The efficacy of caffeine supplementation in collegiate tennis players and the magnitude of improvement in tennis skill mediated by caffeine influenced by a polymorphism of the CYP1A2 gene

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The Efficacy of Caffeine Supplementation in Collegiate Tennis Players and the Magnitude of Improvement in Tennis Skill Mediated by Caffeine Influenced by a Polymorphism of the CYP1A2 Gene

Courtney Shaughnessy Klein

A thesis submitted to the Graduate Faculty of JAMES MADISON UNIVERSITY

In

Partial Fulfillment of the Requirements for the degree of

Master of Science

Kinesiology

May 2010
Acknowledgements

I would like to thank Dr. Christopher Womack with his acceptance as serving as my chairperson and for his support throughout the duration of my thesis. Your generosity in helping provide the necessary resources, knowledge and experience throughout this process was invaluable.

I would also like to thank Dr. Judith Flohr and Dr. Michael Saunders as serving on my thesis committee and offering valuable suggestions throughout this endeavor. Your fastidiousness was highly appreciated.

I’d also like to thank Adam Clawson, Brooke Shafer and Jennifer Wu for their assistance with data collection. Your flexibility and positive attitudes helped enable me to complete the study in a timely fashion.

Lastly, I’d like to thank Maria Malerba and Steve Secord to let me use their players for the basis of my study as well as for the JMU’s men and women tennis players for their cooperation and participation in this study.
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Abstract

**Purpose:** This study examined the efficacy of caffeine supplementation on tennis performance in male and female collegiate tennis players and whether a polymorphism of the CYP1A2 gene influenced the ergogenic response to caffeine.

**Methods:** Eighteen collegiate tennis players (9 male; 9 female) completed two separate trials. Each test occurred one hour after the administration of either 6 mg/kg of caffeine or a placebo, administered in double-blind fashion. The treadmill portion was comprised of 15 minutes at a velocity corresponding with 50% \( \text{VO}_{2\text{max}} \) followed by 30 minutes of intermittent sprints (5 seconds at 80% \( \text{VO}_{2\text{max}} \), 15 seconds at 50% \( \text{VO}_{2\text{max}} \)). 90 second resting periods were administered after the 18th sprint and every twelve sprints thereafter, mirroring the discontinuous nature of tennis. RPE and HR was taken before each 90 second resting period. 120 seconds following the treadmill test, the tennis skills test was administered. A 6-ball drill comprised with 4 groundstrokes, 1 approach shot and 1 volley were fed, followed by a 20 second resting period. The 6-ball drill was repeated 6 times, and every 2 sessions were followed by a 90 second break. Nine full sessions were performed with RPE and HR recorded prior to resting periods. Subjects completed these methods for both treatments, in a randomly counterbalanced, double-blind protocol. DNA was obtained from whole blood samples and analyzed for presence of the C variant using polymerase chain reaction with allele-specific primers. Subjects were classified as AA homozygotes (N=7) or C allele carriers (N=9).

**Results:** Caffeine significantly improved performance during the Tennis Skills test. There was no genotype effect (P=.029), regardless of gender. There was no main effect for
treatment on RPE during the treadmill portion of the test and no significant main
effects or interaction effects for RPE following ingestion of caffeine during the
Tennis Skills Test. A significant gender effect existed during the treadmill portion of
the test (P=0.020) and Tennis Skills test (P=0.027) for RPE with women exhibiting a
lower RPE men. Furthermore, on average, caffeine elevated peak HR in AA
homozygotes and lowered peak HR for C allele carriers. **Conclusion:** Caffeine
supplementation positively impacts tennis performance regardless of genotype.
Prior studies have recognized notable ergogenic benefits of a large dose (5-6 mg/kg) of caffeine supplementation for both aerobic and anaerobic performances (1,3,4,5,12,18,20,21,29,46,51,60)). However, the effect of caffeine supplementation on tennis, a sport that combines aspects of aerobic and anaerobic activities, remains unknown. One study by Hornery et al. (2007) included highly trained male subjects who performed simulated tennis matches against a ball machine in which caffeine was shown to partly attenuate the effects of fatigue and increased serve velocity (35). On the contrary, a study by Ferrauti et al. (1997) included male and female subjects who ingested caffeine during the match. Results indicated that caffeine did not positively affect perception ratings or hitting accuracy (26).

Caffeine is metabolized mainly by the polymorphic cytochrome P450 1A2 (CYP1A2) enzyme (15) and the rate of metabolism varies between individuals (34). Studies have shown the presence of a C/A polymorphism in intron 1 of the CYP1A2 gene (57), with slower caffeine metabolism following caffeine ingestion in individuals possessing the C allele (57,15). In an unpublished study, subjects homozygous for the A variant of the studied polymorphism had a more prominent ergogenic effect for 40 kilometer cycling time during the caffeine trial than subjects possessing the C variant (67), suggesting AA homozygotes have an elevated caffeine response. Whether possession of this genotype results in a greater ergogenic effect on tennis performance is unknown. The purpose of this study is to determine the efficacy of caffeine
supplementation in collegiate tennis players and whether the polymorphism may explain the different response to caffeine supplementation as an ergogenic aid in tennis performance. The hypothesis was that caffeine will improve tennis performance in collegiate tennis players and that subjects homozygous for the CYP1A2*1A allele will have the greatest ergogenic response.
Chapter II

Review of Literature

The ingestion of caffeine prior to physical activity has elicited ergogenic effects for both aerobic and anaerobic sports. A substantial amount of evidence exists supporting the ergogenic effects of caffeine on aerobic sports which are addressed in Table 1. Anaerobic performance improvements are less established than endurance performance. Equivocal effects of caffeine intake on performance during anaerobic exercise have been observed (Table 2). Literature exists demonstrating enhanced performance following caffeine supplementation; no effect on performance following caffeine ingestion; as well as varied effect on performance following the ingestion of caffeine during anaerobic exercise.

