an athlete's antioxidant capacity and reduce production ROS may therefore be beneficial in attenuating inflammation.

Central Nervous System Stimulants and Exercise

A number of nutritional interventions have been employed to reduce fatigue with repeated sprinting. Oral creatine was described earlier. Study of caffeine and ephedrine is of most relevance to our hypotheses. While not targeted at inhibiting inflammation or ROS management, these compounds are utilized for their ability to offset fatigue, presumably eliciting their effects through enhancing catecholamine secretion and stimulation of the central nervous system (CNS) (79). Bell et al. studied the effects of caffeine, ephedrine, and their combination

on intense cycling performance in eight healthy males. Following a warm up, subjects cycled at 125 % VO_{2peak} to exhaustion. The combination of caffeine and ephedrine increased time to exhaustion by about 38 %) and reduced RPE while caffeine alone increased time to exhaustion by about 8 % compared to placebo (PCB). The authors attributed this to enhanced stimulation of CNS-mediated functions, associated with the significant increases in plasma epinephrine (0.29±0.16 versus 0.50±0.22 nM) and norepinephrine (27.3±8.6 versus 34.3±7.0 nM) compared to the PCB post exercise (13). Jackman et al. examined the effects of caffeine on 14 active male and female subjects performing exhaustive cycling tests. Subjects were given 6 mg/kg caffeine then required to cycle for 2 x 2 min at VO_{2max} power with 6 min rest between, then cycle to exhaustion. They found a significant ($p<0.05$) increase in mean time to exhaustion in the caffeine group compared to the PCB group (4.93±0.60 versus 4.12±0.36 min), and significantly increased plasma epinephrine throughout the exercise protocol (57).

Davis et al. injected male Wistar rats in the CNS with caffeine, NECA (an adenosine receptor agonist, countering caffeine's adenosine receptor antagonism), caffeine + NECA, and vehicle. The rats were then made to run until exhaustion. It was found that caffeine significantly ($p<0.004$) increased run time to exhaustion by 60% when compared to the vehicle (119.05±12.28 versus 76.46±8.98 min), NECA reduced this time by 68% (24.25±6.99 min, $p<0.001$), and there was no difference in the caffeine + NECA group compared to the vehicle group. This study supports the notion of performance enhancement via CNS stimulation by caffeine (30). Wiles et al. gave eight trained male cyclists 5 mg/kg caffeine and measured performance in a 1-km time trial. They found a significant decrease in time to completion in the caffeine group compared to the PCB and control (71.1±2.0 versus 73.4±2.3 versus 73.3±2.7 s, $p<0.02$), mean power (523±43 versus 505±46 versus 504±38 W, $p=0.007$), peak power (940±83 versus 864±107 versus 830±87

W, p=0.027), and mean speed (50.7±1.4 versus 49.1±1.5 versus 49.2±1.7 km/hr, p=0.0005) (142). This illustrates a clear advantage in short term high intensity exercise performance.

These performance benefits are also observed in repeated sprints. Schneiker et al. investigated the effects of caffeine on repeated sprint performance on 10 male team sport athletes. Subjects were given 5 mg/kg and performed 18 x 4 s cycling sprints with 2 min in between, and 5 x 2 s sprints with 18 s in between, in two 36 min halves. Caffeine administration resulted in a significant ($p<0.001$) 8.5 % increase in work performed in the first half, and 7.6 % increase ($p<0.05$) in the second half, and significantly ($p<0.01$) greater peak power in the intermitted sprints by 7.0 % in the first half and 6.6 % in the second half (117). Glaister et al. investigated the effects of 5 mg/kg in 21 healthy active men. Subjects performed 12 x 30 m sprints on 35 s intervals. It was found that the caffeine supplement resulted in significantly ($p<0.05$) faster sprint times, though there was an accompanied greater percent fatigue decrement ($p<0.001$) and HR ($p<0.004$) (41).

There is, however, conflicting research regarding caffeine and performance. Paton et al. found that in 16 male team sport athletes given 6 mg/kg caffeine and made to perform 10 x 20 m sprints with 20 s rest in between, there was a slight but insignificant decrease in sprint speed and increase in fatigue in the caffeine group. It is possible that this lack of effect was due to too short of sprints to elicit fatigue, and errors in the timing system set up (105). Crowe et al. found that in 17 healthy male and female subjects performing 2 x 60 s maximal cycling sprints following 6 mg/kg caffeine ingestion that there was no significant effect on peak power, total work, RPE, plasma epinephrine or norepinephrine, or cognitive parameters (reaction time and number recall) (27). In this case, the sprint may have been too long to observe an ergogenic effect.

From the above literature, one may deduce that in order to gauge an intervention's effect on performance and still have real world implications, an RST should include subjects trained in anaerobic activity (i.e. team sport athletes) and should be of sufficient intensity to induce fatigue while providing enough recovery to allow for fuel substrate regeneration. This will help to isolate the mechanisms of interest influencing performance. Additionally, it may be assumed that a beneficial nutritional intervention in RST have an effect on some metabolic, immune or neurological limiting factor for performance. For example, regulating ion flux may benefit mechanical performance; stimulation of the CNS through enhanced catecholamine secretion may function to delay fatigue; reducing the inflammatory response may facilitate recovery; and addressing muscle damage may reduce the pain and benefit subsequent short term performance.

CAPSAICIN

Background

Capsaicin, 8-methyl-N-vanillyl-6-nonenamide, is the major active component of hot peppers (genus *Capsicum*) gives the fruit its characteristic pungent flavor. A typical chili pepper contains about 0.14 % CAP, though this certainly varies based upon species. Chili peppers are believed to have originated in Central America around 5000 BC, but CAP was not isolated until 1876 (24). It has been used for hundreds of years to enhance the palatability of food or mask un-agreeable flavors, and as a preservative. CAP has been applied in the treatment a variety of ailments including backaches, rheumatism, sore throats and coughs, abdominal issues and ulcers. It has been used to facilitate weight loss through increased metabolism, apatite suppression, and aiding digestion (32). A major aspect of its therapeutic application is that of pain attenuation as a topical analgesic agent. Joint pain, muscle strain, osteoarthritis, and a number of skin ailments

are improved with CAP treatment (24). Oral CAP is readily absorbed through the stomach and intestines to the blood (56), and is also capable of crossing the blood-brain barrier (116).

TRPV1 Receptor

Transient receptor potential vanilloid type 1 (TRPV1) is a CAP sensitive receptor. It is found in neural tissues such as various regions of the brain and spinal cord (26), throughout the gastrointestinal tract, endothelial cells, and other tissues of the body (96). It is believed to work in response to noxious stimuli such as acids, heat, pollutants, and pro-inflammatory substances. TRPV1 has multiple binding sites for CAP, and its activation opens ion channels allowing for the intrarcellular influx of cations. This influx is followed by an initial sensation of pain, but upon prolonged activation desensitization occurs and the characteristic analgesic effect of CAP in exerted (26). TRPV1 also modulates the release of certain neuropeptides such as CGRP, which is a potent vasodilator (67), and substance-P (51), which has implications on catecholamine secretion (148).

Nociception

Nociception is the detection and processing of noxious stimuli. CAP can be used to induce pain, dependent upon dose, duration of exposure, and method and location of application. However, it can also be used to attenuate pain. Berger et al. examined pain in 11 male and female cancer patients exhibiting oral mucositis from therapy. They were given a taffy containing 5-8 mg of CAP (or ½ strength in the case of two subjects) and asked to report pain sensation. It was found that oral pain was significantly ($p<0.01$) reduced, though temporarily and not completely. The authors speculate that a regime building up to higher concentrations of CAP may prolong these analgesic effects (15). Callsen et al. studied cold perception following topical application of 200 µl of CAP in 14 healthy male and female subjects. Thirty min

following treatment, they were subjected to a number of cold stimuli tests. It was found that there was a significant ($p<0.01$) increase in cold detection and cold pain thresholds. Sensation of pain in the primary area of application was reduced and cold tolerance was enhanced following CAP when compared to the control (18).

Anti-inflammatory and Antioxidant Properties

The ability of CAP to reduce inflammation has been demonstrated in several in vitro and animal studies. Kang et al. investigated the influence of CAP on macrophage infiltration induced inflammation in adipose tissue. Adipose tissue was collected from obese mice and cultured with and without CAP. They found that CAP significantly ($p<0.01$) inhibited the gene expression and production of MCP-1 (marker of inflammation) and IL-6 while enhancing adiponectin (an adipokine negatively related to inflammation). CAP inhibited MCP-1 induced macrophage activation ($p<0.05$) and migration ($p<0.01$) into adipose tissue. It suppressed DNA binding activity of NF-κB ($p<0.05$) which regulates expression of TNF-α (a pro-inflammatory cytokine), IL-6, and adiponectin. Their *in vivo* study with obese mice injected with 2 mg/kg CAP for 10 days showed that CAP decreased adipose tissue IL-6 (trend) and MCP-1 ($p<0.01$) production, COX-2 ($p<0.05$), increased adiponectin ($p<0.05$), and reduced the macrophage population in adipose tissue (63). The same research group earlier had found that *in vitro,* lipopolysaccharide induced TNF-α formation in RAW 264.7 cells was significantly ($p<0.01$) inhibited by CAP in a dose-dependent manner (36 % for 10 μM, 42 % for 30 μM, and 59 % for 50 μM). CAP also significantly ($p<0.05$) activated PPAR-γ (a suppressor of inflammatory cytokines) in a dose-dependent manner, indicating that CAP elicits its anti-inflammatory effects through ligation of PPAR-γ thereby inhibiting TNF-α production (104).

Spiller et al. examined C. *baccatum* juice (pepper juice containing CAP) on inflammation. Male Wistar rats were pretreated with the pepper juice 30 min before injection with carrageenan (to induce inflammation). The pepper juice significantly ($p<0.05$) reduced leukocyte migration, exudates (fluids associated with inflammation), neutrophil content, and exudates LDH release in the inflamed area. The pepper juice treatment also reduced TNF-α and IL-1β (a pro-inflammatory marker) levels in immunized mice injected with methylated bovine serum albumin (to induce an immune response) (123). Demirbilek et al. also investigated the effects of CAP on inflammation in rats. Sepsis was induced in female Sprague-Dawley rats by cecal ligation and puncture then the rats received either a high dose (150 mg/kg) or low dose (1 mg/kg) CAP injections and markers of inflammation measured. It was found that the low dosage of CAP in septic rats significantly ($p<0.05$) reduced TNF-α IL-6, and increased IL-10 (an anti-inflammatory cytokine) when compared to the non-CAP treated septic mice (149). These anti-inflammatory properties of CAP may have implications on exercise-induced inflammation, particularly in repeated sprints regarding recovery.

CAP also exhibits antioxidant and anti-atherosclerotic activity in animal models. Manjunatha et al. examined the influences of CAP and curcumin on LDL oxidation induced by iron and copper and also inflammation. Male Wistar rats were fed a diet of 0.2% curcumin, 0.015% CAP, the combination, or control, for 10 weeks then carrageenan was injected to induce inflammation. Iron (II) sulfate (30 mg iron) was injected into the rats to measure *in vivo* LDL oxidation. LDL was isolated from the aforementioned rats and treated with copper (II) sulfate to measure *in vitro* LDL oxidation. It was found that CAP significantly ($p<0.05$) inhibited iron induced LDL oxidation by 62 % when compared to the control, and reduced *in vitro* oxidation. CAP also reduced the carrageenan-induced inflammation by 9 % (80). Choi et al. studied the

effects of CAP on superoxide production and its influence on calcium concentrations in human cells *in vitro*. In a dose-dependent manner, CAP reduced superoxide production by PAF, fMLP (immunomodulators controlling phospholipase-C activation in neutrophils and macrophages) and extracellular ATP (22). Naidu et al. investigated the influence of an assortment of spice components on LDL oxidation, including CAP. Plasma LDL was used from healthy male volunteers and incubated with the individual active spice principles and copper (II) sulfate. It was found that 10 μM CAP significantly ($p<0.02$) inhibited LDL oxidation *in vitro* at 3, 6, and 12 hr, and also decreased the relative electrophoretic mobility of LDL (97). These antioxidant properties of CAP may function in the management of ROS and help to combat oxidative stress and cellular damage.

CNS Stimulation, Catecholamines, and Exercise

CAP has been shown to stimulate catecholamine secretion from the adrenal glands, which can have important implications in the CNS and possibly in exercise performance. Watanabe et al. used male Wistar rats to study the effects of CAP on adrenal catecholamine secretion. CAP injections caused a dose-dependent increase in adrenal activity starting at 20 μg/kg and was significantly ($p<0.05$) above the control at 200 μg/kg. As administration of a cholinergic blocker prevented the increase in epinephrine secretion, and the perfusion of adrenal glands with CAP did not induce an increase in epinephrine levels, this demonstrated that CAP's ability to induce adrenal secretion of catecholamines is not direct but through CNS stimulation (138). Marinelli et al. examined CAP and dopamine in 2-3 week old male Wistar rat brains. Brain slices were prepared and treated with CAP. In a dose-dependent manner, CAP significantly increased dopamine neuron firing rates (27.50±4 % for 1 μM, $p<0.05$, 46 % for 3 μM, and 240 % for 10 μM, $p<0.01$). The application of a TRPV1 antagonist (300 nM IRTX)

significantly reduced firing spike frequencies, indicating CAP's effects are mediated through TRPV1. Injection of CAP also significantly ($p<0.005$) increased dopamine concentrations while CAP+IRTX did not (81).

Kim et al. studied the effect of CAP on endurance capacity and catecholamine secretion in rats. Male rats were given 3-15 mg/kg oral CAP via stomach tube and made to swim to exhaustion in a controlled flow swimming pool 2 hr after administration. Muscle glycogen, serum fatty acids, and epinephrine were measured following exercise. It was found that 10 mg/kg CAP significantly ($p<0.05$) increased swim time to exhaustion and epinephrine concentrations ($p<0.01$). CAP increased swimming endurance capacity by 50 % in intact rats. However, if the adrenal glands were removed, endurance capacity was not significantly increased by CAP. When injected with epinephrine, endurance increased 58 % in the adrenalectomized rats (69). This indicates that performance enhancement obtained through CAP may be mediated through adrenal catecholamine secretions. Oh et al. also studied the effect of CAP on the swimming endurance capacity in rats. Forty nine male Sprague-Dawley rats were fed 6, 10, or 15 mg/kg CAP 2-hrs prior to exercise. A 3 % bodyweight weight was tied to their tales and they were then made to swim to exhaustion. It was found that 15 mg/kg CAP significantly ($p<0.05$) increased time to exhaustion by 219 %, and increased norepinephrine levels at the end of exercise above the control. Fifteen mg/kg CAP also increased blood non-esterified fatty acids (NEFA) and decreased glucose compared to the control, and resulted in higher muscle and liver glycogen content following exercise ($p<0.05$) (101). In another swim-to-exhaustion test, Trudeau et al. neonatally pretreated male Sprague-Dawley rats with CAP to deactivate its receptors. They found that epinephrine ($p=0.0123$) and norepinephrine ($p=0.006$) levels were significantly higher in the normal rats at exhaustion compared to at rest and the

capsaicinized rats, while the capsaicinized rats' epinephrine and norepinephrine levels were not higher at exhaustion than at rest. They also found a longer though not significant time to exhaustion in normal rats (199.33±21.86 min) than in capsaicinized rats (175.33±96.50 min) (132). These results imply that exercise induced catecholamine secretion may involve CAP sensitive neurons.

In one of the few human studies involving CAP and exercise, Shin et al. studied energy metabolism and heart function during exercise in 10 healthy non-obese but sedentary men. Subjects consumed a standard meal, rested for 3 hr, then 1 hr prior to exercise consumed 150 mg CAP or PCB. They then cycled for 30-min at 50% V_{Tmax}. Electrocardiogram, respiratory gas exchange, and cardiac depolarization-repolarization data was collected. It was found that CAP did not affect HR or cardiac depolarization-repolarization at rest or during exercise. It also significantly ($p<0.05$) lowered respiratory quotient values; increased fat oxidation and decreased carbohydrate oxidation during exercise (119). The same research group examined the effect of 150 mg/kg CAP in nine obese male subjects during exercise following a similar protocol, and also found that cardiac depolarization-repolarization was not affected by CAP during exercise (120). Though limited, these studies help to illustrate that while CAP may be capable of stimulating catecholamine secretion similar to caffeine and ephedrine (see table 1 and 2), it may not induce the elevated cardiac responses of these drugs, which may be important to physical performance, as well as cardiovascular disease.

Table 1. Effect of Capsaicin on Catecholamine Levels

Author	Subjects	Treatment	Catecholamine (* = sig.dif from pre, # = sig.dif. from PL)
Kim et al 1997	6wk old male Std ddY mice	10mg/kwbw oral CAP (tube)	[ADR] 71.0→174.7nM (CAP)* 81.9→109.2nM (PCB) 30min post-capsule
Kim et al 1997	6wk old male Std ddY mice	10mg/kwbw oral CAP (via stomach tube)	[ADR] 71.0→98.3nM (CAP)*,# 81.9→49.1Nm (PCB) 120min post-capsule
Oh et al 2003	4wk old male Sprague-Dawley rats	6mg/kgbw oral CAP (via stomach tube)	[ADR] 3.82nM (CAP) 1.64nM (PCB) [NOR] 0.88nM (CAP) 0.88nM (PCB) 120min post-capsule
Oh et al 2003	4wk old male Sprague-Dawley rats	10mg/kgbw oral CAP (via stomach tube)	[ADR] 1.36nM (CAP) 1.64nM (PCB) [NOR] 0.88nM (CAP) 0.88nM (PCB) 120min post-capsule
Oh et al 2003	4wk old male Sprague-Dawley rats	15mg/kgbw oral CAP (via stomach tube)	[ADR] 3.82nM (CAP) 1.64nM (PCB) [NOR] 3.55nM (CAP) 0.88nM (PCB)# 120min post-capsule
Lim et al 1997	8 healthy male mid-long distance runners	30mg oral CAP (from red pepper)	[ADR] 0.60→1.58nM (CAP)# 0.96→0.87nM (PCB) [NOR] 2.96→7.39nM (CAP)# 2.96→4.14nM (PCB) 30 min post-meal

ADR: adrenaline
NOR: noradrenaline
CAP: Capsaicin treatment
PCB: Placebo treatment

Table 2. Effect of Caffeine on Catecholamine Levels

Author	Subjects	Treatment	Catecholamine (* = sig.dif from pre, # = sig.dif. from PL)
Battram et al 2005	9 healthy active males	5mg/kgbw caffeine capsule	[ADR] 0.26→0.26nM (PCB) 0.3→ 0.58±0.02nM (CAF)* [NOR] 0.26→0.36mN (PCB) 0.43→ 0.5 (CAF) 30min post-capsule (rest)
Battram et al 2007	8 healthy male caffeine users	5mg/kgbw caffeine capsule	[ADR] 0.33→0.23nM (PCB) 0.3→ 0.55±0.05nM (CAF)* 30min post-capsule (rest)
Bell et al 1998	8 healthy active males caffeine users	5mg/kgbw caffeine capsule	[ADR] 0.29±0.16nM (PCB) 0.45±0.17nM (CAF) [NOR] 3.3±0.7nM (PCB) 3.0±1.0nM (CAF) 90min post-capsule (rest)
Bell et al2000	16 healthy males (11 caffeine users)	5mg/kgbw caffeine capsule	[ADR] 0.3483±24.6nM (PCB) 0.4516±31.3nM (CAF)# [NOR] 2.7±0.21nM (PCB) 3.3±0.20nM (CAF)# 90min post-capsule (rest)
Bell et al 2000	8 healthy male caffeine users	5mg/kgbw caffeine capsule	[ADR] 0.2674±37.8nM (PCB) 0.4824±56.0nM (CAF)# [NOR] 2.01±0.28nM (PCB) 2.34±0.36nM (CAF)# 90min post-capsule (rest)
Bishop et al 2005	8 endurance trained male low to high caffeine users	6mg/kgbw caffeine capsule	[ADR] 0.075→0.075nM (PCB) 0.1→0.275nM(CAF)*,# [NOR] 1.1±0.2→1.1±0.3nM (PCB) 1.2±0.2→1.6±0.2nM (CAF)* 60min post-capsule (rest)
Bishop et al 2006	11 endurance trained male low to mid caffeine users	6mg/kgbw caffeine dissolved in 3mL/kg flavored water	[ADR] 0.11±0.03→0.14±0.03nM (PCB) 0.11±0.01→0.47±0.08nM (CAF)*,# [NOR] 1.57±0.25→2.02±0.21nM (PCB) 1.70±0.26→2.29±0.23nM (CAF) 60min post-capsule (rest)
Crowe et al 2006	17 male/female team sport low-mid caffeine users	6mg/kgbw caffeine dissolved in 400mL diet soda	[ADR] No differences [NOR] No differences 90min post-capsule (rest)
Laurent et al 2000	20 healthy active males in Navy/Marines	6mg/kgbw caffeine capsule	[ADR] 0.136→0.109nM(PCB) 0.164→0.655nM (CAF)*,# [NOR] 1.18→1.77nM (PCB)* 1.12→2.07nM (CAF)* 90min post-capsule (rest)

Graham et al 1991	5 male + 1 female trained distance runners, non to high caffeine users	9mg/kgbw caffeine capsule	[ADR] 0.2→0.2nM (PCB) 0.2→0.4nM (CAF)*,# [NOR] 1.7→1.7nM (PCB) 1.7→2.25nM (CAF)* 60min post-capsule (rest)
Graham et al 1995	8 male healthy well trained distance runners, non to high caffeine users	3mg/kgbw caffeine capsule	[ADR] 0.4nM (PCB) 0.43nM (CAF) [NOR] 1.9nM (PCB) 3.0nM (CAF)*,# 60min post-capsule (rest)
Graham et al 1995	8 male healthy well trained distance runners, non to high caffeine users	6mg/kgbw caffeine capsule	[ADR] 0.4nM (PCB) 0.62nM (CAF)*,# [NOR] 1.9nM (PCB) 2.9nM (CAF)*,# 60min post-capsule (rest)
Graham et al 1995	8 male healthy well trained distance runners, non to high caffeine users	9mg/kgbw caffeine capsule	[ADR] 0.4nM (PCB) 0.6nM (CAF)*,# [NOR] 1.9nM (PCB) 3.3nM (CAF)*,# 60min post-capsule (rest)
Graham et al 1998	8 male + 1 female healthy trained distance runners, non to mid caffeine users	4.45mg/kgbw caffeine capsule	[ADR] 0.3→0.3nM (PCB) 0.3→0.65nM (CAF)*,# [NOR] No differences 60min post-capsule (rest)
Graham et al 2000	10 healthy males	6mg/kgbw caffeine capsule	[ADR] 0.48±0.055→0.671±0.066nM (PCB) 0.59±0.071→1.46±0.17nM (CAF) [NOR] 1.16±0.12→1.39±0.15nM (PCB) 1.31±0.17→2.41±0.21nM (CAF) 60min post-capsule (rest)
Greer et al 1998	9 healthy active males	6mg/kgbw caffeine tablets	[ADR] 0.3→0.27±0.01nM (PCB) 0.3→0.62±0.08nM (CAF) [NOR] No differences 60min post-tablet (rest)
Greer et al 2000	8 healthy active males	6mg/kgbw caffeine capsule	[ADR] 0.218→0.218nM(CAF) 0.218→0.60nM (CAF)*,# [NOR] 1.4→1.4nM (PCB) 1.2→2.36nM (CAF) 90min post-capsule (rest)
Jackman et al 1996	11 male + 3 female healthy active	6mg/kgbw caffeine capsule	[ADR] 0.25→0.75nM (CAF)*,# 0.25→0.25nM (PCB) [NOR] 1.5→2.3nM (CAF) 1.5→2.0nM (PCB) 60min post-capsule (rest)
Kamimori et al 2000	13 healthy male moderate caffeine	2.1mg/kgbw caffeine capsule	[ADR] 0.11→0.14nM (CAF) 0.06→.08nM (PCB)

	users		[NOR] 1.85→2.25nM (CAF) 1.5→1.65nM (PCB) 60min post-capsule (rest)
Kamimori et al 2000	12 healthy male moderate caffeine users	4.3mg/kgbw caffeine capsule	[ADR] 0.07→0.2nM (CAF) 0.06→0.8nm (PCB) [NOR] 1.7→1.8nM (CAF) 1.5→1.65nM (PCB) 60min post-capsule (rest)
Kamimori et al 2000	13 healthy male moderate caffeine users	8.6mg/kgbw caffeine capsule	[ADR] 0.065→0.36nM (CAF) 0.06→0.8nM (PCB) [NOR] 1.5→2.4nM (CAF) 60min post-capsule (rest)
Lane et al 1990	25 healthy male moderate caffeine users	3.5mg/kgbw caffeine dissolved in water	[ADR] 0.118±0.014→0.201±0.026nM (CAF) 0.147±0.023→0.161±0.028nM (PCB) [NOR] 1.34±0.1→1.95±0.21nM (CAF)* 1.31±0.07→1.51±0.1nM (PCB) 45min post-capsule (rest)
Thong et al 2002	7 healthy moderately active males	5mg/kgbw caffeine capsule	[ADR] 0.25±0.03→0.49±0.07nM (CAF)*,# 0.22±0.01→0.24±0.01nM (PCB) [NOR] 1.61±0.09→2.21±0.32nM (CAF) 1.85±0.31→1.79±0.17nM (PCB) 90min post-capsule (rest)
Van Soeren et al 1993	7 healthy active male caffeine users	5mg/kgbw caffeine capsule	[ADR] 0.25→0.20nM (CAF) 0.25→0.20nM (PCB) [NOR] 1.5→3.5nM (CAF) 1.5→3.5nM (CAF) 60min post-capsule (rest)
Van Soeren et al 1993	7 healthy active male caffeine non-users	5mg/kgbw caffeine capsule	[ADR] 0.20→0.35nM (CAF) 0.20→0.20nM (PCB) [NOR] 1.5→2.5nM (CAF) 2.0→2.7nM (PCB) 60min post-capsule (rest)
Whitham et al 2006	10 healthy endurance trained male cyclists	6mg/kgbw caffeine dissolved in 3ml/kg flavored drink	[ADR] 0.2→0.5nM (CAF) 0.2→0.2nM (PCB) [NOR] 1.9→2.2nM (CAF) 1.8→2.1nM (PCB) 60min post-capsule (rest)

ADR: adrenaline
NOR: noradrenaline
CAF: Caffeine treatment
PCB: Placebo treatment

TRPV1, Capsaicin, and Intercellular Calcium

As previously mentioned, a major contributing factor to fatigue in repeated sprints is the attenuation of calcium release from the SR to intensely working muscle fibers for contraction (40). Central to this phenomenon is the inhibition of the ryanodine receptor calcium channels in the SR membrane by accumulated magnesium ions (76), the intracellular concentration of which increases during intense anaerobic exercise (92). TRPV1 is also expressed in the membrane of SR and endoplasmic reticulum (ER) (145). When CAP binds to TRPV1, it is activated and cations, particularly calcium ions, are allowed to flow through its widely opened channels (24). Indeed, Wisnoskey et al. showed that *in vitro* incubation of 10 μM CAP (well within safe physiological range) with cells induced calcium release via activated TRPV1 from ER (143). Xin et al. found that 30 μM CAP applied to rat skeletal muscle elicited SR-release of calcium (145). Morita et al. found that CAP is able to penetrate plasma cell membranes and bind with ER TRPV1 to induce intracellular calcium release, while some more lipophilic CAP analogs are not as capable (93). While it is well beyond the scope of this study to determine, it is possible that CAP may enhance SR calcium release into muscle cells during repeated sprints and help to offset fatigue.

Vasodilation

Vasoconstriction may affect exercise; influencing blood flow, metabolite clearance, and delivery of oxygen and nutrients to working muscles. Harms et al. studied blood flow in seven male competitive cyclists performing 14 x 2.5 min repeated cycling sprints at VO_{2max} with inspiratory loading and unloading (assistance or resistance) to control respiratory work. They found that with increased work of breathing there was a decreased blood flow in locomotor muscles and lower volume oxygen delivered. This reduction in blood flow was due to

vasoconstriction (48). Unfortunately the authors did not measure performance. However, in that oxygen delivery is important to recovery from intense exercise to restore myoglobin oxygen content (115), it would seem that optimal vascular diameter during exercise would be beneficial.

CAP may provide the stimulus for vasodilation during exercise. Van der Schueren et al. investigated transdermal CAP and dermal blood flow. Varying doses (100, 300, and 1000 μg) of CAP was topically applied to the forearm of 12 healthy male subjects. Blood flow was measured via laser Doppler scans at 10, 20, 30, 45, and 60-min following application. It was found that 300 and 1000 μg CAP induced a significant ($p<0.001$) increase in dermal blood flow through vasodilation (136). Potenza et al. studied CAP and vasodilation in rats. The mesenteric vascular bed of male Wistar rats was exposed and perfused with norepinephrine (to induce vasoconstriction) and then 1-6 nmol of CAP was infused. It was found that CAP had a vasodilating effect in a dose-dependent manner starting at 3.5 nmol, and perfusion with CGRP (8-37) (a CGRP antagonist) inhibited this effect. The authors concluded that the vasodilation ability of CAP is probably mediated through the neural release of CGRP (110). In a human study, Fragasso et al. examined the vasodilation effects of CAP in 12 male subjects with stable coronary disease following exercise. Subjects were given either a 3 g oleic CAP transdermal patch or PCB and performed a treadmill exercise of increasing speed and inclination to fatigue (or other indicator of cessation). It was found that eight subjects significantly ($p<0.01$) increased time to 1 mm ST depression (an indication of myocardial ischemia; oxygen deprivation to the heart) in the CAP group, and at 6-hrs post CAP nitric oxide (a vasodilator) increased significantly ($p<0.05$). Thus the authors suggested that the improved ischemic threshold is probably due to the CAP induced arterial and venous vasodilation (37).

Summary

The dynamic nature of team sports makes it difficult to simulate, however RST is a valid, reliable, and sensitive protocol when performed using tested protocols with dual beam timing equipment. Reduced performance and fatigue in RST can be due to metabolic factors (such as calcium flux, phosphocreatine availability, and ATP resynthesis) psychological factors (involving catecholamines and CNS stimulation) and mechanical factors (involving, muscle damage, inflammation, and recovery). A nutritional intervention designed to enhance performance should address one or a combination of these variables. CAP is the spicy component of chili peppers with many physiological effects. It can serve as a CNS stimulant enhancing catecholamine secretion. It is an anti-inflammatory inhibiting expression and production of pro-inflammatory cytokines and as an antioxidant attenuates ROS production. It enhances blood flow as a vasodilator through CGRP and it may promote calcium release from SR to intensely exercising muscle. The limited research available concerning CAP and exercise has focused on endurance exercise, presumably due to its ability to shift fuel utilization from carbohydrate to fat. Macronutrient fuel usage is of marginal consequence in anaerobic activity. In addition, most of the research on this compound has utilized animal models or in vitro approaches. Since, CAP supplements are currently being marketed for the general population, it is important to verify any benefits of this compound as well as side effects. For all of the aforementioned properties, it is within reason to hypothesize that CAP may prove to be beneficial to the performance of and response to brief high intensity exercise.

CHAPTER 3: JOURNAL MANUSCRIPT

Effect of Capsaicin Supplementation on Performance of and Physiological Response to Repeated Sprinting.

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ABSTRACT

Aim: Fatigue during team sports requiring multiple sprints can result from the combined effects of metabolic, mechanical, neurological, and immune factors. The purpose of this study was to investigate the influence of capsaicin on performance of and the physiological response to an exercise test simulating the fitness demands of team sport game conditions. **Methods:** This study was a placebo-controlled, crossover design. Nineteen healthy male experienced athletes age 18-30 yr consumed either 3 g/d cayenne (25.8 mg/d capsaicin) or placebo for 1 wk. Directly following the supplementation period, they completed a repeated sprint test consisting of 15 30 m maximal effort sprints on 35 s intervals. Sprint times were recorded via electronic dual-beam timing system. Fasted blood draws for interleukin-6 (IL-6) were taken at baseline prior to supplementation, 45 min pretest, and immediately post test. Heart rate (HR), blood pressure (BP), rate of perceived exertion (RPE), muscle soreness (MS), and gastrointestinal distress (GD) were measured 1-min pretest, during, posttest, and 1-min posttest. MS was also measured for 3 d posttest. **Results:** Relative to the placebo, capsaicin significantly reduced maximum HR by 9.3%, total average HR by 8.5%, and sprinting average HR by 6.0% ($P<0.05$). Capsaicin caused GD of at least 2/5 in 24.5% of subjects. There was no difference between treatments in fastest or mean sprint time, fatigue, percent change or difference in IL-6, BP, RPE, sprint or posttest MS. **Conclusion:** Capsaicin did not influence repeated sprint performance or the inflammatory response, but reduced HR during intense activity and causes substantial GD.

Keywords: Capsaicin, Repeated Sprint Test, Inflammation, Heart Rate, Gastrointestinal Distress

INTRODUCTION

The combination of aerobic endurance, anaerobic speed, and power requirements in sports involving multiple sprint efforts such as soccer, lacrosse, rugby, or tennis can make it difficult to simulate the conditions experienced during a game and assess performance. Repeated sprint tests (RST) are considered to be a valid, reliable, and sensitive assessment of these fitness components for such sports (25, 69). An RST typically involves a set number of sprints, distance, and time interval between sprints allowing for recovery. As the RST progresses, sprint times increase due to fatigue, which is the result of a complicated interaction of many systems. These may include substrate availability and regeneration (22, 83), ion management (30, 57, 59), and neurological signaling (51, 63). Nutritional strategies for improving RST have included stimulants such as caffeine or ephedrine (7, 24), supplements enhancing substrate regeneration such as creatine (55), or buffering agent such as sodium bicarbonate (43). However, research is conflicting regarding the benefit of these interventions towards repeated sprint performance.

High intensity exercise such as repeated sprinting can induce muscle damage, resulting in pain which may hinders subsequent performance (3, 75). Interleukin-6 (IL-6) is an inflammatory cytokine, with a dual role as both pro and anti-inflammatory (31, 61). While inflammation is important in the recovery process, prolonged elevated IL-6 levels may be detrimental, delaying recovery and leading to muscle atrophy (29). During excessive training and intense competition situations, the constant elevated inflammation could be deleterious. Non-steroidal anti-inflammatory drugs such as ibuprofen or aspirin are commonly used to reduce the pain associated with exercise. However, there can be side effects in taking these drugs (5).

Capsaicin (CAP), 8-methyl-N-vanillyl-6-nonenamide, is the major active component of hot peppers (genus *Capsicum, Solanaceae*) which gives the fruit its characteristic pungent flavor.

It is readily absorbed through the gastrointestinal tract (33) and can cross the blood brain barrier (64). It has a broad range of biological effects that are mediated by agonism of the transient receptor potential vanilloid receptor type 1 (TRPV1) (13), which is widely distributed throughout the body (14, 46, 54). Activation by CAP results in the opening of its channel allowing for the intercellular influx of cations (13). This influx is responsible for the analgesic properties (9, 80). Additionally, the TRPV1 receptor is present on the sarcoplasmic reticulum and upon activation by CAP has been shown to induce the flux of calcium ions *in vitro* (53, 81, 82). CAP also induces adrenal catecholamine secretion through excitation of the central nervous system (41, 45, 58, 74, 78), which is similar to the increase plasma catecholamines following caffeine administration, but without the associated increased heart rate (67). CAP has been shown to inhibit inflammation *in vitro* (36, 60) and *in vivo* rodent models (36, 17, 70). Further, it induces the release of calcitonin gene-related peptide (CGRP) which results in vasodilation (21, 62, 76,). CGRP has also been shown to play a role in muscle excitability and force recovery (47).

There is very limited research pertaining to CAP in exercise, particularly in humans. Most of the research involving CAP and metabolism has focused on its ability to decrease respiratory quotients and shift fuel utilization from carbohydrate to fat, aiding in weight loss and endurance exercise (41, 44, 45, 58). Based on *in vitro* and animal research, CAP may reduce inflammation, affect pain perception, and affect the cardiovascular response to intense exercise. To the authors' knowledge, there is no research currently on the effects of CAP on brief intense exercise. Therefore, the purpose of this study is to investigate whether chronic supplementation of CAP benefits the performance of and physiological response to repeated sprinting in a healthy population.

METHODS

Design and Subjects

This study was designed as a single-blinded, placebo controlled cross-over study. Subjects were recruited from the Virginia Tech campus and surrounding area via fliers, email, and classroom presentation. Initial screening criteria were healthy males, ages 18-30 yr that were participating in a sport requiring repeated sprints (such as soccer, rugby, field hockey, etc.) or runners that trained with intervals or speed work. Exclusion factors included smoking, medical conditions, recent illnesses or injuries, or medications that could interfere with completing the testing protocol, recent weight fluctuations (±10 % body weight), or supplements being taken for the purpose of improving anaerobic performance. Prior to participation, eligible subjects provided written informed consent as approved by the Virginia Tech Institutional Review Board.

Treatment

Subjects were randomly selected to receive either the placebo (PCB) or CAP intervention first. The PCB consisted of gelatin capsules of 500 mg of toasted wheat flour to replicate appearance/texture of the treatment. The CAP was administered in the form of a commercially available cayenne supplement (GNC Nature's Fingerprint Cayenne Herbal Supplement, Pittsburgh, PA), providing 4.3 mg of CAP per 500 mg capsule (100,000 SHU). Subjects consumed six treatment capsules with food per day for a total of 25.8 mg of CAP per day, for seven d. Subjects were instructed not to consume any additional sources of CAP throughout either intervention period.

Baseline Measurements, VO2max Estimate, and Testing Familiarization

Subjects reported to the lab on three separate mornings following a 12 h fast, refraining from exercise and alcohol for 24 h prior. On the first visit 3-day diet records were collected for nutrient content analysis (Nutrition Data System for Research software version, 2009, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN). Height, weight, and body fat percentage via skin fold caliper were measured. Baseline 5 min post resting heart rate (HR) and blood pressure (BP) via wrist cuff monitor (Prevention WS-820PV) were assessed. A baseline blood draw (B-0) was taken and stored for future analysis of serum IL-6. Subjects then completed a progressive shuttle-run test (PST) to estimate VO_2max and were familiarized with the RST protocol to be encountered during testing. They were given their randomly assigned treatments and instructed as to when to initiate supplementation.

RST Protocol

On the morning of testing following the week of their first treatment, subjects arrived at the lab between 6AM and 9AM under 12 hr fasted conditions, not having exercised or consumed alcohol for 24 hr prior or competing for 72 hr prior to testing, and having consumed 500 ml of water before going to bed that night and when they woke in the morning. Upon arrival, they filled out compliance, activity, and health questionnaires to insure lack of acute infection and had their blood drawn (B-1). They were given a dose of their treatment along with at least 500 ml of water and rested for 45 min.

Testing took place in the same gymnasium on a hard court-type floor under similar climate conditions (23.0±1.8 °C and 53.6±8.4 % humidity). Subjects prepared for testing by following a standardized warm up and stretching routine. The RST protocol consisted of 15 30 m sprints on 35 s intervals based on similar procedures described by Glaister et al (42). One minute prior to the test initiation, HR, BP, rate of perceived exertion (RPE), muscle soreness

(MS), and gastrointestinal distress (GD) were measured. Upon verbal countdown, subjects sprinted down the length of the 30 m run course to the second set of timing gates. They were allowed to rest for the remainder of the 35 s interval before initiating the next sprint. Subjects repeated this procedure for a total 15 sprints. They were instructed to run at maximal effort for each sprint but were not given verbal encouragement, timing feedback, or indication of sprint number in order to prevent pacing strategies and motivation biases.

Following the final sprint, subjects were taken to the laboratory where their last blood sample (B-2) was drawn within 5 minutes. Subjects completed posttest MS surveys 24, 48, and 72 hr following the test. There was at least a one wk washout period, after which they initiated their alternate treatment. Subjects returned to the testing facility following the second supplement period and repeated the protocol described above.

Measurements

BP was measured at one min pretest, following the last sprint, and one min posttest. HR was measured via chest strap HR monitor (Timex Ironman Triathlon Bodylink®, Timex Group, USA) at one min pretest, immediately following every third sprint, and one min posttest. Subjects self reported RPE at one min pretest and after every third sprint by indicating their perception on a visual 20 point Borg scale.

GD was self reported by subjects at one min pretest, after the seventh sprint, following the last sprint, and one min posttest. Subjects were shown a visual scale of the five symptoms; intestinal cramping, diarrhea, nausea, flatulence, and stomach discomfort and as asked to indicate on the scale of zero to five the degree of the symptom; zero indicating none and five indicating severe. MS for region A – front upper legs (quadriceps), region B – back upper legs (hamstrings), region C – front lower legs (shins), and region D – back lower legs (calves) was

self reported by subjects at one min pretest, after the seventh sprint, following the last sprint, and one min posttest. Subjects were shown a visual diagram of a person with the muscle regions A through D indicated. They were asked to gauge on a 100 mm visual scale the degree of MS experienced in each section after lightly shaking the legs while standing.