Tennis is a unique sport in that it includes all three energy systems - high energy phosphates, anaerobic-glycolytic processes and aerobic energy system (55). Although there is an abundance of literature surrounding caffeine’s effect on aerobic and anaerobic sports, few studies exists which cover sports that combine aspects of both aerobic and anaerobic activities and also include a skill component. Furthermore, an even more limited amount of information exists that is directly related to caffeine and tennis performance (Table 3).

Lastly, evidence illustrating that the mode of exercise, dosage of caffeine and characteristics of the subjects - trained versus untrained, may have an effect on caffeine’s ability to enhance performance during aerobic or anaerobic activities is addressed in Table 4.
Table 1 illustrates the ergogenic effects of caffeine during endurance activities. Twelve of these studies were conducted using a cycle ergometer. Some of these studies used time trials varying from sprint distances to exercise trials lasting 3 or more hours. Furthermore, male subjects were used in ten of these studies. All of these studies elicited a performance enhancement following caffeine ingestion.

There are five studies that observed a performance enhancement following caffeine ingestion using a treadmill protocol. Each of these high-intensity endurance studies, regardless of distance, demonstrated an ergogenic effect of caffeine. In one study, an ergogenic effect was observed at a dosage of 150 ml/l and even greater with the medium dosage of 225 mg/l. However, the highest dosage (320 mg/l) did not further improvement as compared to the medium dosage (46).

Although the methodology and protocols used for each study varies, the ingestion of caffeine prior to activity has been identified as the catalyst supporting the enhanced performance.