Sprint performance was measured using a Speedlight TT twin beam timing system (Swift Performance Equipment, Ltd.). Fatigue was calculated using sprint times as described by Glaister et al (25): %FD = [(Total Sum/Ideal)*100]-100, where Ideal = (15*fastest).

Blood was drawn at baseline B-0 prior to supplementation, pretest B-1, and posttest B-2 to be analyzed for markers of inflammation. Samples were taken by a certified phlebotomist via venepuncture. Serum was separated and stored. Serum IL-6 concentrations were determined using an enzyme-linked immunosorbant assay (R&D Systems, Quantikine HS ELISA, Human IL-6) in duplicate. Samples with a coefficient of variance greater than 10% were re-run.

Statistics

Statistical analysis was performed using JMP® 7.0 (SAS Institute Inc. 2007). Descriptive statistics were used to characterize subjects' baseline measurements, profiles, and the testing conditions. Sprint time, IL-6, HR, BP, RPE, GD, and sprinting and posttest MS were evaluated using a multivariate ANOVA with repeated measures, evaluating time and treatment effect and time by treatment interaction. If a significant effect was found, a post hoc student's t-test was conducted to compare individual means to locate the source of differences. One-way ANOVA was used to analyze average and fastest sprint time, fatigue, difference and percent change in IL-6, maximum, total average, and sprint average HR, sprinting and posttest MS average and sum score, and GD average and sum score. All data presented as mean ± standard deviation. A p-value of less than 0.05 was considered significant.

RESULTS

Subjects

Due to withdrawal of four subjects for inability to tolerate treatment due to GD or schedule conflicts, 19 subjects completed the study (see table 1 for mean subject characteristics). Treatment compliance was 93.0 % during the placebo supplementation and 86.5 % during the capsaicin supplementation.

Sprint Performance and Fatigue

During the RST, there was a significant time effect in sprint performance ($P=0.0477$); mean sprint times increased with time and were significantly longer (2.62 %) than the first sprint starting at sprint 5 ($P<0.05$) and 5.77 % longer by the last sprint ($P<0.0001$) (see figure 1). There was not a significant treatment effect or time by treatment interaction nor was there a difference between groups in average sprint time, fastest sprint time, or percent fatigue decrement.

Inflammation

There was a significant time effect in the serum concentration of IL-6 ($P<0.0001$); mean IL-6 was significantly elevated by 59.0 % ($P<0.0005$) from B1 to B2 and by 36.3 % ($P<0.0001$) from B0 to B2 (see table 2). There was no significant difference from B0 to B1. There was not a significant treatment effect or time by treatment interaction for IL-6 concentration nor was there a significant difference between groups when change score or percent change was analyzed.

Cardiac Measures

There was a significant time effect in mean HR during the RST ($P<0.0001$); HR was significantly elevated above pre RST values at all sprint time points by at least 79.4 % and by 39.3 % at post, and sprint 12 HR was 6.7 % higher than sprint 3 ($P<0.0052$). There was a trend for a treatment effect ($P=0.0668$) in that HR tended to be lower following CAP treatment. There was no significant time by treatment interaction. Compared to the PCB, following CAP

treatment values were 9.3 % lower in maximum HR (171.12±15.23 bpm versus 187.73±14.67 bpm, P=0.0026), 8.5 % lower in total average HR for the PCB (144.57± 15.40 bpm versus 157.40±11.28 bpm, P=0.0370) and 6.0 % lower in sprint average HR for the PCB (163.89± 16.28 bpm versus 173.96±11.59 bpm, P=0.0330) (see figure 2).

There was no significant time effect, treatment effect, or time by treatment interaction for systolic blood pressure. There was a trend (p=0.0544) for time effect in diastolic blood pressure, with 1-minute post 14.10% lower than 1-minute pre (P=0.0003) and post 13.17% lower than 1-minute pre (P=0.0007).

RPE

There was a significant time effect in RPE during the RST (P<0.0001); RPE was significantly different from sprint 3 at all following time points (P≤0.0065). There was no significant treatment effect or time by treatment interaction.

Sprint Muscle Soreness

There was a significant effect of time in MS during the RST (P<0.0001); average MS was greater than pretest values at all subsequent time points (P≤0.0012), and post was greater than mid (P=0.0371) (see figure 3). There was no significant treatment effect or time by treatment interaction. There was no significant difference in the total average MS or sum score between the PCB and CAP treatments.

Post Test Muscle Soreness

There was a significant time effect in MS following the RST (P<0.0001); average MS was greater than pretest values up to 2 days following the RST (P≤0.0020) and day 1 MS was greater than Day 3 (P=0.0057) (see figure 4). There was no significant treatment effect or time

by treatment interaction. There was no significant difference in the posttest MS total average or sum score between the PCB and CAP treatments.

Gastrointestinal Distress

There was a significant time effect (P=0.0005) and treatment effect (P<0.0001) in average GD (see figure 5). GD was significantly elevated above pre values at posttest (P=0.0198) and a trend to be higher at mid (P=0.0542). Average GD was significantly higher after CAP treatment at all time points compared to the PCB (P≤0.0005) and in all categories (P<0.05). There was no significant time by treatment interaction. Following the placebo, the occurrence of some symptom of GD during the RST was: 91.3 % for 0/5, 4.7 % for 1/5, 3.4 % for 2/5, 0.5 % for 3/5, and 0 % for 4/5 and 5/5. Following the capsaicin treatment, symptom occurrence was 60.0 % for 0/5, 15.5 % for 1/5, 11.1 % for 2/5, 8.2 % for 3/5, 4.7% for 4/5, and 0.5 % for 5/5. Subjects indicated a rating of at least 2 out of 5 in some symptom of GD during the RST 3.9 % of the time when taking the placebo 24.5 % of the time when taking capsaicin. Intestinal cramps and stomach discomfort contributing the most to GD.

DISCUSSION

The objective of this study was to determine the effect of CAP consumption on repeated sprint performance, and the inflammatory and cardiovascular response to this exercise. The RST was sufficient to induce fatigue, as indicated by the significant increase in sprint time with both treatments by the fifth sprint. This is consistent with the findings of other studies utilizing a similar protocol (20, 24, 32) and unpublished research by our lab group. Fatigue was quantified by the percent fatigue decrement calculation. We found the fatigue decrement, which is the most valid and reliable gauge for fatigue in RST (25), to be 4.84 (±3.05) %. This finding is also in agreement with the literature for this protocol (32). However, we found that there was not an

effect of CAP on individual, fastest, or average sprint times, or in fatigue. This lack of difference in any indices of performance does not support our hypothesis of CAP as ergonenic for repeated sprints.

There is a very limited amount of available research concerning CAP and exercise, particularly in humans. Most studies involving CAP use supplements containing a combination of bioactive ingredients (77) or do not gauge performance (45, 67, 68). Since it is exceptionally rare to find animal studies evaluating intense exercise outside of thoroughbred horses and sled dogs, the only studies investigating CAP and exercise in rodents have been on its influence on fatigue in endurance type activity (41, 48, 58). Limited research supports the value of CAP for endurance exercise with the suggested mechanism being its ability to shift fuel utilization towards fat oxidation. However, in brief intense exercise as in that of the current study, these fuel sources are of marginal consequence to the onset of fatigue.

Catecholamines stimulate the CNS and may reduce fatigue during exercise. In human and animal studies, CAP has been shown to stimulate the adrenal secretion of catecholamines at rest (34, 41, 45, 58, 38, 78, 79) and following exercise (41, 45, 58). While likely through a different mechanism (34), the increase in circulating catecholamines is similar in magnitude to that observed following exercise with caffeine consumption (7, 11, 26, 27, 28, 72). Although there is conflicting research as to the ergogenic effect of caffeine (2, 12, 66, 72), a similar increase in catecholamines with CAP predicts a similar performance benefit. Those studies supporting the benefit of caffeine to exercise performance suggest that in addition to effects on catecholamines, antagonism of adenosine receptors and excitation of the CNS may reduce work perception, i.e. RPE (10, 15, 16, 19). In our study, we did not observe a difference in RPE or performance between the CAP and placebo treatments. This does not support the theoretical connection

between CAP and CNS stimulation. However, we did not measure plasma catecholamines and thus cannot confirm that this treatment successfully stimulated release of these hormones. It is possible that the dose of CAP was not sufficient to induce adequate CNS stimulation to have any influence over work perception. On the other hand, it is possible that the benefits of CNS excitation were compromised by the GD following CAP treatment.

The major finding of this study was that CAP supplementation caused a significant reduction in HR during high intensity exercise without a coinciding reduction in performance or increase in BP. This is contradictory to what was expected. Through activation of the TRPV1 receptor, capsaicin is known to induce the release of CGRP, a potent vasodilator (1, 21, 35, 40, 76). Though reducing BP, vascular dilation would result in an increase in HR, rather than the decrease observed in this study. Further, as mentioned CAP consumption causes the release of catecholamines, which elevate HR and BP (41, 45, 58, 74, 78). A possible explanation for the reduction in HR we observed is that CAP activates TRPV1 receptors which stimulate parasympathetic nervous activity, which suppresses HR (4), though parasympathetic modulation of heart rate is considered to be mostly withdrawn at rates above 120-130 bpm (86) which is below that of the current study. These results are not entirely clear. It is possible that a slight variability in timing of measurements might have contributed to this inconsistency. Additional investigation may be warranted to confirm that the observed difference was due to capsaicin supplementation and not an equipment or timing matter. In light of these results, it is interesting that in some studies during sub-maximal exercise, capsaicin was not reported to affect HR (45, 67).

Although a reduction in exercise HR could be considered to have a negative effect on performance due to lower cardiac output and oxygen delivery, this would not likely influence

performance of this relatively brief (~ 9 min) high intensity exercise bout (23). However, it is possible that a lower exercise HR, if maintained throughout a longer game, could have implications for performance.

There was a significant increase in IL-6 due to the RST. Though the observed increases in IL-6 were similar to our previous lab results (unpublished), they were not as pronounced as in some studies involving repeated sprints. These studies typically involve a longer test and/or are of a different mode of exercise, i.e. cycling sprints (52, 84, 85, 87, 88) and these variations in protocol may contribute to this discrepancy.

We did not observe a difference in IL-6 levels between treatments. CAP has been shown *in vitro* and *in vivo* to reduce inflammation in adipose tissue (17, 36, 60, 70). The mechanism through which CAP is believed to work is through ligation of PPAR-γ and subsequent NF-κB suppression involving immune cells (36, 37). However, it is now accepted that the pronounced increase in IL-6 due to exercise is not an immune cell derived response but instead due to muscle contraction and through pathways independent of NF-κB (61). IL-6 functions in inflammation, but can also serve as an anti-inflammatory cytokine, inducing IL-10, IL-1ra, and inhibition of TNF-α (65, 71). It can also function in signaling for fuel economy (6, 39). It may be that these roles drive the prominent increases in IL-6 observed following exercise, and would therefore not be attenuated by an anti-inflammatory agent such as CAP which targets different pathways.

In any case, we only measured IL-6. There may have been an unobserved difference in other cytokines known to increase following brief intense exercise, such as IL-1β (8, 52), or there may have been significant differences between treatments at time points beyond our measurements once the IL-6 induced via muscle contraction subsided. Mechanistic determinations were beyond the scope of this study. It should also be noted that a post-hoc

power analysis revealed that our sample size was not sufficient to achieve adequate power to detect a minimum effect size.

The RST protocol employed in this study resulted in MS that lasted up to two days following the exercise. This is in agreement with research employing a similar protocol by Howatson et al. (32), though our study was not specifically designed to induce muscle damage and did not result in as pronounced an MS response. Indeed, the MS following our test was less than half of that observed in their study. The observed significant increase may be indicative of exercise induced muscle damage, most likely due to the eccentric breaking components of the RST. This study only measured MS rather than the other indicators of damage such as lower strength, joint angle, or serum creatine kinase. However, MS that continues beyond hours after the exercise is most likely due to muscle injury rather than some immediate metabolic consequence of the exercise bout. CAP is used as the active component of analgesic topical ointments and patches for the alleviation of inflammatory, joint, and muscle pain (18, 42). In our study, the lack of effect of oral CAP on RPE or MS suggests that the mode of administration is important. Our delivery strategy likely did not provide CAP in sufficient amounts to the specific location to elicit analgesic or anti-inflammatory effects.

It is not surprising that the rating of GD in the CAP group was significantly higher than the placebo group at all time points. Oral CAP has been reported to induce severe nausea and cramping for several hours following ingestion of 2 mg/kg (56). Though subjects following the capsaicin treatment indicated 60 % of the time no symptom of GD during the RST, there was still a 24.5 % occurrence of some symptom rating of at least 2 out of 5. One short coming of this study was that our subjects were required to consume their supplement under fasted conditions. The dosage, despite being within the manufacturer's recommendation, was still sufficiently

potent to induce indigestion, stomach discomfort, nausea, intestinal cramping, flatulence, and burning bowel movements in all but one subject. Indeed, these side effects resulted in several subjects withdrawing from the study. There exists a great disparity between the literature and practical application in terms of CAP doses. Studies describe upwards of 150 mg (67, 68) in a single acute dose and 135 mg/d chronic ingestion (44). While well below the oral LD.50 in rats for CAP of 47.2 mg/kgbw LD.50 (MSDS), these amounts are far beyond our observed tolerable doses. Additionally, all subjects received the same dose, yet there was not a relationship between gastric distress and body mass. It may be that tolerance is more a factor of an individual's TRPV1 receptor density in the gastrointestinal tract; an increase in receptors leading to an increase in sensitivity (49). What is remarkable was that despite significantly elevated GD in the CAP group, there was no difference in performance or RPE between groups. One would expect that with GD there would be an associate decline in performance of repeated sprints (73). It could be that CAP has ergogenic potential, but the benefit in this study was undercut by GD.

CONCLUSION

In summary, capsaicin supplementation lowered HR during repeated sprinting, but did not affect performance, fatigue, inflammation, MS, BP, or RPE during repeated sprint testing, despite drastically increasing GD. There was also no effect on MS up to three days after the exercise. This was the first study to assess CAP in terms of high intensity exercise performance in humans. Future research should investigate endurance mode of exercise in humans, building on our results for reduced HR, expanding upon the already demonstrated benefit of CAP in animal studies on endurance, and utilizing a more tolerable supplementation protocol.

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Manuscript Tables and Figures

Table 1. Subject Characteristics

Age	22.63 (±2.56)
Height (cm)	179.05 (±7.36)
Weight (kg)	75.87 (±9.80)
Body Mass Index	23.66 (±2.64)
Body Fat %	13.10 (±4.54)
Ave Resting Heart Rate	65 (±9)
Ave Resting Blood Pressure	134/84
Est. VO2max	45.44 (±5.40)
Training (d/wk)	4.88 (±1.11)
Training (hr/d)	1.80 (±0.54)
kcal/kgbw	42.30 (±9.40)
Diet % carbohydrate	47.37 (±10.19)
Diet %protein	17.79 (±4.84)
Diet %fat	32.95 (±7.89)

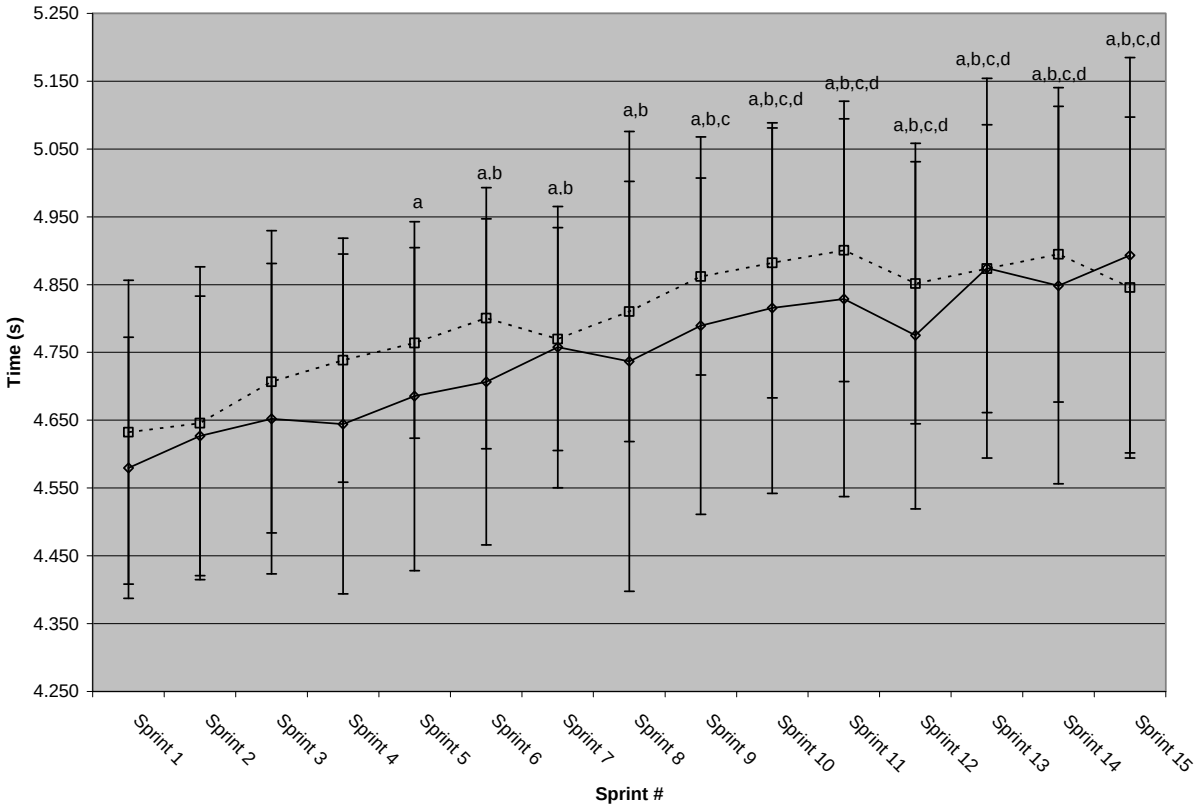
All values mean (±SD)

Table 2. IL-6 (pg/ml)

Time Point	B0	B1	B2	Difference	% Change
Placebo	0.520 (±0.283)	0.667 (±0.365)	1.126 (±0.675)	0.407 (±0.487)	76.9 (±96.3)
Capsaicin	0.520 (±0.283)	0.700 (±0.386)	0.983 (±0.475)	0.248 (±0.300)	41.2 (±53.3)
Mean	0.520 (±0.283)	0.683 (±0.370)	1.055 (±0.580)[a,b]	0.328 (±0.406)	59.0 (±78.8)

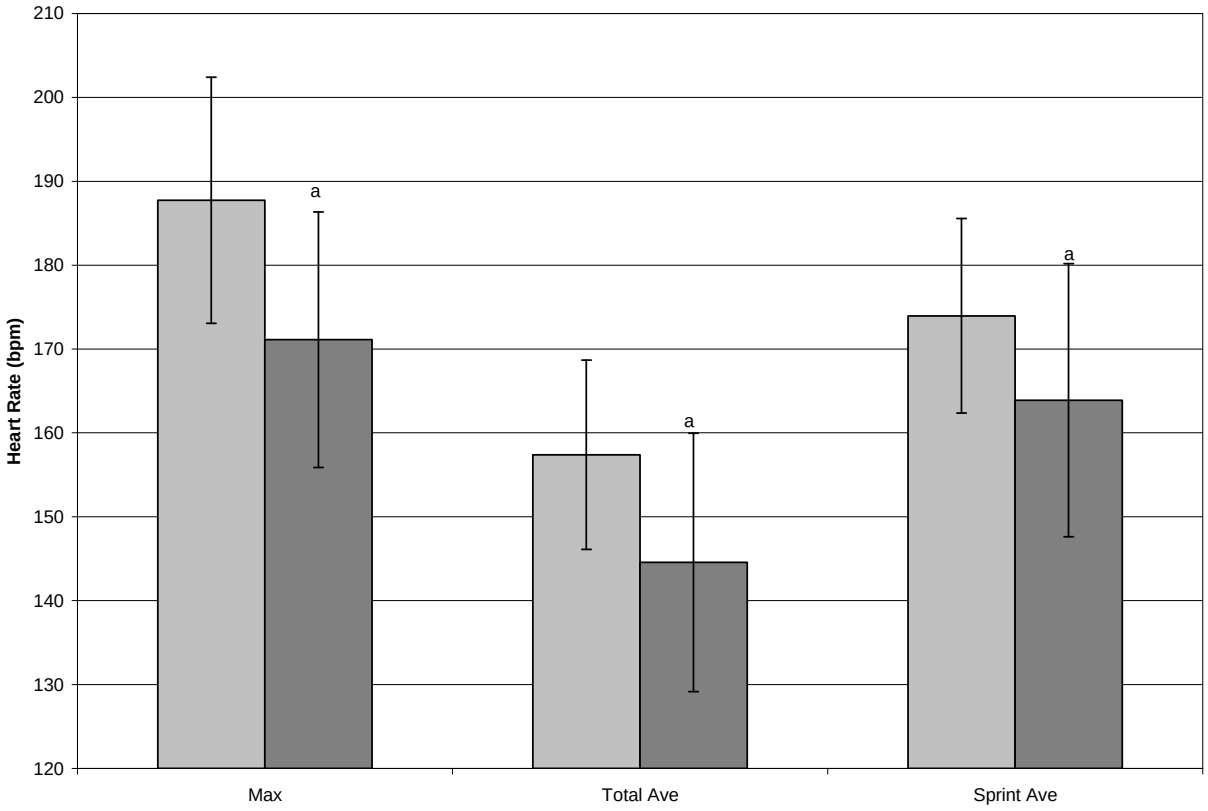
B0 – baseline blood draw prior to supplementation week
B1 – blood draw 1 hr prior to RST
B2 – blood draw immediately following RST
All values mean (±SD)
[a] Significantly higher than B0, $P<0.0001$
[b] Significantly higher than B1, $P<0.0005$

Figure 1. Sprint Times



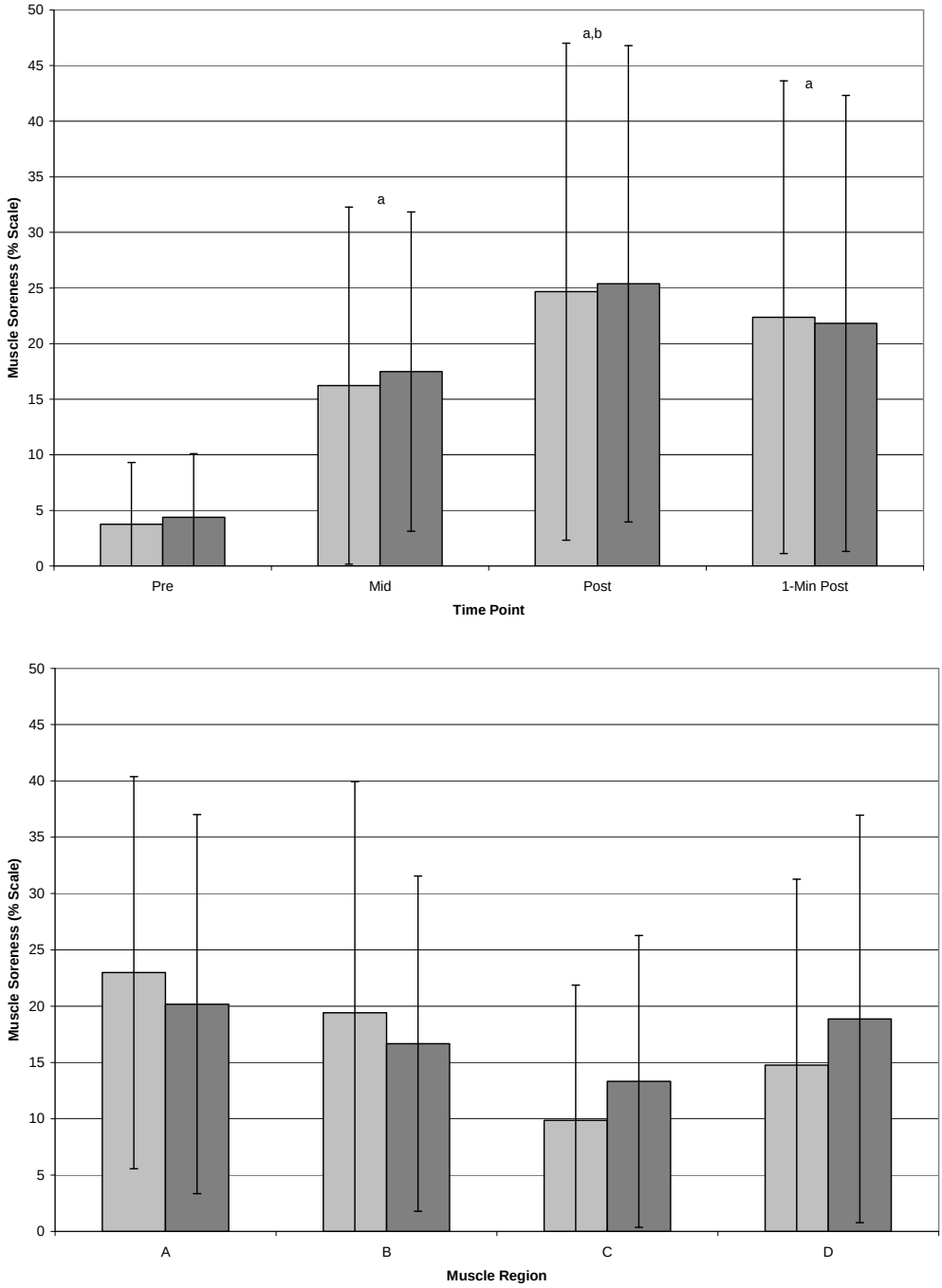
Mean Sprint Times (±SD)
Placebo (—◇—), Capsaicin (--□--).
[a]Significantly longer than sprint 1 (P<0.05)
[b] Significantly longer than sprint 2 (P<0.05)
[c] Significantly longer than sprint 3 (P<0.05)
[d] Significantly longer than sprint 4 (P<0.05)

Figure 2. Heart Rate



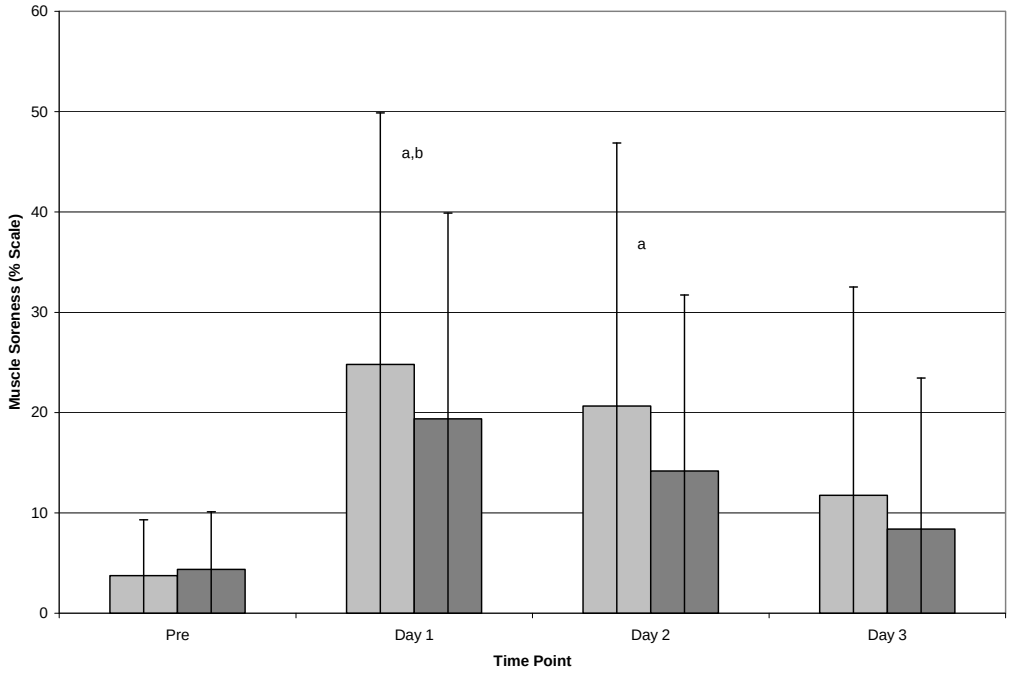
Mean Heart Rate Summary (±SD)
Placebo ▁, Capsaicin ▁
[a] Significantly lower than placebo (P≤0.037)
Total Average Heart Rate includes measurements at 1-min pretest, after every 3rd sprint, and 1-min posttest
Sprint Average Heart Rate includes measurements after every 3rd sprint

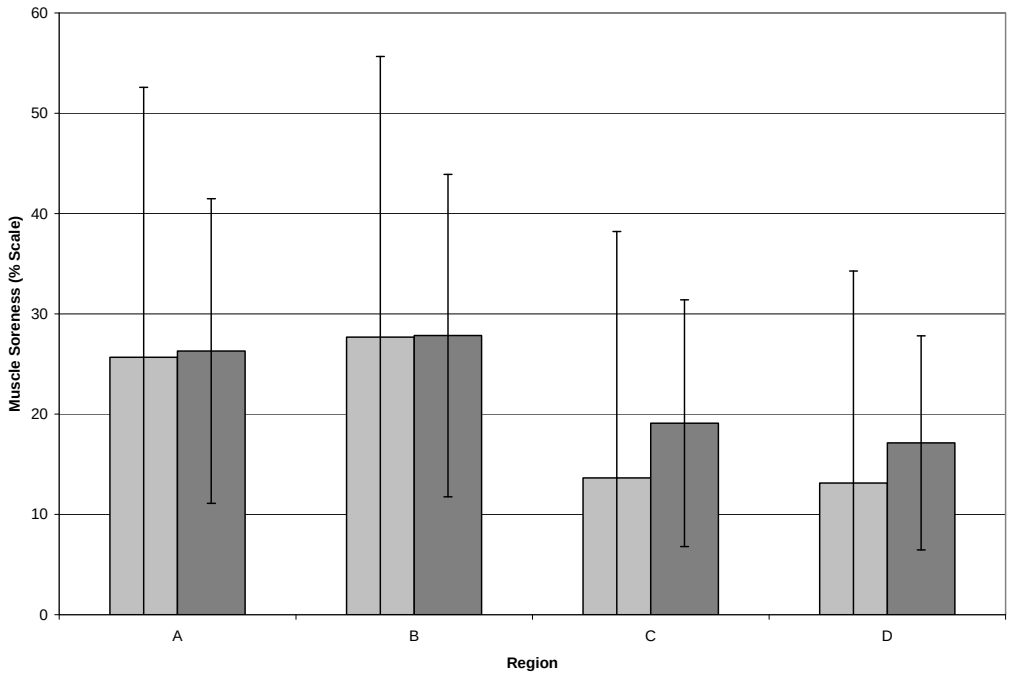
Figure 3. Sprint Muscle Soreness



Mean Sprint Muscle Soreness (±SD)
Placebo ▁, Capsaicin ▆
[a] Significantly higher than Pre ($P<0.002$)
[b] Significantly higher than Mid ($P=0.0371$)
Region A = front upper legs (quadriceps), Region B = back upper legs (hamstrings), Region C = front lower legs (shins), Region D = back lower legs (calves)

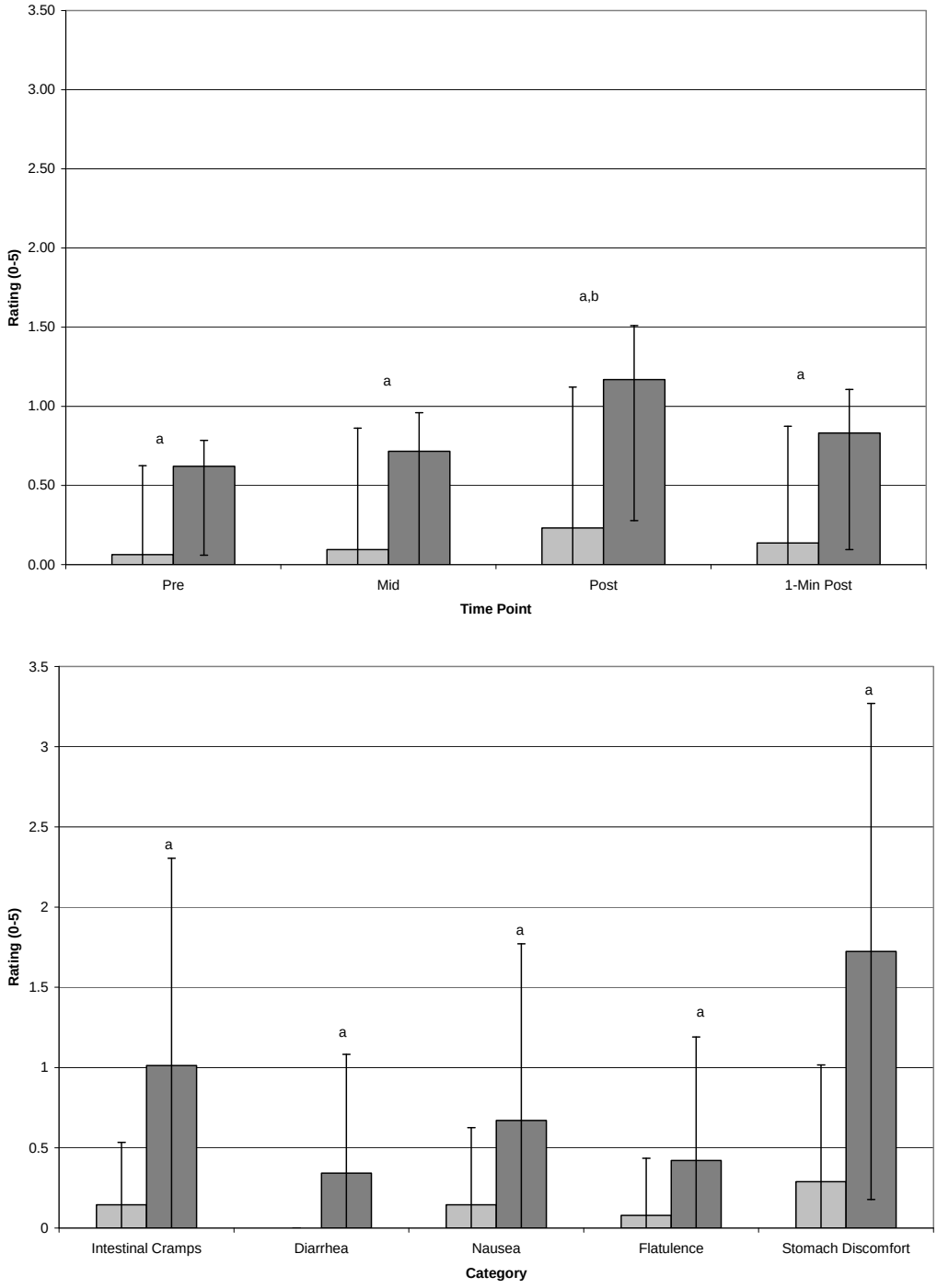
Figure 4. Posttest Muscle Soreness





Mean Posttest Muscle Soreness (±SD)
Placebo ▁, Capsaicin ▆
[a] Significantly higher than Pre (P<0.002)
[b] Significantly higher than Day (P=0.0057)
Region A = front upper legs (quadriceps), Region B = back upper legs (hamstrings), Region C = front lower legs (shins), Region D = back lower legs (calves)

Figure 5. Gastrointestinal Distress



Mean Gastrointestinal Distress (±SD)
Placebo ▁, Capsaicin ▁
[a] Significantly higher than placebo ($P<0.05$)
[b] Significantly higher than Pre ($P=0.0198$)

CHAPTER 4: SUMMARY AND RECOMMENDATIONS

SUMMARY

The purpose of this study was to examine the potential benefit of CAP supplementation in performance of a repeated sprinting protocol and the consequential inflammatory, cardiovascular, gastrointestinal, and hyperalgesic response to this exercise. CAP has been shown to reduce inflammation (63), pain (139), and improve endurance capacity (69), but most research has been in animal models, and its effects on maximal intensity exercise have yet to be investigated.

It was hypothesized that as compared to the PCB, following CAP supplementation: 1.) there would be an improvement in performance as indicated by reduced average sprint time and percent fatigue decrement, 2.) there would be a reduction in inflammation as indicated by serum IL-6, 3.) there would not be a difference in HR or BP, 4.) MS would be reduced during and post test, and 5.) perception of exertion would be reduced.

Nineteen healthy male athletes active in sports requiring repeated maximal sprint efforts were recruited for this study. Subjects underwent a baseline session to have their anthropometric data and blood collected, their fitness assessed, and were familiarized with testing protocol. Subjects then consumed three g per day of either a cayenne supplement to provide 25.8 mg of CAP or a PCB for one week along with their regular diet except avoiding all spicy foods. After the supplementation week, they returned for their first test session. Fasted blood was taken followed by a dose of their treatment and a 45 minute rest period. They then performed a repeated sprint protocol consisting of 15 30 m sprints on 35 s intervals. Sprint time, HR, BP, RPE, GD, and MS were measured and fatigue quantified. Blood was taken immediately post test, and MS measured for three days following the protocol. Subjects had a one week washout

period, followed by a one week supplementation period on the alternate treatment and then repeated the test protocol.

Sprint time, HR, BP, MS, GD, and IL-6 were analyzed by multivariate ANOVA with repeated measures, using post-hoc student-t test to identify source of significance if found. One way ANOVA was used to analyze fatigue, average and maximum sprint time, difference and percent change in IL-6, average and maximum HR, average GD and total sum score, and average MS and total sum score.

Performance

There was not a difference in sprint times, average sprint time, maximal sprint time, fatigue, or RPE among groups. CAP did not attenuate the decline in performance typically observed in RST (unpublished lab data, 41, 52). The hypothesis that performance may be enhanced following CAP supplementation was in part based on its ability to induce adrenal catecholamine secretion, i.e. through excitation of the CNS perceived exertion would be reduced. One rationale for this ergonenic effect was that an individual exercising at a reduced RPE may be able to perform at greater intensity for longer (33). However, we did not observe any difference between treatments in RPE. Another theoretical basis was that increased CNS activity may reduce central fatigue and attenuate the reduction of neuromuscular activity exhibited in repeated sprints (87). CAP is known to induce the adrenal secretion of catecholamines in similar magnitude to that of caffeine, which has been shown ergogenic in repeated sprints (117, 41), though research is conflicting. However, in this study we did not measure catecholamines. It is possible that the oral dose of CAP was not sufficient to elicit the adrenal stimulatory effects necessary to improve performance. We administered one g of cayenne supplement, providing 8.6 mg of CAP prior to testing. This was less than the literature suggests for adrenal stimulation,

but a greater dose would not have been tolerable. Without measuring catecholamine levels or neuromuscular firing rates, it is not possible to definitively state whether or not CAP influenced CNS activity during the RST.

Inflammation

We observed a significant time effect in the serum concentration of IL-6, which was to be expected following RST (84, 89, unpublished lab data). However, we did not observe a difference between groups. In contrast to most of the effects elicited by CAP, its influence on inflammation is not controlled by the TRPV1 receptor, rather mediation of NF-κB (68). While the precise mechanisms underling these results could not be determined in this study, some theoretical explanations may provide some insight. *In vitro* and *in vivo* mouse studies involving adipose tissue have characterized the anti-inflammatory properties of CAP. One of the asserted mechanisms involves CAP ligation of PPAR-γ and the resulting inhibition of NF-κB activation (63, 64, 104). Activated PPAR-γ can inhibit pro-inflammatory gene expression by binding to co-factors required for certain transcription factors (129). Activated PPAR-γ also binds directly to subunits of NF-κB inhibiting function (1). Additional CAP induced anti-inflammatory mechanisms include reduced MCP-1 and IL-6 and increased adiponectin mRNA expression and the subsequent protein production, as well as reduced macrophage infiltration into adipocytes and the release of pro-inflammatory cytokines by macrophages (63). Briefly, macrophage dependent inflammatory cytokine production is as follows: an inflammatory stimulant (lipopolysaccharide for example) binds to the TLR-4 receptor of a macrophage. TLR-4 recruits MyD88, which initiates a sequence of cellular signaling events resulting in the activation of IKK. Activated IKK phosphorylates IκB, which is the inhibitory subunit of NF-κB. With IκB phosphorylated, the inhibition of NF-κB is lifted and it able to then elicit its transcriptional

effects on inflammatory genes including IL-6, TNF-α, and IL-1β (106). The distillation of all of this is that the in vitro data suggests that the anti-inflammatory effects of CAP are mediated via PPAR-γ and macrophage activity. Thus, if CAP enters the cells in sufficient concentration, this theoretically could occur in humans. This has not been validated via oral consumption in humans.

Though CAP is readily absorbed in the stomach and upper intestinal tract (56) and is capable of crossing cell membranes (93), it is possible that the circulating plasma CAP level attainable via tolerable oral supplementation may not be adequate to elicit the effects. Studies using rodent models have observed the anti-inflammatory effects following 2 mg/kg CAP injections (63). Yet in humans an oral dose of 2 mg/kg (which would be ~ 160 mg CAP) has been found to not be tolerable (98), and it is suggested that there is an 80% gastrointestinal absorption of CAP (56). Thus, it is likely that our smaller dose of oral CAP would not have provided the circulating plasma levels reached in the rodent studies.

It is now believed that the increase in IL-6 due to muscle contraction is the result of mechanisms that are independent of immune cells and the NF-κB pathways (106, 144). One hypothesis is that intracellular Ca^{2+} triggers expression of IL-6. Muscular contraction induces the release of Ca^{2+} from the sarcoplasmic reticulum, which results in calcineurin activation and subsequent IL-6 gene expression (5). Another possibility is that activation of p38 MAPK via phosphorylation causes transcription of IL-6 in skeletal muscle (20). Additionally, nitric oxide release resulting from muscular contraction has been found to play a role in the expression of inflammatory genes, including IL-6 (127). As with most things in biology, the IL-6 response to exercise is most likely not due to a single pathway or mechanism but a combination of many.

But the increase in IL-6 following exercise has been observed in the absence of muscle damage (103) and independent of monocytes and macrophages (124, 125, 90, 135).

We may have not observed any difference in IL-6 following CAP supplementation compared to the placebo since CAP elicits its anti-inflammatory effects through the regulation of immune cell activity and NF- κB (63) yet the majority of exercise induced IL-6 production is independent of these (106). As previously stated, IL-6 can play a dual role in inflammation; serving as a pro-inflammatory but also anti-inflammatory by suppression of TNF-α and also induction of IL-10 and IL-1, both anti-inflammatory cytokines (144, 106). It is speculative but it may be that exercise induced increase in IL-6 as a result of contracting muscle is of a protective anti-inflammatory nature rather than pro-inflammatory as governed by immune cells. Thus, the interpretation of the increase we observed may be an indicator of the stimulation of the inflammatory response but additionally can dampen the production of downstream inflammatory factors to limit the extent of inflammation.

Cardiovascular Implications

We observed a lower HR following CAP treatment compared to the PCB during the RST. After CAP, total average sprint HR which included the measurement shortly before and after the RST was significantly lower by 8.50 % (P=0.037), sprinting average HR which only included measures during the RST was lower by 6.0 % (P=0.0330), and maximal HR attained during the RST was lower by 9.3% (P=0.0026). We did not observe a difference in BP between treatments during the RST. These results were not entirely expected, and there are multiple possible explanations.

CAP is known to stimulate the local release of endogenous CGRP and substance P and subsequently induce vasodilation through activation of the TRPV1 receptor in CAP-sensitive c-

fibers and Aδ-fibers, which also function in baroreception (3, 51, 53, 67, 110, 136, 137, 140, 150). General vasodilation would result in a decrease in BP and a subsequent increase in HR (133). CAP also stimulates adrenal release of catecholamines which are vasoconstrictive, and elevate HR and BP (69, 78, 101, 132, 138). However, it's been shown the CGRP release induced by CAP can ablate the vasoconstriction of infused norepinephrine (110). But, TRPV1 receptor activation also stimulates parasympathetic nervous activity, thereby suppressing HR (7) though this effect is considered to be mostly withdrawn at rates above 120-130 bpm (154). Nevertheless during sub-maximal activity CAP does not affect HR (119). Taken together, CAP can have opposite effects on vasodilation and HR. The different nervous and hormonal effects observed in vitro and animal studies make it difficult to characterize the influence of CAP on cardiovascular responses in human subjects in the novel application of intense activity. It may be that during maximal exertion one system is dominant and obscures the other, or that they cancel one another. In this study we only measured HR and BP. Without additional measurements such as flow mediated dilation, plasma catecholamines, or nervous system activity, it is only by speculation that we can attempt to offer explanation of these observations.

A lower HR response to the same exercise bout may have important implications in terms of continued exercise. Even though the RST utilized in this study was designed to simulate the fitness demands of team sporting events such as soccer or rugby, it only consisted of 15 sprints and lasted less than nine minutes rather than the 90 minutes or more of exercise for many team events. Theoretically, an athlete, performing just as well but with a reduced HR, may find an advantage towards the end of a game in managing fatigue with a heart less taxed.

Gastrointestinal Distress

GD was significantly elevated following CAP treatment during the RST ($P<0.0001$). The most severe average GD symptom was stomach discomfort with 42 % having a distress rating greater than 2, whereas it was only 5 % following the PCB and most subjects (53 %) without symptom. All subjects, regardless of body mass, received the same treatment dose, though subject compliance for consuming their CAP treatment was only 86 %, largely attributed to GD. Contrary to popular belief, the size of the individual did not appear to have any influence on GD during testing (correlation of 0.1763). Only one subject did not complain of some symptom due to the CAP during the supplementation week. See appendix B for detailed table of compliance and symptoms. All subjects prior to participation completed entrance surveys and confirmed that they were, in fact, tolerant of spicy foods in terms of "heat" or burning mouth sensation and gastric upset. The supplements provided were intended to remain intact until broken or dissolved in the stomach and to avoid any taste of chili peppers. Each capsule was roughly equivalent to two cayenne peppers and the amount consumed daily was about half that of the Korean daily average (78). It may have been that subjects' reported perception of "tolerance" was based upon the amounts of CAP they normally consume which was dictated by mouth burn, not gastric upset, and that the amount consumed during treatment was more than typical thereby inducing an unaccustomed degree of GD.

An important issue in interpreting this high degree of GD was that subjects consumed their treatment under fasted conditions. This was a major source of GD, enough to prevent several subjects from continuing with testing. Typically, chili peppers are consumed with a meal, not stand alone. For the subjects for which the GD was too great to continue on the morning of testing, ingestion of something bland such as a bagel or banana alleviated the

problem. There are TRPV1 receptors in the gastrointestinal tract (96). Association of CAP with these receptors induces the sensation of pain (25). Indeed, there is an increased TRPV1 receptor density in the esophagus of individual suffering from gastro-esophageal reflux disease (82). CAP ingestion results in the stomach production of HCl and protective secretions (94, 35). The complexity of gastric hormonal processes is far beyond the scope of this project. However, it was certain that some subjects exhibited greater GD than others. The purpose of this study was not to elucidate the mechanisms underlying CAP induced GD, and we could not have established this without invasive measures (e.g. gastric biopsies), but this may have been due, in part, to a greater TRPV1 receptor density and CAP sensitivity within the gut. However, on a practical level, use of supplements such as those in our study should be used with substantial caution because of the GI intolerance.

It is worth noting that despite the significant increase in GD observed following CAP treatment, there was not a difference in performance, fatigue, or RPE between the treatments. One would expect a substantial impairment of performance in repeated sprints related to even mild GD (131). It is possible that CAP may very well have improved the systems hypothesized to benefit performance, e.g. catecholamine secretion, but these effects were undermined by the induced GD.

Muscle Soreness

MS was significantly elevated by the RST in all muscle groups and remained for up to two days following, though our study was not specifically designed to induce MS. The research protocol was similar to that of Howatson et al, except that we did not restrict the deceleration zone following sprinting to 10 m (52). Our subjects were allowed to break as they felt necessary and also received a shorter recovery period. Accordingly, our MS results were about one forth

that observed by Howatson et al. However, the observed significant increase may still indicate exercise induced muscle damage, most likely due to this eccentric component of the RST. CAP is a known analgesic agent used in topical applications for the treatment of muscle, joint, and inflammatory pain (31, 70). We did not observe a difference in MS however between treatments. This is likely due to the mode of administration. When CAP binds to the TRPV1 receptor, its ion channel is opened allowing for cation flux, which is initially perceived as pain. Upon prolonged activation and subsequent cation depletion, the result is the analgesic "numbing" effect (24). The pain reduction would cease following dissociation of CAP from the receptor and resulting deactivation. CAP patches designed for pain relieve are applied for up to 24 hours of slow release. Since the CAP in this study was given orally, and as such has a circulating half life of about 25 minutes, it may have been that any pain reducing benefits were too short lived to benefit post-RST MS. Additionally, as previously covered, we did not observe a reduction in inflammation following CAP treatment compared to the PCB, which may contribute to the lack of difference between groups in MS.

RECOMMENDATIONS

In order for CAP to elicit its effects, it needs to be distributed and bind with the TRPV1 receptor (24). CAP is readily absorbed in the stomach and small intestine, up to 80% of the administered dose within an hour of administration (56, 65). Maximum plasma CAP is reported at ~ 47 min post-ingestion, with a half life of ~ 25 min (19). Further, the adrenal stimulatory effects of CAP are reported to be optimal at 60 to 90 minutes post ingestion (119). The cayenne capsules used in this study should have released their contents in the stomach within five minutes or so of ingestion. The subjects had at least 45 minutes to rest after the time of ingestion before heading to the gym for their warm up, which typically lasted about 15 minutes. So there was at

least an hour for the CAP to be absorbed, circulated, and associate with its receptor. It was assumed that subjects would process and absorb the supplements at the same rate. However, in this study we did not measure the pharmacokinetics or plasma concentration of CAP. It may therefore be of benefit to the interested researcher to measure plasma CAP content, to verify its absorption and distribution.

It was also assumed that the supplements were of the same CAP content. The manufacturer's analysis of content reported 4.3 mg CAP per 500 mg capsule. However, this was not a pure CAP treatment, but an herbal supplements derived from cayenne pepper. As such, the CAP content of cayenne peppers can vary (95) and it was not possible to ensure that the supplements came from the same production lot number as we were not capable of analyzing for CAP content. Being of dried and ground *capsicum* fruit, other capsinoids would also be present in the cayenne supplements, though these are not the principle active components of *capsicum* fruit and their contributions are of marginal consequence in terms of the biological parameters of interest (93). However, if absolute certainly is desired as to the purity of the supplement and attributing the effects were exclusive to CAP, pure CAP to within 95% could be procured through pepper fruit distillation and alcohol extraction methods, then the content verified by HPLC.

As suggested, GD may have been a limiting factor on performance following CAP ingestion. Future investigations should focus on a supplementation protocol that does not cause such upset, for instance, CAP with food. Another possibility may be utilizing one of the commercially available cayenne supplements claimed to be "cool" on the stomach (e.g. Solaray Cool Cayenne, Nutraceutical Corp., Park City UT), though these often contain other active ingredients including ginger root and annatto, which may have their own effects. Some CAP

analogs not considered “hot” or as distressing, such as capsiate derived from the CH-19 sweet pepper plant, may be attractive options. However, although they activate the TRPV1 receptor, they are more rapidly broken down and do not as readily cross cell membranes (54, 93), so these compounds may not have similar effects in terms of magnitude.

CAP does induce the secretion of HCl, and indigestion was one of the major complaints of subjects following ingestion of the cayenne supplement. If only interested in performance enhancement and not necessarily isolating the contributor, an interesting approach to an ergogenic treatment may be a combined supplement of CAP and sodium bicarbonate. Sodium bicarbonate may benefit performance by improving blood buffering capacity (75) but also functions to neutralize stomach acid and thereby reduce some of the GD caused by CAP.

We selected IL-6 as the marker for inflammation as it is the major cytokine produced in response to exercise. However, it is only a single indicator of inflammation, and the mechanisms underlying its elevation following exercise, i.e. muscle contraction rather than immune cell function, may not be affected by CAP. Accordingly, future research may wish to measure additional indicators of inflammation such as TNF-α or NF-κB following CAP treatment and RST. Similarly, in terms of the pain associated with exercise induced muscle damage and the resulting inflammation following repeated sprinting, additional measures beyond MS could be taken, such as muscle girth or maximal voluntary contraction.

For many of the addressed mechanisms, it is within reason to predict that CAP may be of benefit to endurance exercise in humans. However, to the authors’ knowledge to date the only studies investigating CAP and endurance have been of rodents. In these, endurance capacity was improved by an increase of up to 219 % in time to exhaustion following CAP treatment (101). In humans CAP has been shown to lower respiratory quotient, increasing fat oxidation and

sparing muscle glycogen (119). Considering these results in conjunction with the results of the current study in terms of reduced HR, future investigations could examine the effects of CAP on endurance capacity in humans.

It is possible though that in the practical oral doses of CAP dictated by tolerance, it may not be of benefit to humans. As mentioned, much of the research has involved rodents and/or injections that achieve plasma CAP levels that may not be attainable through oral supplementation in humans. In the only study on CAP pharmacokinetics in humans, Chaiyasit et al found that following an acute dose of five g of capsicum providing 26.6 mg of CAP, plasma CAP reached a peak of 2.47 ng/ml which was sufficient to induce a reduction in plasma glucose (19). This dose was roughly three times that of the current study, and though the authors did not report GD, based on the interpretation of our observations, would be too high to be of practical application.

Conclusion

In conclusion, the results of this study showed that CAP supplementation did not affect performance of repeated sprinting in terms of sprint time or fatigue despite a drastic increase in GD. It did not influence perceived exertion or BP during sprinting, nor did it affect the inflammatory response or MS resulting from this exercise. However, it did appear to lower HR during this maximal intensity activity. This was the first study to address the influence of CAP on exercise performance in humans. Future research should consider measuring plasma CAP and catecholamines, additional measures of inflammation and MS, using a more tolerable supplement protocol, and investigating the effects of CAP on endurance exercise.

Appendix A: Detailed Description of Research Procedures

Baseline Session

- Subject arrives at lab under conditions described in "subject preparation for testing".
- Collect 3-day diet record and any remaining forms not previously turned in.
- Have subject sit quietly for five minutes resting to prepare for resting heart rate and blood pressure.
- Place wrist cuff blood pressure monitor on subject's left wrist, hold at heart level, press "start", record blood pressure and heart rate. Repeat 2 more times for a total of 3 readings.
- Measure height and weight on digital balance.
- Using skinfold calipers, measure skinfold thickness as 7 sites described in body fat percent determination document. Measure in circuit of 7 sites a total of 3 times.
- Escort subject to phlebotomist for baseline blood draw (B0).
- Allow subject to rest for at least 10 minutes.
- Escort subject to gymnasium for $VO2_{max}$ test.

Progressive Shuttle-Run Test (PST) Protocol

- Warm-up
    - Subject allowed to jog at self-selected pace for 5 minutes in gymnasium.
    - Subject instructed to perform standardized warm up exercises:
        - 2 x 10 m heel flicks
        - 2 x 10 m high knees
        - 2 x 10 m walking lunges
        - practice sprints
    - Subject allowed to stretch for 5 minutes until ready to start test.
    - Subject allowed water ad lib.
    - Brief subject in testing protocol.
- PST course set up
    - Measure and set 2 cones 10 m apart.
    - Measure 20 m from cones.
    - Set another pair of cones 10 m apart.
    - Place 5 m tape line between cones to indicate crossing line.
    - Volunteer at each end of course.
    - Set up cd player/stereo within clear audible distance from both sets of cones.
- Testing
    - When subject ready at starting line, start audio CD with instructions for PST.
    - 4 tone countdown to start of test, followed by 3 tones to indicate start of level.
    - Subject runs from first set of cones to next.
    - Must reach crossing line by 2^{nd} tone.
    - Each tone indicates start of next run.
    - Triple tone indicates next level, and decrease in allowed time.
    - Volunteer marks off successful completion of run on PST form.
    - Volunteer indicates on PST form if subject misses crossing line by the tone.
    - Three consecutive missed crossing lines indicate test is over.
    - Subject allowed to cool down as necessary.
    - Use PST performance $VO2_{max}$ chart to sync subjects sprint number level with estimated $VO2_{max}$.

Elite Male Soccer Player Body Fat % Equation (Withers 1987)

Body Density (BD) = 1.0988 – 0.0004(Σ tripceps + subscapular + biceps + supraspinale + abdominal + thigh + calf skinfolds) (in mm)

Body Fat % = ((4.95/BD) – 4.5)x100
Or
Body Fat % = ((4.57/BD) – 4.142)x100

Most accurate equation when compared with DXA according to (Reilly 2009)

Skinfold sites (Romero 2009)

- Triceps (H3): a vertical fold halfway between the acromion process and the superior head of the radius, in the posterior aspect of the arm

- Biceps: at the same level as the triceps skinfold and directly above the centre of the cubital fossa.

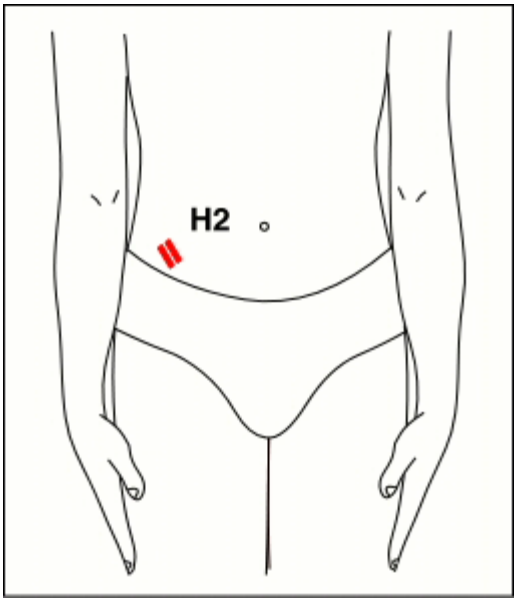
- Subscapular (H1): about 20mm below the inferior angle of the scapula and 45° to the lateral side of the body.

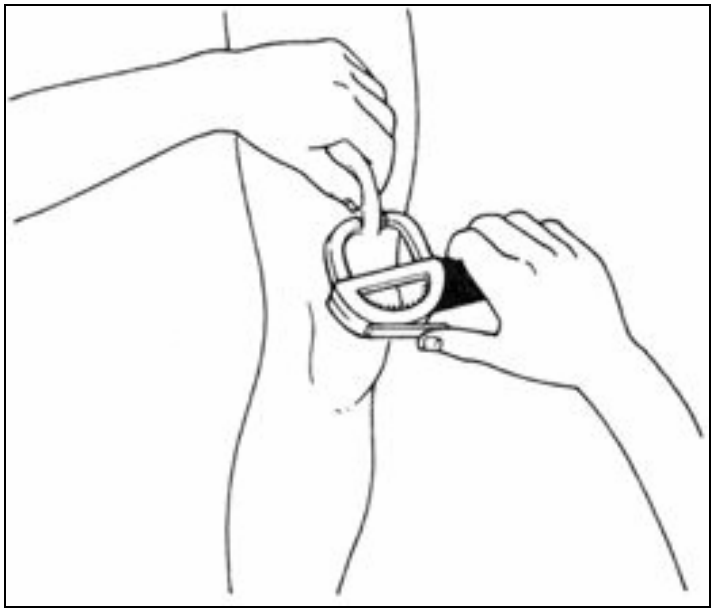
- Supraspinale (H2): which is raised at the intersection of two lines, the line from the marked iliospinale (most inferior or undermost part of the tip of the anterior iliac crest) to the anterior axillary border, and the horizontal line at the level of the marked iliac crest skinfold. The supraspinale skinfold runs medially downward at about a 45° angle.

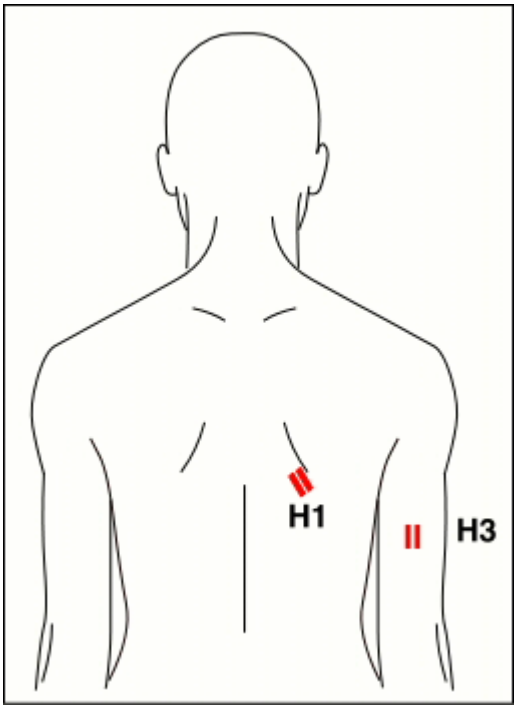
- Abdominal: a vertical fold raised 5cm from the right-hand side of the navel.

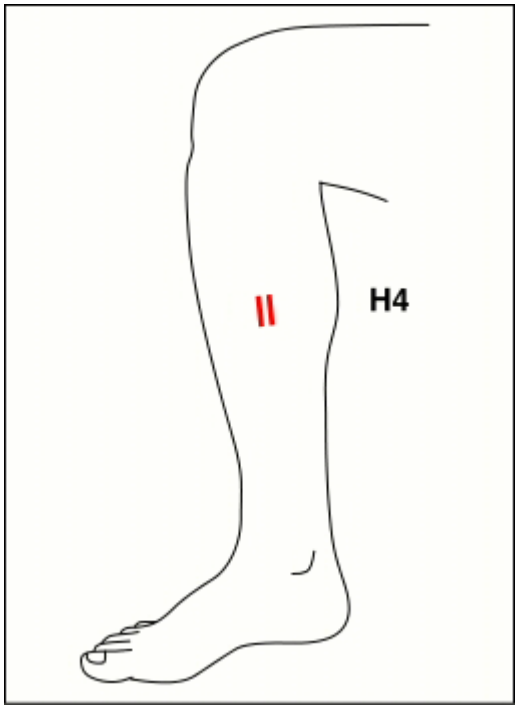
- Thigh: at the midline of the anterior aspect of the thigh, midway between the inguinal crease and the proximal border of the patella.

- Medial calf (H4): at the level of the maximum calf circumference, on the medial aspect of the calf.


H2




H1
H3


H4

Subject Prep for Testing Sessions

- No spicy food for week prior to testing.
- No competing in sport for 72 hours prior to testing.
- No exercise for 24 hours prior to testing.
- No alcohol for 24 hours prior to testing.
- No caffeine for 12 hours prior to testing.
- No food prior for 12 hours prior to testing.
- Drink 16 oz water before going to bed and when wake up.
- Wear comfortable work out clothing (i.e. gym shorts tee shirt) and running shoes, but no compression garments.
- Wear same type clothing and same footwear for each test.
- Subject arrives at lab and provides compliance form surveying health, activity, and treatment.
- If compliance results acceptable, escort to phlebotomist for blood draw.
- Give subject one dose of treatment (2 capsules) and record time.
- Subject may rest for 45 minutes and drink water and use restroom as desired.
- Escort subject from lab area to gymnasium for testing.

Repeated Sprint Test (RST) Protocol

- Warm-up
    - Subject allowed to jog at self-selected pace for 5 minutes in gymnasium.
    - Subject instructed to perform standardized warm up exercises:
        - 2 x 10 m heel flicks
        - 2 x 10 m high knees
        - 2 x 10 m walking lunges
        - practice sprints
    - Subject allowed to stretch for 5 minutes until ready to start test.
    - Subject allowed water ad lib.
    - Remind subject of testing protocol and scales, forms to be encountered.
- RST course set up
    - Open Swift SpeedlightTT wireless timing gate system storage box and remove contents.
    - Open and extent tripods to full length.
    - Attach timing sensors and reflectors to tripods.
    - Attach transmission antennas to timing sensors.
    - Set a timing sensor and reflector 5 m apart and place tape line between.
    - Measure 30 cm back from tape line and place 2nd tape line down as starting line.
    - Measure 30 m from sensor/reflector set and place the 2nd sensor/reflector set in same fashion.
    - Press and hold “on” button on sensor for 2 seconds.
    - Align sensor and reflector so that sensor does not beep and both red lights are illuminated.
    - Place a laptop with Swift SpeedlightTT wireless timing system software between timing gates but out of the way from run course.
    - Connect the transmission receiver to USB cable and antenna.
    - Plug transmitter into computer USB port and open Swift SpeedlightTT wireless timing system software.
    - Click “connect to base unit”, click “connect to gates”.
    - Make sure that the battery level indicator is adequate and “timing gates clear” indicator bars are green. If they are red, realign sensors/reflectors.
    - Click “select athlete” and chose the subject, if he/she does not already exist, select “new athlete” and create his/her profile.

- Click “select test name” and chose the RST protocol, if it does not exist, select “new test” and set up the appropriate test.
- Provide volunteer with clipboard, RST Sheet, timing sheet, pen, and stopwatch, locate at each sensor/reflector line.

- Testing
  - Have subject put on heart rate (HR) monitor chest strap and wrist receiver on right wrist, ensure that HR is registering.
  - One minute prior to test initiation: place wrist cuff blood pressure (BP) monitor on left wrist, hold wrist at heart level, and press “start”, record BP and remove wrist cuff.
  - Record HR.
  - Hold up rate of perceived exertion (RPE) scale for subject to gauge RPE.
  - Hold up muscle soreness (MS) form for subject to gauge MS.
  - Hold up gastrointestinal distress (GD) form for subject to gauge GD.
  - Line up subject at timing gates behind the starting line.
  - Click “start timing new event”.
  - Give subject a 10 second countdown to test initiation.
  - When subject starts sprinting, start stop watch.
  - Subject sprints at maximum intensity down 30 m run course to 2nd timing gate.
  - Subject should not start decelerating until through the timing gate.
  - Subject may break from sprinting as necessary but is encouraged not to “hammer” stop.
  - Subject may rest once through the timing gate but not within 5 feet of timing gate to prevent false triggering.
  - Subject may walk or stand but not sit or “actively recover” by jogging.
  - Do not provide verbal encouragement, performance feedback, or indicator of sprint number to subject.
  - Subject is to initiate sprint on 35 second intervals, i.e. at 0:00, 0:35, 1:10, 1:45…. For a total of 15 sprints.
  - Notify subject 10 seconds before next interval to make way to starting line.
  - Give 5 second countdown to start of next sprint.
  - After every 3rd sprint, hold up RPE scale for subject to gauge RPE.
  - After every 3rd sprint, record HR.
  - After the 7th sprint, hold up GD and MS scales for subject to gauge GD and MS.
  - After the final (15th) sprint, place wrist cuff BP monitor on wrist and measure/gauge BP, RPE, HR, GD, and MS.
  - One minute after final sprint, repeat above step.
  - Escort subject to phlebotomist for blood draw.
  - Click “stop timing event” in Swift SpeedlightTT software, save test data.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: ***Alkaline phosphatase is detectable in saliva.*** *Take precautionary measures (e.g. wear a mask and gloves) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 100 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 1000 mL of Wash Buffer.

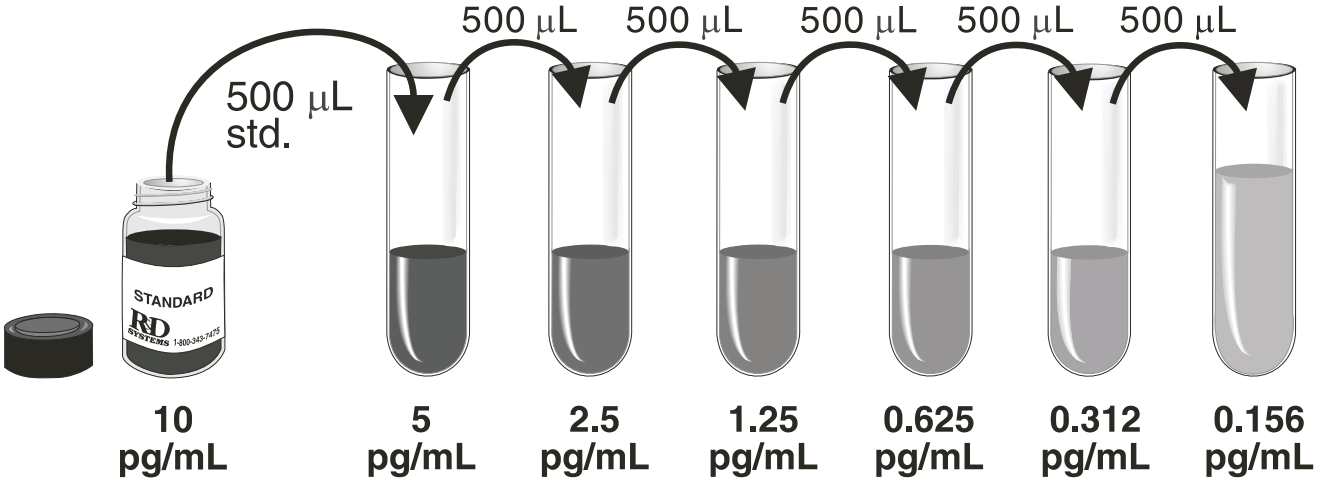
Substrate Solution - Reconstitute the lyophilized Substrate with 6.0 mL of Substrate Diluent at least 10 minutes before use. **Re-stopper and re-cap the vial**, and mix thoroughly. Avoid contamination.

Amplifier Solution - Reconstitute the lyophilized Amplifier with 6.0 mL of Amplifier Diluent at least 10 minutes before use. **Re-stopper and re-cap the vial**, and mix thoroughly. Avoid contamination.

Calibrator Diluent RD6-11 (1X) - Dilute 10 mL of Calibrator Diluent RD6-11 Concentrate into 10 mL of deionized or distilled water to prepare 20 mL of Calibrator Diluent RD6-11 (1X) (*for urine samples*).

IL-6 Standard - Reconstitute the IL-6 Standard with 5.0 mL of the appropriate Calibrator Diluent (*Calibrator Diluent RD6-11 Concentrate for serum/plasma samples or Calibrator Diluent RD6-11 (1X) for urine samples*). This reconstitution produces a stock solution of 10 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of the Calibrator Diluent RD6-11 Concentrate (*for serum/plasma samples*) or Calibrator Diluent RD6-11 (1X) (*for urine samples*) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The appropriate reconstituted standard serves as the high standard (10 pg/mL). The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

Note: ***Alkaline phosphatase is detectable in saliva.*** *Take precautionary measures to protect reagents (e.g. wear a mask and gloves).*

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-75 to each well. Assay Diluent RD1-75 may contain a precipitate. Mix well before and during use.
4. Add 100 µL of Standard or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Wash

   Notes on washing

   - *Excessive drying of the wells can lead to poor assay performance and imprecision. Subsequent reagents should be added immediately after washing the plate, and the wells not allowed to completely dry. Also avoid prolonged exposure of the wells to vacuum aspiration apparatus.*
   - *Inclusion of a 30 second soak between each addition of Wash Buffer and decanting of the plate contents will improve the precision of the assay.*

   Wash Procedure

   a. Remove liquid from the wells by inverting the plate and decanting the contents.
   b. Remove excess liquid by grasping the plate firmly and smartly rapping the plate inverted on a clean paper towel at least 5 times.
   c. Fill each well with 400 µL of Wash Buffer using a squirt bottle, manifold dispenser or autowasher.
   d. Remove liquid from the wells by inverting the plate and decanting the contents or by aspirating the contents with an autowasher.
   e. Repeat steps b - d five times for a total of 6 washes. After the last wash, smartly rap the inverted plate on a clean paper towel at least 10 times to remove excess Wash Buffer.
6. Add 200 µL of IL-6 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the wash as in step 5.
8. Add 50 µL of Substrate Solution to each well. Cover with a new adhesive strip. Incubate for 60 minutes at room temperature **on the benchtop. Do not wash the plate.**
9. Add 50 µL of Amplifier Solution to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature **on the benchtop.**

   Note: *Addition of Amplifier Solution initiates color development.*
10. Add 50 µL of Stop Solution to each well. Addition of Stop Solution does not affect color in the wells.
11. Determine the optical density of each well within 30 minutes using a microplate reader set to 490 nm. If wavelength correction is available, set to 650 nm or 690 nm. If wavelength correction is not available, subtract readings at 650 nm or 690 nm from the readings at 490 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 490 nm without correction may be higher and less accurate.

ASSAY PROCEDURE SUMMARY

1. Prepare all reagents and standards as instructed.

2. Add 100 µL Assay Diluent RD1-75 to each well. Assay Diluent RD1-75 may contain a precipitate. Mix well before and during use.

3. Add 100 µL Standard or sample to each well. Incubate 2 hours at RT on the shaker.

4. Wash 6 times.

5. Add 200 µL Conjugate to each well. Incubate 2 hours at RT on the shaker.

6. Wash 6 times.

7. Add 50 µL Substrate Solution to each well. Incubate 60 minutes at RT **on the benchtop.**

8. Add 50 µL Amplifier Solution to each well. Incubate 30 minutes RT **on the benchtop.**

9. Add 50 µL Stop Solution to each well. Read at 490 nm within 30 minutes.
λ correction 650 or 690 nm

CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density.

Plot the optical density of the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation.

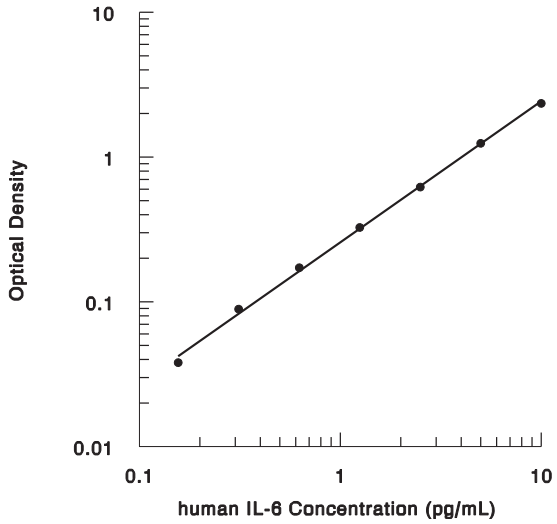
To determine the IL-6 concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding IL-6 concentration.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL STANDARD CURVES

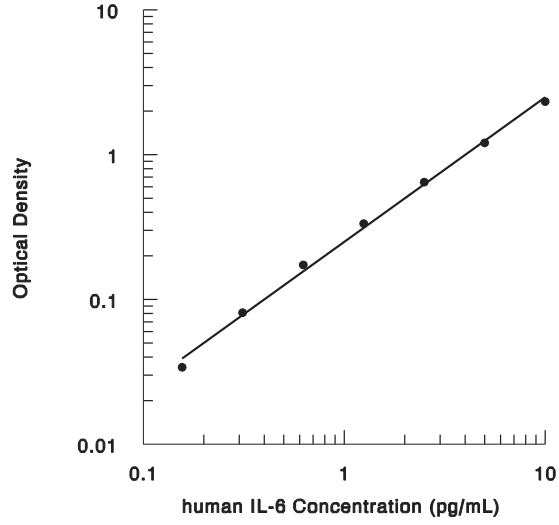
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Calibrator Diluent RD6-11



pg/mL	O.D.	Average	Corrected
0	0.130 0.134	0.132	——
0.156	0.166 0.173	0.170	0.038
0.312	0.218 0.223	0.221	0.089
0.625	0.296 0.311	0.304	0.172
1.25	0.447 0.469	0.458	0.326
2.5	0.731 0.773	0.752	0.620
5	1.348 1.401	1.375	1.243
10	2.389 2.566	2.478	2.346

Calibrator Diluent RD6-11 (1X)



pg/mL	O.D.	Average	Corrected
0	0.133 0.133	0.133	——
0.156	0.165 0.168	0.167	0.034
0.312	0.210 0.217	0.214	0.081
0.625	0.301 0.310	0.306	0.173
1.25	0.445 0.489	0.467	0.334
2.5	0.752 0.806	0.779	0.646
5	1.331 1.346	1.339	1.206
10	2.429 2.488	2.459	2.326

Appendix B: Raw Data

Table 1.

Baseline Measurements

Subject #	Age	Height (cm)	Weight (kg)	BMI	Body Fat %
1	22	174.0	79.7	26.3	18.2
2	22	179.5	76.7	23.8	16.3
4	20	186.7	78.0	22.4	15.6
6	21	178.0	62.6	19.8	10.9
7	21	170.0	86.1	29.8	25.3
8	25	184.5	76.9	22.6	13.7
9	23	172.7	75.4	25.3	17.1
10	26	180.3	76.1	23.4	10.7
11	30	175.3	69.8	22.7	8.3
12	23	191.0	99.9	27.4	9.1
13	20	166.0	62.9	22.8	9.4
15	24	189.0	81.6	22.8	16.6
16	23	173.0	71.5	23.9	8.7
17	19	177.0	65.5	20.9	8.0
18	21	167.0	66.7	23.9	9.7
19	21	183.0	81.0	24.2	13.7
20	25	185.5	91.1	26.5	16.5
22	22	183.5	76.0	22.6	12.2
23	22	186.0	64.0	18.5	8.9
Mean:	**22.63**	**179.05**	**75.87**	**23.66**	**13.10**
SD:	**2.56**	**7.36**	**9.80**	**2.64**	**4.54**

Subject #	Ave RHR	Ave BP-S	Ave BP-D	Ave RBP	Estimated VO2max
1	54.0	142.0	83.0	142/83	39.2
2	59.0	138.0	82.0	138/82	44.2
4	68.0	137.0	88.0	137/88	44.9
6	68.0	140.0	88.0	140/88	47.1
7	69.0	140.0	85.0	140/85	37.5
8	66.0	153.0	93.0	153/93	45.5
9	60.0	138.0	90.0	138/90	51.4
10	65.0	140.0	87.0	140/87	51.1
11	60.0	132.0	93.0	132/93	54.6
12	53.3	126.3	69.7	126/70	41.5
13	46.3	121.3	74.0	121/74	46.3
15	78.7	109.3	69.0	109/69	38.5
16	81.3	142.3	95.0	142/95	45.2
17	67.0	128.0	84.3	128/84	54.3
18	63.3	142.0	83.7	142/84	40.8
19	67.0	122.3	78.0	122/78	47.7
20	60.7	132.3	86.7	132/87	40.2
22	78.0	138.3	90.3	138/90	41.1
23	62.0	128.3	82.3	128/82	52.5
Mean:	**64.56**	**134.25**	**84.32**	**134/84**	**45.44**
SD:	**8.77**	**9.90**	**7.38**		**5.40**

Table 2.

Subject Health Survey

Subject #	Food Restrictions	Supplements	Recent Injury/Illness	Medical Conditions
1	No	No	No	No
2	No	No	No	No
4	Sulfa	Fiber	No	No
6	Milk	No	No	No
7	No	No	No	No
8	No	No	No	No
9	No	No	No	No
10	No	No	No	No
11	No	No	No	No
12	No	M.V., E.F.A.	No	No
13	Shark	No	Shin splints	No
15	No	M.V., Glucosamine	Shin splints	No
16	No	No	No	No
17	Amoxicillin, Sulfa	M.V., Ca, Flax, E.F.A.	No	No
18	No	M.V.	No	No
19	No	No	No	No
20	No	No	Perforated Eardrum	No
22	No	No	No	No
23	No	No	No	No

Subject #	Medication	Smoke	Weight Stable	Fear of Needles or Blood Draws
1	No	No	Yes	No
2	No	No	Yes	No
4	Strattera, Oxcarbazepine	No	Yes	No
6	No	No	Yes	No
7	No	No	Yes	No
8	No	No	Yes	No
9	No	No	Yes	No
10	No	No	Yes	No
11	No	No	Yes	No
12	No	No	Yes	No
13	No	No	Yes	No
15	Flonase	No	Yes	No
16	No	No	Yes	No
17	No	No	Yes	No
18	No	No	Yes	No
19	No	No	Yes	No
20	Advair, Flonase, Albuterol	No	Yes	No
22	No	No	Yes	No
23	No	No	Yes	No

M.V. = Multivitamin
E.F.A = Essential Fatty Acids
Ca = Calcium

Table 3.

Subject Profiles

Subject #	Age	Sport	Position	Years Played	Level	Training (d/wk)	Training (hr/d)	Alcohol (drinks/wk)	Caffeine (drinks/wk)	Tolerant of Spicy Food
1	22	Tennis	N/A	15	Club	5	1.5	6	1	Yes
2	22	Running	N/A		N/A	5	1	4	14	Yes
4	20	Crew	Varsity	2	Club	5	1.5	0	0	Yes
6	21	Running	N/A	6	Rec	3			0	Yes
7	21	Soccer	N/A	8	Rec	3	3	6	14	Yes
8	25	Soccer	Midfield	17	Club				0	Yes
9	23	Soccer	Midfield	13	Rec			2	1	Yes
10	26	Running	N/A	12	Rec	7	1.75	1	1	Yes
11	30	Running	N/A	15	Rec	5	1	6	14	Yes
12	23	Running	N/A	1	Rec	4	2	1	0	Yes
13	20	Rugby	Wing	3	Club	6	2	14	14	Yes
15	24	Triathlon	N/A	3	Club	5	2	3	7	Yes
16	23	Soccer	Midfield	15	Club	3	2	18	0	Yes
17	19	Triathlon	N/A	1	Club	6	2.5	8	0	Yes
18	21	Rugby	Center	2	Club	5	2	10	14	Yes
19	21	Rugby	Wing	5	Club	5	2	20	0	Yes
20	25	Rugby	Flanker	3	Club	5	2	10	0	Yes
22	22	Soccer	Midfield	10	Club	5	1	3	0	Yes
23	22	Running	N/A	8	Club	6	1.5	8	21	Yes
Ave:	**22.63**			**7.72**		**4.88**	**1.80**	**7.06**	**5.32**	
SD:	**2.56**			**5.58**		**1.11**	**0.54**	**5.86**	**7.21**	

Table 4.