Table 1: The Ergogenic Effects of Caffeine on Aerobic Sports

<table>
<thead>
<tr>
<th>Articles</th>
<th>Problem/Question studied</th>
<th>Participants</th>
<th>Procedures</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine increases exogenous carbohydrate oxidation during exercise (68)</td>
<td>To determine the effect of combined ingestion of glucose and caffeine during 2h of cycling on exogenous CHO oxidation rates.</td>
<td>Eight trained male cyclists</td>
<td>3 exercise trials consisting of 120 min of cycling at 55% Wmax. Received either a glucose drink, glucose + caffeine or plain water. Trials separated by 1 wk</td>
<td>Glu + Caf during exercise resulted in higher exogenous CHO oxidation than Glu ingestion alone</td>
</tr>
<tr>
<td>Study Title</td>
<td>Objective</td>
<td>Participants</td>
<td>Methods</td>
<td>Conclusion</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------</td>
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</tbody>
</table>
| Effect of caffeinated coffee on running speed, respiratory factors, blood lactate and perceived exertion during 1500-m treadmill running (66) | To study the effects of low doses of caffeine (~2 cups of strong coffee) on a number of factors  | 1. Eighteen male local middle distance athletes  
2. Ten athletes  
3. Six male middle distance athletes | 1. 9 time trials of 1500m on treadmill  
2. run for 1100m at 1km/h, final 400m bursts for final minute  
3. 9 runs - 3 test and 6 assessment: 3 three following caffeinated coffee and 3 with decaffeinated coffee | Small doses of caffeine (3g) had ergogenic effect upon sustained high-intensity exercise       |
| Effects of Caffeine on Prolonged Intermittent-Sprint Ability in Team-Sport Athletes (60) | To examine the effects of acute caffeine ingestion on prolonged intermittent-sprint performance | 10 male team-sport athletes | Two exercise trials, separated by 7d, 60 min after ingestion of either 6 mg/kg caff or placebo - trials performed on cycle ergometer 2 x 36-min halves, each composed of 18 x 4-s sprints with 2-min active recovery at 35% VO2peak between sprints | Acute caffeine ingestion can significantly enhance performance of prolonged, intermittent-sprint ability in competitive, team-sport male athletes |
| Caffeine Ingestion Attenuates the VO2 Slow Component during Intense Exercise (58) | To analyze the effects of caffeine ingestion on the slow component of oxygen uptake during high-intensity endurance exercise | Nine subjects (8 male; 1 female) | Two 9-min tests on a treadmill at 90% VO2max, 60 min after ingesting either Pla or Caf capsule | Ergogenic effect of caffeine in a high-intensity endurance exercise may be partly mediated by attenuation of VO2 slow component |
| Caffeine as a Lipolytic Food Component Increases Endurances Performance in Rats and Athletes (56) | To investigate the effects of caffeine ingestion on exercise performance in rats and athletes | 1. 48 male rats 3 wk of age  
2. 5 healthy rugby players | 1. Rats ingested Caff 1 h before exercise, run on treadmill at 20m/min  
2. Humans ingested Caff 1 h before exercise, exercised on cycle ergometer at 60% VO2max for 45 min then intensity increased to 80% until exhaustion | RER Caff < Pla in athletes; FFA Caff > Pla for both rats and athletes; Lactate levels increased for both during exercise; FFA and glycerol concentrations reduced glycogen utilization during ex vs. Pla for rats; time to exhaustion significantly increased by Caff for both rats and athletes |
<table>
<thead>
<tr>
<th>Study Title</th>
<th>Objective</th>
<th>Participants</th>
<th>Design</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of Caffeine on Muscle Glycogen Utilization and the Neuroendocrine Axis during Exercise (48)</td>
<td>To examine the effect of caffeine ingestion on muscle glycogen utilization and the neuroendocrine axis during exercise</td>
<td>Twenty healthy men</td>
<td>Glycogen-loaded subjects given Pla or Caff in double blinded manner 90 min before cycling for 2 h at 65% their VO2max</td>
<td>Caff ingestion 90 min before prolonged exercise does not exert muscle glycogen-sparing effect in athletes with high muscle glycogen content; Caff lowers threshold for B-endorphin and cortisol release leading to Caff benefits on exercise endurance</td>
</tr>
<tr>
<td>Effect of Caffeine Ingestion on Lactate and EMG thresholds in men during graded exercise at room temperature and cold environment (47)</td>
<td>To determine whether caffeine ingestion prior to graded exercise influences the lactate and electromyography thresholds</td>
<td>Seven Finnish students</td>
<td>Incremental exercise tests until exhaustion on a cycle ergometer at room temp (22 degrees C or in the cold -20 degrees C). Ingestion of either 2 cups of caffeinated or decaff coffee 1 hr prior to exercise</td>
<td>Beneficial effect of caffeine on the LA threshold occurs in majority of subjects exercising at room temp, but none exercising in cold environment</td>
</tr>
<tr>
<td>Effects of Ephedrine, Caffeine, and Their Combination of Muscular Endurance (41)</td>
<td>To investigate the effects of ingesting caffeine, ephedrine, and their combination on muscular endurance</td>
<td>13 male subjects</td>
<td>90 min after ingesting either C, E, or a combination of C+E or a placebo, performed weight-training circuit consisting of three supersets, each consisting of leg press, followed by bench press with 2 min rest between supersets</td>
<td>Trials involving C+E and E, when compared to C and P, caused significant increases in the mean number of repetitions completed for both the leg-press and bench-press exercises but only during the first SS; performance enhancement attributed mainly to E, no additive effect of C</td>
</tr>
<tr>
<td>Metabolic, catecholamine, and endurance responses to caffeine during intense exercise (39)</td>
<td>To examine the possible effects of caffeine ingestion on muscle metabolism and endurance during brief intense exercise</td>
<td>Fourteen young adults</td>
<td>Ingested either Pla or Caf (6 mg/kg), cycled for 2 min, rested 6 min, cycled 2 min, rested 6 min, and then cycled to exhaustion.</td>
<td>Caffeine ingestion can be an effective ergogenic aid for exercise around 5 minutes in duration</td>
</tr>
<tr>
<td>Comparison of caffeine and theophylline ingestion: exercise metabolism and endurance (32)</td>
<td>To investigate the ergogenic and metabolic effects of theophylline and caffeine: a) the ergogenic potential of theophylline on endurance exercise b) effects of Theo on muscle metabolism were investigated and compared with caffeine</td>
<td>a) Eight men b) Seven men</td>
<td>a) cycle at 80% VO2max to exhaustion 90 min after ingesting either placebo, caffeine, or theophylline b) cycle at 70% VO2max, followed by same treatment as Part A</td>