3 Day Diet Record

Day 1

Subject #	Cal (kcal)	Fat (g)	Sat Fat (g)	Chol (mg)	Na+ (mg)	CHO (g)	Fiber (g)	Sugars (g)	Protein (g)
1	3487.0	112.6	35.2	285.0	6866.0	344.8	17.2	82.9	166.9
2	4618.0	231.6	33.6	77.0	5416.0	535.6	49.8	85.4	98.3
4	5066.0	95.5	31.3	575.0	8002.0	766.9	69.4	120.0	314.8
6	2703.0	115.8	40.2	191.0	4450.0	209.7	9.7	9.7	84.5
7	2730.0	154.4	58.6	1070.0	8618.0	200.0	10.2	57.9	138.8
8	2833.0	75.1	26.1	250.0	6190.0	453.1	37.3	184.9	113.1
9	3869.0	60.2	23.5	86.0	5553.0	728.5	37.3	325.5	118.4
10	3758.0	160.4	60.7	335.0	7179.0	445.7	15.5	192.6	130.6
11	3708.0	169.2	76.8	410.0	3907.0	462.8	22.4	225.7	105.1
12	5164.0	74.9	17.7	239.0	8238.0	813.7	116.3	172.9	333.1
13	2305.0	73.6	26.0	389.0	3448.0	290.1	24.4	74.9	83.7
15	2016.0	65.1	24.2	365.0	2955.0	225.2	26.6	79.1	149.8
16	2074.0	103.5	44.1	324.0	3268.0	148.1	11.0	22.4	133.0
17	1922.0	68.8	27.2	468.0	4329.0	255.9	21.7	71.2	78.1
18	3203.0	118.3	36.0	487.0	3928.0	280.5	37.1	100.3	201.0
19	3228.0	130.6	46.5	337.0	5977.0	284.5	15.8	30.6	135.5
20	2545.0	111.4	47.3	305.0	4232.0	265.5	23.7	111.2	122.3
22	2874.0	80.9	29.8	193.0	3621.0	455.8	18.8	276.9	89.1
23	4070.0	169.0	71.0	688.0	4690.0	542.8	23.0	259.6	110.4

Day 2

Subject #	Cal (kcal)	Fat (g)	Sat Fat (g)	Chol (mg)	Na+ (mg)	CHO (g)	Fiber (g)	Sugars (g)	Protein (g)
1	3190.0	113.4	64.1	362.0	6273.0	361.1	20.2	101.3	181.4
2	2801.0	112.8	35.4	1128.0	5466.0	308.9	15.1	193.6	116.3
4	4338.0	86.9	38.3	371.0	7599.0	727.7	59.2	160.0	192.9
6	1617.0	105.3	27.7	732.0	4528.0	109.0	7.8	6.7	58.3
7	4770.0	236.6	113.6	707.0	8662.0	488.5	22.7	179.1	175.8
8	3377.0	128.3	27.4	260.0	4128.0	422.7	34.3	131.0	107.6
9	2564.0	104.5	16.5	379.0	3540.0	287.1	33.3	92.8	134.4
10	2705.0	86.4	30.2	313.0	5515.0	390.6	17.1	117.3	96.6
11	3303.0	104.8	59.1	350.0	3139.0	528.9	24.8	274.6	79.1
12	3896.0	93.2	27.0	508.0	6083.0	549.2	130.3	167.1	273.7
13	3502.0	105.7	45.3	187.0	3547.0	425.2	31.0	168.2	97.3
15	2016.0	65.1	24.2	365.0	2955.0	225.2	26.6	79.1	149.8
16	2438.0	65.3	27.4	210.0	4678.0	367.7	25.6	142.6	104.8
17	2870.0	105.1	39.1	577.0	7578.0	375.3	30.7	79.7	114.2
18	3261.0	162.8	54.1	448.0	4240.0	295.7	28.3	168.6	166.3
19	2598.0	119.0	52.7	506.0	4673.0	269.3	20.2	74.7	114.4
20	4144.0	167.1	56.9	1241.0	7091.0	507.7	19.3	273.2	161.1
22	1894.0	75.0	18.1	145.0	3668.0	227.2	11.5	48.5	75.9
23	3474.0	142.7	79.3	622.0	3942.0	427.0	20.2	204.9	131.3

Table 4.

3 Day Diet Record (cont)

				Day 3					
Subject #	**Cal (kcal)**	**Fat (g)**	**Sat Fat (g)**	**Chol (mg)**	**Na+ (mg)**	**CHO (g)**	**Fiber (g)**	**Sugars (g)**	**Protein (g)**
1	5883.0	211.8	72.8	589.0	11301.0	585.5	30.8	188.3	306.2
2	3298.0	185.0	43.6	774.0	3903.0	316.6	55.9	89.1	120.6
4	5678.0	100.3	36.9	505.0	7625.0	926.6	86.0	162.1	304.6
6	2838.0	121.7	48.2	449.0	5548.0	226.1	10.6	13.8	109.1
7	1552.0	63.2	22.4	296.0	2639.0	98.6	11.4	32.6	157.3
8	2734.0	64.3	25.4	295.0	4340.0	447.1	23.6	139.5	99.6
9	2462.0	83.9	35.7	143.0	3419.0	344.3	27.0	190.0	96.3
10	4573.0	207.7	88.9	585.0	8131.0	466.2	22.6	165.1	216.1
11	1750.0	50.2	23.7	162.0	3608.0	239.3	9.3	86.2	87.1
12	4552.0	111.0	34.2	205.0	3498.0	683.5	120.9	334.0	259.2
13	2768.0	107.3	33.8	187.0	5903.0	384.4	19.4	63.6	79.8
15	2016.0	65.1	24.2	365.0	2955.0	225.2	26.6	79.1	149.8
16	2357.0	118.7	45.3	868.0	4692.0	210.5	18.3	56.4	109.9
17	2757.0	156.1	66.6	711.0	5921.0	209.2	25.8	62.7	135.7
18	2712.0	115.8	35.8	770.0	4217.0	267.0	41.2	134.6	171.4
19	3138.0	75.2	31.2	299.0	2120.0	193.5	2.2	4.8	106.6
20	4167.0	111.9	54.3	427.0	3911.0	333.3	11.0	81.5	143.0
22	2585.0	76.1	28.8	700.0	4401.0	358.4	25.8	182.9	126.2
23	3602.0	130.7	69.7	450.0	4291.0	478.6	14.2	276.8	141.0

Table 4.

3 Day Diet Record (cont)

Subject #	Cal (kcal)	CHO (g)	% CHO	Protein (g)	% Protein	Fat (g)	% Fat
			Average				
1	4186.67	430.47	41.13	218.17	20.84	145.93	31.37
2	3572.33	387.03	43.34	111.73	12.51	176.47	44.46
4	5027.33	807.07	64.21	270.77	21.54	94.23	16.87
6	2386.00	181.60	30.44	83.97	14.08	114.27	43.10
7	3017.33	262.37	34.78	157.30	20.85	151.40	45.16
8	2981.33	440.97	59.16	106.77	14.32	89.23	26.94
9	2965.00	453.30	61.15	116.37	15.70	82.87	25.15
10	3678.67	434.17	47.21	147.77	16.07	151.50	37.07
11	2920.33	410.33	56.20	90.43	12.39	108.07	33.30
12	4537.33	682.13	60.14	288.67	25.45	93.03	18.45
13	2858.33	366.57	51.30	86.93	12.17	95.53	30.08
15	2016.00	225.20	44.68	149.80	29.72	65.10	29.06
16	2289.67	242.10	42.29	115.90	20.25	95.83	37.67
17	2516.33	280.13	44.53	109.33	17.38	110.00	39.34
18	3058.67	281.07	36.76	179.57	23.48	132.30	38.93
19	2988.00	249.10	33.35	118.83	15.91	108.27	32.61
20	3618.67	368.83	40.77	142.13	15.71	130.13	32.37
22	2451.00	347.13	56.65	97.07	15.84	77.33	28.40
23	3715.33	482.80	51.98	127.57	13.73	147.47	35.72
Mean:	**3199.18**	**385.91**	**47.37**	**143.11**	**17.79**	**114.16**	**32.95**
SD:	**789.78**	**155.20**	**10.19**	**58.69**	**4.84**	**30.13**	**7.89**

Subject #	Sat Fat (g)	Chol (mg)	Na+ (mg)	Fiber (g)	Sugars (g)	kcal/kgbw
1	57.37	412.00	8146.67	22.73	124.17	52.53
2	37.53	659.67	4928.33	40.27	122.70	46.58
4	35.50	483.67	7742.00	71.53	147.37	64.45
6	38.70	457.33	4842.00	9.37	10.07	38.12
7	64.87	691.00	6639.67	14.77	89.87	35.04
8	26.30	268.33	4886.00	31.73	151.80	38.77
9	25.23	202.67	4170.67	32.53	202.77	39.32
10	59.93	411.00	6941.67	18.40	158.33	48.34
11	53.20	307.33	3551.33	18.83	195.50	41.84
12	26.30	317.33	5939.67	122.50	224.67	45.42
13	35.03	254.33	4299.33	24.93	102.23	45.44
15	24.20	365.00	2955.00	26.60	79.10	24.71
16	38.93	467.33	4212.67	18.30	73.80	32.02
17	44.30	585.33	5942.67	26.07	71.20	38.42
18	41.97	568.33	4128.33	35.53	134.50	45.86
19	43.47	380.67	4256.67	12.73	36.70	36.89
20	52.83	657.67	5078.00	18.00	155.30	39.72
22	25.57	346.00	3896.67	18.70	169.43	32.25
23	73.33	586.67	4307.67	19.13	247.10	58.05
Mean:	**42.35**	**443.25**	**5098.16**	**30.67**	**131.40**	**42.30**
SD:	**14.53**	**147.76**	**1426.04**	**26.10**	**62.71**	**9.40**

Table 5.

Subject Compliance

Treatment A

Subject #	# Pills Consumed (out of 42)	Exercise in Last 24 Hours?	Compete in Last 72 Hours?	Drink Alcohol Last 24 Hours?	Drink Caffeine in Last 12 Hours?
1	42	No	No	No	No
2	42	No	No	No	No
4	42	No	No	No	No
6	42	No	No	No	No
7	40	No	No	No	No
8	42	No	Yes (1)	No	No
9	42	No	No	No	No
10	42	No	No	No	No
11	26	Yes (2)	Yes (3)	No	No
12	38	No	No	No	No
13	42	No	No	No	No
15	38	No	No	Yes (4)	No
16	42	Yes (5)	Yes (6)	No	No
17	36	No	No	No	No
18	36	No	No	No	No
19	38	No	Yes (8)	No	No
20	42	No	No	No	No
22	30	No	Yes (11)	No	No
23	40	No	No	No	No
%:	**92.98**	**89.47**	**73.68**	**94.74**	**100.00**
Mean:	**39.05**				
SD:	**4.49**				

Subject #	Eat Spicy Food in Last Week?	Sick/Injured in Last Week?	Any Side Effects or Symptoms from Pills?
1	No	No	No
2	No	No	No
4	No	No	No
6	No	No	No
7	No	No	No
8	No	No	No
9	No	No	No
10	No	No	No
11	No	No	No
12	No	No	No
13	No	No	No
15	No	No	No
16	No	No	No
17	No	No	No
18	Yes (7)	No	No
19	No	Yes (9)	No
20	No	Yes (10)	No
22	No	Yes (12)	No
23	No	No	No
%:	**94.74**	**84.21**	**100.00**

Subject Compliance (cont)

Treatment B

Subject #	# Pills Consumed (out of 42)	Exercise in Last 24 Hours?	Compete in Last 72 Hours?	Drink Alcohol Last 24 Hours?	Drink Caffeine in Last 12 Hours?
1	40	No	No	No	No
2	42	No	No	No	No
4	42	No	No	No	No
6	42	No	No	No	No
7	42	No	No	No	No
8	42	No	No	No	No
9	42	No	No	No	No
10	21	No	No	No	No
11	37	Yes (16)	No	No	No
12	42	No	No	No	No
13	23	No	No	No	No
15	34	Yes (17)	No	Yes (18)	No
16	28	No	Yes (19)	No	No
17	21	No	No	No	No
18	42	No	Yes (21)	No	No
19	36	No	Yes (22)	No	No
20	34	No	Yes (24)	No	No
22	42	No	No	No	No
23	38	No	No	No	No
%:	**86.47**	**89.47**	**78.95**	**94.74**	**100.00**
Mean:	**36.32**				
SD:	**7.58**				

Subject #	Eat Spicy Food in Last Week?	Sick/Injured in Last Week?	Any Side Effects or Symptoms from Pills?
1	Yes (13)	No	Yes: Acid reflux - for 45min after dose
2	No	Yes (14)	Yes: Heartburn, stomach ache, G.I. cramping - 10min
4	No	No	Yes: Slight indigestion (if without food), spicy burps
6	No	No	Yes: Slight morning sickness, gas
7	No	No	Yes: Slight upset stomach and burning stool, not often
8	No	No	Yes: Burping, upset stomach - for 1hr after dose
9	No	No	No
10	Yes (15)	No	Yes: Heartburn/stomach ache, burning stool - for 1hr
11	No	No	Yes: Upset stomach
12	No	No	Yes: Slight indigestion, not bad
13	No	No	Yes: Indigestion, upset stomach - for 1hr after dose
15	No	No	Yes: Stomach ache - once, for 2hrs
16	No	Yes (20)	Yes: Heartburn, diarrhea
17	No	No	Yes: Threw up if more than 1 without food
18	No	No	Yes: Heartburn, spicy burps 5-15min, burning stool
19	Yes (23)	No	Yes: Upset stomach, heartburn - for 30min after dose
20	No	No	Yes: Heart burn, burning stool
22	No	No	Yes: Upset stomach sometimes - for 2hrs after dose
23	No	Yes (25)	Yes: Stomach ache, loose stools
%:	**84.21**	**84.21**	

Table 5.

Subject Compliance (cont)

Compliance Explanation Codes

1 - Unorganized pickup soccer game
2 - Upper body lifting, ~20hrs prior
3 - No explanation
4 - 1 Corona
5 - 40 min soccer game
6 - 40 min soccer game (K6)
7 - Homemade chili, 36hrs prior
8 - Rugby game
9 - Minor cold 6 days prior
10 - Seasonal allergies
11 - Soccer game 3 days prior
12 - Allergies
13 - 2 hot wings 2 days prior
14 - Minor cold symptoms
15 - Salsa with chicken dinner
16 - Easy jog, 24 minutes
17 - Yoga, 1.5hrs
18 - 2 beers
19 - 40 minute soccer game
20 - Sore throat, sinus
21 - 1/2 rugby game 48hrs prior
22 - Rugby game 48hrs prior
23 - 1 hot wing 2 days prior
24 - Rugby game 48hrs prior
25 - Minor sinus infection

Table 6.

Test Climate

RST Climate by Treatment

PCB	Temp (°C)	% Humidity
7/24/2009	23.0	50.0
7/24/2009	23.0	50.0
7/24/2009	23.0	50.0
7/29/2009	22.6	55.0
8/5/2009	24.0	60.0
8/7/2009	22.0	58.0
8/12/2009	25.0	63.0
8/12/2009	25.0	63.0
8/14/2009	25.0	66.0
9/11/2009	23.0	50.0
9/14/2009	23.0	58.0
9/17/2009	24.9	58.0
9/21/2009	23.5	59.0
9/25/2009	26.6	65.0
9/29/2009	19.6	39.0
10/2/2009	21.0	44.0
10/5/2009	21.0	42.0
10/9/2009	21.0	44.0
10/9/2009	21.0	44.0
Mean:	**23.01**	**53.58**
SD:	**1.80**	**8.39**

CAP	Temp (°C)	% Humidity
7/10/2009	23.1	58.0
7/10/2009	23.1	58.0
7/17/2009	24.4	68.0
7/24/2009	23.0	50.0
7/24/2009	23.0	50.0
7/31/2009	24.7	67.0
8/7/2009	22.0	58.0
8/7/2009	22.0	58.0
8/12/2009	25.0	63.0
8/28/2009	23.0	53.0
9/18/2009	24.8	59.0
9/21/2009	23.5	59.0
9/21/2009	23.5	59.0
9/25/2009	26.6	65.0
9/25/2009	26.6	65.0
9/29/2009	19.6	39.0
10/5/2009	21.0	42.0
10/9/2009	21.0	44.0
10/9/2009	21.0	44.0
Mean:	**23.21**	**55.74**
SD:	**1.88**	**8.73**

PST Climate by Subject

Subject #	Temp (°C)	% Humidity
1	23.0	47.0
2	23.0	47.0
4	23.0	47.0
6	23.1	58.0
7	23.1	58.0
8	23.1	58.0
9	23.1	58.0
10	24.4	68.0
11	24.4	68.0
12	23.0	50.0
13	24.7	67.0
15	24.0	60.0
16	23.0	57.0
17	23.0	50.0
18	23.0	58.0
19	23.0	58.0
20	23.0	58.0
22	24.8	59.0
23	26.6	65.0
Mean:	**23.59**	**57.42**
SD:	**0.98**	**6.75**

Table 7.

Sprint Time Data

PCB

Subject #	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5	Sprint 6	Sprint 7	Sprint 8
1	4.572	4.543	4.598	4.620	4.688	4.730	4.689	4.615
2	4.348	4.261	4.320	4.294	4.400	4.416	4.454	4.467
4	4.870	5.019	5.032	5.103	5.059	5.003	4.993	5.082
6	4.614	4.615	4.687	4.617	4.726	4.665	4.708	4.790
7	4.681	4.666	4.691	4.696	4.836	4.939	4.956	4.882
8	4.266	4.321	4.405	4.442	4.513	4.523	4.602	4.660
9	4.524	4.773	4.712	4.662	4.625	4.652	4.718	4.660
10	4.577	4.652	4.719	4.714	4.703	4.668	4.708	4.667
11	4.574	4.664	4.728	4.663	4.611	4.619	4.672	4.640
12	4.889	4.956	4.896	4.975	4.956	5.080	4.890	4.997
13	4.678	4.704	4.876	4.778	4.787	4.824	4.888	4.755
15	5.048	5.001	5.032	4.980	4.878	4.967	5.076	5.064
16	4.170	4.216	4.239	4.316	4.287	4.371	4.363	4.460
17	4.768	4.714	4.578	4.613	4.554	4.585	4.556	4.561
18	4.376	4.501	4.561	4.585	4.731	4.604	4.757	4.844
19	4.423	4.641	4.348	4.549	4.568	4.616	4.554	4.446
20	4.642	4.619	4.752	4.732	4.779	4.848	4.801	4.870
22	4.355	4.328	4.527	4.629	4.596	4.636	4.761	4.825
23	4.641	4.716	4.691	4.735	4.702	4.679	4.857	4.714
Mean:	**4.580**	**4.627**	**4.652**	**4.644**	**4.685**	**4.707**	**4.758**	**4.737**
SD:	**0.224**	**0.231**	**0.223**	**0.180**	**0.140**	**0.193**	**0.164**	**0.192**
H Outlier:	5.028	5.088	5.098	5.077	5.054	5.092	5.104	5.120
L Outlier:	4.132	4.166	4.207	4.260	4.315	4.321	4.370	4.353

Subject #	Sprint 9	Sprint 10	Sprint 11	Sprint 12	Sprint 13	Sprint 14	Sprint 15
1	4.813	4.592	4.745	4.727	4.847	4.988	4.893
2	4.658	4.689	4.799	4.665	5.012	5.130	4.800
4	5.033	5.055	5.033	5.032	5.076	5.132	5.131
6	4.921	4.705	4.943	4.767	4.711	4.741	4.865
7	5.023	5.059	5.125	5.113	5.349	5.447	5.352
8	4.733	4.790	4.807	4.956	4.893	4.931	5.096
9	4.600	4.665	4.637	4.634	4.831	4.642	4.625
10	4.697	4.687	4.706	4.728	4.723	4.703	4.753
11	4.651	4.710	4.678	4.632	4.724	4.677	4.646
12	4.994	5.077	4.990	4.991	5.060	4.998	5.029
13	4.770	4.887	4.812	4.825	4.792	5.041	4.845
15	4.890	5.095	4.926	4.975	5.009	5.076	5.106
16	4.368	4.431	4.448	4.518	4.517	4.513	4.531
17	4.565	4.598	4.582	4.541	4.587	4.575	4.557
18	4.703	4.821	4.709	4.392	4.634	4.691	4.648
19	4.676	4.719	4.743	4.509	4.875	4.571	4.741
20	4.886	5.109	5.110	5.013	5.079	5.095	5.326
22	4.869	4.974	5.156	4.898	5.133	5.047	5.183
23	4.728	4.828	4.799	4.812	4.756	4.718	4.844
Mean:	**4.789**	**4.815**	**4.829**	**4.775**	**4.874**	**4.848**	**4.893**
SD:	**0.145**	**0.199**	**0.194**	**0.207**	**0.212**	**0.218**	**0.251**
H Outlier:	5.109	5.213	5.216	5.189	5.298	5.385	5.396
L Outlier:	4.425	4.417	4.441	4.361	4.450	4.375	4.390

Table 7.

Sprint Data (cont.)

CAP

Subject #	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5	Sprint 6	Sprint 7	Sprint 8
1	4.722	4.852	4.918	4.919	5.168	5.274	5.006	5.315
2	4.260	4.337	4.300	4.308	4.381	4.456	4.443	4.465
4	4.793	5.012	5.151	5.116	5.222	5.079	5.132	5.278
6	4.665	4.721	4.814	5.194	4.641	5.041	4.829	5.309
7	4.750	4.712	4.896	4.989	5.131	5.111	5.251	5.296
8	*****	*****	*****	*****	*****	*****	*****	*****
9	4.552	4.544	4.741	4.748	4.697	4.624	4.660	4.385
10	4.495	4.511	4.548	4.610	4.580	4.616	4.648	4.156
11	4.871	4.902	4.878	4.927	4.866	4.908	4.838	4.999
12	4.835	4.834	4.826	4.911	4.940	4.966	4.974	4.985
13	5.005	4.803	4.935	4.787	4.881	4.911	4.917	4.918
15	4.795	4.829	4.882	4.891	5.085	4.929	4.921	4.843
16	4.390	4.356	4.382	4.330	4.302	4.319	4.357	4.416
17	4.636	4.576	4.630	4.691	4.620	4.640	4.768	4.658
18	4.491	4.405	4.466	4.662	4.632	4.710	4.587	4.706
19	4.537	4.508	4.647	4.639	4.699	4.753	4.766	4.531
20	4.464	4.572	4.582	4.380	4.689	4.676	4.576	4.634
22	4.380	4.336	4.333	4.457	4.516	4.577	4.722	4.888
23	4.739	4.810	4.790	4.734	4.700	4.819	4.939	4.805
Mean:	**4.632**	**4.646**	**4.707**	**4.739**	**4.764**	**4.801**	**4.770**	**4.810**
SD:	**0.193**	**0.206**	**0.229**	**0.251**	**0.257**	**0.241**	**0.207**	**0.339**
H Outlier:	5.017	5.058	5.164	5.240	5.279	5.282	5.245	5.489
L Outlier:	4.247	4.233	4.249	4.237	4.249	4.319	4.348	4.132

Subject #	Sprint 9	Sprint 10	Sprint 11	Sprint 12	Sprint 13	Sprint 14	Sprint 15
1	5.532	5.143	5.355	5.490	5.943	5.813	5.546
2	4.494	4.483	4.551	4.487	4.576	4.515	4.511
4	5.171	5.389	5.511	5.206	5.190	5.375	5.213
6	5.068	4.921	5.013	4.853	4.943	4.924	4.808
7	5.379	5.542	5.345	5.342	5.555	5.516	5.554
8	*****	*****	*****	*****	*****	*****	*****
9	4.714	4.728	4.752	4.753	4.702	4.850	4.610
10	4.718	4.693	4.687	4.695	4.693	4.689	4.695
11	4.860	4.882	4.858	4.965	4.912	4.858	4.835
12	4.942	5.084	5.004	5.070	4.976	5.006	5.026
13	4.884	4.884	4.921	4.823	*****	4.879	4.968
15	4.910	4.818	4.939	4.969	4.948	4.893	4.857
16	4.410	4.485	4.446	4.436	4.470	4.545	4.467
17	4.669	4.612	4.710	4.635	4.755	4.641	4.809
18	4.724	4.690	4.627	4.770	4.618	4.810	4.476
19	4.737	4.785	4.883	4.696	4.916	4.881	4.754
20	4.688	4.825	4.579	4.687	4.637	4.687	4.809
22	4.949	5.076	5.224	5.253	5.244	5.422	5.267
23	4.664	4.835	4.806	4.834	4.840	4.720	4.719
Mean:	**4.862**	**4.882**	**4.901**	**4.851**	**4.873**	**4.895**	**4.846**
SD:	**0.278**	**0.273**	**0.291**	**0.256**	**0.280**	**0.292**	**0.292**
H Outlier:	5.419	5.429	5.483	5.451	5.664	5.639	5.521
L Outlier:	4.305	4.335	4.318	4.323	4.208	4.253	4.248

Table 7.

Sprint Data (cont.)

		PCB		
Subject #	Average	Fastest	Slowest	Difference
1	4.711	4.543	4.988	0.445
2	4.581	4.261	5.130	0.869
4	5.044	4.870	5.132	0.262
6	4.738	4.614	4.943	0.329
7	4.988	4.666	5.447	*****
8	4.663	4.266	5.096	0.830
9	4.664	4.524	4.831	0.307
10	4.694	4.577	4.753	0.176
11	4.659	4.574	4.728	0.154
12	4.985	4.889	5.080	0.191
13	4.817	4.678	5.041	0.363
15	5.008	4.878	5.106	0.228
16	4.383	4.170	4.531	0.361
17	4.596	4.541	4.768	0.227
18	4.637	4.376	4.844	0.468
19	4.599	4.348	4.875	0.527
20	4.911	4.619	5.326	0.707
22	4.794	4.328	5.183	0.855
23	4.748	4.641	4.857	0.216
Mean:	**4.748**	**4.545**	**4.956**	**0.418**
SD:	**0.174**	**0.211**	**0.198**	**0.243**
H Outlier:	5.097	4.968	5.429	0.938
L Outlier:	4.399	4.123	4.535	-0.065

		CAP		
Subject #	Average	Fastest	Slowest	Difference
1	5.266	4.722	5.943	1.221
2	4.438	4.260	4.576	0.316
4	5.189	4.793	5.511	0.718
6	4.916	4.641	5.309	0.668
7	5.225	4.712	5.555	0.843
8	*****	*****	*****	*****
9	4.671	4.385	4.850	0.465
10	4.602	4.156	4.718	0.562
11	4.891	4.835	4.999	0.164
12	4.959	4.826	5.084	0.258
13	4.894	4.787	5.005	0.218
15	4.901	4.795	5.085	0.290
16	4.407	4.302	4.545	0.243
17	4.670	4.576	4.809	0.233
18	4.625	4.405	4.810	0.405
19	4.715	4.508	4.916	0.408
20	4.632	4.380	4.825	0.445
22	4.843	4.333	5.422	1.089
23	4.784	4.664	4.939	0.275
Mean:	**4.813**	**4.560**	**4.998**	**0.407**
SD:	**0.240**	**0.215**	**0.302**	**0.200**
H Outlier:	5.293	4.991	5.766	1.087
L Outlier:	4.332	4.129	4.335	-0.106

Table 7.

Sprint Data (cont.)

PCB Mean:	**4.748**
PCB SD:	**0.217**
CAP Mean:	**4.797**
CAP SD:	**0.273**
Ave Mean:	**4.780**
Ave SD:	**0.212**
Fastest Mean:	**4.553**
Fastest SD:	**0.213**

Sprint #:	**Sprint 1**	**Sprint 2**	**Sprint 3**	**Sprint 4**	**Sprint 5**	**Sprint 6**	**Sprint 7**	**Sprint 8**
Time Mean:	4.605	4.636	4.679	4.691	4.726	4.752	4.764	4.773
Time SD:	0.211	0.219	0.227	0.224	0.214	0.223	0.184	0.278
Sprint #:	**Sprint 9**	**Sprint 10**	**Sprint 11**	**Sprint 12**	**Sprint 13**	**Sprint 14**	**Sprint 15**	
Time Mean:	4.826	4.848	4.864	4.811	4.874	4.871	4.871	
Time SD:	0.227	0.242	0.250	0.254	0.242	0.254	0.268	
Total Mean:	4.772							
Total SD:	0.247							

outliers defined at mean ± 2 SD, H = high limit, L = low limit
red values indicate outlier
outliers excluded in calculations
missing data (*****) due to equipment failure

Table 8

Fatigue Data

	PCB				CAP		
Subject #	Total Time	Ideal Time	%FD	Subject #	Total Time	Ideal Time	% FD
1	70.660	68.145	3.69	1	56.204	51.942	*****
2	68.713	63.915	7.51	2	66.567	63.900	4.17
4	65.491	63.310	3.44	4	77.838	71.895	8.27
6	71.075	69.210	2.69	6	73.744	69.615	5.93
7	69.368	65.324	6.19	7	73.118	65.968	10.84
8	69.938	63.990	9.30	8	*****	*****	*****
9	69.960	67.860	3.09	9	70.060	65.775	6.51
10	70.405	68.655	2.55	10	69.034	62.340	10.74
11	69.889	68.610	1.86	11	73.359	72.525	1.15
12	74.778	73.335	1.97	12	74.379	72.390	2.75
13	72.262	70.170	2.98	13	68.516	67.018	2.24
15	75.123	73.170	2.67	15	73.509	71.925	2.20
16	52.730	50.040	*****	16	66.111	64.530	2.45
17	68.934	68.115	1.20	17	70.050	68.640	2.05
18	69.557	65.640	5.97	18	69.374	66.075	4.99
19	68.979	65.220	5.76	19	70.732	67.620	4.60
20	73.661	69.285	6.32	20	69.485	65.700	5.76
22	71.917	64.920	10.78	22	72.644	64.995	11.77
23	71.220	69.615	2.31	23	71.754	69.960	2.56
Mean:	**70.663**	**67.694**	**4.09**	**Mean:**	**71.193**	**67.698**	**5.23**
SD:	**2.319**	**2.965**	**2.30**	**SD:**	**3.011**	**3.209**	**3.39**
H Outlier:	79.100	76.706	8.69	**H Outlier:**	79.528	76.515	12.01
L Outlier:	60.338	56.823	-0.51	**L Outlier:**	61.191	57.131	-1.54
Total Mean:	70.031	66.793	**4.84**				
Total SD:	4.586	4.842	**3.05**				

% fatigue decrement calculated by: %FD = [(Total Sum/Ideal)*100]-100, where Ideal = (15*fastest)
outliers defined at mean ± 2 SD, H = high limit, L = low limit
red values indicate outlier
outliers excluded in calculations
missing data (*****) due to equipment failure

Table 9.

Inflammation Data

		PCB		CAP	
Subject #	**B0**	**B1**	**B2**	**B1**	**B2**
1	0.821	0.895	1.223	0.695	1.067
2	0.475	0.382	1.797	0.457	1.781
4	0.246	1.202	1.499	0.700	0.337
6	0.294	0.181	0.324	0.272	0.266
7	0.247	0.332	0.680	0.307	0.565
8	0.524	0.831	1.922	0.751	1.202
9	1.164	0.557	0.569	0.679	0.475
11	*****	*****	*****	*****	*****
10	0.802	0.719	2.987	1.028	1.027
12	0.459	0.272	0.729	0.520	0.944
13	0.226	0.501	0.520	0.480	0.557
15	0.285	0.506	0.542	0.315	0.614
16	0.320	0.226	0.810	3.052	3.778
17	0.430	1.329	1.326	0.302	0.802
18	0.503	0.865	1.125	0.915	1.153
19	1.025	1.616	1.852	0.874	1.529
20	1.478	1.257	2.779	1.170	1.499
22	0.661	0.856	1.134	0.649	1.157
23	0.365	0.425	0.324	1.788	1.745
Mean:	**0.520**	**0.667**	**1.126**	**0.700**	**0.983**
SD:	**0.283**	**0.365**	**0.675**	**0.386**	**0.475**
H Outlier:	1.284	1.557	2.806	2.168	2.747
L Outlier:	-0.137	-0.119	-0.347	-0.507	-0.469

B1 Mean:	0.683
B1 SD:	0.370
B2 Mean:	1.055
B2 SD:	0.580
PCB Mean:	0.897
PCB SD:	0.583
CAP Mean:	0.842
CAP SD	0.450
Total Mean:	0.799
Total SD:	0.499

Table 9.

Inflammation Data (cont.)

	Normalized (B2/B1)		Difference (B2-B1)		% Change	
	PCB	CAP	PCB	CAP	PCB	CAP
Subject #	B2/B1	B2/B1	B2-B1	B2-B1		
1	1.37	1.54	0.328	0.372	36.65	53.53
2	4.71	3.90	1.416	1.325	371.04	290.14
4	1.25	0.48	0.297	-0.363	24.72	-51.82
6	1.79	0.98	0.143	-0.006	78.73	-2.21
7	2.05	1.84	0.348	0.259	104.98	84.34
8	2.31	1.60	1.091	0.451	131.23	60.05
9	1.02	0.70	0.013	-0.204	2.25	-29.99
11	*****	*****	*****	*****	*****	*****
10	4.15	1.00	2.268	-0.001	315.37	-0.10
12	2.68	1.82	0.457	0.424	168.32	81.54
13	1.04	1.16	0.019	0.077	3.69	16.06
15	1.07	1.95	0.036	0.299	7.12	94.92
16	3.59	1.24	0.585	0.727	259.20	23.81
17	1.00	2.66	-0.002	0.501	-0.19	166.00
18	1.30	1.26	0.260	0.238	30.00	26.01
19	1.15	1.75	0.236	0.655	14.60	74.89
20	2.21	1.28	1.522	0.329	121.13	28.12
22	1.32	1.78	0.278	0.508	32.48	78.27
23	0.76	0.98	-0.101	-0.043	-23.79	-2.40
Mean:	**1.769**	**1.412**	**0.407**	**0.248**	**76.852**	**41.236**
SD:	**0.963**	**0.533**	**0.487**	**0.300**	**96.319**	**53.336**
H Outlier:	4.26	3.12	1.799	1.080	325.92	211.52
L Outlier:	-0.40	-0.01	-0.778	-0.464	-139.52	-101.39
Total Mean:	**1.590**		**0.328**		**59.04**	
Total SD:	**0.788**		**0.406**		**78.766**	

outliers defined at mean ± 2 SD, H = high limit, L = low limit
red values indicate outlier
calculations exclude outliers
missing data (*****) due to inability to draw blood from subject

Table 10.

Sprint Heart Rate

PCB Heart Rate

Subject #	1-Min Pre	Sprint 3	Sprint 6	Sprint 9	Sprint 12	Sprint 15	1-Min Post
1	112	218	184	187	177	173	133
2	82	190	*****	*****	166	*****	144
4	103	152	158	186	170	172	119
6	84	179	209	194	183	189	144
7	106	187	190	192	192	192	167
8	128	187	187	169	184	177	146
9	81	153	160	169	196	198	130
10	96	157	176	179	183	186	146
11	85	148	155	156	159	159	130
12	93	189	152	156	161	164	115
13	105	179	187	181	190	190	126
15	96	151	179	169	167	174	123
16	93	170	173	179	170	198	129
17	80	159	161	160	172	170	84
18	91	157	165	165	176	176	123
19	106	*****	*****	*****	*****	147	137
20	96	*****	164	*****	166	*****	132
22	103	228	222	192	190	*****	166
23	96	130	155	177	166	*****	119
Mean:	**94.89**	**169.13**	**172.19**	**175.69**	**176.00**	**179.86**	**134.94**
SD:	**9.69**	**22.08**	**16.13**	**12.71**	**11.36**	**12.34**	**14.90**
H Outlier:	121	224	215	201	199	207	169
L Outlier:	72	121	136	150	153	148	95

CAP Heart Rate

Subject #	1-Min Pre	Sprint 3	Sprint 6	Sprint 9	Sprint 12	Sprint 15	1-Min Post
1	75	155	153	144	*****	130	126
2	128	177	*****	184	189	192	160
4	99	156	159	164	160	164	109
6	78	172	172	170	170	176	133
7	101	190	193	177	192	193	153
8	109	174	179	179	179	179	143
9	61	144	159	156	156	157	99
10	96	172	180	181	186	185	137
11	57	125	135	139	139	139	106
12	84	143	151	157	157	160	98
13	72	153	148	149	170	160	101
15	93	151	160	156	*****	174	121
16	85	164	174	170	181	176	136
17	101	161	157	152	157	149	81
18	80	*****	*****	*****	*****	128	91
19	101	157	*****	155	*****	146	123
20	87	144	*****	156	*****	142	116
22	96	188	192	204	189	181	142
23	109	144	140		172	156	109
Mean:	**88.00**	**159.44**	**163.47**	**161.81**	**173.69**	**162.47**	**120.21**
SD:	**15.28**	**17.03**	**17.61**	**13.65**	**13.31**	**19.97**	**21.81**
H Outlier:	125	194	199	198	203	202	164
L Outlier:	55	125	128	131	140	123	77

Table 10.

Sprint Heart Rate (cont)

PCB Heart Rate Subject #	Max	Total Average	Sprint Average	CAP Heart Rate Subject #	Max	Total Average	Sprint Average
1	218	169.14	187.80	1	155	130.50	145.50
2	*****	*****	*****	2	192	*****	185.50
4	186	151.43	167.60	4	164	144.43	160.60
6	209	168.86	190.80	6	176	153.00	172.00
7	192	175.14	190.60	7	193	171.29	189.00
8	187	175.00	180.80	8	179	163.14	178.00
9	198	155.29	175.20	9	159	133.14	154.40
10	186	160.43	176.20	10	186	162.43	180.80
11	159	141.71	155.40	11	139	116.83	134.50
12	189	147.14	164.40	12	160	135.71	153.60
13	190	165.43	185.40	13	170	136.14	156.00
15	179	151.29	168.00	15	174	142.50	160.25
16	198	158.86	178.00	16	181	155.14	173.00
17	172	150.33	164.40	17	161	136.86	155.20
18	176	150.43	167.80	18	*****	*****	*****
19	*****	*****	*****	19	*****	*****	*****
20	*****	*****	*****	20	156	129.00	147.33
22	*****	*****	*****	22	192	164.67	187.50
23	177	140.50	157.00	23	172	138.33	153.00
Mean:	**187.73**	**157.40**	**173.96**	**Mean:**	**171.12**	**144.57**	**163.89**
SD:	**14.67**	**11.28**	**11.59**	**SD:**	**15.23**	**15.40**	**16.28**

PCB Sprint Mean:	**174.48**
PCB Sprint SD:	**15.44**
CAP Sprint Mean:	**163.65**
CAP Sprint SD:	**16.99**
PCB Total Mean:	**155.99**
PCB Total SD:	**33.19**
CAP Total Mean:	**145.12**
CAP Total SD:	**33.84**
Total Sprint Mean:	**169.03**
Total Sprint SD:	**17.07**
Total Mean:	**150.51**
Total SD:	**33.89**
Total Max Mean:	**178.91**
Total Max SD:	**16.97**
Total Total Ave Mean:	**150.78**
Total Total Ave SD:	**14.85**
Total Sprint Ave Mean:	**168.61**
Total Sprint Ave SD:	**14.95**

Time Point:	**1-Min Pre**	**Sprint 3**	**Sprint 6**	**Sprint 9**	**Sprint 12**	**Sprint 15**	**1-Min Post**
Mean:	**91.44**	**164.00**	**167.97**	**168.75**	**175.03**	**169.85**	**127.38**
SD:	**13.09**	**19.87**	**17.16**	**14.76**	**12.05**	**19.03**	**19.96**

Total average heart rate includes measurements at 1-min pretest, after every 3rd sprint, and 1-min posttest
Sprint average heart rate includes measurements after every 3rd sprint
outliers defined at mean ± 2 SD, H = high limit, L = low limit
red values indicate outlier
calculations exclude outliers
missing data (*****) due to equipment failure

Table 11.