<td>Ingestion of Theo resulted in 14% increase in performance during whole body cycling exercise at 80% VO2max; illustrate that muscle glycogen sparing not always observed after ingestion of caffeine or theophylline</td>
</tr>
<tr>
<td>Caffeine and Exercise: metabolism, endurance and performance (30)</td>
<td>To review existing literature regarding caffeine and athletic performance</td>
<td>A review of literature</td>
<td>A summarization of existing literature</td>
<td>Caffeine ingestion prior to exercise often improves performance during prolonged endurance cycling/running &amp; short-term intense cycling &amp; decrease swim time for 1500m. These results are reported in well-trained athletes but field studies are lacking to confirm effects in the athletic world</td>
</tr>
<tr>
<td>Effects of caffeine on time to exhaustion in exercise performed below and above the anaerobic threshold (20)</td>
<td>To compare the effects of caffeine ingestion on endurance performance during exercise on a bicycle ergometer, 10% above and 10% below anaerobic threshold.</td>
<td>Eight untrained males</td>
<td>Cycled to exhaustion - once 10% above AT and another 10% below AT. Each intensity performed twice following ingestion of Caf (5 mg/kg) or placebo.</td>
<td>Caffeine can improve endurance performance during prolonged exercise below AT for untrained subjects</td>
</tr>
<tr>
<td>Effect of different protocols of caffeine intake on metabolism and endurance performance (18)</td>
<td>To examine the effects of different protocols of caffeine on cycling performance</td>
<td>a) Twelve subjects b) Eight subjects</td>
<td>a) Subjects received either decaf 1hr before exercise, 6 doses every 20 min, 2 doses between 100 and 120 min SS, or placebo b) Subjects received 3 doses of caf during last 40 min SS and TT</td>
<td>Coke enhanced TT due to caffeine; 6 mg/kg enhanced TT performance independent of timing of intake; replacing sports drink with Coke during latter stages of exercise equally effective in enhancing endurance performance due to low intake of caffeine</td>
</tr>
</tbody>
</table>
| **Effect of divided caffeine dose on endurance cycling performance, postexercise urinary caffeine concentration, and plasma paraxanthine**  
(14) | To compare the effects of a single and divided dose of caffeine on endurance performance and on postexercise urinary caffeine and plasma paraxanthine concentrations | Nine male cyclists | Cycle for 90 min at 68% VO2max, followed by self-paced time trial at 80% VO2max for 30 min with three interventions: placebo, single caffeine dose or divided caffeine dose 60 min before and 45 min during exercise | TT faster with caffeine compared to placebo; postexercise urinary caffeine concentration lower in CC compared with CP; plasma paraxanthine increased in dose-dependent with no peak during exercise: dividing caffeine dose does not provide an ergogenic effect over a single dose but reduces postexercise urinary concentration |
|---|---|---|---|---|
| **The effect of caffeine ingestion of 8km run performance in a field setting**  
(8) | To investigate the effect of caffeine ingestion on 8km run performance using an ecologically valid field test | Eight trained male distance runners | Three trials separated by one week of an 8 km race run as quickly as possible 1 h after ingesting placebo, caffeine capsule (3 mg/kg) or no supplement | Caffeine ingestion improved absolute 8 km run performance in a valid race setting. |
| **Physiological effects of caffeine on cross-country runners**  
(9) | To determine the physiological effects of caffeine on cross-country runners during submaximal exercise | Ten college-aged subjects (5 male, 5 female) | 2 30-min runs at 70% VO2max on the treadmill, 1 h after ingesting caffeine or placebo | Decreased perception of effort with caffeine ingestion; caffeine had effect on pulmonary function during submaximal exercise |
| **Effect of Repeated Caffeine Ingestion on Repeated Exhaustive Exercise Endurance**  
(4) | To examine the effect of repeated doses of caffeine on repeated exercise endurance | Nine male subjects | Subjects ingested either caffeine or placebo 1 hr before exercise ride; ER to exhaustion 80% VO2max two times per day - 4 treatments with different combination of caffeine dosage and/or placebo | Redosing with caffeine after exhaustive exercise in the morning wasn't necessary to maintain ergogenic effect of the drug during subsequent exercise 6 h later |
| **Exercise endurance 1,3, and 6 h after caffeine ingestion in caffeine users and nonusers**  
(5) | To examine the duration of caffeine's ergogenic effect and whether it differs between users and nonusers of the drug. | Twenty-one subjects | Six randomized exercise rides to exhaustion at 80% of maximal oxygen consumption after ingesting either a placebo or caffeine - 1,3, or 6 h after placebo or drug ingestion | Exercise times 1 and 3 h after caffeine intake greater than placebo trials; both duration and magnitude of ergogenic effect following 5 mg/kg caffeine greater in nonusers compared with users. |
| **Effect of caffeine on sport-specific endurance performance: a systematic review (27)** | To objectively evaluate studies that have examined the effect of caffeine on time-trial endurance performance (>5 min) | Twenty-one studies with 33 identifiable caffeine treatments that measured endurance performance with a time-trial aspect | Each study objectively analyzed with PEDro scale | Abstaining from caffeine at least 7 days before use will elicit greatest chance of optimizing ergogenic effect. |
| **Effect of caffeine on metabolism, exercise endurance, and catecholamine responses after withdrawal (64)** | To test whether caffeine-induced changes in metabolic and catecholamine responses are less pronounced during periods of chronic caffeine ingestion, thus influencing the ergogenic response to caffeine ingestion | Six recreational male athletes who were habitual caffeine users | 7 exercise trials to exhaustion, 10 days between trials. First 2 trials without caffeine withdrawal - assigned randomly with placebo or caffeine. Subsequent trials after 2 or 4 day caffeine withdrawal | short-term withdrawal from caffeine had no effect on caffeine-induced increases in endurance during high-intensity exercise vs. no withdrawal; responses to acute caffeine ingestion during w/d on FFAs and NE but no effect on plasma EP or RER |
| **Effect of caffeinated drinks on substrate metabolism, caffeine excretion, and performance (46)** | To determine the effect of addition of different dosages of Caf to a CHO-electrolyte solution on metabolism, Caf excretion and performance | Fifteen healthy and well-trained male subjects | Ingestion of Pla, 7% Pla-CES, or 7% CS with 150, 225, and 320-mg/l Caf during 20-m warm up protocol and 3 ml/kg at 1/3 and 2/3 of a 1-h time trial | Performance was improved with Caf supplementation (Greatest results with medium dosage; highest dosage did not result in further improvement) |