Sprint Blood Pressure Data

PCB Blood Pressure

Subject #	1-Min Pre S	1-Min Pre D	Sprint 15 S	Sprint 15 D	1-Min Post S	1-Min Post D
1	151	94	*****	*****	87	51
2	144	98	137	71	131	81
4	157	107	125	86	126	84
6	134	85	153	102	159	105
7	152	103	127	86	85	24
8	157	105	*****	*****	71	41
9	146	96	149	95	140	83
10	134	95	125	88	133	94
11	134	90	148	95	139	92
12	126	83	131	86	144	98
13	149	87	122	72	133	82
15	119	77	137	68	159	94
16	149	99	123	73	133	59
17	130	90	131	62	109	63
18	156	160	124	74	166	89
19	148	93	127	78	132	76
20	143	100	144	81	145	95
22	175	114	*****	*****	123	59
23	112	67	120	85	120	85
Mean:	**142.88**	**93.50**	**132.69**	**81.38**	**131.33**	**79.50**
SD:	**11.43**	**11.25**	**10.68**	**10.97**	**21.77**	**17.81**
H Outlier:	173.04	134.54	154.05	103.31	178.73	119.55
L Outlier:	112.86	59.46	111.32	59.44	77.59	33.61

CAP Blood Pressure

Subject #	1-Min Pre S	1-Min Pre D	Sprint 15 S	Sprint 15 D	1-Min Post S	1-Min Post D
1	166	111	148	49	143	91
2	156	91	139	77	*****	*****
4	143	105	158	103	*****	*****
6	142	92	160	99	115	65
7	131	96	115	74	*****	*****
8	165	107	131	75	125	77
9	149	93	169	111	186	113
10	144	102	*****	*****	105	59
11	121	80	161	107	136	89
12	138	97	131	54	143	98
13	124	77	116	63	125	76
15	132	90	131	85	146	104
16	150	97	158	108	147	98
17	120	75	132	83	124	76
18	151	99	136	76	148	65
19	134	84	153	93	154	81
20	158	98	131	63	144	87
22	96	106	*****	*****	*****	*****
23	148	93	123	41	129	82
Mean:	**142.89**	**94.37**	**140.71**	**80.06**	**134.57**	**84.07**
SD:	**13.99**	**10.07**	**16.68**	**21.49**	**14.30**	**15.24**
H Outlier:	175.10	114.50	174.06	123.04	176.28	114.55
L Outlier:	105.75	74.23	107.35	37.08	99.72	53.58

Table 11.

Sprint Blood Pressure Data (cont.)

PCB	
S Mean:	**135.61**
S SD:	**16.26**
D Mean:	**84.92**
D SD:	**14.95**
CAP	
S Mean:	**139.76**
S SD:	**15.14**
D Mean:	**86.57**
D SD:	**16.99**
Total	
S Mean:	**137.64**
S SD:	**15.78**
D Mean:	**85.74**
D SD:	**15.94**

Time Point:	**1-Min Pre S**	**1-Min Pre D**	**Sprint 15 S**	**Sprint 15 D**	**1-Min Post S**	**1-Min Post D**
Mean:	**142.89**	**93.95**	**136.82**	**80.70**	**132.75**	**81.58**
SD:	**12.62**	**10.52**	**14.46**	**16.96**	**18.67**	**16.60**

outliers defined at mean ± 2 SD, H = high limit, L = low limit
outliers indicated by red
calculations exclude outliers
missing data (*****) due to equipment failure

Table 12.

Rate of Percieved Effort (RPE) Data

PCB

Subject #	1-Min Pre	Sprint 3	Sprint 6	Sprint 9	Sprint 12	Sprint 15
1	7	10	12	13	16	16
2	9	17	19	19	19	20
4	6	11	12	13	14	14
6	6	13	15	16	18	18
7	6	6	8	10	13	15
8	6	9	13	14	16	18
9	6	7	9	13	14	13
10	6	12	13	14	14	15
11	6	11	13	14	14	15
12	6	8	11	13	15	16
13	6	10	12	14	15	15
15	6	9	12	15	16	18
16	6	9	13	14	14	17
17	6	8	9	12	13	13
18	6	8	10	12	14	16
19	6	11	13	15	17	18
20	7	10	11	13	14	17
22	6	13	15	16	17	18
23	6	10	14	16	19	20
Mean:	**6.26**	**10.11**	**12.32**	**14.00**	**15.37**	**16.42**
SD:	**0.73**	**2.51**	**2.52**	**1.94**	**1.89**	**2.06**
H Outlier:	7.73	15.13	17.35	17.89	19.15	20.55
L Outlier:	4.80	5.08	7.28	10.11	11.58	12.29

CAP

Subject #	1-Min Pre	Sprint 3	Sprint 6	Sprint 9	Sprint 12	Sprint 15
1	7	9	13	14	16	17
2	7	14	15	16	17	18
4	6	11	13	15	15	16
6	6	14	15	15	17	18
7	6	9	13	14	16	19
8	7	9	13	14	16	18
9	6	7	7	11	12	13
10	6	14	15	15	17	18
11	6	11	12	13	12	13
12	6	10	13	15	16	16
13	7	11	12	12	15	17
15	6	10	12	13	15	16
16	6	10	12	13	15	16
17	6	9	11	12	13	13
18	6	9	10	13	14	15
19	6	8	11	15	16	18
20	6	8	11	13	14	16
22	6	8	12	16	18	19
23	6	9	12	14	17	19
Mean:	**6.21**	**10.00**	**12.21**	**13.84**	**15.32**	**16.58**
SD:	**0.42**	**2.08**	**1.87**	**1.38**	**1.70**	**1.98**
H Outlier:	7.05	14.16	15.96	16.61	18.72	20.54
L Outlier:	5.37	5.84	8.46	11.07	11.91	12.62

Table 12.

Rate of Perceived Effort (RPE) Data (cont.)

PCB Subject #	Total Average	Sprint Average	Max	Min	Difference
1	12.33	13.4	16.00	7.00	9.00
2	17.17	18.8	20.00	9.00	11.00
4	11.67	12.8	14.00	6.00	8.00
6	14.33	16	18.00	6.00	12.00
7	9.67	10.4	15.00	6.00	9.00
8	12.67	14	18.00	6.00	12.00
9	10.33	11.2	14.00	6.00	8.00
10	12.33	13.6	15.00	6.00	9.00
11	12.17	13.4	15.00	6.00	9.00
12	11.50	12.6	16.00	6.00	10.00
13	12.00	13.2	15.00	6.00	9.00
15	12.67	14	18.00	6.00	12.00
16	12.17	13.4	17.00	6.00	11.00
17	10.17	11	13.00	6.00	7.00
18	11.00	12	16.00	6.00	10.00
19	13.33	14.8	18.00	6.00	12.00
20	12.00	13	17.00	7.00	10.00
22	14.17	15.8	18.00	6.00	12.00
23	14.17	15.8	20.00	6.00	14.00
Mean:	12.41	13.64	16.47	6.26	10.21
SD:	1.74	2.00	1.98	0.73	1.81
H Outlier:	15.88	17.64	20.44	7.73	13.84
L Outlier:	8.94	9.65	12.51	4.80	6.58

CAP Subject #	Total Average	Sprint Average	Max	Min	Difference
1	12.67	13.8	17.00	7.00	10.00
2	14.50	16	18.00	7.00	11.00
4	12.67	14	16.00	6.00	10.00
6	14.17	15.8	18.00	6.00	12.00
7	12.83	14.2	19.00	6.00	13.00
8	12.83	14	18.00	7.00	11.00
9	9.33	10	13.00	6.00	7.00
10	14.17	15.8	18.00	6.00	12.00
11	11.17	12.2	13.00	6.00	7.00
12	12.67	14	16.00	6.00	10.00
13	12.33	13.4	17.00	7.00	10.00
15	12.00	13.2	16.00	6.00	10.00
16	12.00	13.2	16.00	6.00	10.00
17	10.67	11.6	13.00	6.00	7.00
18	11.17	12.2	15.00	6.00	9.00
19	12.33	13.6	18.00	6.00	12.00
20	11.33	12.4	16.00	6.00	10.00
22	13.17	14.6	19.00	6.00	13.00
23	12.83	14.2	19.00	6.00	13.00
Mean:	12.36	13.59	16.58	6.21	10.37
SD:	1.27	1.50	1.98	0.42	1.92
H Outlier:	14.89	16.58	20.54	7.05	14.21
L Outlier:	9.83	10.59	12.62	5.37	6.53

Table 12.

Rate of Perceived Effort (RPE) Data (cont.)

PCB Sprint Mean:	**13.64**					
PCB Sprint SD:	**3.12**					
CAP Sprint Mean:	**13.59**					
CAP Sprint SD:	**2.93**					
PCB Mean:	**12.41**					
PCB SD:	**3.98**					
CAP Mean:	**12.36**					
CAP SD:	**3.85**					
Total Sprint Mean:	**13.62**					
Total Sprint SD:	**3.02**					
Total Sprint Ave Mean:	**13.62**					
Total Sprint Ave SD:	**1.74**					
Total Mean:	**12.39**					
Total SD:	**3.90**					
Time Point:	**1-Min Pre**	**Sprint 3**	**Sprint 6**	**Sprint 9**	**Sprint 12**	**Sprint 15**
Mean:	**6.24**	**10.05**	**12.26**	**13.92**	**15.34**	**16.50**
SD:	**0.59**	**2.28**	**2.19**	**1.67**	**1.77**	**2.00**

Total average includes measurements at 1-min pre and after every 3rd sprint
Sprint average includes measurements after every 3rd sprint
outliers defined at mean ± 2 SD, H = high limit, L = low limit
red values indicate outlier

Table 13.

Sprint Muscle Soreness Data

PCB Muscle Soreness

	Pre				Mid			
Subject #	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
1	0	0	0	18	0	0	0	0
2	0	0	0	0	0	0	0	0
4	0	0	0	12	0	0	0	0
6	0	0	0	0	25	0	0	0
7	0	0	0	0	18	18	18	20
8	5	0	0	5	20	20	10	25
9	0	0	0	0	30	0	12	0
10	0	0	0	0	0	30	0	0
11	0	0	0	0	50	50	50	50
12	0	0	0	0	0	0	0	0
13	10	0	0	0	20	0	0	0
15	0	0	0	25	45	60	60	35
16	25	25	0	0	48	48	0	0
17	0	0	0	0	10	10	10	10
18	0	0	0	0	0	0	0	0
19	20	40	5	15	40	50	15	20
20	30	20	0	0	55	30	10	10
22	0	20	0	0	20	45	20	45
23	5	0	5	0	22	15	22	12
Mean:	**5.0**	**5.5**	**0.5**	**3.9**	**21.2**	**19.8**	**11.9**	**11.9**
SD:	**9.4**	**11.7**	**1.6**	**7.6**	**19.1**	**21.5**	**17.1**	**16.3**
H Outlier:	23.86	28.83	3.68	19.20	59.33	62.83	46.15	44.63
L Outlier:	-13.86	-17.78	-2.63	-11.31	-16.91	-23.25	-22.26	-20.74

	Post				1-Min Post			
Subject #	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	12
6	45	12	12	12	50	5	5	5
7	50	20	25	60	60	20	35	65
8	45	40	25	48	50	52	55	68
9	72	22	8	2	48	0	0	0
10	30	50	0	0	28	30	0	0
11	65	45	35	35	60	60	30	30
12	0	0	0	0	0	0	0	0
13	10	0	5	0	10	0	0	0
15	85	80	32	60	55	62	45	38
16	55	55	0	45	50	50	0	32
17	32	10	0	0	15	15	0	0
18	0	0	0	0	0	0	0	0
19	30	50	10	45	35	50	15	30
20	65	40	15	15	30	30	10	10
22	75	90	55	78	48	85	50	75
23	30	10	15	25	20	12	30	30
Mean:	**36.3**	**27.6**	**12.5**	**22.4**	**29.4**	**24.8**	**14.5**	**20.8**
SD:	**28.8**	**28.4**	**15.5**	**26.2**	**23.0**	**27.2**	**19.6**	**25.5**
H Outlier:	93.80	84.40	43.57	74.77	75.42	79.14	53.76	71.78
L Outlier:	-21.27	-29.24	-18.62	-30.03	-16.58	-29.56	-24.81	-30.20

Table 13.

Sprint Muscle Soreness Data (cont.)

CAP Muscle Soreness

	Pre				Mid			
Subject #	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	20	0
4	0	0	0	0	10	10	10	10
6	35	8	10	10	40	20	25	25
7	0	0	0	0	0	0	0	0
8	8	10	2	5	15	18	15	15
9	0	0	0	0	15	10	10	10
10	0	25	0	0	18	30	12	12
11	0	0	0	0	35	35	35	35
12	0	0	0	0	0	0	0	0
13	0	0	0	0	15	15	15	15
15	0	0	0	0	52	40	30	50
16	0	0	0	50	0	0	0	70
17	8	20	0	0	85	20	20	20
18	5	5	5	5	15	15	15	15
19	20	20	10	20	25	25	10	25
20	5	5	5	20	10	10	10	12
22	0	0	0	0	28	55	50	55
23	0	0	8	8	0	0	8	8
Mean:	**4.3**	**4.9**	**2.1**	**6.2**	**19.1**	**15.9**	**15.0**	**19.8**
SD:	**9.0**	**8.1**	**3.6**	**12.4**	**21.9**	**15.7**	**13.0**	**19.8**
H Outlier:	22.26	21.11	9.31	31.07	62.95	47.28	41.07	59.41
L Outlier:	-13.73	-11.32	-5.10	-18.65	-24.74	-15.38	-11.07	-19.73

	Post				1-Min Post			
Subject #	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
1	0	0	0	0	0	0	0	0
2	0	40	0	0	0	25	0	0
4	20	10	10	10	0	0	0	0
6	45	35	20	20	50	15	10	10
7	25	0	0	0	0	0	0	0
8	30	25	30	30	30	30	25	25
9	35	30	30	35	50	30	20	40
10	15	25	5	10	8	15	5	5
11	60	60	60	60	52	52	52	52
12	0	0	0	0	0	0	0	0
13	20	20	20	20	15	15	15	15
15	75	75	70	80	80	75	60	75
16	30	0	0	55	30	0	0	75
17	60	5	5	5	45	5	5	5
18	20	20	20	20	20	20	15	15
19	20	20	10	25	25	25	0	25
20	20	8	18	25	18	10	12	22
22	70	75	70	70	75	70	40	50
23	25	18	30	30	22	18	30	30
Mean:	**30.0**	**24.5**	**20.9**	**26.1**	**27.4**	**21.3**	**15.2**	**23.4**
SD:	**22.5**	**23.7**	**23.0**	**24.4**	**25.2**	**22.6**	**18.5**	**24.6**
H Outlier:	75.09	71.92	66.98	74.88	77.80	66.46	52.26	72.61
L Outlier:	-15.09	-22.87	-25.09	-22.78	-23.06	-23.83	-21.84	-25.88

Table 13.

Sprint Muscle Soreness Data (cont.)

	PCB Region Average				CAP Region Average			
Subject #	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
1	0.00	0.00	0.00	4.50	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	16.25	5.00	0.00
4	0.00	0.00	0.00	6.00	7.50	5.00	5.00	5.00
6	30.00	4.25	4.25	4.25	42.50	19.50	16.25	16.25
7	32.00	14.50	19.50	36.25	6.25	0.00	0.00	0.00
8	30.00	28.00	22.50	36.50	20.75	20.75	18.00	18.75
9	37.50	5.50	5.00	0.50	25.00	17.50	15.00	21.25
10	14.50	27.50	0.00	0.00	10.25	23.75	5.50	6.75
11	43.75	38.75	28.75	28.75	36.75	36.75	36.75	36.75
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	12.50	0.00	1.25	0.00	12.50	12.50	12.50	12.50
15	46.25	50.50	34.25	39.50	51.75	47.50	40.00	51.25
16	44.50	44.50	0.00	19.25	15.00	0.00	0.00	62.50
17	14.25	8.75	2.50	2.50	49.50	12.50	7.50	7.50
18	0.00	0.00	0.00	0.00	15.00	15.00	13.75	13.75
19	31.25	47.50	11.25	27.50	22.50	22.50	7.50	23.75
20	45.00	30.00	8.75	8.75	13.25	8.25	11.25	19.75
22	35.75	60.00	31.25	49.50	43.25	50.00	40.00	43.75
23	19.25	9.25	18.00	16.75	11.75	9.00	19.00	19.00
Mean:	**22.97**	**19.42**	**9.86**	**14.76**	**20.18**	**16.67**	**13.32**	**18.87**
SD:	**17.41**	**20.52**	**12.01**	**16.51**	**16.83**	**14.89**	**12.95**	**18.09**
H Outlier:	57.79	60.47	33.87	47.79	53.84	46.45	39.22	55.04
L Outlier:	-11.84	-21.63	-14.16	-18.26	-13.47	-13.10	-12.59	-17.30

PCB Sum Score Muscle Soreness Score	
Subject #	
1	18
2	0
4	24
6	171
7	409
8	468
9	194
10	168
11	560
12	0
13	55
15	682
16	433
17	112
18	0
19	470
20	370
22	706
23	253
Mean:	**268.05**
SD:	**237.95**
H Outlier:	743.95
L Outlier:	-207.84
MS Score Mean:	272.11
MS Score SD:	224.50

CAP Sum Score Muscle Soreness Score	
Subject #	
1	0
2	85
4	90
6	378
7	25
8	313
9	315
10	185
11	588
12	0
13	200
15	762
16	310
17	308
18	230
19	305
20	210
22	708
23	235
Mean:	**276.16**
SD:	**216.68**
H Outlier:	709.52
L Outlier:	-157.21

Table 13.

Sprint Muscle Soreness Data (cont.)

PCB Time Point Average

Subject #	Pre	Mid	Post	1-Min Post
1	4.50	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00
4	3.00	0.00	0.00	3.00
6	0.00	6.25	20.25	16.25
7	0.00	18.50	38.75	45.00
8	2.50	18.75	39.50	56.25
9	0.00	10.50	26.00	12.00
10	0.00	7.50	20.00	14.50
11	0.00	50.00	45.00	45.00
12	0.00	0.00	0.00	0.00
13	2.50	5.00	3.75	2.50
15	6.25	50.00	64.25	50.00
16	12.50	24.00	38.75	33.00
17	0.00	10.00	10.50	7.50
18	0.00	0.00	0.00	0.00
19	20.00	31.25	33.75	32.50
20	12.50	26.25	33.75	20.00
22	5.00	32.50	74.50	64.50
23	2.50	17.75	20.00	23.00
Mean:	**3.75**	**16.22**	**24.67**	**22.37**
SD:	**5.56**	**16.05**	**22.35**	**21.25**
H Outlier:	14.88	48.32	69.37	64.88
L Outlier:	-7.38	-15.87	-20.03	-20.14

CAP Time Point Average

Subject #	Pre	Mid	Post	1-Min Post
1	0	0	0	0
2	0	5	10	6.25
4	0	10	12.5	0
6	15.75	27.5	30	21.25
7	0	0	6.25	0
8	6.25	15.75	28.75	27.5
9	0	11.25	32.5	35
10	6.25	18	13.75	8.25
11	0	35	60	52
12	0	0	0	0
13	0	15	20	15
15	0	43	75	72.5
16	12.5	17.5	21.25	26.25
17	7	36.25	18.75	15
18	5	15	20	17.5
19	17.5	21.25	18.75	18.75
20	8.75	10.5	17.75	15.5
22	0	47	71.25	58.75
23	4	4	25.75	25
Mean:	**4.37**	**17.47**	**25.38**	**21.82**
SD:	**5.74**	**14.36**	**21.42**	**20.51**
H Outlier:	15.84	46.19	68.23	62.83
L Outlier:	-7.11	-11.24	-17.47	-19.20

Table 13.

Sprint Muscle Soreness Data (cont.)

PCB		A	B	C	D
Mean:		23.0	19.4	9.9	14.8
SD:		23.9	24.2	15.9	21.3
CAP		A	B	C	D
Mean:		20.2	16.7	13.3	18.9
SD:		22.6	19.7	17.4	21.9
Total		A	B	C	D
Mean:		21.7	18.2	11.7	16.9
SD:		23.2	22.1	16.7	21.6
Time Point:		Pre			
Region:		A	B	C	D
Mean:		4.6	5.2	1.3	5.1
SD:		9.1	9.9	2.9	10.2
Time Point:		Mid			
Region:		A	B	C	D
Mean:		20.2	17.9	13.5	15.9
SD:		20.3	18.7	15.1	18.3
Time Point:		1-Min Post			
Region:		A	B	C	D
Mean:		28.4	23.1	14.8	22.1
SD:		23.8	24.7	18.8	24.8
Time Point:		Post			
Region:		A	B	C	D
Mean:		33.1	26.1	16.7	24.2
SD:		25.7	25.8	19.8	25.0

	Total Average	
Subject #	PCB	CAP
1	1.13	0.00
2	0.00	5.31
4	1.50	5.63
6	10.69	23.63
7	25.56	1.56
8	29.25	19.56
9	12.13	19.69
10	10.50	11.56
11	35.00	36.75
12	0.00	0.00
13	3.44	12.50
15	42.63	47.63
16	27.06	19.38
17	7.00	19.25
18	0.00	14.38
19	29.38	19.06
20	23.13	13.13
22	44.13	44.25
23	15.81	14.69
Mean:	**16.75**	**17.26**
SD:	**14.87**	**13.54**
H Outlier:	46.50	44.35
L Outlier:	-12.99	-9.83
Total:	17.01	
SD:	14.03	

A = Front Upper Thighs (qudriceps)
B = Back Upper Thighs (hamstrings)
C = Front Lower Legs (shins)
D = Back Lower Legs (calves)

outliers defined at mean ± 2 SD, H = high limit, L = low limit, red values indicate outlier
Red values indicate outlier

Table 14.

Post-Test Muscle Soreness

PCB Posttest Muscle Soreness

	Day 1				Day 2			
Subject #	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
1	0	0	0	20	0	0	0	0
2	50	65	15	15	30	30	0	0
4	12	10	0	15	18	12	0	20
6	20	15	2	2	12	2	2	2
7	25	65	0	0	18	35	0	0
8	38	22	5	12	42	32	10	18
9	35	25	5	15	15	5	0	2
10	0	35	18	12	0	0	20	30
11	95	95	95	95	95	95	95	95
12	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0
15	70	75	45	55	70	70	55	35
16	50	50	0	30	50	50	0	0
17	78	78	55	25	100	100	80	0
18	30	35	0	0	20	10	0	0
19	0	0	0	0	0	0	0	0
20	32	25	2	2	15	2	2	2
22	45	70	18	48	10	75	15	25
23	8	10	8	8	8	18	10	12
Mean:	**32.67**	**37.50**	**14.89**	**18.56**	**27.94**	**29.78**	**16.06**	**13.39**
SD:	**28.54**	**30.92**	**25.04**	**24.40**	**31.25**	**33.89**	**28.76**	**23.09**
H Outlier:	89.75	99.35	64.98	67.36	90.45	97.57	73.57	59.57
L Outlier:	-24.42	-24.35	-35.20	-30.25	-34.56	-38.01	-41.46	-32.79

	Day 3				Average			
Subject #	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
1	0	0	0	0	0.00	0.00	0.00	6.67
2	0	5	0	0	26.67	33.33	5.00	5.00
4	12	12	0	15	14.00	11.33	0.00	16.67
6	0	0	0	0	10.67	5.67	1.33	1.33
7	15	25	0	0	19.33	41.67	0.00	0.00
8	38	15	10	10	39.33	23.00	8.33	13.33
9	0	0	0	0	16.67	10.00	1.67	5.67
10	0	0	8	2	0.00	11.67	15.33	14.67
11	80	80	80	80	90.00	90.00	90.00	90.00
12	0	0	0	0	0.00	0.00	0.00	0.00
13	0	0	0	0	0.00	0.00	0.00	0.00
15	60	60	40	25	66.67	68.33	46.67	38.33
16	20	20	0	0	40.00	40.00	0.00	10.00
17	50	50	35	0	76.00	76.00	56.67	8.33
18	0	0	0	0	16.67	15.00	0.00	0.00
19	0	0	0	0	0.00	0.00	0.00	0.00
20	15	2	2	2	20.67	9.67	2.00	2.00
22	5	15	5	0	20.00	53.33	12.67	24.33
23	0	0	0	0	5.33	9.33	6.00	6.67
Mean:	**16.39**	**15.78**	**10.00**	**7.44**	**25.67**	**27.69**	**13.65**	**13.13**
SD:	**24.02**	**23.46**	**20.70**	**18.88**	**26.91**	**27.99**	**24.55**	**21.14**
H Outlier:	64.42	62.69	51.40	45.19	79.50	83.66	62.74	55.41
L Outlier:	-31.65	-31.13	-31.40	-30.31	-28.16	-28.29	-35.45	-29.15

Table 14.

Post-Test Muscle Soreness (cont.)

CAP Posttest Muscle Soreness

	Day 1				Day 2			
Subject #	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
1	0	0	0	0	0	0	0	0
2	65	50	30	8	30	12	0	0
4	0	30	0	0	0	25	0	0
6	35	5	5	5	15	10	10	10
7	15	0	35	0	10	0	25	0
8	15	20	2	8	20	15	2	5
9	70	65	5	25	55	45	0	10
10	78	80	40	40	50	72	30	30
11	0	52	0	0	0	50	0	0
12	8	8	0	0	5	5	0	0
13	0	0	0	0	0	0	0	0
15	80	85	60	65	80	85	65	35
16	*****	*****	*****	*****	*****	*****	*****	*****
17	25	10	0	0	25	0	0	0
18	0	0	0	0	0	0	0	0
19	15	15	15	25	8	8	8	8
20	25	15	0	8	15	8	0	0
22	20	25	20	18	5	2	5	5
23	12	12	22	25	30	30	25	32
Mean:	**25.72**	**26.22**	**13.00**	**12.61**	**19.33**	**20.39**	**9.44**	**7.50**
SD:	**28.14**	**28.06**	**17.90**	**17.79**	**22.60**	**26.12**	**17.09**	**12.01**
H Outlier:	81.99	82.34	48.80	48.18	64.54	72.64	43.62	31.51
L Outlier:	-30.55	-29.90	-22.80	-22.96	-25.87	-31.86	-24.73	-16.51

	Day 3				Average			
Subject #	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
1	0	0	0	0	0.00	0.00	0.00	0.00
2	10	10	0	0	35.00	24.00	10.00	2.67
4	0	0	0	0	0.00	18.33	0.00	0.00
6	30	18	8	15	26.67	11.00	7.67	10.00
7	0	0	12	0	8.33	0.00	24.00	0.00
8	20	10	2	5	18.33	15.00	2.00	6.00
9	20	12	0	0	48.33	40.67	1.67	11.67
10	10	45	10	15	46.00	65.67	26.67	28.33
11	0	28	0	0	0.00	43.33	0.00	0.00
12	0	0	0	0	4.33	4.33	0.00	0.00
13	0	0	0	0	0.00	0.00	0.00	0.00
15	75	78	65	30	78.33	82.67	63.33	43.33
16	*****	*****	*****	*****	*****	*****	*****	*****
17	0	0	0	0	16.67	3.33	0.00	0.00
18	0	0	0	0	0.00	0.00	0.00	0.00
19	0	0	0	0	7.67	7.67	7.67	11.00
20	2	2	0	0	14.00	8.33	0.00	2.67
22	0	0	0	0	8.33	9.00	8.33	7.67
23	18	18	18	18	20.00	20.00	21.67	25.00
Mean:	**10.28**	**12.28**	**6.39**	**4.61**	**18.44**	**19.63**	**9.61**	**8.24**
SD:	**18.71**	**20.54**	**15.58**	**8.80**	**21.46**	**23.81**	**16.09**	**12.25**
H Outlier:	47.70	53.37	37.55	22.21	61.36	67.25	41.78	32.73
L Outlier:	-27.14	-28.81	-24.77	-12.99	-24.47	-27.99	-22.56	-16.25

Table 14.

Post-Test Muscle Soreness (cont.)

PCB Region Average

Subject #	A	B	C	D	Average
1	0	0	0	18	4.5
2	0	0	0	0	0
4	0	0	0	12	3
6	0	0	0	0	0
7	0	0	0	0	0
8	5	0	0	5	2.5
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	0	0	0	0	0
13	10	0	0	0	2.5
15	0	0	0	25	6.25
16	25	25	0	0	12.5
17	0	0	0	0	0
18	0	0	0	0	0
19	20	40	5	15	20
20	30	20	0	0	12.5
22	0	20	0	0	5
23	5	0	5	0	2.5
Mean:	**5.00**	**5.53**	**0.53**	**3.95**	**3.75**
SD:	**9.43**	**11.65**	**1.58**	**7.63**	**5.56**
H Outlier:	23.86	28.83	3.68	19.20	
L Outlier:	-13.86	-17.78	-2.63	-11.31	

CAP Region Average

Subject #	A	B	C	D	Average
1	0	0	0	0	0
2	0	0	0	0	0
4	0	0	0	0	0
6	35	8	10	10	15.75
7	0	0	0	0	0
8	8	10	2	5	6.25
9	0	0	0	0	0
10	0	25	0	0	6.25
11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
15	0	0	0	0	0
16	*****	*****	*****	*****	*****
17	8	20	0	0	7
18	5	5	5	5	5
19	20	20	10	20	17.5
20	5	5	5	20	8.75
22	0	0	0	0	0
23	0	0	8	8	4
Mean:	**4.26**	**4.89**	**2.11**	**6.21**	**4.37**
SD:	**9.00**	**8.11**	**3.60**	**12.43**	**5.74**
H Outlier:	22.26	21.11	9.31	31.07	
L Outlier:	-13.73	-11.32	-5.10	-18.65	

Table 14.

Post-Test Muscle Soreness (cont.)

PCB Day Average

Subject #	Day 1	Day 2	Day 3
1	5	0	0
2	36.25	15	1.25
4	9.25	12.5	9.75
6	9.75	4.5	0
7	22.5	13.25	10
8	19.25	25.5	18.25
9	20	5.5	0
10	16.25	12.5	2.5
11	95	95	80
12	0	0	0
13	0	0	0
15	61.25	57.5	46.25
16	32.5	25	10
17	59	70	33.75
18	16.25	7.5	0
19	0	0	0
20	15.25	5.25	5.25
22	45.25	31.25	6.25
23	8.5	12	0
Mean:	**24.80**	**20.64**	**11.75**
SD:	**25.06**	**26.22**	**20.76**
H Outlier:	74.92	73.09	53.27
L Outlier:	-25.31	-31.80	-29.77

CAP Day Average

Subject #	Day 1	Day 2	Day 3
1	0	0	0
2	38.25	10.5	5
4	7.5	6.25	0
6	12.5	11.25	17.75
7	12.5	8.75	3
8	11.25	10.5	9.25
9	41.25	27.5	8
10	59.5	45.5	20
11	13	12.5	7
12	4	2.5	0
13	0	0	0
15	72.5	66.25	62
16	*****	*****	*****
17	8.75	6.25	0
18	0	0	0
19	17.5	8	0
20	12	5.75	1
22	20.75	4.25	0
23	17.75	29.25	18
Mean:	**19.39**	**14.17**	**8.39**
SD:	**20.50**	**17.55**	**15.05**
H Outlier:	60.40	49.27	38.49
L Outlier:	-21.62	-20.94	-21.71

Table 14.

Post-Test Muscle Soreness (cont.)

	Sum Score			Total Average		
Subject #	**PCB**	**CAP**	**Subject #**	**PCB**	**CAP**
1	20	0	**1**	1.67	0.00
2	210	215	**2**	17.50	17.92
4	126	55	**4**	10.50	4.58
6	57	166	**6**	4.75	13.83
7	183	97	**7**	15.25	8.08
8	252	124	**8**	21.00	10.33
9	102	307	**9**	8.50	25.58
10	125	500	**10**	10.42	41.67
11	1080	130	**11**	90.00	10.83
12	0	26	**12**	0.00	2.17
13	0	0	**13**	0.00	0.00
15	660	803	**15**	55.00	66.92
16	270	*****	**16**	22.50	*****
17	651	60	**17**	54.25	5.00
18	95	0	**18**	7.92	0.00
19	0	102	**19**	0.00	8.50
20	103	75	**20**	8.58	6.25
22	331	100	**22**	27.58	8.33
23	82	260	**23**	6.83	21.67
Mean:	**228.79**	**167.78**	**Mean:**	**19.07**	**13.98**
SD:	**281.54**	**202.74**	**SD:**	**23.46**	**16.90**
H Outlier:	791.86	573.26	**H Outlier:**	65.99	47.77
L Outlier:	-334.28	-237.70	**L Outlier:**	-27.86	-19.81

PCB	**A**	**B**	**C**	**D**
Ave:	**24.32**	**26.23**	**12.93**	**12.79**
SD:	**28.35**	**30.45**	**24.73**	**22.36**
CAP	**A**	**B**	**C**	**D**
Ave:	**18.44**	**19.63**	**9.61**	**8.24**
SD:	**23.90**	**25.30**	**16.78**	**13.55**
Day 1	**A**	**B**	**C**	**D**
Mean:	**28.41**	**31.00**	**13.57**	**15.70**
SD:	**28.07**	**29.53**	**21.57**	**21.36**
Day 2	**A**	**B**	**C**	**D**
Mean:	**23.00**	**24.41**	**12.41**	**10.16**
SD:	**27.25**	**30.21**	**23.66**	**18.48**
Day 3	**A**	**B**	**C**	**D**
Mean:	**12.97**	**13.65**	**7.97**	**5.86**
SD:	**21.47**	**21.82**	**18.20**	**14.70**
Total	**A**	**B**	**C**	**D**
Mean:	**21.46**	**23.02**	**11.32**	**10.58**
SD:	**26.33**	**28.13**	**21.21**	**18.66**

MS Score Mean:	199.11
MS Score SD:	244.94

A = Front Upper Thighs (qudriceps)
B = Back Upper Thighs (hamstrings)
C = Front Lower Legs (shins)
D = Back Lower Legs (calves)

outliers defined at mean ± 2 SD, H = high limit, L = low limit, red values indicate outlier
missing data (*****) due to subject failure to return survey

Table 15.

Gastric Distress Data

Subject #	Intestinal Cramps	Diarrhea	PCB Pre Nausea	Flatulence	Stomach Discomfort
1	0	0	0	0	0
2	0	0	0	0	0
4	0	0	0	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	0	0	0	0	0
9	0	0	0	0	0
10	0	0	0	2	0
11	0	0	0	1	0
12	0	0	0	0	0
13	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	0	0	0
19	1	0	0	0	2
20	0	0	0	0	0
22	0	0	0	0	0
23	0	0	0	0	0
Mean:	**0.05**	**0.00**	**0.00**	**0.16**	**0.11**
SD:	**0.23**	**0.00**	**0.00**	**0.50**	**0.46**
H Outlier:	0.51	0.00	0.00	1.16	1.02
L Outlier:	-0.41	0.00	0.00	-0.85	-0.81

Subject #	Intestinal Cramps	Diarrhea	Mid Nausea	Flatulence	Stomach Discomfort
1	0	0	0	0	0
2	0	0	0	0	0
4	0	0	0	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	1	0	0	0	0
9	0	0	0	0	0
10	0	0	0	2	0
11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	1	0	0
19	1	0	1	0	3
20	0	0	0	0	0
22	0	0	0	0	0
23	0	0	0	0	0
Mean:	**0.11**	**0.00**	**0.11**	**0.11**	**0.16**
SD:	**0.32**	**0.00**	**0.32**	**0.46**	**0.69**
H Outlier:	0.74	0.00	0.74	1.02	1.53
L Outlier:	-0.53	0.00	-0.53	-0.81	-1.22

Table 15.

Gastric Distress Data (cont.)

			PCB Post		
Subject #	Intestinal Cramps	Diarrhea	Nausea	Flatulence	Stomach Discomfort
1	0	0	0	0	0
2	2	0	0	0	2
4	0	0	0	0	0
6	0	0	0	0	0
7	1	0	0	0	1
8	1	0	1	0	0
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	0	0	0	0	1
13	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	2	0	0
19	1	0	0	0	2
20	0	0	0	0	2
22	1	0	2	0	3
23	0	0	0	0	0
Mean:	**0.32**	**0.00**	**0.26**	**0.00**	**0.58**
SD:	**0.58**	**0.00**	**0.65**	**0.00**	**0.96**
H Outlier:	1.48	0.00	1.57	0.00	2.50
L Outlier:	-0.85	0.00	-1.04	0.00	-1.34

			1-Min Post		
Subject #	Intestinal Cramps	Diarrhea	Nausea	Flatulence	Stomach Discomfort
1	0	0	0	0	0
2	0	0	0	0	0
4	0	0	0	0	0
6	0	0	0	0	0
7	1	0	0	0	1
8	0	0	0	0	0
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	2	1	0
19	0	0	0	0	2
20	0	0	0	0	1
22	1	0	2	0	2
23	0	0	0	0	0
Mean:	**0.11**	**0.00**	**0.21**	**0.05**	**0.32**
SD:	**0.32**	**0.00**	**0.63**	**0.23**	**0.67**
H Outlier:	0.74	0.00	1.47	0.51	1.66
L Outlier:	-0.53	0.00	-1.05	-0.41	-1.03

Table 15.

Gastric Distress Data (cont.)

Subject #	Intestinal Cramps	Diarrhea	CAP Pre Nausea	Flatulence	Stomach Discomfort
1	2	0	0	0	3
2	1	0	0	1	0
4	0	0	0	1	0
6	0	0	0	0	1
7	0	0	0	0	1
8	1	0	0	0	2
9	2	0	1	0	2
10	3	1	0	1	3
11	0	0	0	2	0
12	0	0	0	0	0
13	0	0	0	0	0
15	0	0	0	1	2
16	0	3	0	0	2
17	0	0	0	0	0
18	0	0	0	0	0
19	1	1	0	0	3
20	0	0	1	0	1
22	2	0	2	1	3
23	2	2	2	1	1
Mean:	**0.74**	**0.37**	**0.32**	**0.42**	**1.26**
SD:	**0.99**	**0.83**	**0.67**	**0.61**	**1.19**
H Outlier:	2.72	2.03	1.66	1.64	3.65
L Outlier:	-1.25	-1.29	-1.03	-0.79	-1.13

Subject #	Intestinal Cramps	Diarrhea	Mid Nausea	Flatulence	Stomach Discomfort
1	4	0	0	0	4
2	1	0	0	0	0
4	0	0	2	0	1
6	1	0	3	0	4
7	1	0	0	0	1
8	2	1	0	1	2
9	3	0	0	0	3
10	3	0	0	0	3
11	0	0	0	3	0
12	0	0	0	0	0
13	0	0	0	0	1
15	0	0	0	0	0
16	0	3	0	0	1
17	0	0	0	0	0
18	0	0	0	0	0
19	1	1	0	1	3
20	0	0	0	0	0
22	3	2	3	2	4
23	0	0	0	0	0
Mean:	**1.00**	**0.37**	**0.42**	**0.37**	**1.42**
SD:	**1.33**	**0.83**	**1.02**	**0.83**	**1.57**
H Outlier:	3.67	2.03	2.46	2.03	4.57
L Outlier:	-1.67	-1.29	-1.61	-1.29	-1.73

Table 15.

Gastric Distress Data (cont.)

Subject #	Intestinal Cramps	Diarrhea	CAP Post Nausea	Flatulence	Stomach Discomfort
1	4	0	0	0	5
2	2	0	0	0	0
4	0	0	3	0	2
6	1	0	2	0	5
7	0	0	0	0	4
8	3	2	0	1	2
9	4	0	4	0	4
10	3	0	1	0	4
11	2	0	1	3	3
12	0	0	0	0	2
13	1	0	2	0	2
15	0	0	0	1	2
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	1	0	0
19	1	1	0	1	4
20	0	0	1	0	1
22	4	2	4	3	4
23	2	2	2	1	2
Mean:	**1.42**	**0.37**	**1.11**	**0.53**	**2.42**
SD:	**1.54**	**0.76**	**1.37**	**0.96**	**1.71**
H Outlier:	4.50	1.89	3.85	2.45	5.84
L Outlier:	-1.66	-1.15	-1.63	-1.40	-1.00

Subject #	Intestinal Cramps	Diarrhea	1-Min Post Nausea	Flatulence	Stomach Discomfort
1	4	0	0	0	4
2	0	0	0	0	0
4	0	0	3	0	2
6	1	0	1	0	4
7	0	0	0	0	3
8	2	2	1	2	3
9	3	0	3	0	3
10	1	0	0	0	2
11	1	0	1	1	1
12	0	0	0	0	0
13	0	0	2	0	0
15	0	0	0	1	2
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	1	0	0
19	1	1	0	1	4
20	0	0	0	0	1
22	3	1	3	2	3
23	1	1	1	0	2
Mean:	**0.89**	**0.26**	**0.84**	**0.37**	**1.79**
SD:	**1.24**	**0.56**	**1.12**	**0.68**	**1.51**
H Outlier:	3.38	1.39	3.08	1.74	4.81
L Outlier:	-1.59	-0.86	-1.40	-1.00	-1.23

Table 15.

Gastric Distress Data (cont.)