There exists an abundance of information illustrating the effect of caffeine ingestion on anaerobic sports. Although the majority of information suggests an enhanced performance following caffeine ingestion, some studies show no effect. According to a study on male cyclists, caffeine did not affect RPE or heart rate during the first stages of the test. The ingestion of caffeine did, however, have an ergogenic-effect during the last minute of the preloaded cycle test (24). A study by Beck et al., (2006) also reported significant increase in anaerobic performance following caffeine ingestion (2). This study noted an increase in bench press 1RM, suggesting caffeine ingestion to be an effective supplement for increasing upper-body strength. Furthermore, Kalmar et al.,
(1999), reported increased isometric leg extension strength and maintenance of submaximal leg extension contractions following caffeine intake (44).

Some studies show no effect on performance following caffeine ingestion for healthy subjects. Collomp et al. (1991), reported that caffeine supplementation an hour and a half prior to testing had no effect on peak power during a Wingate Anaerobic Test (13). Although this study incorporated a caffeine intake of 5 mg/kg, which is the approximate level of caffeine recorded to elicit an ergogenic effect in most studies, the extended timeframe of an hour and a half between intake and activity may explain the lack of effect.

Other studies show a varied effect of caffeine supplementation on performance dependent on the skill level of the athlete. Collomp et al. (1992) reported an increase in maximal swimming velocity following caffeine ingestion for trained subjects, but untrained swimmers did not experience this benefit (12).

Table 2: The Effects of Caffeine Supplementation During Anaerobic Sports

<table>
<thead>
<tr>
<th>Articles</th>
<th>Problem/Question studied</th>
<th>Participants</th>
<th>Procedures</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine potentiates low frequency skeletal muscle force in habitual and nonhabitual caffeine consumers (62)</td>
<td>To examine the involuntarily elicited and volitional neuromuscular contractile and electrical properties of skeletal muscle in men with habitual (&gt;500 mg/day) and nonhabitual caffeine</td>
<td>Twelve men with habitual (&gt;500 mg/day) and nonhabitual caffeine consumption (&lt;50 mg/day)</td>
<td>Subjects randomly assigned to Caf or Pla in double-blind study, 100 min before a 2-min tetanic stimulation of the common peroneal nerve - 2 trials each of 20 and 40 Hz</td>
<td>Caffeine potentiated the force of contraction during the final minute of the 20-Hz stimulation with no effect of habituation; no effect of Caf on 40-Hz stimulation strength nor maximal voluntary contraction or peak twitch torque</td>
</tr>
<tr>
<td>Multiple Effects of Caffeine on Simulated High-Intensity Team-Sport Performance (61)</td>
<td>To investigate effects of caffeine in a performance test simulating physical and skill demands of a rugby union game</td>
<td>Nine high-level amateur rugby union players</td>
<td>double-blind, randomized, crossover design - Ingested either Caf or Pla 70 min before test (seven circuits in each of two 40-min halves with 10-min half-time rest)</td>
<td>sprint speeds; first-drive power; passing accuracy all improved with Caf</td>
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</tr>
<tr>
<td>Caffeine increases time to fatigue by maintaining force and not by altering firing rates during submaximal isometric contractions (51)</td>
<td>To examine single motor unit firing rates during intermittent submaximal contractions and to determine whether administering caffeine increased time to fatigue (Tlim) by maintaining higher firing rates</td>
<td>Ten male volunteers</td>
<td>randomized, double-blind, repeated measures - 2 separate days 50% max voluntary contractions of quads to Tlim, 1 h after ingesting Caff or Pla</td>
<td>Rates didn't decline during intermittent contractions in control condition and the increase in Tlim in Caff couldn't be explained by firing rates</td>
</tr>
<tr>
<td>Effect of caffeine on neuromuscular function (44)</td>
<td>To examine the effects of caffeine on neuromuscular function</td>
<td>Eleven male volunteers</td>
<td>10 x 1-ms stimulation of the tibial nerve to elicit maximal voluntary contraction of the right knee extensors, six brief submax contractions and 50% MVC held to fatigue</td>
<td>Voluntary activation at MVC increased, but there was no change in H-reflex amplitude - Caff increased max voluntary activation at a supraspinal level</td>
</tr>
<tr>
<td>Caffeine lowers perceptual response and increases power output during high-intensity cycling (24)</td>
<td>To determine the effects of caffeine ingestion on a preloaded protocol</td>
<td>Eleven male cyclists</td>
<td>2 min constant rate of 100% max power output followed by 1-min all out effort - conducted test after consuming placebo or caffeine</td>
<td>Caffeine ingestion didn't affect heart rate or RPE during 12-min warmup. Moderate amount of caffeine can have ergogenic effect during last min of preloaded cycle test in trained cyclists</td>
</tr>
<tr>
<td>Benefits of caffeine ingestion on sprint performance in trained and untrained swimmers (12)</td>
<td>To determine whether specific training (trained vs. untrained) is necessary to produce benefits from acute ingestion of caffeine by improving maximal anaerobic capacity during a spring swimming test</td>
<td>Fourteen subjects - 7 trained subjects and 7 untrained subjects</td>
<td>each subject swam on two different occasions 2 x 100 m distances of freestyle at maximum speed and separated by 20 min passive recovery, once after ingestion of Caf (250 mg) and once after placebo</td>
<td>Benefits of the ingestion of caffeine during supramaximal exercise requiring high anaerobic capacity observed in trained subjects</td>
</tr>
</tbody>
</table>
The acute effects of a caffeine-containing supplement on strength, muscular endurance, and anaerobic capabilities (2)

To examine the acute effects of a caffeine-containing supplement on upper and lower body strength and muscular endurance and anaerobic capabilities

Thirty-seven resistance-trained men

Subjects randomly assigned to supplement group or placebo group - after 1 hr of ingestion, subjects performed 2 Anaerobic Tests and 1RM and muscular endurance tests

Significant increase in 1RM for Supp group; caffeine-containing supplement may be effective supplement for increasing upper-body strength (no effect on lower-body strength, muscular endurance, anaerobic capabilities or upper-body muscular strength) and could be beneficial for competitive/recreational athletes performing resistance training.

Effects of Caffeine on Prolonged Intermittent-Sprint Ability in Team-Sport Athletes (60)

To examine the effects of acute caffeine ingestion on prolonged intermittent-sprint performance

10 male team-sport athletes

Two exercise trials, separated by 7d, 60 min after ingestion of either 6 mg/kg caff or placebo - trials performed on cycle ergometer 2 x 36-min halves, each composed of 18 x 4-s sprints with 2-min active recovery at 35% VO2peak between sprints

Acute caffeine ingestion can significantly enhance performance of prolonged, intermittent-sprint ability in competitive, team-sport male athletes

Effects of caffeine ingestions on performance and anaerobic metabolism during the Wingate test (13)

To determine the effects of caffeine ingestion on performance and metabolic responses during supramaximal exercise

Six healthy volunteers

60 min before each trial (two Wingate Anaerobic Tests), ingested 5 mg/kg caffeine or placebo - single blind, randomized procedure

Caffeine modifies glycolytic metabolism but fails to improve performance during Wingate Anaerobic Test in healthy subjects

A limited number of studies study the effect of caffeine supplementation which incorporates aspects of aerobic activities, anaerobic activities as well as a skill component. To-date, there are two studies that observe the effect of caffeine ingestion on tennis performance, a sport containing these aforementioned qualities. In one of these studies using a group of male tennis players, caffeine ingestion prior to activity enhanced serve velocity and attenuated the effects of fatigue (35). The study by Ferrauti et al.
(1997), found that caffeine didn’t significantly affect RPE or hitting accuracy, however female subjects won more games during the caffeine treatment (26).

<table>
<thead>
<tr>
<th>Articles</th>
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<th>Participants</th>
<th>Procedures</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine, carbohydrate, and cooling use during prolonged simulated tennis (35)</td>
<td>To determine the effects of prolonged simulated tennis on performance and the ergogenic potential of caffeine, carbohydrates, and cooling.</td>
<td>12 highly trained male tennis players (18.3 years old; +/-3 years)</td>
<td>4 simulated matches against a ball machine. Experimental trials included caffeine supplementation (3 mg/kg), CHO supplementation (6% solution), pre-cooling and intermittent cooling, and placebo.</td>
<td>Caffeine increased serve velocity in the final set of the match compared with placebo and CHO conditions. Caffeine also partly attenuated effects of fatigue</td>
</tr>
<tr>
<td>Metabolic and ergogenic effects of carbohydrate and caffeine beverages in tennis (26)</td>
<td>To investigate the metabolic and ergogenic effects of carbohydrate and caffeine concentrations</td>
<td>8 male &amp; 8 female tournament players</td>
<td>Three double-blind occasions, subjects ingested placebo, carbohydrate or caffeine drink at court changeover and during the resting period during 4 hours interrupted tennis match. Men (women) total intake was 2.8 l (2.0 l) fluid, supplemented with 243 g (182 g) carbohydrates or with 364 mg (260 mg) caffeine. Post-exercise, players performed a ball-machine test</td>
<td>RPE and hitting accuracy were not affected by treatment. Under caffeine, women won significantly more games than during other treatments.</td>
</tr>
</tbody>
</table>

Studies have observed the effect of caffeine ingestion on performance during aerobic and anaerobic activities by incorporating a variety of exercise modes. The majority of these studies used a cycling or running protocol. There are other studies using a swimming protocol as well as one study that observed the ergogenic effect of caffeine during a game of rugby that enhanced the subjects’ sprint speeds, drive power and passing performance (61).
The caffeine dosage also fluctuated throughout each study providing information relative to the optimal level of caffeine to be ingested in order to enhance performance. Studies have recognized notable ergogenic benefits when ingesting doses around 5-6mg/kg of caffeine for both aerobic and anaerobic performances, while lower or higher dosages have fewer instances in which an enhanced performance is recognized. A study by Graham et al. (1995), using a treadmill protocol observed that supplementation with 9 mg/kg caffeine did not further improve performance as compared to the 3 and 6 mg/kg doses, while the 6 mg/kg dosage elicited the greatest performance enhancement (31).

Few studies exist supporting the effect of habitual caffeine intake on the ergogenic effect of caffeine. Bell et al. (2002) found that both duration and magnitude of exercise are enhanced following 5 mg/kg caffeine in nonusers versus habitual caffeine users (5). Another study found that caffeine administered to low to moderate caffeine-consuming athletes was not associated with neuromuscular or metabolic changes that could be considered ergogenic to running performance (63). This could be due to the subjects’ tolerance to habitual caffeine consumption.