Subject #	Pre	Mid	Post	1-Min Post	PCB Average	Subject #	PCB Sum Score
1	0	0	0	0	0	1	0
2	0	0	0.8	0	0.2	2	4
4	0	0	0	0	0	4	0
6	0	0	0	0	0	6	0
7	0	0	0.4	0.4	0.2	7	4
8	0	0.2	0.4	0	0.15	8	3
9	0	0	0	0	0	9	0
10	0.4	0.4	0	0	0.2	10	4
11	0.2	0	0	0	0.05	11	1
12	0	0	0.2	0	0.05	12	1
13	0	0	0	0	0	13	0
15	0	0	0	0	0	15	0
16	0	0	0	0	0	16	0
17	0	0	0	0	0	17	0
18	0	0.2	0.4	0.6	0.3	18	6
19	0.6	1	0.6	0.4	0.65	19	13
20	0	0	0.4	0.2	0.15	20	3
22	0	0	1.2	1	0.55	22	11
23	0	0	0	0	0	23	0
Mean:	**0.06**	**0.09**	**0.23**	**0.14**	**0.13**	**Mean:**	**2.63**
SD:	**0.16**	**0.24**	**0.34**	**0.28**	**0.19**	**SD:**	**3.82**
H Outlier:	0.39	0.58	0.91	0.69	0.51	**H Outlier:**	10.27
L Outlier:	-0.26	-0.39	-0.45	-0.41	-0.25	**L Outlier:**	-5.00

Subject #	Pre	Mid	Post	1-Min Post	CAP Average	Subject #	CAP Sum Score
1	1	1.6	1.8	1.6	1.5	1	30
2	0.4	0.2	0.4	0	0.25	2	5
4	0.2	0.6	1	1	0.7	4	14
6	0.2	1.6	1.6	1.2	1.15	6	23
7	0.2	0.4	0.8	0.6	0.5	7	10
8	0.6	1.2	1.6	2	1.35	8	27
9	1	1.2	2.4	1.8	1.6	9	32
10	1.6	1.2	1.6	0.6	1.25	10	25
11	0.4	0.6	1.8	0.8	0.9	11	18
12	0	0	0.4	0	0.1	12	2
13	0	0.2	1	0.4	0.4	13	8
15	0.6	0	0.6	0.6	0.45	15	9
16	1	0.8	0	0	0.45	16	9
17	0	0	0	0	0	17	0
18	0	0	0.2	0.2	0.1	18	2
19	1	1.2	1.4	1.4	1.25	19	25
20	0.4	0	0.4	0.2	0.25	20	5
22	1.6	2.8	3.4	2.4	2.55	22	51
23	1.6	0	1.8	1	1.1	23	22
Mean:	**0.62**	**0.72**	**1.17**	**0.83**	**0.83**	**Mean:**	**16.68**
SD:	**0.56**	**0.77**	**0.89**	**0.74**	**0.66**	**SD:**	**13.19**
H Outlier:	1.74	2.25	2.95	2.31	2.15	**H Outlier:**	43.07
L Outlier:	-0.50	-0.82	-0.61	-0.64	-0.48	**L Outlier:**	-9.70

Table 15.

Gastric Distress Data (cont.)

PCB Average

Subject #	Intestinal Cramps	Diarrhea	Nausea	Flatulence	Stomach Discomfort
1	0	0	0	0	0
2	0.5	0	0	0	0.5
4	0	0	0	0	0
6	0	0	0	0	0
7	0.5	0	0	0	0.5
8	0.5	0	0.25	0	0
9	0	0	0	0	0
10	0	0	0	1	0
11	0	0	0	0.25	0
12	0	0	0	0	0.25
13	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	1.25	0.25	0
19	0.75	0	0.25	0	2.25
20	0	0	0	0	0.75
22	0.5	0	1	0	1.25
23	0	0	0	0	0
Mean:	**0.14**	**0.00**	**0.14**	**0.08**	**0.29**
SD:	**0.25**	**0.00**	**0.36**	**0.24**	**0.58**
H Outlier:	0.65	0.00	0.86	0.55	1.46
L Outlier:	-0.36	0.00	-0.57	-0.39	-0.88

CAP Average

Subject #	Intestinal Cramps	Diarrhea	Nausea	Flatulence	Stomach Discomfort
1	3.5	0	0	0	4
2	1	0	0	0.25	0
4	0	0	2	0.25	1.25
6	0.75	0	1.5	0	3.5
7	0.25	0	0	0	2.25
8	2	1.25	0.25	1	2.25
9	3	0	2	0	3
10	2.5	0.25	0.25	0.25	3
11	0.75	0	0.5	2.25	1
12	0	0	0	0	0.5
13	0.25	0	1	0	0.75
15	0	0	0	0.75	1.5
16	0	1.5	0	0	0.75
17	0	0	0	0	0
18	0	0	0.5	0	0
19	1	1	0	0.75	3.5
20	0	0	0.5	0	0.75
22	3	1.25	3	2	3.5
23	1.25	1.25	1.25	0.5	1.25
Mean:	**1.01**	**0.34**	**0.67**	**0.42**	**1.72**
SD:	**1.20**	**0.57**	**0.89**	**0.68**	**1.35**
H Outlier:	3.41	1.48	2.44	1.78	4.42
L Outlier:	-1.38	-0.79	-1.10	-0.93	-0.97

Table 15.

Gastric Distress Data (cont.)

	Intestinal Cramps	Diarrhea	Nausea	Flatulence	Stomach Discomfort
Pre Mean:	0.39	0.18	0.16	0.29	0.68
Pre SD:	0.79	0.61	0.49	0.57	1.07
Mid Mean:	0.55	0.18	0.26	0.24	0.79
Mid SD:	1.06	0.61	0.76	0.68	1.36
Post Mean:	0.87	0.18	0.68	0.26	1.50
Post SD:	1.28	0.56	1.14	0.72	1.66
1-Min Post Mean:	0.50	0.13	0.53	0.21	1.05
1-Min Post SD:	0.98	0.41	0.95	0.53	1.37
PCB Mean:	0.14	0.00	0.14	0.08	0.29
PCB SD:	0.39	0.00	0.48	0.36	0.73
CAP Mean:	1.01	0.34	0.67	0.42	1.72
CAP SD:	1.29	0.74	1.10	0.77	1.55
Total Mean:	0.58	0.17	0.41	0.25	1.01
SD:	1.05	0.55	0.89	0.62	1.40
GD Score Mean:	9.66				
GD Score SD:	11.94				

outliers defined at mean ± 2 SD, H = high limit, L = low limit
red values indicate outlier

Appendix C: Statistics Summary

Table 16. RMANOVA for Sprint Time

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0012291	0.0356	1	29	0.8516

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	806.04572	23375.326	1	29	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0012291	0.0356	1	29	0.8516

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	1.0226511	1.1687	14	16	0.3789

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	2.1018881	2.4022	14	16	0.0477*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	1.0226511	1.1687	14	16	0.3789

Table 17. RMANOVA for IL-6

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0031644	0.0918	1	29	0.7641

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	6.2651995	181.6908	1	29	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0031644	0.0918	1	29	0.7641

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0082068	0.1149	2	28	0.8919

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.9327262	13.0582	2	28	<.0001*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0082068	0.1149	2	28	0.8919

Table 18. RMANOVA for Total Heart Rate

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1494736	2.9895	1	20	0.0992

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	181.98587	3639.7175	1	20	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1494736	2.9895	1	20	0.0992

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.2342203	0.5856	6	15	0.7368

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	94.189178	235.4729	6	15	<.0001*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.2342203	0.5856	6	15	0.7368

Table 19. RMANOVA for Sprint Heart Rate

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1189883	2.6177	1	22	0.1199

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	222.91628	4904.1581	1	22	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1189883	2.6177	1	22	0.1199

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0793548	0.3769	4	19	0.8222

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.3359907	1.5960	4	19	0.2163

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0793548	0.3769	4	19	0.8222

Table 20. RMANOVA for Blood Pressure

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1189883	2.6177	1	22	0.1199

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	222.91628	4904.1581	1	22	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1189883	2.6177	1	22	0.1199

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0793548	0.3769	4	19	0.8222

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.3359907	1.5960	4	19	0.2163

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0793548	0.3769	4	19	0.8222

Table 21. RMANOVA for Rate of Perceived Exertion

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0002346	0.0084	1	36	0.9273

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	62.807998	2261.0879	1	36	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0002346	0.0084	1	36	0.9273

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0185836	0.1533	4	33	0.9602

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	7.73869	63.8442	4	33	<.0001*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0185836	0.1533	4	33	0.9602

Table 22. RMANOVA for Average Total Sprint Muscle Soreness

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0003348	0.0121	1	36	0.9132

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	1.509215	54.3317	1	36	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0003348	0.0121	1	36	0.9132

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0123632	0.1401	3	34	0.9353

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	1.0077855	11.4216	3	34	<.0001*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0123632	0.1401	3	34	0.9353

Table 23. RMANOVA for Region A Sprint Muscle Soreness

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0070051	0.2522	1	36	0.6186

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	1.6768341	60.3660	1	36	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0070051	0.2522	1	36	0.6186

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0420271	0.4763	3	34	0.7009

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	1.2756795	14.4577	3	34	<.0001*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0420271	0.4763	3	34	0.7009

Table 24. RMANOVA for Region B Sprint Muscle Soreness

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0062083	0.2235	1	36	0.6392

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	1.0693858	38.4979	1	36	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0062083	0.2235	1	36	0.6392

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.015722	0.1782	3	34	0.9104

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.7555671	8.5631	3	34	0.0002*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.015722	0.1782	3	34	0.9104

Table 25. RMANOVA for Region C Sprint Muscle Soreness

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0202585	0.7293	1	36	0.3988

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.9082711	32.6978	1	36	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0202585	0.7293	1	36	0.3988

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.2337125	2.6487	3	34	0.0645

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.7494043	8.4932	3	34	0.0002*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.2337125	2.6487	3	34	0.0645

Table 26. RMANOVA for Region D Sprint Muscle Soreness

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.014832	0.5340	1	36	0.4697

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.9954314	35.8355	1	36	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.014832	0.5340	1	36	0.4697

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0724774	0.8214	3	34	0.4911

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.6155379	6.9761	3	34	0.0009*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0724774	0.8214	3	34	0.4911

Table 27. RMANOVA for Average Total Posttest Muscle Soreness

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0160664	0.5623	1	35	0.4583

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.8053178	28.1861	1	35	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0160664	0.5623	1	35	0.4583

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0423469	0.4658	3	33	0.7081

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	1.0739867	11.8139	3	33	<.0001*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0423469	0.4658	3	33	0.7081

Table 28. RMANOVA for Region A Posttest Muscle Soreness

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0161587	0.5656	1	35	0.4571

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.9348718	32.7205	1	35	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0161587	0.5656	1	35	0.4571

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0169552	0.1865	3	33	0.9048

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.821109	9.0322	3	33	0.0002*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0169552	0.1865	3	33	0.9048

Table 29. RMANOVA for Region B Posttest Muscle Soreness

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0173701	0.6080	1	35	0.4408

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.9399839	32.8994	1	35	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0173701	0.6080	1	35	0.4408

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0418717	0.4606	3	33	0.7117

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	1.0560565	11.6166	3	33	<.0001*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0418717	0.4606	3	33	0.7117

Table 30. RMANOVA for Region C Posttest Muscle Soreness

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0046181	0.1616	1	35	0.6901

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.3351739	11.7311	1	35	0.0016*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0046181	0.1616	1	35	0.6901

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0930342	1.0234	3	33	0.3948

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.4483874	4.9323	3	33	0.0061*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0930342	1.0234	3	33	0.3948

Table 31. RMANOVA for Region D Posttest Muscle Soreness

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0176908	0.6192	1	35	0.4366

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.4647893	16.2676	1	35	0.0003*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0176908	0.6192	1	35	0.4366

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0395653	0.4352	3	33	0.7292

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.6472893	7.1202	3	33	0.0008*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0395653	0.4352	3	33	0.7292

Table 32. RMANOVA for Total Gastrointestinal Distress

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.5526622	19.8958	1	36	<.0001*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	1.0441655	37.5900	1	36	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.5526622	19.8958	1	36	<.0001*

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1982889	2.2473	3	34	0.1006

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.6683017	7.5741	3	34	0.0005*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1982889	2.2473	3	34	0.1006

Table 33. RMANOVA for Gastrointestinal Distress – Intestinal Cramps

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.2656098	9.5620	1	36	0.0038*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.4721951	16.9990	1	36	0.0002*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.2656098	9.5620	1	36	0.0038*

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1141908	1.2942	3	34	0.2922

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.5444337	6.1702	3	34	0.0018*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1141908	1.2942	3	34	0.2922

Table 34. RMANOVA for Gastrointestinal Distress – Diarrhea

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1924829	6.9294	1	36	0.0124*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1924829	6.9294	1	36	0.0124*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1924829	6.9294	1	36	0.0124*

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0709258	0.8038	3	34	0.5005

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0709258	0.8038	3	34	0.5005

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0709258	0.8038	3	34	0.5005

Table 35. RMANOVA for Gastrointestinal Distress - Nausea

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1602564	5.7692	1	36	0.0216*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.385016	13.8606	1	36	0.0007*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1602564	5.7692	1	36	0.0216*

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1284568	1.4558	3	34	0.2439

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.406806	4.6105	3	34	0.0082*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1284568	1.4558	3	34	0.2439

Table 36. RMANOVA for Gastrointestinal Distress - Flatulence

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1200284	4.3210	1	36	0.0448*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.256392	9.2301	1	36	0.0044*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1200284	4.3210	1	36	0.0448*

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1347059	1.5267	3	34	0.2253

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.0288727	0.3272	3	34	0.8057

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1347059	1.5267	3	34	0.2253

Table 37. RMANOVA for Gastrointestinal Distress – Stomach Discomfort

All Between

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.5024104	18.0868	1	36	0.0001*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.9898934	35.6362	1	36	<.0001*

Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.5024104	18.0868	1	36	0.0001*

All Within Interactions

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1017513	1.1532	3	34	0.3418

Time

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.5991936	6.7909	3	34	0.0010*

Time*Treatment

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1017513	1.1532	3	34	0.3418

Table 38. Average Sprint Time

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	0.0381515	0.038151	0.8417	0.3652
Error	35	1.5864406	0.045327		
C. Total	36	1.6245921			

Table 39. Maximum Sprint Time

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	0.0019646	0.001965	0.0420	0.8388
Error	35	1.6380046	0.046800		
C. Total	36	1.6399692			

Table 40. Fatigue

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	12.11545	12.1154	1.4055	0.2448
Error	31	267.21394	8.6198		
C. Total	32	279.32939			

Table 41. IL-6 Difference

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	0.2148885	0.214888	1.3141	0.2602
Error	32	5.2328222	0.163526		
C. Total	33	5.4477107			

Table 42. IL-6 Percent Change

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	10782.17	10782.2	1.7789	0.1917
Error	32	193952.31	6061.0		
C. Total	33	204734.48			

Table 43. Maximum Heart Rate

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	2331.8432	2331.84	10.7367	0.0026*
Error	31	6732.7022	217.18		
C. Total	32	9064.5455			

Table 44. Sprint Muscle Soreness Sum

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	624.1	624.1	0.0121	0.9132
Error	36	1864259.5	51785.0		
C. Total	37	1864883.6			

Table 45. Sprint Muscle Soreness Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	2.4354	2.435	0.0120	0.9132
Error	36	7282.9433	202.304		
C. Total	37	7285.3786			

Table 46. Sprint Muscle Soreness Region A Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	73.921	73.921	0.2522	0.6186
Error	36	10552.467	293.124		
C. Total	37	10626.388			

Table 47. Sprint Muscle Soreness Region B Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	71.844	71.844	0.2235	0.6392
Error	36	11572.138	321.448		
C. Total	37	11643.982			

Table 48. Sprint Muscle Soreness Region C Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	113.7648	113.765	0.7293	0.3988
Error	36	5615.6447	155.990		
C. Total	37	5729.4095			

Table 49. Sprint Muscle Soreness Region D Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	160.105	160.105	0.5340	0.4697
Error	36	10794.605	299.850		
C. Total	37	10954.711			

Table 50. Posttest Muscle Soreness Sum

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	34407.3	34407.3	0.5666	0.4567
Error	35	2125484.3	60728.1		
C. Total	36	2159891.6			

Table 51. Posttest Muscle Soreness Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	86.0256	86.026	0.5113	0.4795
Error	34	5720.9737	168.264		
C. Total	35	5806.9993			

Table 52. Posttest Muscle Soreness Region A Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	310.817	310.817	0.8441	0.3647
Error	34	12520.173	368.240		

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
C. Total	35	12830.990			

Table 53. Posttest Muscle Soreness Region B Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	431.254	431.254	0.9618	0.3336
Error	34	15244.237	448.360		
C. Total	35	15675.492			

Table 54. Posttest Muscle Soreness Region C Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	37.5973	37.597	0.2156	0.6453
Error	34	5927.9933	174.353		
C. Total	35	5965.5906			

Table 55. Posttest Muscle Soreness Region D Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	44.8007	44.8007	0.5077	0.4810
Error	34	3000.5010	88.2500		
C. Total	35	3045.3017			

Table 56. Gastrointestinal Distress Sum

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	1876.0263	1876.03	19.8958	<.0001*
Error	36	3394.5263	94.29		
C. Total	37	5270.5526			

Table 57. Gastrointestinal Distress Total Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	4.690066	4.69007	19.8958	<.0001*
Error	36	8.486316	0.23573		
C. Total	37	13.176382			

Table 58. Gastrointestinal Distress Pretest Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	2.9568421	2.95684	17.2921	0.0002*
Error	36	6.1557895	0.17099		
C. Total	37	9.1126316			

Table 59. Gastrointestinal Distress Mid Test Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	3.664211	3.66421	11.3183	0.0018*
Error	36	11.654737	0.32374		
C. Total	37	15.318947			

Table 60. Gastrointestinal Distress Posttest Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	8.337895	8.33789	18.3451	0.0001*
Error	36	16.362105	0.45450		
C. Total	37	24.700000			

Table 61. Gastrointestinal Distress 1-Minute Posttest Average

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	4.585263	4.58526	14.8107	0.0005*
Error	36	11.145263	0.30959		
C. Total	37	15.730526			

Table 62. Gastrointestinal Distress – Intestinal Cramping

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	7.164474	7.16447	9.5620	0.0038*
Error	36	26.973684	0.74927		
C. Total	37	34.138158			

Table 63. Gastrointestinal Distress – Diarrhea

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	1.1118421	1.11184	6.9294	0.0124*
Error	36	5.7763158	0.16045		
C. Total	37	6.8881579			

Table 64. Gastrointestinal Distress – Flatulence

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	2.631579	2.63158	5.7692	0.0216*
Error	36	16.421053	0.45614		
C. Total	37	19.052632			

Table 65. Gastrointestinal Distress – Nausea

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	1.111842	1.11184	4.3210	0.0448*
Error	36	9.263158	0.25731		
C. Total	37	10.375000			

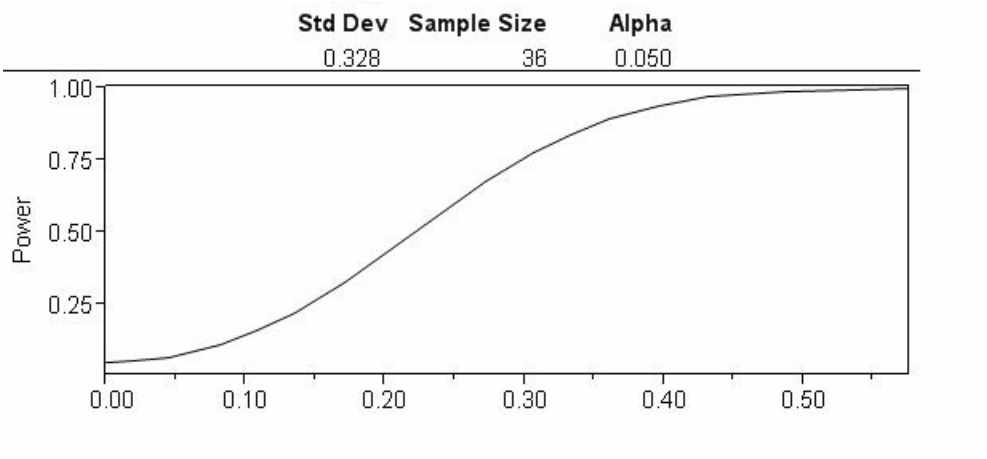
Table 66. Gastrointestinal Distress – Stomach Discomfort

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	19.541118	19.5411	18.0868	0.0001*
Error	36	38.894737	1.0804		
C. Total	37	58.435855			

Table 67. Power Analysis

$\alpha = 0.05$
Sample size: 36 (2 x 18 subject in cross-over design)
Pooled standard deviation: 0.328
Power: 0.80
Difference to detect: 0.315



Appendix D: Institutional Review Board Request and Approval

Once complete, upload this form as a Word document to the IRB Protocol Management System: https://secure.research.vt.edu/irb

Section 1: General Information

1.1 DO ANY OF THE INVESTIGATORS OF THIS PROJECT HAVE A REPORTABLE CONFLICT OF INTEREST? (http://www.irb.vt.edu/pages/researchers.htm#conflict)

☒ **No**
☐ **Yes,** explain:

1.2 WILL THIS RESEARCH INVOLVE COLLABORATION WITH ANOTHER INSTITUTION?

☒ **No,** go to question 1.3
☐ **Yes,** answer questions within table

IF YES
Provide the name of the institution [for institutions located overseas, please also provide name of country]**:**
Indicate the status of this research project with the other institution's IRB: ☐ Pending approval ☐ Approved ☐ Other institution does not have a human subject protections review board ☐ Other, explain:
Will the collaborating institution(s) be engaged in the research? (http://www.hhs.gov/ohrp/humansubjects/guidance/engage08.html) ☐ No ☐ Yes
Will Virginia Tech's IRB review all human subject research activities involved with this project? ☐ No, provide the name of the primary institution: ☐ Yes *Note: primary institution = primary recipient of the grant or main coordinating center*

1.3 IS THIS RESEARCH FUNDED?

☒ **No,** go to question 1.4
☐ **Yes,** answer questions within table

IF YES
Provide the name of the sponsor [if NIH, specify department]:
Is this project receiving federal funds? ☐ No ☐ Yes **If yes,**

Does the grant application, OSP proposal, or "statement of work" related to this project include activities involving human subjects that are <u>not</u> covered within this IRB application?

- ☐ No, all human subject activities are covered in this IRB application
- ☐ Yes, however these activities will be covered in future VT IRB applications, these activities include:
- ☐ Yes, however these activities have been covered in past VT IRB applications, the IRB number(s) are as follows:
- ☐ Yes, however these activities have been or will be reviewed by another institution's IRB, the name of this institution is as follows:
- ☐ Other, explain:

Is Virginia Tech the primary awardee or the coordinating center of this grant?

- ☐ No, provide the name of the primary institution:
- ☐ Yes

1.4 DOES THIS STUDY INVOLVE CONFIDENTIAL OR PROPRIETARY INFORMATION (OTHER THAN HUMAN SUBJECT CONFIDENTIAL INFORMATION), OR INFORMATION RESTRICTED FOR NATIONAL SECURITY OR OTHER REASONS BY A U.S. GOVERNMENT AGENCY?

For example – government / industry proprietary or confidential trade secret information

- ☒ **No**
- ☐ **Yes,** describe:

1.5 DOES THIS STUDY INVOLVE SHIPPING ANY TANGIBLE ITEM, BIOLOGICAL OR SELECT AGENT OUTSIDE THE U.S?

- ☒ **No**
- ☐ **Yes**

Section 2: Justification

2.1 DESCRIBE THE BACKGROUND, PURPOSE, AND ANTICIPATED FINDINGS OF THIS STUDY:

The purpose of this study is to establish capsaicin's potential role as a nutritional supplement in sports requiring repeated bouts of high intensity exercise in terms of delaying fatigue and attenuating inflammation. The combination of aerobic endurance and anaerobic speed and power requirements in sports involving multiple sprint efforts can make it difficult to simulate the conditions experienced during a game and assess performance (1). Repeated sprint testing (RST) is considered to be a valid, reliable, and sensitive assessment of the fitness component in such sports. The impairment of performance as exercise progresses during RST can be attributed to fatigue. This can be due to the complex interaction of metabolic, mechanical, neurological, and immune factors (2, 3).

High intensity exercise can induce muscle damage and hinder performance (4). The inflammatory response to muscle damage is largely mediated via the cytokine IL-6 (5). While inflammation is important in the recovery process, prolonged elevated IL-6 levels can be detrimental, delaying recovery and leading to muscle atrophy (6). Additionally, reactive oxygen species (ROS) can cause cellular damage and oxidative stress if unbalanced by antioxidants. ROS production is increased during intense exercise and contributes to the pronounced inflammatory response (7, 8). Non-steroidal anti-inflammatory drugs are commonly used to reduce the pain associated with exercise induced muscle damage and resulting inflammation. However, there can be side effects in taking these drugs (9). If the initial exercise-induced muscle damage and inflammation can be reduced, it may be to the athletes' benefit in facilitating recovery, and thereby improve subsequent performance.

Intracellular calcium uptake and release by the sarcoplasmic reticulum (SR) is imperative in muscular contraction. Inhibition of this flux due to metabolite accumulation can result in impaired muscle function and lead to fatigue (10, 11). Providing an additional mechanism for calcium flux in active muscle may delay

the onset of fatigue. Vasodilation may also help through enhanced blood flow; accelerating recovery and metabolite clearance (12, 13). Another ergogenic strategy for repeated sprints could be through central nervous system (CNS) stimulation. This neurological component of fatigue regulated by catecholamines is thought to affect variables such as motivation, drive, mood, alertness, reaction time, and rating of perceived exertion (RPE) (14, 15). Additionally, voluntary muscle contraction is controlled via input from the CNS, and reduced neuromuscular activity is correlated with impaired performance in repeated bouts of intense exercise (16, 17). It may therefore be of benefit to ensure optimal CNS activity through increased catecholamine levels to delay the onset of fatigue.

Ephedrine and caffeine are popular drugs used as ergonenic aids in endurance and intense exercise, including repeated sprints. Both ephedrine, caffeine, and their combination have been shown to reduce RPE during exercise (18, 19), which is likely a consequence of their effect on catecholamines. Of concern regarding the use of caffeine and ephedrine are the cardio-stimulatory effects (increased blood pressure and heart rate) that accompany ingestion of these compounds (20, 21). These concerns are part of the reason that ephedrine has been banned by the FDA (22) and should be taken into consideration when recommending a supplement for sports performance.

Capsaicin is the spicy component of the chili pepper that gives it its characteristic pungent flavor. Its physiological effects are similar to that of ephedrine and caffeine in that it induces catecholamine secretion and stimulates the CNS, though the mechanism is through activation of TRPV1 receptors (23). It does not have the cardiac effects of the aforementioned stimulants, and may have a variety of other physiological effects that may prove beneficial to intense exercise. It has been shown to activate ion channels in the SR of muscle cells, encouraging intercellular calcium flux (24) and improves ischemic threshold through calcitonin gene-related peptide mediated vasodilation (25-27). It can also function as an anti-inflammatory; activating PPAR-γ, suppressing macrophage infiltration, and reducing cytokine production (28-32). Additionally, it is an antioxidant, inhibiting LDL oxidation and formation of ROS (33-36). Most of the research involving capsaicin and metabolism in humans has focused on its ability to decrease respiratory quotients and shift fuel utilization from carbohydrate to fat, aiding in weight loss and endurance exercise (37, 38). However, for the above properties, it is within reason to expect that it may be beneficial to brief intense exercise as well.

This will be the first study to the investigators' knowledge to investigate the effect of capsaicin on short, intense exercise in humans. The results may provide evidence for the use of capsaicin as an ergogenic aid in sports requiring repeated sprints; improving performance through delaying the onset of fatigue and facilitating recovery via reducing exercise induced inflammation.

2.2 EXPLAIN WHAT THE RESEARCH TEAM PLANS TO DO WITH THE STUDY RESULTS:

For example - publish or use for dissertation

The study results will contribute to the participating graduate student's thesis and will be submitted for publication.

Section 3: Recruitment

3.1 DESCRIBE THE SUBJECT POOL, INCLUDING INCLUSION AND EXCLUSION CRITERIA AND NUMBER OF SUBJECTS:

Examples of inclusion/exclusion criteria - gender, age, health status, ethnicity

Twenty healthy male athletes, ages 18 - 30 years old, and recreationally trained in sports involving repeated bouts of sprinting (e.g. soccer, rugby, lacrosse, tennis) will be recruited. Exclusion criteria will include reports of a history of inflammatory conditions (e.g. inflammatory bowel syndrome, arthritis), gastric conditions (e.g. ulcers, acid reflux), or other organ anomalies (e.g. liver or kidney diseases), currently taking anxiety or psychological medication, supplements to improve intense activity (e.g. creatine, phosphatidylserine), and recent/chronic musculoskeletal injury or illness that may interfere with participation or place the subject at risk, recent weight fluctuation (>10% bw), and smoking.

3.2 WILL EXISTING RECORDS BE USED TO IDENTIFY AND CONTACT / RECRUIT SUBJECTS?

Examples of existing records - directories, class roster, university records, educational records

☒ **No,** go to question 3.3

☐ **Yes,** answer questions within table

IF YES
Are these records private or public? ☐ Public ☐ Private, describe the researcher's privilege to the records:
Will student, faculty, and/or staff records or contact information be requested from the University? ☐ No ☐ Yes, visit the following link for further information: http://www.policies.vt.edu/index.php (policy no. 2010)

3.3 DESCRIBE RECRUITMENT METHODS, INCLUDING HOW THE STUDY WILL BE ADVERTISED OR INTRODUCED TO SUBJECTS:

Recreational athletes with experience in sports involving repeated sprints will be recruited from the surrounding area. E-mails will be sent out over sport club listservs, coaches/captains of recreational/intramural teams will be contacted, and flyers will be posted in local shops and bulletin boards.

3.4 PROVIDE AN EXPLANATION FOR CHOOSING THIS POPULATION:

Note: the IRB must ensure that the risks and benefits of participating in a study are distributed equitably among the general population and that a specific population is not targeted because of ease of recruitment.

Since the purpose of this study is to investigate the influence of a supplement on athletic performance and the inflammatory response to an exercise protocol simulating repeated sprint sports, it is important to use a study population that reflects the target audience. The sex and age range of this study are similar to those previously used in work by our lab group. The study protocol involves repeated high intensity sprints, so the subjects should be regular exercisers accustomed to brief bouts of high maximal sprinting, as opposed to sedentary individuals to decrease the risk of injury. The exclusion criteria was chosen to minimize the potential risk to subjects and eliminate possible confounding influences on performance and inflammation.

Section 4: Consent Process

For more information about consent process and consent forms visit the following link: http://www.irb.vt.edu/pages/consent.htm

If feasible, researchers are advised and may be required to obtain signed consent from each participant unless obtaining signatures leads to an increase of risk (e.g., the only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting in a breach of confidentiality). Signed consent is typically not required for low risk questionnaires (consent is implied) unless audio/video recording or an in-person interview is involved. If researchers will not be obtaining signed consent, participants must, in most cases, be supplied with consent information in a different format (e.g., in recruitment document, at the beginning of survey instrument, read to participant over the phone, information sheet physically or verbally provided to participant).

4.1 CHECK ALL OF THE FOLLOWING THAT APPLY TO THIS STUDY'S CONSENT PROCESS:

☒ Verbal consent will be obtained from participants
☒ Written/signed consent will be obtained from participants
☐ Consent will be implied from the return of completed questionnaire. Note: The IRB recommends providing consent information in a recruitment document or at the beginning of the questionnaire (if the study only involves implied consent, skip to Section 5 below)
☐ Other, describe:

4.2 PROVIDE A GENERAL DESCRIPTION OF THE PROCESS THE RESEARCH TEAM WILL USE TO OBTAIN AND MAINTAIN INFORMED CONSENT:

Interested volunteers that have contacted the investigators will be sent a copy of the Informed Consent Form and invited to attend a preliminary informational meeting. At the meeting, the investigators will explain the purpose and details of the study, along with the requirements and responsibilities of the subjects as well as the potential risks and benefits. The investigators will provide and go over the Informed Consent Form, and ensure that the participants thoroughly understand the study and what will be involved before signature consent will be accepted. Participants may sign at that time or will be allowed to take the form home to further contemplate participation. Once it has been submitted, the subjects will be be asked to complete a screening survey which will collect information on their exercise/sports history, medical/health history, and schedule availability. They may complete these surveys at the meeting or return them in the days following the meeting. Subjects will be selected from those who volunteer and qualify for this study. Upon selection, they will be asked to submit a form detailing personal, medical, and emergency contact information.

4.3 WHO, FROM THE RESEARCH TEAM, WILL BE OVERSEEING THE PROCESS AND OBTAINING CONSENT FROM SUBJECTS?

Maximilian Opheim

4.4 WHERE WILL THE CONSENT PROCESS TAKE PLACE?

The consent process will take place at the preliminary informational meeting, or subjects may return the Informed Consent Form to the investigators in the days following the meeting.

4.5 DURING WHAT POINT IN THE STUDY PROCESS WILL CONSENTING OCCUR?

Note: unless waived by the IRB, participants must be consented before completing any study procedure, including screening questionnaires.

Consent will occur before administration of any screening questionnaires, treatments, or testing.

4.6 IF APPLICABLE, DESCRIBE HOW THE RESEARCHERS WILL GIVE SUBJECTS AMPLE TIME TO REVIEW THE CONSENT DOCUMENT BEFORE SIGNING:

Note: typically applicable for complex studies, studies involving more than one session, or studies involving more of a risk to subjects.

Once potential subjects have indicated interest in participating in this study, they will be sent an electronic copy of the Informed Consent Form via e-mail and invited to a preliminary informational meeting. If they completely understand the project, their responsibilities, and what will be required of them as subjects, they will have to option to sign and return the form at the meeting. Subjects may also take the form home to further contemplate participation before returning the signed form. Open ended questions surveying the subjects' understanding of the project will be asked prior to accepting the signed consent.

☐ Not applicable

Section 5: Procedures

5.1 PROVIDE A STEP-BY-STEP THOROUGH EXPLANATION OF ALL STUDY PROCEDURES EXPECTED FROM STUDY PARTICIPANTS, INCLUDING TIME COMMITMENT & LOCATION:

Following informed consent, subjects will be required to report for testing and measurements at three separate times. On the first occasion, subjects will arrive in the morning under 12-hour fasting conditions. They will undergo anthropometric measurements (height, weight, body fat) prior to the experimental period. Resting blood pressure and heart rate will be measured. A 10 ml baseline blood withdrawal (B-0) will be taken and stored for future analysis of serum IL-6 prior to the capsaicin/placebo supplement period. Following blood draw subjects will be taken to the facility where the testing will take place. They will perform a progressive shuttle run test to estimate their VO2peak, involving repeated 20 m sprints at increasing intensity to exhaustion.

Subjects will be familiarized with the testing equipment and procedures (audible sprint count down, timing gates, run course, heart rate and blood pressure monitors, rate of perceived exertion scale, gastric distress scale, muscle soreness scale, warm up protocol, and sprint test) prior to the experimental period. They will run through a mock-up of the full protocol (described below) consisting of the warm-up and stretching routine and a third (five sprints) of the repeated sprint test (RST). Subjects will then be given their randomly assigned supplements and, based upon their schedule, instructions as to when to initiate treatment. Treatment will consist of one week of supplementation. Subjects are to consume 8.6 mg of capsaicin in the form of commercially available cayenne supplements (GNC Nature's Fingerprint Cayenne Herbal Supplement 500 mg capsules) gelatin capsules, or placebo, three times per day (morning, afternoon, evening) for a total of 25.8 mg/d of capsaicin for seven days. This will be a placebo controlled cross over study. After one week on either capsaicin or placebo treatment, there will be a one week washout period, followed by one week of the alternate treatment.

Subjects will be given three day dietary record sheets and instructed on how to complete them (measuring portion size, necessary detail of record, etc.). They will also be given a list of foods containing capsaicin and asked to refrain from consuming them throughout the study.

All testing will take place in the same gymnasium on a court-type floor under a constant temperature (roughly 25°C). Subjects will be required to wear comfortable fitness clothing (i.e. gym shorts and shirt) and running shoes, but no compression garments, and to wear the same type of clothing across all tests. On the morning of the test, subjects will report to the facility, complete a brief questionnaire surveying their dietary compliance, physical activity, and illness/injury, and provide their three-day dietary record. They are to arrive in a fasted state having had nothing to eat for 12 hours prior to the test. They are to follow a standardized diet for the day prior to testing and not to have consumed alcohol for 24 hours or caffeine for 12 hours prior to arrival. Additionally, they are not to have engaged in physical activity for 24 hours or have competed in their sport for 72 hour prior to testing, to control for leg fatigue. They will be asked to drink 500 ml of water before going to bed the night before and 500 ml of water when they wake up, to control for dehydration. When they arrive, a baseline blood draw (B-1) will be taken, followed by ingestion of a single 30 mg dose of their assigned treatment and 500 ml of water. Subjects will rest for 45 minutes in a designated area, followed by a baseline heart rate and blood pressure measure. They will then be taken to the gymnasium for the RST. Subjects will perform a standard warm-up consisting of 400 m of jogging at their own pace, followed by a set of 2x10 m high knees, 2x10 m heel-flicks, 2x10 m walking lunges, and 2x10 m practice sprints, then be allowed to stretch on their own for 5 minutes.

Subjects will start at 30 cm behind the starting line to prevent false starting of the timing gates. Upon a pre-recorded audible countdown, subjects will hear three tones and then a starting beep, thus initiating the sprint. They are to run along the length of the 30 m run course at maximal effort until reaching the second set of timing gates. They will be allowed to rest no closer than 5 ft from the timing gate (to avoid accidental triggering) for the remainder of the 35 second interval. Subjects will be notified at 10 and 5 seconds prior to the start of the next sprint to make their way to the starting line. They will begin their next sprint back to the first set of timing gates and in this manner proceed to complete a total of 15 sprints. They will not be given verbal encouragement, timing feedback or indication of sprint number to prevent pacing strategies. After the final sprint, subjects will be taken to the designated room where the blood sample (B2) will be drawn. The subjects will be given their next treatment and dose/timing instructions and then allowed to leave.

Pilot work will be completed using volunteers from our lab group and associates to ensure all equipment is opertational, timing is appropriate, and protocol runs smoothly. This will include trial runs of the progressive shuttle run test, use of the heart rate and blood pressure monitors and scales, and trial runs of the RST.

5.2 DESCRIBE HOW DATA WILL BE COLLECTED AND RECORDED:

Subjects will submit questionnaires surveying health/medical history, personal/emergency/medical contact information, and physical activity and athletic involvement. Dietary information will be collected using three day food records and analyzed by diet analysis software (NutritionistPro, Axxya Systems Stafford, TX). Height and weight will be measured on a standard balance beam scale. Body fat will be assessed using skin fold calipers and the Withers equation as it has been shown to have the highest agreement with DXA scans in elite soccer players (39). Blood pressure will be measured via wrist cuff monitor (Prevention WS-820PV). Heart rate will be measured using a chest strap heart rate monitor (Polar S610i, Polar Electro Inc., NY). Blood pressure will be measured after the 45 minute rest period, at 1 minute before the start of the RST and within 1 minute upon completion. Heart rate will be measured after the 45 minute rest period and monitored continuously throughout the test, starting at 1 minute prior to initiation and concluding 1 minute following completion. Subjects will be asked to gauge their rating of perceived exertion by pointing to their estimated level on a 20-point Borg scale immediately following every third sprint. Subjects will be asked to gauge any gastric distress, abdominal pains, or discomfort upon arrival, 1 minute before the start of the test, between

sprint seven and eight, and after the last sprint by indicating level of discomfort on a scale. They will gauge muscle soreness by indicating on a 10-point scale before and after the sprint test, and at 24, 48, and 72 hours after the test (emails will be sent to remind subjects to complete the brief muscle soreness survey). Sprint performance will be measured using Speedlight TT twin beam timing system (Swift Performance Equipment, Ltd.). Sprint time will be recorded and performance assessed by fastest and average sprint times. Serum IL-6 will be measured in duplicate using an enzyme-linked immunosorbant assay (R&D Systems, Quantikine HS ELISA, Human IL-6). Blood samples will be drawn from subjects via venepuncture by a certified phlebotomist. Subjects will rest in a supine position in a chair in a designated location for blood withdrawal. Samples of 10 ml each will be collected in a serum separator tube at the familiarization session and twice at each of the two test sessions (once 45 minutes before the RST and once immediately following)and allowed to clot for 30-min. Samples will be frozen for future analysis.

5.3 DOES THE PROJECT INVOLVE ONLINE RESEARCH ACTIVITES (INCLUDES ENROLLMENT, RECRUITMENT, SURVEYS)?

View the "Policy for Online Research Data Collection Activities Involving Human Subjects" at *http://www.irb.vt.edu/documents/onlinepolicy.pdf*

☐ **No,** go to question 6.1
☒ **Yes,** answer questions within table

IF YES
Identify the service / program that will be used: ☐ www.survey.vt.edu, go to question 6.1 ☐ Blackboard, go to question 6.1 ☐ Center for Survey Research, go to question 6.1 ☒ Other **IF OTHER:** Name of service / program: **Virginia Tech Webmail** URL: **https://webmail.vt.edu/** This service is… ☐ Included on the list found at: http://www.irb.vt.edu/pages/validated.htm ☐ Approved by VT IT Security ☒ An external service with proper SSL or similar encryption (https://) on the login (if applicable) and all other data collection pages. ☐ None of the above (note: only permissible if this is a collaborative project in which VT individuals are only responsible for data analysis, consulting, or recruitment)

Section 6: Risks and Benefits

6.1 WHAT ARE THE POTENTIAL RISKS (E.G., EMOTIONAL, PHYSICAL, SOCIAL, LEGAL, ECONOMIC, OR DIGNITY) TO STUDY PARTICIPANTS?

The American College of Sports Medicine (ACSM) states that the risk of death during or immediately after a maximal exercise test is less than 0.01%, and the risk of myocardial infarction (heart attack) is less than 0.04% (40). Since most of the studies that contribute to these statistics have involved testing subjects at risk of disease, it is likely that the risk will be even lower for the young, healthy subjects in our study. A 1979 study involving more than one million exercise tests on athletes reported no fatal or nonfatal complications with testing (41). The subjects we intend to use are at very low risk because of their young age, trained condition, and screening to eliminate those with elevated risk. According to ACSM guidelines, males under 45 years of age and women under 55 years of age fall into the "low risk" stratification if they are asymptomatic of disease, and are positive for no more than one of the following risk factors: family history, cigarette smoking, hypertension, hypercholesterolemia, impaired fasting glucose, obesity, and sedentary lifestyle (40). Fatigue, nausea, muscle soreness, and musculoskeletal injuries could result from the exercise protocols employed in this study, though the use of subjects who are currently training and the warm up and stretching protocol should descrease these risks.

Additionally, blood draws have a minimal risk. Occasionally, a bruise may result from blood collection procedures with no known detrimental effects to health or well being of the participant. The amount of blood collected per time point is approximately 10 ml. There will be five total time points at which blood will be drawn (B0 at the familiarization session, and B1 and B2 at each of the two test sessions), so approximately 50 ml total blood will be drawn over approximately three weeks. This amount is well below the level considered to present minimal risk to study participants (limit of 550 ml within an eight week period).
Capsaicin is a common dietary component for some ethnic groups and is the active ingredient in cayenne supplements. There is no daily recommended dosage for capsaicin. However, the acute oral LD50 for capsaicin has been reported to range from 60 - 75 mg/kg in mice to 148.1 - 161.2 mg/kg in rats (42). In human studies, the maximum acute dose administered was 150 mg (43) and chronic capsaicin supplementation of 135 mg/d (45 mg three times per day) for three months (37). It has been advised that acute administration should not exceed 2 mg/kg (44). The doses used in this study will be 8.6 mg capsaicin three times per day for seven days (25.8 mg/d). Considering subjects' weight will likely not be below 60 kg, this would be at most 0.143 mg/kg acute dose. If it is found that treatment results in any gastric upset in the subjects, they are to notify the investigators immediately and appropriate steps taken, which may include reduction of dosage or termination of the subjects' treatment.
Any costs associated with medical services rendered or transportation to a medical facility, including ambulance service, will be borne by the individual and not by Virginia Tech.

6.2 EXPLAIN THE STUDY'S EFFORTS TO REDUCE POTENTIAL RISKS TO SUBJECTS:

To minimize risk, subjects will be monitored throughout the performance tests by the experimenters for signs and symptoms of cardiovascular problems (e.g. abnormal gait, pale, shortness of breath, angina). The gradual build-up in intensity over the course of the performance test and the use of experienced, fit subjects accustomed to intense activity should reduce the risk of injury. To minimize the risk of injury, all testing will be performed on an even court surface that will be inspected prior to testing to ensure safety. Additionally, all subjects will be closely observed throughout testing for signs of musculoskeletal strain and will be instructed in an appropriate warm-up procedure prior to tesing (15 minutes of jogging, warm up exercises, and stretching). At least two investigators will be on hand to observe the subjects during all performance testing. In the event of an injury, the subject will be instructed to terminate the testing procedure immediately. A first aid kit will be on site at all times, and a cell phone will be on hand at all testing. The university rescue squad will be called in the case of an emergency.
To minimize bruising from blood draws, a certified medical laboratory technician will draw all blood samples and universal precautions will be taken in collection and handling of all blood samples. Participants will be allowed to sit or recline in the most comfortable position for themselves during blood draws to minimize dizziness or lightheadedness, which may occur with some individuals. There is a risk of fainting before, during, or after blood draws. If this occurs, the individual will be placed supine with legs slightly elevated. They will be watched and the rescue squad will be called if there is any concern.
All personnel involved in blood draws and blood handling will have undergone training for Blood Borne Pathogen Exposure Control administered by the Environmental Health and Safety Services of the Occupational Health Lab Safety Division at Virginia Tech or other medical facility (e.g. nurse hired from hospital staff). Standard operating procedures set by Virginia Tech's governing body will be executed in the event that blood exposure occurs to one of the experimenters. Specifically, it will be required that the individual's blood be tested for infectious disease (HIV, AIDS, hepatitis) to determine if the experimenter has been exposed to an infectious agent.
In the event of adverse side effects from capsaicin treatment, individuals will be required to notify the investigators immediately and report symptoms. If necessary, treatment will be ceased and the subject recommended to a health care professional.

6.3 WHAT ARE THE DIRECT OR INDIRECT ANTICIPATED BENEFITS TO STUDY PARTICIPANTS AND/OR SOCIETY?

Participants will be provided with any of their own data if they choose, including performance feedback and any measurements taken during the study. They may also be provided with a summary of the research results once the study is completed. It is hopeful that new scientific knowledge will be gained through this study regarding the value of capsaicin in intense exercise performance and the resulting inflammatory resonse.

Section 7: Full Board Assessment

7.1 DOES THE RESEARCH INVOLVE MICROWAVES/X-RAYS, OR GENERAL ANESTHESIA OR SEDATION?

☒ **No**
☐ **Yes**

7.2 DO RESEARCH ACTIVITIES INVOLVE PRISONERS, PREGNANT WOMEN, FETUSES, HUMAN IN VITRO FERTILIZATION, OR MENTALLY DISABLED PERSONS?

☒ **No,** go to question 7.3
☐ **Yes,** answer questions within table →

IF YES
This research involves: ☐ Prisoners ☐ Pregnant women ☐ Fetuses ☐ Human in vitro fertilization ☐ Mentally disabled persons

7.3 DOES THIS STUDY INVOLVE MORE THAN MINIMAL RISK TO STUDY PARTICIPANTS?

Minimal risk means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily activities or during the performance of routine physical or psychological examinations or tests. Examples of research involving greater than minimal risk include collecting data about abuse or illegal activities. Note: if the project qualifies for Exempt review (http://www.irb.vt.edu/pages/categories.htm), it will not need to go to the Full Board.

☐ **No**
☒ **Yes**

IF YOU ANSWERED "YES" TO ***ANY ONE*** OF THE ABOVE QUESTIONS, 7.1, 7.2, OR 7.3, THE BOARD MAY REVIEW THE PROJECT'S APPLICATION MATERIALS AT ITS MONTHLY MEETING. VIEW THE FOLLOWING LINK FOR DEADLINES AND ADDITIONAL INFORMATION: http://www.irb.vt.edu/pages/deadlines.htm

Section 8: Confidentiality / Anonymity

For more information about confidentiality and anonymity visit the following link: http://www.irb.vt.edu/pages/confidentiality.htm

8.1 WILL PERSONALLY IDENTIFYING STUDY RESULTS OR DATA BE RELEASED TO ANYONE OUTSIDE OF THE RESEARCH TEAM?

For example – to the funding agency or outside data analyst, or participants identified in publications with individual consent

☒ **No**
☐ **Yes,** to whom will identifying data be released?

8.2 WILL ANY STUDY FILES CONTAIN PARTICIPANT IDENTIFYING INFORMATION (E.G., NAME, CONTACT INFORMATION, VIDEO/AUDIO RECORDINGS)?

Note: if collecting signatures on a consent form, select "Yes."

☐ **No,** go to question 8.3
☒ **Yes,** answer questions within table →

IF YES
Describe if/how the study will utilize study codes: A code number will be assigned to each individual participating in the study. All questionnaires, data collection sheets, data analysis sheets, blood collection and storage containers, and treatment containers will be identified by code numbers.
If applicable, where will the key [i.e., linked code and identifying information document (for instance, John Doe = study ID 001)] **be stored and who will have access? A master list of participants' code numbers will be kept in a secure filing cabinet separate from completed data, which will also be maintained in a secure filing cabinet. Only investigators involved in this study or future students of the principal investigator (faculty member) will be allowed access to any data.** *Note: the key should be stored separately from subjects' completed data documents and accessibility should be limited.*
The IRB strongly suggests and may require that all data documents (e.g., questionnaire responses, interview responses, etc.) do not include or request identifying information (e.g., name, contact information, etc.) from participants. If you need to link subjects' identifying information to subjects' data documents, use a study ID/code on all data documents.

8.3 WHERE WILL DATA BE STORED?

Examples of data - questionnaire, interview responses, downloaded online survey data, observation recordings, biological samples

All hard copies of data documents (e.g. food records, activity and health questionnaires, and performance sheets) will be stored in a secure filing cabinet separate from completed data.

8.4 WHO WILL HAVE ACCESS TO STUDY DATA?

Only investigators involved in this study or future students of the principal investigator (faculty member) will be allowed access to any data.

8.5 DESCRIBE THE PLANS FOR RETAINING OR DESTROYING THE STUDY DATA

Study data will be kept in a secure filing cabinet for at least one year following the publication of the study or no longer than three years after completion of the study. Documents will be shredded after one of these conditions is met.

8.6 DOES THIS STUDY REQUEST INFORMATION FROM PARTICIPANTS REGARDING ILLEGAL BEHAVIOR?

☒ **No,** go to question 9.1
☐ **Yes,** answer questions within table

IF YES
Does the study plan to obtain a Certificate of Confidentiality? ☐ No ☐ Yes (Note: participants must be fully informed of the conditions of the Certificate of Confidentiality within the consent process and form) *For more information about Certificates of Confidentiality, visit the following link:* *http://www.irb.vt.edu/pages/coc.htm*

Section 9: Compensation

For more information about compensating subjects, visit the following link: http://www.irb.vt.edu/pages/compensation.htm

9.1 WILL SUBJECTS BE COMPENSATED FOR THEIR PARTICIPATION?

☐ **No,** go to question 10.1
☒ **Yes,** answer questions within table

IF YES
What is the amount of compensation? Subjects will receive a maximum of $40 for participation in the study.
Will compensation be prorated? ☒ Yes, please describe: **Subjects will receive $10 per trial (familiarization trial + 2 test trials = $30) and an additional $10 for completing the study. The maximum amount of compensation is $40 per subject.** ☐ No, explain why and clarify whether subjects will receive full compensation if they withdraw from the study? *Unless justified by the researcher, compensation should be prorated based on duration of study participation. Payment must not be contingent upon completion of study procedures. In other words, even if the subject decides to withdraw from the study, he/she should be compensated, at least partially, based on what study procedures he/she has completed.*

Section 10: Audio / Video Recording

For more information about audio/video recording participants, visit the following link: http://www.irb.vt.edu/pages/recordings.htm

10.1 WILL YOUR STUDY INVOLVE VIDEO AND/OR AUDIO RECORDING?

☒ **No,** go to question 11.1
☐ **Yes,** answer questions within table

IF YES
This project involves: ☐ Audio recordings only ☐ Video recordings only ☐ Both video and audio recordings
Provide compelling justification for the use of audio/video recording:
How will data within the recordings be retrieved / transcribed?
How and where will recordings (e.g., tapes, digital data, data backups) be stored to ensure security?
Who will have access to the recordings?
Who will transcribe the recordings?
When will the recordings be erased / destroyed?

Section 11: Research Involving Students

11.1 DOES THIS PROJECT INCLUDE STUDENTS AS PARTICIPANTS?

☐ **No,** go to question 12.1
☒ **Yes,** answer questions within table

IF YES
Does this study involve conducting research with students of the researcher? ☐ No ☒ Yes, describe safeguards the study will implement to protect against coercion or undue influence for participation: **Since the study will be advertised among students within the researchers' department, it is possible that students of the researchers may volunteer to participate in the study. To protect against coercion, the investigators will not directly advertise the study in their classes or indicate any academic benefit from participation.** *Note: if it is feasible to use students from a class of students not under the instruction of the researcher, the IRB recommends and may require doing so.*
Will the study need to access student records (e.g., SAT, GPA, or GRE scores)? ☒ No ☐ Yes

11.2 DOES THIS PROJECT INCLUDE ELEMENTARY, JUNIOR, OR HIGH SCHOOL STUDENTS?

☒ **No,** go to question 11.3
☐ **Yes,** answer questions within table

IF YES
Will study procedures be completed during school hours? ☐ No ☐ Yes **If yes,** **Students not included in the study may view other students' involvement with the research during school time as unfair. Address this issue and how the study will reduce this outcome:** **Missing out on regular class time or seeing other students participate may influence a student's decision to participate. Address how the study will reduce this outcome:**
Is the school's approval letter(s) attached to this submission? ☐ Yes ☐ No, project involves Montgomery County Public Schools (MCPS) ☐ No, explain why: *You will need to obtain school approval (if involving MCPS, click here: http://www.irb.vt.edu/pages/mcps.htm). Approval is typically granted by the superintendent, principal, and classroom teacher (in that order). Approval by an individual teacher is insufficient. School approval, in the form of a letter or a memorandum should accompany the approval request to the IRB.*

11.3 DOES THIS PROJECT INCLUDE COLLEGE STUDENTS?

☐ **No,** go to question 12.1
☒ **Yes,** answer questions within table

IF YES
Some college students might be minors. Indicate whether these minors will be included in the research or actively excluded: ☐ Included ☒ Actively excluded, describe how the study will ensure that minors will not be included: **Subjects will be required to provide their date of birth on the initial screening forms.**
Will extra credit be offered to subjects? ☒ No ☐ Yes **If yes,** **What will be offered to subjects as an equal alternative to receiving extra credit without participating in this study?** **Include a description of the extra credit (e.g., amount) to be provided within question 9.1 ("IF YES" table)**

Section 12: Research Involving Minors

12.1 DOES THIS PROJECT INVOLVE MINORS (UNDER THE AGE OF 18 IN VIRGINIA)?

Note: age constituting a minor may differ in other States.

☒ **No,** go to question 13.1
☐ **Yes,** answer questions within table

IF YES
Does the project reasonably pose a risk of reports of current threats of abuse and/or suicide? ☐ No ☐ Yes, thoroughly explain how the study will react to such reports: *Note: subjects and parents must be fully informed of the fact that researchers must report threats of suicide or suspected/reported abuse to the appropriate authorities within the Confidentiality section of the Consent, Assent, and/or Permission documents.*
Are you requesting a waiver of parental permission (i.e., parent uninformed of child's involvement)? ☐ No, **both** parents/guardians will provide their permission, if possible. ☐ No, **only one** parent/guardian will provide permission. ☐ Yes, describe below how your research meets **all** of the following criteria (A-D): Criteria A - The research involves no more than minimal risk to the subjects: Criteria B - The waiver will not adversely affect the rights and welfare of the subjects: Criteria C - The research could not practicably be carried out without the waiver: Criteria D - (Optional) Parents will be provided with additional pertinent information after participation:
Is it possible that minor research participants will reach the legal age of consent (18 in Virginia) while

enrolled in this study?

☐ No

☐ Yes, will the investigators seek and obtain the legally effective informed consent (in place of the minors' previously provided assent and parents' permission) for the now-adult subjects for any ongoing interactions with the subjects, or analysis of subjects' data? If yes, explain how:

For more information about minors reaching legal age during enrollment, visit the following link: http://www.irb.vt.edu/pages/assent.htm

*The procedure for obtaining assent from minors and permission from the minor's guardian(s) must be described in **Section 4** (Consent Process) of this form.*

Section 13: Research Involving Deception

For more information about involving deception in research and for assistance with developing your debriefing form, visit our website at http://www.irb.vt.edu/pages/deception.htm

13.1 DOES THIS PROJECT INVOLVE DECEPTION?

☒ **No,** go to question 14.1

☐ **Yes,** answer questions within table

IF YES
Describe the deception:
Why is the use of deception necessary for this project?
Describe the debriefing process:
Provide an explanation of how the study meets <u>all</u> the following criteria (A-D) for an alteration of consent: Criteria A - The research involves no more than minimal risk to the subjects: Criteria B - The alteration will not adversely affect the rights and welfare of the subjects: Criteria C - The research could not practicably be carried out without the alteration: Criteria D - (Optional) Subjects will be provided with additional pertinent information after participation (i.e., debriefing for studies involving deception): *By nature, studies involving deception cannot provide subjects with a complete description of the study during the consent process; therefore, the IRB must allow (by granting an alteration of consent) a consent process which does not include, or which alters, some or all of the elements of informed consent.*
The IRB requests that the researcher use the title "Information Sheet" instead of "Consent Form" on the document used to obtain subjects' signatures to participate in the research. This will adequately reflect the fact that the subject cannot fully consent to the research without the researcher fully disclosing the true intent of the research.

Section 14: Research Involving Existing Data

14.1 WILL THIS PROJECT INVOLVE THE COLLECTION OR STUDY/ANALYSIS OF EXISTING DATA DOCUMENTS, RECORDS, PATHOLOGICAL SPECIMENS, OR DIAGNOSTIC SPECIMENS?

Please note: it is not considered existing data if a researcher transfers to Virginia Tech from another institution and will be conducting data analysis of an on-going study.

☒ **No,** you are finished with the application
☐ **Yes,** answer questions within table

IF YES
From where does the existing data originate?
Provide a detailed description of the existing data that will be collected or studied/analyzed:
Is the source of the data public? ☐ No, continue with the next question ☐ Yes, you are finished with this application
Will any individual associated with this project (internal or external) have access to or be provided with existing data containing information which would enable the identification of subjects: ▪ **Directly** (e.g., by name, phone number, address, email address, social security number, student ID number), or ▪ **Indirectly through study codes** even if the researcher or research team does not have access to the master list linking study codes to identifiable information such as name, student ID number, etc or ▪ **Indirectly through the use of information that could reasonably be used in combination to identify an individual** (e.g., demographics) ☐ No, collected/analyzed data will be completely de-identified ☐ Yes, **If yes,** *Research will not qualify for exempt review; therefore, if feasible, written consent must be obtained from individuals whose data will be collected / analyzed, unless this requirement is waived by the IRB.* **Will written/signed or verbal consent be obtained from participants prior to the analysis of collected data?** -select one-

This research protocol represents a contract between all research personnel associated with the project, the University, and federal government; therefore, must be followed accordingly and kept current.

Proposed modifications must be approved by the IRB prior to implementation except where necessary to eliminate apparent immediate hazards to the human subjects.

Do not begin human subjects activities until you receive an IRB approval letter via email.

It is the Principal Investigator's responsibility to ensure all members of the research team who interact with research subjects, or collect or handle human subjects data have completed human subjects protection training prior to interacting with subjects, or handling or collecting the data.

----------END----------

Office of Research Compliance
Institutional Review Board
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Blacksburg, Virginia 24061
540/231-4991 Fax 540/231-0959
e-mail moored@vt.edu
www.irb.vt.edu
FWA00000572(expires 1/20/2010)
IRB # is IRB00000667

DATE: May 18, 2009

MEMORANDUM

TO: Janet W. Rankin
Maximilian Opheim

Approval date: 5/18/2009
Continuing Review Due Date:5/3/2010
Expiration Date: 5/17/2010

FROM: David M. Moore

SUBJECT: **IRB Expedited Approval:** "Effect of Capsaicin Supplementation on Performance of and Inflammatory Response to Repeated Sprinting" , IRB # 09-455

This memo is regarding the above-mentioned protocol. The proposed research is eligible for expedited review according to the specifications authorized by 45 CFR 46.110 and 21 CFR 56.110. As Chair of the Virginia Tech Institutional Review Board, I have granted approval to the study for a period of 12 months, effective May 18, 2009.

As an investigator of human subjects, your responsibilities include the following:

1. Report promptly proposed changes in previously approved human subject research activities to the IRB, including changes to your study forms, procedures and investigators, regardless of how minor. The proposed changes must not be initiated without IRB review and approval, except where necessary to eliminate apparent immediate hazards to the subjects.
2. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.
3. Report promptly to the IRB of the study's closing (i.e., data collecting and data analysis complete at Virginia Tech). If the study is to continue past the expiration date (listed above), investigators must submit a request for continuing review prior to the continuing review due date (listed above). It is the researcher's responsibility to obtain re-approval from the IRB before the study's expiration date.
4. If re-approval is not obtained (unless the study has been reported to the IRB as closed) prior to the expiration date, all activities involving human subjects and data analysis must cease immediately, except where necessary to eliminate apparent immediate hazards to the subjects.

Important:
If you are conducting **federally funded non-exempt research**, please send the applicable OSP/grant proposal to the IRB office, once available. OSP funds may not be released until the IRB has compared and found consistent the proposal and related IRB applicaton.

cc: File

Office of Research Compliance
Institutional Review Board
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e-mail moored@vt.edu
www.irb.vt.edu
FWA00000572(expires 1/20/2010)
IRB # is IRB00000667

DATE: July 7, 2009

MEMORANDUM

TO: Janet W. Rankin
Maximilian Opheim

Approval date: 5/18/2009
Continuing Review Due Date:5/3/2010
Expiration Date: 5/17/2010

FROM: David M. Moore

SUBJECT: **IRB Amendment 1 Approval:** "Effect of Capsaicin Supplementation on Performance of and Inflammatory Response to Repeated Sprinting" , IRB # 09-455

This memo is regarding the above referenced protocol which was previously granted approval by the IRB on May 18, 2009. You subsequently requested permission to amend your IRB application. Since the requested amendment is nonsubstantive in nature, I, as Chair of the Virginia Tech Institutional Review Board, have granted approval for requested protocol amendment, effective as of July 7, 2009. The anniversary date will remain the same as the original approval date.

As an investigator of human subjects, your responsibilities include the following:

1. Report promptly proposed changes in previously approved human subject research activities to the IRB, including changes to your study forms, procedures and investigators, regardless of how minor. The proposed changes must not be initiated without IRB review and approval, except where necessary to eliminate apparent immediate hazards to the subjects.
2. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.
3. Report promptly to the IRB of the study's closing (i.e., data collecting and data analysis complete at Virginia Tech). If the study is to continue past the expiration date (listed above), investigators must submit a request for continuing review prior to the continuing review due date (listed above). It is the researcher's responsibility to obtain re-approval from the IRB before the study's expiration date.
4. If re-approval is not obtained (unless the study has been reported to the IRB as closed) prior to the expiration date, all activities involving human subjects and data analysis must cease immediately, except where necessary to eliminate apparent immediate hazards to the subjects.

cc: File

Appendix E: Informed Consent Form

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Informed Consent for Participants
in Research Projects Involving Human Subjects

Title of Project:
Effect of Capsaicin Supplementation on Performance of and Inflammatory Response to Repeated Sprinting

Investigator(s):
Janet W. Rankin, Ph.D. (PI), Maximilian Opheim (MS Candidate)

I. Purpose:

The purpose of this study is to examine the effect of a substance called capsaicin on sprint performance and the body's response to intense activity following consumption. Capsaicin is the compound found in chili peppers that gives them their characteristic spicy flavor. Some studies have shown that it can serve as a stimulant, similar to caffeine, and may improve exercise by delaying fatigue during endurance exercise. It can also act as an anti-inflammatory and antioxidant, and may thereby benefit recovery. Sports such as soccer, rugby, and tennis require multiple sprint efforts with little rest between. The ability to delay the onset of fatigue in performance may offer a competitive advantage to the athlete. We want to determine if capsaicin can affect performance and fatigue in repeated sprints. Additionally, we will measure some physical consequences of repeated sprinting following capsaicin ingestion. We will be recruiting twenty healthy college age males trained in multiple sprint sports for this study.

II. Procedures

In order to be eligible for inclusion in this study, you must be a male non-smoker between the ages of 18 and 30 and trained in a sport requiring repeated sprints (such as soccer, rugby, field hockey or tennis). You must be free of any chronic health conditions (e.g. cardiovascular disease), gastric issues (ulcers, acid reflux, etc.), recent severe weight fluctuation, not have recently suffered any illness or injury that would affect participation in intense activity, be taking any supplements for improving performance (e.g. creatine), or medication for psychological illness or inflammatory conditions (e.g. arthritis, inflammatory bowel disease). If you meet these criteria, you will be asked to attend an informational meeting outlining the details of this study. We will cover your responsibilities and what is expected of you as a subject, the risks and benefits associated with participation, and timeline of events. Once it has been established that you completely understand this study, we will collect your signature. You will be asked to complete a health and activity questionnaire, medical history survey, and personal/medical/emergency contact information form. If you qualify for participation in this study, you will be notified within one week of the informational meeting. You will be given a three-day dietary record form to complete along with instructions prior to the next meeting described below.

If you are selected to participate, you will attend a familiarization and baseline measurement session in War Memorial Hall. You are to arrive wearing comfortable exercise clothing (no compression garments). We will collect the three-day dietary record. Height, weight, body fat, and resting heart rate and blood pressure will be measured. You will complete a maximal fitness test, involving repeated running of 20 meters until exhaustion. This will be followed by a mockup trial of the warm up, stretching, and repeated sprint test to introduce you to the experimental elements you will be encountering during this study, including the test course and procedure (see below), timing gates, heart rate and blood pressure monitors, and visual scales to measure your effort, gastric distress, and muscle soreness during the tests. The familiarization session will then be completed.

You will be given your randomly assigned treatment; either capsules containing capsaicin or placebo (blank) capsules, and specific instructions as to how to consume them. You must take the capsules three times per day (spaced out throughout the day, i.e. morning, afternoon, evening) for seven days (the start of which will depend upon your schedule). It is critical that you do not open the capsules or try to determine which treatment you received. During this period, you may not consume food products containing capsaicin (e.g. hot sauces or spicy foods). A list of these foods will be provided.

For the 24 hours prior to the test, you are to eat a standardized diet consistent with your previously reported dietary record (consume what you usually do; don't experiment with new things). You are not to consume alcohol for 24 hours or caffeine for 12 hours. You will also be asked to refrain from competing for 72 hours and moderate and prolonged or intense exercise 24 hours before the test to control for leg fatigue (testing dates will be assigned to accommodate competition schedules). On the morning of the test, you are to arrive at War Memorial Hall under fasting conditions; not having consumed anything but water for 12 hours prior (i.e. if you are scheduled to arrive at 8 AM, please do not eat anything after 8 PM the previous evening or drink anything except water). You will be asked to drink 500 ml of water (about 16 oz or half a Nalgene bottle) before going to bed and when you wake up. When you arrive, a brief questionnaire surveying your dietary compliance, physical activity, and illness/injury within the last week will be completed. Your blood will then be drawn (about one tablespoon) by a certified medical technician. You will consume one dose of your treatment along with 500 ml of water and may rest for 45 minutes. Your resting heart rate and blood pressure will be measured and you will be escorted to the gymnasium where the test will be conducted.

The warm up will consist of 400 m of jogging at your own pace, followed by 2x10 m high knees, 2x10 m walking lunges, and 2x10 m practice sprints. You will then put on your heart rate monitor (which will be measuring heart rate throughout the test) and be allowed to stretch on your own for five minutes. Just before the test, you will be asked to indicate on separate scales your level of gastric distress (stomach ache) and muscle soreness, and your blood pressure will be measured.

The test will consist of 15 x 30 m sprints, on 35 s intervals. Starting behind a set of electronic timing gates, you will hear a countdown, and then sprint to a second set of timing gates at the other end of the run course. You will have the remainder of the 35 seconds to rest before starting your next sprint back to the first set of timing gates. These are to be maximal all-out efforts, with no pacing strategies. You will be asked periodically during the test to gauge your gastric distress and rate your effort by indicating on the previously used scales. Upon finishing your 15th sprint, your blood pressure, gastric distress, perceived effort, and muscle soreness will be measured. You will then be escorted back to the room where your blood will be drawn again. This will complete your first testing session and you may then leave.

At 24, 48, and 72 hours following the sprint test, you are to indicated on a provided form your muscle soreness (emails will be sent as reminders the night before; each morning for three days following the test you are to gauge muscle soreness, i.e. if you finished your test at 8:30AM on Tuesday, please gauge your muscle soreness on the provided form at 8:30AM on Wednesday, Thursday, and Friday). You will be given a one week break from the supplementation, but will be required to abstain from the hot and spicy foods on the list provided. Depending upon your schedule, following this week you will then begin your alternate treatment and the above process repeats.

In summary, if you are selected to participate in this study, you will attend a familiarization session of about an hour, and two test sessions of about one and a half hours each. You will be asked to adhere to diet abstaining from spicy foods for the duration of the study, about three weeks, and consume a supplement three times a day for two separate weeks. Diet, physical activity, and illness/injury records throughout the study will be collected. Prior to the test you are not to compete

for 72 hours, exercise for 24 hours, consume alcohol for 24 hours, or any caffeine or food for 12 hours. Your blood will be drawn twice at each test, and we will measure your heart rate and blood pressure, and ask you how you feel several times throughout. Your participation in the study will last approximately three weeks. However, the amount if time at each test and duration of the study may vary based upon how smoothly things go. If at any point in time your health or medical status changes or you experience unexpected symptoms, it is important that you notify the investigators. Your ability to give maximal effort and adhere to the guidelines is very important to the successful completion of this study.

III. Risks:

There is a remote risk of cardiovascular complications from maximal exercise testing. The American College of Sports Medicine (ACSM) states that the risk of death during or immediately after a maximal exercise test is less than 0.01%, and the risk of myocardial infarction (heart attack) is less than 0.04% (ACSM Guidelines 2000). Since most of the studies contributing to these statistics have involved testing subjects at risk of disease, it is likely that the risk will be even lower for the young, healthy subjects in this study. A 1979 study involving more than 1 million exercise tests on athletes reported no fatal or nonfatal complications with testing (ACSM Guidelines 2000). You will be monitored throughout the performance test by the experimenters for signs and symptoms of cardiovascular problems (e.g. abnormal gait, pale, shortness of breath, angina).

Fatigue, nausea, muscle soreness, and muscle strains could result from the exercise tests. However, the warm-up prior to the test will decrease the risk of injury. To further minimize this risk, all testing will be performed on an even court surface that will be inspected for your safety. You will be closely observed throughout the testing for signs of musculoskeletal strain. In the event of injury, you will be instructed to terminate the test procedure immediately and appropriate medical care will be provided. In the case of an emergency, a cell phone will be on hand at all testing, and appropriate medical personnel will be contacted. If a minor emergency arises during your participation in this study, you will discontinue your participation and seek care from your personal physician. If a major emergency arises during your participation in this study, emergency personnel will be called (911), and they will care for you. Any costs associated with medical care received or transportation to a medical facility will be at the expense of the individual, and not the investigators or Virginia Tech.

Blood draws have a minimal risk. Occasionally, a bruise may result from blood collection procedures with no known detrimental effects to your health or well-being. To minimize bruising, a certified medical laboratory technician will draw all blood samples and universal precautions will be taken in collection and handling of all blood samples. There is also a small risk of fainting before, during, or after blood draws. Please notify investigators of any history of fainting and/or lightheadedness with blood draws. If you do faint, we will have you lay down with your feet slightly elevated. If you continue to experience problems we will call for medical help.

All personnel involved in drawing and handling blood have undergone training for Blood Borne Pathogen Exposure Control administered by the Environmental Health and Safety Services of the Occupational Health Lab Safety Division at Virginia Tech or other medical facility. Precautions will be taken by research personnel during handling of your blood samples. You understand that the standard operating procedures set by Virginia Tech's governing body will be executed in the event that blood exposure occurs (blood spilled onto open skin of researcher or a needle stick) in that your blood would then be tested for HIV and hepatitis to determine exposure to the experimenter. There are two HIV/AIDS test sites in the area that offer HIV testing. If you are a Virginia Tech student, you have access to the Schiffert Health Center, otherwise, you must use the Montgomery County Health Department. You will have the option of an anonymous test or a confidential test. The confidential test requires that you give your name and social security number to the testing facility, if you are positive, your name will be sent to the State Health Department (state law requires this). Your name

will remain confidential, but this will be on your medical record. Both sites require pre-test and post-test counseling, and you will have to return in person 2 weeks later to get your results. You will not be allowed to call in for your results. Again, this would occur only if someone is exposed to your blood; we will do all that we can to insure this does not occur.

Capsaicin is the "hot" compound found in many spicy foods and is a common dietary component of some ethnic groups. It is the active ingredient in cayenne supplements, and is marketed for a number of applications, including but not limited to aiding in digestion, improving circulation, and boosting energy. While there is not a daily recommended intake or upper limit established for capsaicin, the doses used in this study have been used in previous research and were generally well tolerated. The doses of capsaicin used in this study are safe; about 1/100th the lowest reported toxic amount in mice (about the amount you would get from eating 1 or 2 hot peppers). However, capsaicin has been found to irritate the gastrointestinal tract in individuals with digestive conditions (such as irritable bowel syndrome). If you find that you experience any discomfort or pain as a result of your treatment; (e.g. heartburn, stomach ache, diarrhea), you must notify the investigators immediately.

IV. Benefits:

This study may contribute to the general wealth of knowledge, in that it will be the first study to our knowledge to examine the value of capsaicin in high intensity exercise. Through your participation in this research, new applications for capsaicin may be identified in benefiting intense activity and reducing the inflammatory response. No promises or guarantee of benefits have been made to encourage you to participate. If you would like, we can provide you with your performance results and measurements taken during this study. Baseline measurements will include height, weight, body fat percentage, VO_{2peak} (a measure of maximal fitness), resting blood pressure and heart rate, and average nutritional content of your three-day diet record. Your experimental and performance measurements will include fastest sprint time, average sprint time, percent fatigue decrement, heart rate, blood pressure, and serum IL-6 (a marker of inflammation). If you would like a summary of the research results, please notify the investigators following the conclusion of the study. Please note that there will be a delay in getting this information as it takes months to analyze the samples and data.

V. Extent of Anonymity and Confidentiality:

Due to the nature of this study, we are not able to offer anonymity. However, you responses to surveys and questions taken, as well as measurements and laboratory results will be kept confidential. You will be assigned a code number, and all treatments and documentation corresponding to you in terms of data collection and results will be identified by this number. At no point in time will the researchers release the results of the study to anyone other than individuals working on the project without your written consent. It is possible that the Institutional Review Board (IRB) may view this study's collected data for auditing purposes. The IRB is responsible for the oversight of the protection of human subjects involved in research. If it is found that you as a subject for whatever reason pose a threat to yourself or others, your confidentiality may be broken. In this situation, we will reference a master list of subjects' names and code numbers, which will be kept in a secure location separate from completed data. Following completion of this study, all forms and data collected will be sealed and stored for at least one year and will not be made accessible by anyone other than the investigators.

VI. Compensation:

You will be compensated for participation in this research project. You will be given $10 (in the form of cash or gift card) for the familiarization session and each of the two test sessions you complete. Additionally you will receive another $10 bonus for finishing the entire study, for a

maximum of $40.

VII. Freedom to Withdraw:

You are free to withdraw from this study at any point in time without penalty. You will be compensated for the portion of the study that you have completed (e.g. if you only complete the familiarization and first test session, you will receive $20). You may refuse to answer any questions or to not participate in any procedure included in this study. There may be circumstances, under which the researchers determine that you should not continue to participate in this study. These may include but are not limited to risk to you as a subject or others, or non-compliance with the research procedures outlined in this consent form.

VIII. Subject's Responsibilities

You voluntarily agree to participate in this study. As a subject, you have the following responsibilities:

1. Disclose any medical condition you may have that may present a risk to your or others and affect your participation in this study according to the guidelines set forth.
2. Consume no foods containing capsaicin throughout the course of the study (approximately three week period).
3. Keep and provide a three day diet record prior to the familiarization trial.
4. Allow for measurements to be made (i.e. height, weight, body fat).
5. Attend the familiarization trial and perform to the best of your ability the maximal fitness test.
6. Consume both treatment supplements provided by the investigators according to their instructions (three times per day for seven days each).
7. Follow a standardized diet during the 24 hours prior to the test session.
8. Refrain from consuming alcohol for 24 hours and caffeine 12 hours before the tests.
9. Refrain from exercise for 24 hours and competing for 72 hours before the tests.
10. Arrive on test mornings under 12 hour fasting conditions.
11. Complete all forms and questionnaires to the best of your knowledge.
12. Allow for blood to be drawn by a certified medical technician at all time points (before and after test).
13. Wear a heart rate monitor and allow blood pressure to be measured.
14. Provide honest responses to the surveys taken during the test.
15. Perform the pre-test and test protocols as instructed by the investigators.
16. Perform the repeated sprint test to completion with maximal effort at each sprint (no pacing).
17. Complete your muscle soreness surveys at 24, 48, and 72 hours post-test.
18. Notify the investigators of any changes in health (i.e. illness, injury, etc.) that occur during the study.
19. Report any unexpected or unpleasant symptoms that may result from the study protocol.
20. Cooperate with the investigators and volunteers of this study.

IX. Subject's Permission:

You have read and understand the Informed Consent form and the conditions of this project. You have had all of your questions answered and understand that you should contact the investigators at any point in time that you find there is something that you do not understand. You hereby acknowledge the above and give your voluntary consent for participation in this project. If you participate, you may withdraw without penalty. You agree to abide by the guidelines set forth by this project.

_____ Date _____
Subject's Signature

_____ Date _____
Witness (optional except for certain classes of subjects)

Should I have any pertinent questions about this research or its conduct, and research subjects' rights, and whom to contact in the event of a research-related injury to the subject, I may contact:

Maximilian Opheim	(540) 664-6074, mopheim@vt.edu
Investigator(s)	Telephone/email
Dr. Janet Rankin	(540) 231-6355, jrankin@vt.edu
Faculty Advisor	Telephone/email
_____	_____
Departmental Reviewer/Department Head	Telephone/email

David M. Moore (540) 231-4991, moored@vt.edu
Chair, Virginia Tech Institutional Review
Board for the Protection of Human Subjects
Office of Research Compliance
2000 Kraft Drive, Suite 2000 (0497)
Blacksburg, VA 24060

Appendix F: Health History Form

Health History

Please indicate any of the following health conditions you have or have previously experienced:

	Yes	No
Heart disease or any heart problems		
Rheumatic fever		
Respiratory disease or breathing problems (e.g. asthma)		
Circulation problems		
Kidney disease or problems		
Urinary problems		
Musculoskeletal problems (i.e. orthopedic injuries or osteoporosis)		
Fainting and dizziness		
High cholesterol		
Diabetes		
Thyroid problems		
Mental illness		
Hypoglycemia (i.e. low blood sugar)		
Epilepsy or seizures		
Blood clotting problems (e.g. hemophilia)		
Anemia		
Liver disorders (e.g. hepatitis B)		
Rheumatoid arthritis		
Lupus		
Crohn’s disease		
Gastric problems (e.g. ulcers, acid reflux)		
Intestinal problems (e.g. inflammatory bowel syndrome)		
HIV or AIDS		

If you answered “yes” to any of the above, please indicate the date and describe:

_____
_____
_____
_____
_____

Please list any hospitalizations/operations/recent illnesses (type/date):

_____
_____
_____
_____
_____

Please list all medications (prescription and over-the-counter) you are currently taking or have recently been on and the reason:

_____
_____
_____
_____
_____

Do you ever faint, experience shortness of breath or chest discomfort with exertion? If so, please explain:

_____
_____
_____
_____
_____

Are there any orthopedic limitations you have that may restrict your ability to perform exercise? If yes, please explain:

_____
_____
_____
_____
_____

Has anyone in your family been diagnosed or treated for any of the following?

	Yes	No	Relationship	Age
Heart attack				
Heart disease				
High blood pressure				
Stroke				
Kidney disease				
Diabetes				

Do you smoke or use any tobacco products? If so, at what frequency (i.e. cigarettes or packs/day)?

_____
_____

Do you drink alcohol? If so please estimate frequency (i.e. drinks per day, days per week):

_____
_____

Are you on any kind of special diet? If so, please describe (e.g. vegan, vegetarian, Celiac disease):

_____
_____

Are you allergic to any foods? If so, which ones?

_____
_____

Are you generally tolerant of spicy foods? _____

Do you consume caffeine? If so, please estimate amount (e.g. cups of coffee/day, cans of soda/day):

_____
_____

Are you taking any dietary supplements (including multivitamins)? If so, what kind, for what purpose, and how often?

_____

_____

Has your weight been stable over the past 3 months? If not, please describe the change (amount and from what weight, cause i.e. sickness, diet/exercise):

_____

_____

Do you have a fear of needles or having blood drawn?

_____

Appendix G: Profile Form

Subject Profile

1. Name:

2. Date of Birth:

3. Height:

4. Weight:

5. Sport:

6. Position:

7. How many years have you participated in the above sport?

8. At what level do you participate?

9. Are you a member of a club or team? If so, which:

10. What is the frequency of your training/competition (hours/day, days/week)?

11. Do you have any food restrictions (allergies, aversions, specific diets)?

12. Are you taking any supplements (including a multivitamin)? If so, which:

13. Have you recently sustained any injury or serious illness? If so, please describe:

14. To your knowledge, do you have any gastric, inflammatory, or cardiovascular conditions? If so, please describe:

15. Are you taking any medications (prescription or over-the-counter)? If so, please list:

16. When might you be available for about 2 hours of testing in the mornings during June (does not have to be exact; your best estimate will do)?