Table 4: The effect of various modes of exercise, dosages and frequency of caffeine intake that effect the ergogenic effect of caffeine during aerobic or anaerobic activities

<table>
<thead>
<tr>
<th>Articles</th>
<th>Problem/Question studied</th>
<th>Participants</th>
<th>Procedures</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ergogenic Effects of Low Doses of Caffeine on Cycling Performance (43)</td>
<td>To learn whether low doses of caffeine have ergogenic, perceptual, and metabolic effects during cycling</td>
<td>Thirteen male cyclists</td>
<td>Refrained from exercise 24h &amp; caffeine for 48h before testing. Ingested either placebo or one of 3 treatments (1,2 or 3 mg/kg caffeine). After 60 min, cycled at 80% VO2peak followed by 15 min of cycling at all-out effort</td>
<td>Caffeine doses of 2 and 3 mg/kg enhance cycling performance under these conditions.</td>
</tr>
<tr>
<td>Subject</td>
<td>Description</td>
<td>Participants</td>
<td>Protocol</td>
<td>Conclusion</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
<td>--------------</td>
<td>----------</td>
<td>------------</td>
</tr>
<tr>
<td>Physiological responses to caffeine during endurance running in habitual caffeine users (63)</td>
<td>To describe the effects of caffeine ingestion on the neuromuscular and metabolic responses to endurance running in subjects who were low-mod-intake habitual caffeine consumers</td>
<td>Six varsity level male runners</td>
<td>6-d of preparation, then test day: 2h after consumption of meal arrived. Measurements of maximal voluntary isometric strength and evoked peak twitch torque or the right knee extensors. Subjects then ran for 90 min on a treadmill at 70% VO2max</td>
<td>Caffeine admin in low-mod habitual caffeine-consuming athletes was not associated with neuromuscular or metabolic changes that could be considered ergogenic to endurance running performance - could be due to tolerance to habitual caffeine consumption.</td>
</tr>
<tr>
<td>Metabolic and exercise endurance effects of coffee and caffeine ingestion (29)</td>
<td>The impact of ingestion of the same dose of Caf in coffee consumption or in water</td>
<td>Nine adults</td>
<td>Ingested capsule (Caf or placebo) with water or Caf (decaff, decaff with Caf added, or reg cof); completed 5 trials running to exhaustion on treadmill at 85% VO2max - 1 week between trials</td>
<td>Caffeine ingested in association with coffee does not alter bioavailability of caffeine and fails to enhance endurance.</td>
</tr>
<tr>
<td>Caffeine and Exercise Performance: metabolism, endurance and performance (30)</td>
<td>To review existing literature regarding caffeine and athletic performance</td>
<td>A review of literature</td>
<td>A summarization of existing literature</td>
<td>Caffeine ingestion prior to exercise often improves performance during prolonged endurance cycling/running &amp; short-term intense cycling &amp; decrease swim time for 1500m. These results are reported in well-trained athletes but field studies are lacking to confirm effects in the athletic world</td>
</tr>
<tr>
<td>Exercise endurance 1,3, and 6 h after caffeine ingestion in caffeine users and nonusers (5)</td>
<td>To examine the duration of caffeine's ergogenic effect and whether it differs between users and nonusers of the drug.</td>
<td>Twenty-one subjects</td>
<td>Six randomized exercise rides to exhaustion at 80% of maximal oxygen consumption after ingesting either a placebo or caffeine - 1,3, or 6 h after placebo or drug ingestion</td>
<td>Exercise times 1 and 3 h after caffeine intake greater than placebo trials; both duration and magnitude of ergogenic effect following 5 mg/kg caffeine greater in nonusers compared with users.</td>
</tr>
</tbody>
</table>
Caffeine is metabolized mainly by cytochrome P450 1A2 (CYP1A2) (15) and the rate of metabolism varies between individuals (34). Studies have shown the presence of a C/A polymorphism in intron 1 of the CYP1A2 gene (57), with a slower caffeine metabolism following caffeine ingestion in individuals possessing the C allele (15,57). For subjects possessing the C allele, caffeine intake is associated with increased risk of nonfatal myocardial infarction, unlike those individuals homozygous for the CYP1A2*1A allele (15). According to Womack et al. (2009), subjects homozygous for the A variant of the studied polymorphism had a more prominent ergogenic effect for a cycling time trial during the caffeine trial than subjects possessing the C variant (67), suggesting AA homozygotes have an elevated caffeine response.
**Table 5: Genetic Variability in Response to Caffeine Supplementation as an Ergogenic Aid**