Appendix H: Contact Information Form

Personal, Medical, and Emergency Contact Information

Name: _____

Date of birth: _____

Email: _____

Phone: _____

Person to contact in case of emergency: _____

Relationship: _____

Phone: _____

Primary care physician: _____

Phone: _____

Medical insurance carrier: _____

Policy number: _____

Are you student/faculty/staff at Virginia Tech (if so which)? _____

Are you a U.S. citizen (if not, what nationality)? _____

Appendix I: Screening Survey Form

Thank you for your interest in this study! Your participation will provide valuable information regarding the use of a nutritional supplement in exercise. The purpose of this study is to evaluate the effects of capsaicin, the spicy component of chili peppers, on repeated sprint performance and inflammation. On your part, this will involve a familiarization session, 1 week of consuming a treatment followed by a repeated sprint test, a week off, and then 1 more week of treatment followed by a final sprint test. Each session will require 1 ½ to 2 hours of your time in the morning (much of which will be resting) once a week, and blood draws. Additionally, you will be asked to adhere to certain dietary guidelines throughout the study (no spicy foods). In compensation for your participation, you will receive up to $40 ($10 per session + $10 bonus for completion of the study), information about your sprint performance, the opportunity for good exercise, and the satisfaction of contributing to scientific research!

To participate, you must be a male, between the ages of 18 and 30 years, non-smoker in good health with no recent injuries or illness, and actively participating in a sport such as soccer, rugby, lacrosse, tennis, or a similar sport requiring frequent sprinting. Please fill out the below survey and return it as soon as possible so that we may decide if you qualify as a research subject. We will notify you as soon as possible.

1. Name:
2. Date of Birth:
3. Height:
4. Weight:
5. Sport:
6. Position:
7. How many years have you participated in the above sport?
8. At what level do you participate?
9. Are you a member of a club or team? If so, which:
10. What is the frequency of your training/competition (hours/day, days/week)?
11. Do you have any food restrictions (allergies, aversions, specific diets)?
12. Are you taking any supplements (including a multivitamin)? If so, which:
13. Have you recently sustained any injury or serious illness? If so, please describe:
14. To your knowledge, do you have any gastric, inflammatory, or cardiovascular conditions? If so, please describe:
15. Are you taking any medications (prescription or over-the-counter)? If so, please list:
16. When might you be available for about two hours of testing in the mornings during June/July once a week for three weeks (does not have to be exact; your best estimate will do)?

Appendix J: Three Day Food Record Form

3-Day Food Record

Please provide as much information as possible about your food consumption during 3 days within 1 week. Try to include a weekend as one of your days. Your best estimates will do, but try to be as specific as you can (i.e. instead of "turkey sandwich", list the ingredients, see example below). Try to include all foods you eat—foods often forgotten include condiments or toppings. Please use measuring tools when possible to estimate quantities accurately—for example, measuring cups, tape measures for size of apples, etc.

Date	Food	Preparation/Brand	Amount
05/30/09	Whole wheat bread	Store bought Natures Own	2 slices
	Turkey breast	Roasted, sliced, Oscar Meyer	2 oz
	Mayonnaise	Regular, Hellmann's	2 TBS
	Yelllow Mustard	Regular, French's	1 TBS
	Swiss cheese	Sliced, Sargento	1 slice (1 oz)
	Lettuce	Fresh, Iceberg	2 leaves (1 oz)
	Tomato	Fresh, Beefsteak	1 slice (3" dia)

Date	Food	Preparation/Brand	Amount

Appendix K: Compliance Form

Compliance Form

Please fill out this survey to the best of your recollection. Please be honest; the information you provide is important to this study.

1. Did you consume all of your supplements during the week?

2. If not, how much did you not consume and why?

3. Did you consume your supplements according to your described schedule?

4. If not, please describe how they were consumed:

5. Did you experience any symptoms from consuming your supplement (e.g. upset stomach)?

6. If so, please describe (i.e. when, how long it lasted):

7. Did you participate in any exercise beyond normal day-to-day activity in the last 24 hours?

8. If so please describe (i.e. what activity, for how long, and what intensity, e.g. light, moderate, or heavy lifting, slow and easy, moderate, or fast paced running, etc):

9. Did you compete in any sports activities the last 72 hours?

10. Did you consume any spicy foods during the past week (e.g. foods containing chili peppers)?

11. If so, please describe:

12. Have you consumed anything caffeinated within the past 12 hours?

13. If so, what and how much?

14. Have you consumed any alcohol in the past 24 hours?

15. If so what and how much?

16. Have you been sick within the past week?

17. If so, please describe how, when, and if you are still exhibiting symptoms:

Appendix L: Low Capsaicin Diet

Low Capsaicin Diet

The purpose of this study is to evaluate the effects of a specific quantity of a dietary compound, capsaicin, on sprint performance and inflammation. Therefore, it is very important that you try to consume as little capsaicin as possible outside of what we give you. We realize that this is frequently found in many foods that you may enjoy, and appreciate your best efforts. If you do eat something containing capsaicin, please do not try to compensate by not consuming your supplement! Just let us know.

Foods, sauces, spices, and seasonings to avoid:

- Most Mexican or Tex-Mex foods (please check ingredients)
- Most spicy Oriental foods
- Chili peppers (any and all)
- Hot sauces (any and all)
- Salsa (even mild)
- Picante Sauce
- Taco Sauce
- Most barbeque sauces
- Paprika
- Taco seasoning
- Chili seasoning

Foods that are OK:

- Mustard (as long as it doesn't specifically have paprika, red or chili pepper added)
- Black pepper
- Wasabi
- Horseradish
- Sichuan pepper
- Bell pepper

Basically, if it's spicy and not in the above list, do not eat it! Capsaicin is a compound exclusive to the fruit of the plants in the genus *Capsicum*, which is where we get hot peppers. If you think a food, sauce, spice, or seasoning has hot peppers in it, try to avoid that. If you have any questions about a specific food or seasoning, please let us know. We want this to be as easy for you as possible.

Appendix M: Baseline Data Sheet

Baseline Session				
Date:				
Time:				
Subject Number:				
Height:				
Weight:				
Heart Rate:	1.	2.	3.	Ave.
Blood Pressure:	1.	2.	3.	Ave.
Triceps Skinfold:	1.	2.	3.	Ave.
Subscapular Skinfold:	1.	2.	3.	Ave.
Biceps Skinfold:	1.	2.	3.	Ave.
Supraspinal Skinfold:	1.	2.	3.	Ave.
Abdominal Skinfold:	1.	2.	3.	Ave.
Thigh Skinfold:	1.	2.	3.	Ave.
Calf Skinfold:	1.	2.	3.	Ave.
Mid-Arm Circumference:	1.	2.	3.	Ave.
BodyDensity (1.0988-0.0004*Σ):				
Body Fat % ((4.95/BD)-4.5):				
Completed Shuttles:				
Estimated VO2max:				

Additional Comments:

Appendix N: Progressive Shuttle-Run Test Data Sheet

Subject #:_____
Date:_____

Progressive Shuttle-Run Test

Level	Shuttle								Completed
	1	2	3	4	5	6	7	8	
1									
2									
3									
4									
5									
6									

Level											Completed
	1	2	3	4	5	6	7	8	9	10	
7											
8											

Level												Completed
	1	2	3	4	5	6	7	8	9	10	11	
9												
10												

Level													Completed
	1	2	3	4	5	6	7	8	9	10	11	12	
11													

12													

Level													Completed
	1	2	3	4	5	6	7	8	9	10	11	12	13	
13														
14														
15														

Level														Completed
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
16															
17															

Level															Completed
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
18																
19																

Level																Completed
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
20																	

Estimated VO2 Max:_____

Appendix O: Repeated Sprint Test Data Sheet

Subject #:_____
Date:_____
Time of Start:_____

Sprint Test Data Sheet

R.P.E.	1 minute before test	After sprint 3	After sprint 6	After sprint 9	After sprint 12	After sprint 15

Heart Rate	Resting	1 minute before test	After sprint 3	After sprint 6	After sprint 9	After sprint 12	After sprint 15	1 minute after test

Blood Pressure	Resting	1 minute before test	After Sprint 15	1 minute after test

REMINDERS

Gastric Distress	1 minute before test	After sprint 7	After sprint 15	1 minute after test
Muscle Soreness	1 minute before test	After sprint 7	After sprint 15	1 minute after test

Any Deviations, Abnormalities, Etc.

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Appendix P: Recruitment Materials

The Virginia Tech Human Nutrition, Foods, and Exercise Department is looking for subjects to participate in a research study on nutrition and sprint performance.

- **Eligible candidates must be:**
  - **Male.**
  - **18 – 30 years old.**
  - **Healthy, non-smoker.**
  - **Participate in a sport involving repeated sprinting or runners training with intervals or speed work.**
  - **Able to meet in the morning once a week for three weeks at the Virginia Tech campus.**

- **What does the study involve?**
  - **Subjects will be given a nutritional supplement for one week, perform a repeated sprint test, then given an alternative supplement for another week followed by a 2nd sprint test.**
  - **Blood samples before and after testing.**

- **What are the benefits?**
  - **Financial compensation up to $40.**
  - **Exercise and performance feedback.**
  - **Participation in nutritional research!**

If interested, please email Maximilian Opheim at mopheim@vt.edu. You will be contacted soon and given additional information regarding the study.

The Virginia Tech Human Nutrition, Foods, and Exercise Department is looking for subjects to participate in a research study on nutrition and sprint performance.

- **Eligible candidates must be:**
  - **Male.**
  - **18 – 30 years old.**
  - **Healthy, non-smoker.**
  - **Participate in a sport involving repeated sprinting or runners training with intervals or speed work.**
  - **Able to meet in the morning once a week for three weeks in Blacksburg this summer.**
- **What does the study involve?**
  - **Subjects will be given a nutritional supplement for one week, perform a repeated sprint test, then given an alternative supplement for another week followed by a 2nd sprint test.**
  - **Blood samples before and after testing.**
- **When will this take place?**
  - **Subject recruitment will take place during June.**
  - **Sprint testing will occur in July and August.**
- **What are the benefits?**
  - **Financial compensation up to $40.**
  - **Exercise and performance feedback.**
  - **Participation in nutritional research!**

If interested, please contact Maximilian Opheim at mopheim@vt.edu. You will be contacted soon and given additional information regarding the study.

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Sprint Study mopheim@vt.edu

Hello, my name is Maximilian Opheim, I am a graduate student in the HNFE Department here at Virginia Tech. This summer I am conducting research on nutrition and exercise performance and I am looking for subjects. Eligible candidates must male, between the ages of 18 and 30, healthy non-smokers, and active in a sport requiring repeated sprinting, such as soccer, lacrosse, rugby, ultimate Frisbee, tennis, and so on and so forth. Additionally, if you are a runner and train with intervals and speed work, I can use you as well. Testing will take place in July, and will require 1-2 hours of your time one morning a week for three weeks. I can't offer any academic incentive but there is financial compensation, performance feedback, and the satisfaction of contributing to scientific research. If you think you might be interested in participating, I've got flyers in the back for you to take, as well as my email address up on the board. Send me an email and I'll get back to you very soon with more details of the study. Thank you for your time.

CALL FOR RESEARCH SUBJECTS.

The Human Nutrition, Foods, and Exercise Department is looking for subjects to participate in a research study on nutrition and sprint performance. Eligible candidates must be male, between the ages of 18 and 30, healthy non-smokers, and active in a sport involving repeated sprinting (such as soccer, rugby, ultimate Frisbee, tennis, etc.). Subjects will need to be able to meet in the morning once a week for three weeks to perform a maximal running test. Compensation up $40. If interested, please email Maximilian Opheim at mopheim@vt.edu . You will be contacted soon and given additional information about the study. Thank you.

Appendix Q: Subject Correspondence Materials

Thank you for contacting me. This is a nutritional intervention and physical performance study. We're looking for healthy, non-smoker, male subjects 18-30 years old, that either play a sport involving repeated sprints (like soccer, ultimate frisbee, rugby, tennis, etc.) or runners that train with intervals or speed work. Basically athletes accustomed to sprinting. Briefly, if you participate as a subject, you will come in for testing at three times. The first will be for baseline measurements and fitness assessment. You will then be given supplements (pills) to take for a week. You'll come in again for a sprinting test, be given a week off, then take another supplement for a week with the same test at the end. So, this will require about 1-2 hours of your time in the morning (not before 6AM, and done by 11AM at the latest), once a week, for three weeks (do not need to be consecutive). I've attached an informed consent document which covers the details of the study. Please read through this, and if you are still interested, let me know. I'll be holding a number of informational meetings for potential subjects in the Wallace Hall Atrium. Please let me know of some times you might be available to meet to go over the documentation and signatures.

Thanks!

-Maximilian Opheim

This is just a reminder that you'll be coming in for testing on _____. Here's what all you need to know. You will be coming in on _____ morning at _____ to War Memorial 228/230 for the first session of this study. You need to arrive under 12-hour fasted conditions. So please do not eat anything after _____ on _____ night, and refrain from alcohol that day. If you need to workout, please try to finish on _____ before _____. You may drink water, but no coffee or calorie free beverages after _____ until after the morning blood draw. I will have a light breakfast for you for afterwards. Please drink about a water bottle (16oz) of water before going to bed and when you wake up. When you come in, you will get your height, weight, body fat, resting heart rate and blood pressure measured, then have your blood drawn. After that, we'll go down to the Gym where you will perform the progressive shuttle-run test to estimate your VO2max. We'll run through a brief tutorial on the sprint test and equipment you'll be encountering during the study. You'll then be given a packet of pills and instructions on when/how to take them, and you'll be done for the day. Please bring with you any remaining documents from the meeting yet to be turned in. So, for a quick guide as to what to do:

• (Day before test)

- No alcohol after _____
- No food/beverages (other than water) after _____
- Try not to workout that day after _____
- Do not experiment with new/weird foods that day
- Drink 16oz of water before going to bed

• (Day of test)

- Drink 16oz of water when you wake up
- Do not eat/drink anything until we let you
- Arrive at War Memorial 228/230 at _____
- Wear comfortable athletic clothing (no compression garments)
- Bring completed documents

If you have any questions, please let me know as soon as possible. Thanks, I'll see you _____ morning!

-Max

540.664.6074

Hi _____,

This is just a reminder that you are scheduled to start taking your supplement _____. You need to take 6 pills per day for 7 days. Take them spread out throughout the day. I highly recommend with food (some subjects have done 2 pills with meals, 3 times per day, but I do not suggest more than 2 at a time). Start low, one at a time, and just experiment around to see what works for you. If you miss a dose or don't consume all 6 in a day, that's okay, do not double up to try to catch up, just take note of it. Please do not open them or chew them. There are a few extra pills in the baggy just in case some break, get lost, etc. so do not feel as though you have to finish the whole set. If you experience any unpleasant side effects, (nausea, heart burn, upset stomach, etc.), stop treatment immediately and get a hold of me as soon as possible. This is not to scare you, just accounting for all possibilities. If you have any questions, please let me know. I will send you a reminder about testing prior to next _____.

Have a good week!

-Maximilian

540.664.6074

Hi _____,

Thanks for coming in this morning, great job on the sprint test! This next week is your off week, so no pills to take, but please still stay away from spicy foods as much as possible. I will email you next week when you should start your next round of supplements. Attached is a short (15 seconds) followup survey for the next 3 days. I would like for you to gauge your muscle soreness just like during the sprint test, as close to the time of your sprint test as possible (i.e. 24, 48, and 72 hours afterward); just mark off on the sheets in the same way. So (day 1), (day 2), and (day 3) morning please fill them out when you wake up and record the time/date. I'll collect these the next time you come in.

Thanks again man, enjoy your pill free weekend!

-Max

Hi _____,

Hope everything's going alright with the treatment. This is just a reminder that you'll be coming in for your first test session on _____. You'll need to arrive at _____ under the same conditions as the baseline session. Let me know as soon as possible if that is a problem. Please bring with you the compliance form (attached) and any left over treatment. When you arrive you'll have your blood drawn, take a final dose of your treatment, then rest for 45 minutes before heading down to the gym for testing. So if you want to bring something to read or work on for that time, you can. Below is a reminder guide for the next 2 days:

- (Day before test)
    - No alcohol after _____
    - No food/beverages (other than water) after _____
    - Try not to workout that day after _____
    - Do not experiment with new/weird foods that day
    - Drink 16oz of water before going to bed
- (Day of test)
    - Drink 16oz of water when you wake up
    - Do not eat/drink anything until we let you
    - Arrive at War Memorial 228/230 at _____
    - Wear comfortable athletic clothing (no compression garments)
    - Bring your compliance form and extra treatment

If you have any questions, let me know. Thanks, I'll see you _____ morning!

-Max

540.664.6074

Appendix R: Volunteer Documents

Volunteer Sheet for Repeated Sprint Test

- **Before sprint test starts:**
  - **Ask subject to gauge RPE by pointing to scale, record on data sheet.**
  - **Ask subject to gauge gastric distress by pointing to scales, circle their indicated levels on the sheet (you will have one sheet for each time point).**
  - **Ask subject to gauge muscle soreness. Point to the area on the diagram and ask them to indicate on the scale their level of soreness. Mark where they point with a pen (you will have one sheet for each time point).**
  - **Measure subject's blood pressure: secure cuff around wrist with palm and sensor facing up, hold wrist at heart level, press "start", wait 30 seconds till cuff releases, record blood pressure on data sheet, remove cuff.**
  - **Record heart rate reading from the subject's watch on data sheet.**
  - **Make sure subject is standing behind the 30cm line behind the gates.**
  - **Give subject a 5 second verbal count down to start sprint test, start stopwatch. Record actual time test started on data sheet.**
- **After sprint test starts:**
  - **Do not offer verbal encouragement, performance feedback, or indicate sprint number.**
  - **Keep note of time remaining before the start of the next sprint.**
  - **Once subject has crossed the timing gate after sprinting, make sure he does not wander back across while resting.**
  - **Notify subject at 10 seconds before start of next sprint.**
  - **Make sure he is behind the 30cm line behind the gates.**

- **Give 5 second verbal count down to start of next sprint.**
- **Make sure subject takes off at predetermined time point (35 second intervals – note stopwatch and corresponding list of sprint start time points). You do not need to record the time each of the 15 sprints starts.**
- **Remember subject is going to be very tired, speak clearly and make things as easy for him as possible.**

- **After final sprint:**
  - **Place blood pressure monitor on subject's wrist, press start, while it is processing:**
  - **Ask subject to gauge RPE.**
  - **Record subject's heart rate.**
  - **Record subject's blood pressure, leave monitor on wrist, note time. Wait 1 minute to start monitor again. While waiting:**
  - **Ask subject to gauge gastric distress.**
  - **Ask subject to gauge muscle soreness.**
  - **After 1 minute, start blood pressure monitor, and record heart rate.**
  - **The subject will then be escorted to the blood draw.**

Measurement Time Points

Before Sprint 1	RPE	Gastric Distress	Muscle Soreness	Blood Pressure	Heart Rate
After Sprint 1					
After Sprint 2					
After Sprint 3	RPE			Heart Rate	
After Sprint 4					
After Sprint 5					
After Sprint 6	RPE			Heart Rate	
After Sprint 7	Muscle Soreness			Gastric Distress	
After Sprint 8					
After Sprint 9	RPE			Heart Rate	
After Sprint 10					
After Sprint 11					
After Sprint 12	RPE			Heart Rate	
After Sprint 13					
After Sprint 14					
After Sprint 15	RPE	Heart Rate	Blood Pressure	Gastric Distress	Muscle Soreness
1 minute after end of test	Heart Rate			Blood Pressure	

Sprint Start Time Points

Sprint 1	0:00
Sprint 2	0:35
Sprint 3	1:10
Sprint 4	1:45
Sprint 5	2:20
Sprint 6	2:55
Sprint 7	3:30
Sprint 8	4:05
Sprint 9	4:40
Sprint 10	5:15
Sprint 11	5:50
Sprint 12	6:25
Sprint 13	7:00
Sprint 14	7:35
Sprint 15	8:10

Thanks for getting back to me. I really do appreciate your interest in helping out, this study cannot be completed without volunteers. So here's basically what you'll be doing if you help out with this study. You will be assigned a subject to be in charge of in terms of data collection and direction. They'll arrive in the morning and you will give them a number of surveys and collect their paperwork. You'll guide them through the blood draws and supplement administration, then take them down to the testing area. You'll make sure that they complete their warm up and stretching routine, and properly perform the exercise test. During the test, you will take a number of measurements (most of which are simply visual scales you'll have them point to) at specific time points. And after the test, you'll escort them back for a second blood draw.

You'll be trained in everything that you need to know to do these tasks. There won't be anything too technical or difficult, you'll have a "cheat-sheet" to guide you along, and if you have any questions before or during I'll be there the whole time. Attached is a brief description of the study to look over. I'll be holding an informational meeting with all of the volunteers to go over all of the specific responsibilities and to run through a mock-up test day so that you'll know exactly what to expect and what to do.

As subject recruitment is currently underway and unpredictable, I cannot give you a solid date range for testing. Testing will for about 1.5-2 hours in the mornings and run for about four weeks. You do not need to be there the whole time, if you can only manage one day a week that would still be of great help to me.

Please let me know if you are still interesting in volunteering, and the times you would be able to attend the information meetings (date/time of meeting will depend on volunteers availability). Also, if you have any previous experience with lab and blood work, let me know as you may be able to perform additional duties. Look over the study description, and do not hesitate to contact me if you have any questions at all.

Thanks again!

-Max

Effect of capsaicin supplementation on performance of and inflammatory response to repeated sprinting.

Background

The purpose of this study is to establish capsaicin's potential role as a nutritional supplement in sports requiring repeated bouts of high intensity exercise in terms of delaying fatigue and attenuating inflammation. The combination of aerobic endurance and anaerobic speed and power requirements in sports involving multiple sprint efforts can make it difficult to simulate the conditions experienced during a game and assess performance. Repeated sprint testing (RST) is considered to be a valid, reliable, and sensitive assessment of the fitness component in such sports. The impairment of performance as exercise progresses during RST can be attributed to fatigue. This can be due to the complex interaction of metabolic, mechanical, neurological, and immune factors.

High intensity exercise can induce muscle damage and hinder performance. The inflammatory response to muscle damage is largely mediated via the cytokine IL-6. While inflammation is important in the recovery process, prolonged elevated IL-6 levels can be detrimental, delaying recovery and leading to muscle atrophy. Additionally, reactive oxygen species (ROS) can cause cellular damage and oxidative stress if unbalanced by antioxidants. ROS production is increased during intense exercise and contributes to the pronounced inflammatory response. Non-steroidal anti-inflammatory drugs are commonly used to reduce the pain associated with exercise induced muscle damage and resulting inflammation. However, there can be side effects in taking these drugs. If the initial exercise-induced muscle damage and inflammation can be reduced, it may be to the athletes' benefit in facilitating recovery, and thereby improve subsequent performance.

Intracellular calcium uptake and release by the sarcoplasmic reticulum (SR) is imperative in muscular contraction. Inhibition of this flux due to metabolite accumulation can result in impaired muscle function and lead to fatigue. Providing an additional mechanism for calcium flux in active muscle may delay the onset of fatigue. Vasodilation may also help through enhanced blood flow; accelerating recovery and metabolite clearance. Another ergogenic strategy for repeated sprints could be through central nervous system (CNS) stimulation. This neurological component of fatigue regulated by catecholamines is thought to affect variables such as motivation, drive, mood, alertness, reaction time, and rating of perceived exertion (RPE). Additionally, voluntary muscle contraction is controlled via input from the CNS, and reduced neuromuscular activity is correlated with impaired performance in repeated bouts of intense exercise. It may therefore be of benefit to ensure optimal CNS activity through increased catecholamine levels to delay the onset of fatigue.

Ephedrine and caffeine are popular drugs used as ergonenic aids in endurance and intense exercise, including repeated sprints. Both ephedrine, caffeine, and their combination have been shown to reduce RPE during exercise, which is likely a consequence of their effect on catecholamines. Of concern regarding the use of caffeine and ephedrine are the cardio-stimulatory effects (increased blood pressure and heart rate) that accompany ingestion of these compounds. These concerns are part of the reason that ephedrine has been banned by the FDA and should be taken into consideration when recommending a supplement for sports performance.

Capsaicin is the spicy component of the chili pepper that gives it its characteristic pungent flavor. Its physiological effects are similar to that of ephedrine and caffeine in that it induces catecholamine secretion and stimulates the CNS, though the mechanism is through activation of TRPV1 receptors. It does not have the cardiac effects of the aforementioned stimulants, and may have a variety of other physiological effects that may prove beneficial to intense exercise. It has been shown to activate ion channels in the SR of muscle cells, encouraging intercellular calcium flux and improves ischemic threshold through calcitonin gene-related peptide mediated vasodilation. It can

also function as an anti-inflammatory; activating PPAR-γ, suppressing macrophage infiltration, and reducing cytokine production. Additionally, it is an antioxidant, inhibiting LDL oxidation and formation of ROS. Most of the research involving capsaicin and metabolism in humans has focused on its ability to decrease respiratory quotients and shift fuel utilization from carbohydrate to fat, aiding in weight loss and endurance exercise. However, for the above properties, it is within reason to expect that it may be beneficial to brief intense exercise as well.

This will be the first study to investigate the effect of capsaicin on short, intense exercise in humans. The results may provide evidence for the use of capsaicin as an ergogenic aid in sports requiring repeated sprints; improving performance through delaying the onset of fatigue and facilitating recovery via reducing exercise induced inflammation.

It is hypothesized that following capsaicin treatment: 1. sprint performance, as evidenced by percent fatigue decrement and reduced average sprint time, will improve, 2. blood pressure and heart rate response to sprinting will be the same for the capsaicin group and placebo group, and 3. inflammatory response to the RST will be reduced as indicated by reduced levels of the inflammatory-responsive cytokine, IL-6.

Experimental Design

This study will be a placebo-controlled, double blind, cross-over design. Subjects will be recruited from the surrounding area via contacting local clubs and teams, flyers posted locally, and by emails sent out through academic, recreational, and club listservs. Participants will be healthy males, ages 18 – 30 yrs, recreationally active in repeated sprint sports (soccer, rugby, lacrosse, tennis, etc.). Interested individuals will be contacted via email and requested to complete an initial screening survey regarding eligibility; basic physical characteristics (age, height, weight, etc.), sports participation and physical activity, medical condition and health status, and general schedule. Exclusion factors include smokers, history of gastric conditions (ulcers, acid reflux, etc.) inflammatory conditions (inflammatory bowel disease, arthritis, etc.), liver or kidney anomalies, cardiovascular diseases, history of mental illness or currently taking any anxiety or psychological medication, recent injuries that could interfere with completion of the testing protocol, significant recent weight fluctuations (±10% body weight), or taking supplements for the purposes of improving anaerobic performance (creatine, β-alanine, D-ribose, phosphatidylserine, etc.).

Following the initial screening, eligible volunteers will be invited to an informational meeting outlining the objectives and aspects of the study, benefits and risks associated with the protocol, and their responsibilities and expectations as subjects. They will be given a copy of the Informed Consent Form to complete along with a more specific screening form, asking participants to detail their histories related to physical activity, weight gain/loss, hospitalization/medication, any dietary restrictions, and potential testing date availabilities. From the remaining qualified candidates, 20 subjects will be selected.

Subjects will be required to report for testing and measurements at three separate times. On the first occasion, subjects will arrive in the morning under 12-hr fasting conditions. They will undergo baseline anthropometric measurements (height, weight, body fat) prior to the experimental period. Resting blood pressure and heart rate will be measured and a baseline blood withdrawal will be taken and stored for analysis of serum IL-6.

Following the blood draw, subjects will be taken to the gym where the RST will take place. Subjects will perform a progressive shuttle run test to exhaustion to estimate their $VO_{2peak.}$ They will be familiarized with the testing equipment and procedures (audible sprint countdown, timing gates, run course, heart rate and blood pressure monitors, RPE scale, gastric distress scale, warm up

protocol, and sprint test) prior to the experimental period. They will be made to run through a mock-up of the full protocol (described below) consisting of the warm-up and stretching routine and a third (five sprints) of the RST. Subjects will then be given their randomly assigned supplements and, based upon their schedule, instructions as to when to initiate treatment.

Subjects will be randomly divided into either a treatment group or a placebo group. Treatment will consist of 90 mg/d of capsaicin in a gel capsule or gelatin placebo. They will be given a list of foods containing capsaicin and asked to refrain from consuming them throughout the course of the experimental period. They will be informed not to consume alcohol for 24-hrs or caffeinated beverages for 12-hrs prior to the RST.

All testing will take place in the same gymnasium on a court-type floor under a constant temperature (roughly 25°C). Subjects will be required to wear comfortable fitness clothing and running shoes. On the morning of the test, subjects will report to the facility, provide a three-day food record, and complete a brief questionnaire surveying their dietary compliance, physical activity, and illness/injury. They are to arrive in a fasted state having had nothing to eat for 12-hrs prior to the test. A baseline blood draw will be taken, followed by ingestion of a single dose of their assigned treatment and 500 ml of water. Subjects will rest for 45-min in a designated area, followed by a baseline heart rate and blood pressure measure. They will then be taken to the gymnasium for the RST. Subjects will perform a standard warm-up and then be allowed to stretch on their own for 5 minutes.

The test will consist of 15x30-m sprints on 35-s intervals. Subjects will start at 30-cm behind the starting line to prevent false starting of the timing gates. Upon a pre-recorded audible countdown, subjects will hear three tones and then a starting beep, thus initiating the sprint. They are to run along the length of the 30-m run course at maximal effort until reaching the second set of timing gates. They will be allowed to rest no closer than 5-ft from the timing gate (to avoid accidental triggering) for the remainder of the 35-s interval. Subjects will be notified at 10 and 5-s prior to the start of the next sprint to make their way to the starting line. They will begin their next sprint back to the first set of timing gates and in this manner proceed to complete a total of 15 sprints. They will not be given verbal encouragement, timing feedback or indication of sprint number to prevent pacing strategies. After the final sprint, subjects will be taken to the designated room where a second blood sample will be drawn. They may rest quietly for 1-hr and will be allowed water ad lib but no food. After the 1-hr the third and final blood sample will be taken. The subjects will be given their next treatment and dose/timing instructions and then allowed to leave. There will be at least a 1-wk washout period before subjects will begin their alternate treatment and repeat the protocol. Subjects will be compensated a total of $40 for their participation in the study.

Measurements

Blood pressure will be measured after the 45-min rest period, at 1-min before the start of the RST and within 1-min upon completion. Subjects will be asked to gauge their RPE by pointing to their estimated level on a 15-point Borg scale immediately following every third sprint. Heart rate will be measured after the 45-min rest period and monitored continuously throughout the test, starting at 1-min prior to initiation and concluding 1-min following completion. Subjects will be asked to gauge any gastric distress, abdominal pains, or discomfort 1-min before the start of the test, between sprint seven and eight and after the last sprint by indicating level of discomfort on a visual scale. They will be asked to gauge muscle soreness before and immediately after the test, and to complete a survey of muscle soreness at 24, 48, and 72 hours following. Sprint time will be measured using Speedlight TT twin beam timing system, and performance assessed by fastest and average sprint times. Fatigue will be gauged as percentage decrement, calculated as follows: % fatigue decrement = (100 x (total sprint time/ideal sprint time)) – 100, where ideal sprint time is the fastest sprint time x total number of sprints and total sprint time is the sum of all the sprint times.

Serum IL-6 will be measured in duplicate using an enzyme-linked immunosorbant assay. Blood samples will be drawn from subjects via venepuncture by a certified phlebotomist. Subjects will rest in a supine position in a chair in a designated location for blood withdrawal. Samples of 10 ml each will be collected in a serum separator tube and allowed to clot for 30-min. They will then be centrifuged at 1000 x g for 15-min and serum removed, aliquoted into appropriately labeled conical tubes, and frozen at -20°C for future analysis.

Appendix S: Rate of Perceived Exertion Scale

R.P.E.


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

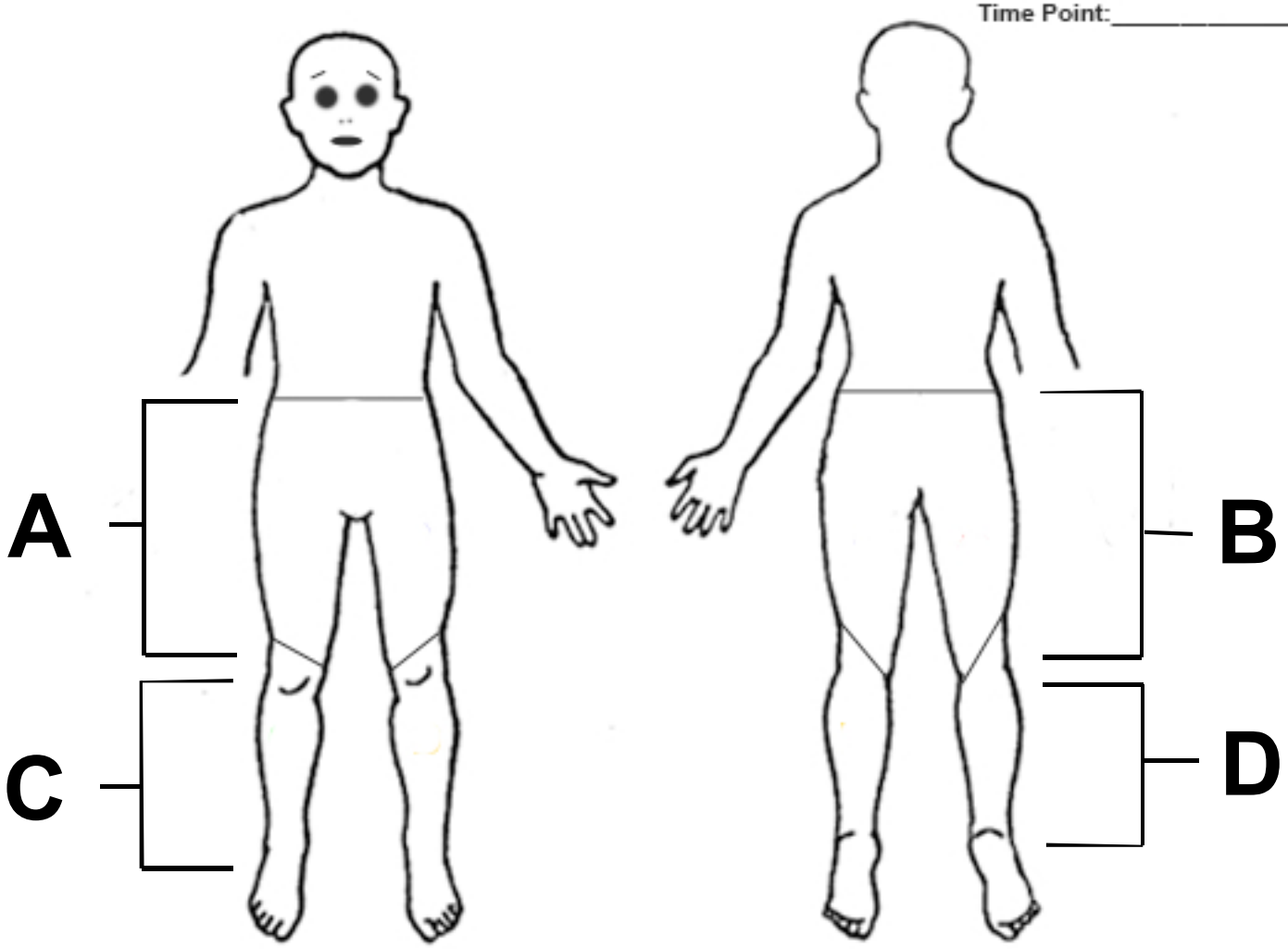
Appendix T: Muscle Soreness Scale

Muscle Soreness

Subject #: _____

Date: _____

Time Point: _____



A: Upper Front of Legs

No Soreness _____ Unbearably Sore

B: Upper Back of Legs

No Soreness _____ Unbearably Sore

C: Lower Front of Legs

No Soreness _____ Unbearably Sore

D: Lower Back of Legs

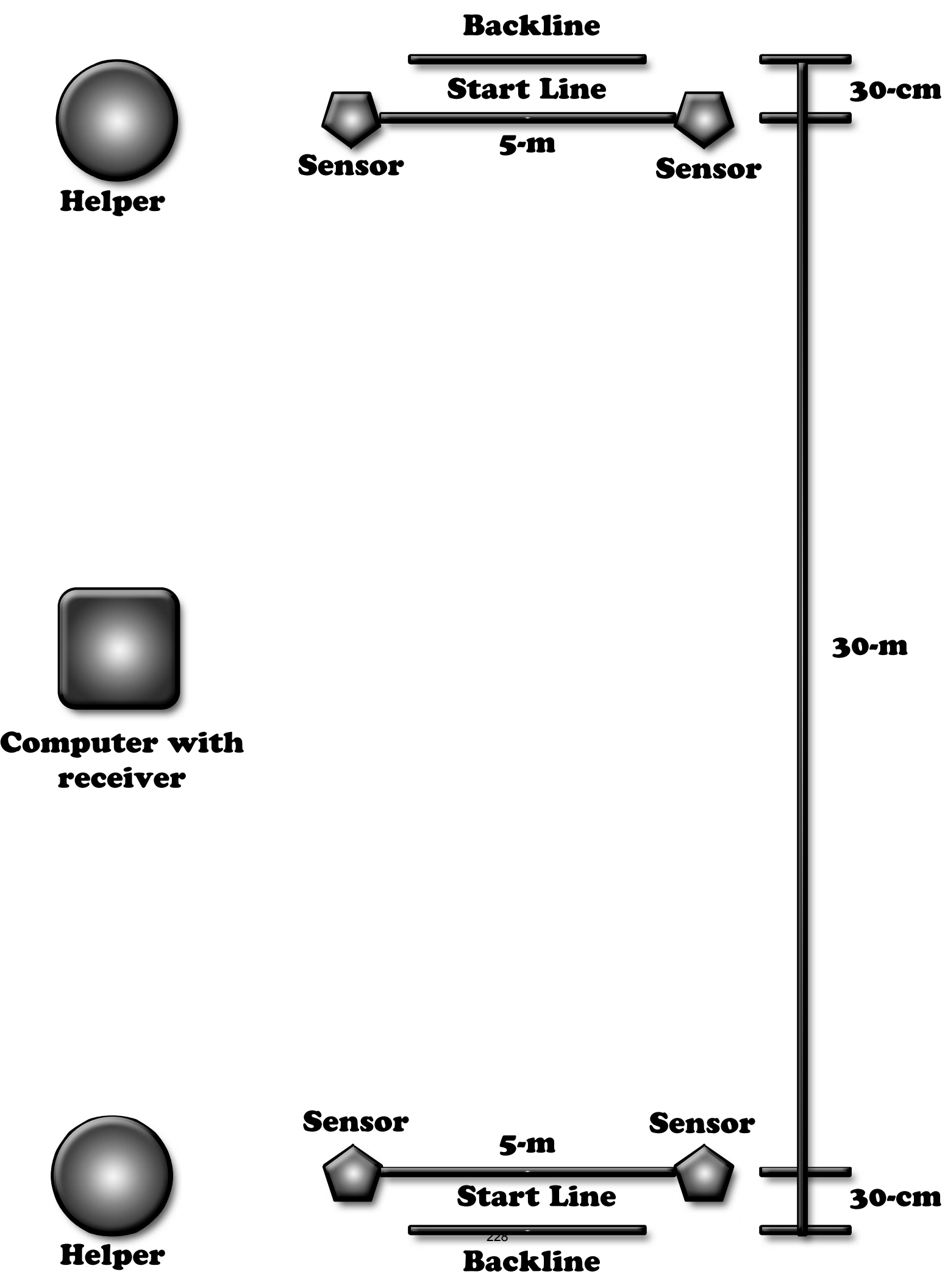
No Soreness _____ Unbearably Sore

Appendix U: Gastrointestinal Distress Scale

Subject #:_____
Date:_____
Timepoint:_____

No Intestinal Cramps	0	1	2	3	4	5	Severe Intestinal Cramps
No Diarrhea	0	1	2	3	4	5	Severe Diarrhea
No Nausea	0	1	2	3	4	5	Severe Nausea
No Flatulence	0	1	2	3	4	5	Severe Flatulence
No Stomach Discomfort	0	1	2	3	4	5	Severe Stomach Discomfort

Appendix V: Repeated Sprint Test Diagram


Backline
Start Line
30-cm
5-m
Sensor
Sensor
Helper
30-m
Computer with receiver
Sensor
Sensor
5-m
Start Line
30-cm
Helper
Backline

Appendix W: GNC Cayenne Supplement


GNC
Nature's Fingerprint®
Cayenne
500mg
HERBAL SUPPLEMENT
Supports Healthy
Digestive Function*
100,000 Scoville Heat Units
100 CAPSULES
* This statement has not been evaluated by the Food and Drug Administration.
This product is not intended to diagnose, treat, cure, or prevent any disease.

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