<table>
<thead>
<tr>
<th>Articles</th>
<th>Problem/Question studied</th>
<th>Participants</th>
<th>Procedures</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional significance of a C → A polymorphism in intron I of the cytochrome P450 CYP1A2 gene tested with caffeine (57)</td>
<td>To determine how much of the variability of CYP1A2 activity is due to a newly discovered gene polymorphism in intron I</td>
<td>185 healthy Caucasian non-smokers and 51 smokers</td>
<td>1. Using DNA sequence analysis by genotyping and phenotyping using 100 mg oral dose of caffeine immediately following emptying of the bladder and collecting urine for the subsequent 5 hours. Urine concentrations of caffeine (137X) and the CYP1A2 catalyzed metabolites 1,7-dimethylxanthine (17X) and 1,7-dimethyluric acid (17U) was quantified by h.p.l.c. the urine ratio was used as the index of CYP1A2 activity 2) 51 smokers received 100 mg oral dose of caffeine and venous blood sample take 5 hours after administration. Molar metabolic concentration ration 17X/137X was used as the index of CYP1A2 activity.</td>
<td>The A/A genotype, which could represent a CYP1A2 high inducibility genotype, may be a direct cause of increased CYP1A2 activity or genetically linked to polymorphisms giving high inducibility.</td>
</tr>
<tr>
<td>Genetic polymorphism of CYP1A2 increases the risk of myocardial infarction (16)</td>
<td>To determine the effects of CYP1A1 and CYP1A2 genotypes on risk of myocardial infarction and whether smoking interacts with genotype to modify risk.</td>
<td>873 subjects with a first acute non-fatal MI and population based controls (932)</td>
<td>Subjects matched for age, sex, and area of residence were genotyped for CYP1A1<em>2A and CYP1A2</em>1F by restriction-fragment length polymorphism (RFLP)-PCR, and smoking status was identified by questionnaire.</td>
<td>The low inducibility genotype for CYP1A2 was associated with an increased myocardial infarction, which was unrelated to smoking status and suggests that a substrate of CYP1A2 that is detoxified rather than activated may play a role in CHD.</td>
</tr>
<tr>
<td>Cytochrome P450 IA2 activity in man measured by caffeine metabolism: effect of smoking, broccoli and exercise (65)</td>
<td>To investigate factors influencing the enzymes - cytochrome P450IA2, N-acetyl transferase and xanthine oxidase, measured by the metabolite ratios of dietary caffeine.</td>
<td>1) Cross-sectional: Urine samples collected from 335 healthy volunteers - 171 women 2) Longitudinal: urine samples collected from 23 men before and after 30 days with vigorous exercise, 8 hours per day 3) Longitudinal: urine samples collected from 9 healthy subjects after two 10-day periods with a diet supplemented with 500 g green beans or 500 g broccoli</td>
<td>1) Cross-sectional: Urine samples collected from 335 healthy volunteers - 171 women 2) Longitudinal: urine samples collected from 23 men before and after 30 days with vigorous exercise, 8 hours per day 3) Longitudinal: urine samples collected from 9 healthy subjects after two 10-day periods with a diet supplemented with 500 g green beans or 500 g broccoli</td>
<td>The ratio reflecting P450IA2 activity normally distributed. With smokers, ratio was 66% higher for male and 71% higher for female as compared to non-smokers. Ratio reflecting the XO-activity was normally distributed and smoking increases XO activity. 30 days of vigorous exercise increased the IA2 ratio by 58%, NAT-ratio remained unchanged. IA2 ratio increased with bean and broccoli supplementation and 19% induction of P450IA2 activity by broccoli.</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Plasma caffeine metabolite ratio (17X/137X) in vivo associated with G-2964A and C734A polymorphisms of human CYP1A2 (34)</td>
<td>To observe the association between phenotypes and genotypes of CYP1A2 with respect to the two genetic polymorphisms.</td>
<td>163 healthy Chinese volunteers living in Qidong</td>
<td>The ratio of plasma 17X/137X at 6 hours after orally ingesting 300 mg caffeine. Genotyping analysis conducted by polymerase chain reaction-restriction fragment length polymorphism.</td>
<td>The allele frequencies of A at 2964 were 0.25 and A at 734 was 0.67 in 139 non-smokers. The A/A-2964, C/C-734, G/A-2964, C/C-734, A/A-2964, C/A-734 genotype that was believed to have lower activity of CYP1A2 than other genotypes did not exist in the subjects. The G-2964 and A734 was associated with the high activity of CYP1A2 in non-smokers and smokers, using the caffeine ratio of 17X/137X.</td>
</tr>
<tr>
<td>Coffee, CYP1A2 Genotype, and Risk of Myocardial Infarction (15)</td>
<td>To determine whether CYP1A2 genotype modifies the association between coffee consumption and and risk of acute nonfatal MI</td>
<td>2014 cases with a first acute nonfatal MI and population-based controls living in Costa Rica, matched for age, sex, and area of residence.</td>
<td>Subjects were genotyped by restriction fragment-length polymorphism polymerase chain reaction. Food frequency questionnaire sued to measure caffeinated coffee consumption.</td>
<td>55% of subjects and 54% of controls were carriers of the slow *1F allele. Intake of coffee associated with an increased risk of nonfatal MI only among individuals with slow caffeine metabolism.</td>
</tr>
</tbody>
</table>
The Influence of a CYP1A2 Polymorphism on the Ergogenic Effects of Caffeine (67)

To determine if a single nucleotide polymorphism (C/A) in the DNA sequence of the CYP1A2 gene influences the ergogenic effect of caffeine supplementation.

24 trained cyclists

1 h following ingestion of 6 mg/kg of anhydrous caffeine or placebo (administered in a double-blind fashion), subjects completed two short-duration tests to fatigue (150% of VO$_{2\text{peak}}$) and two 40k time trials on a cycle ergometer. DNA obtained from whole blood samples and analyzed for presence or absence of C variant.

Possession of the C variant negatively impacts the ergogenic effect of caffeine on cycling performance.
Chapter III

Methodology

Subjects

Twenty NCAA Division I tennis players (10 male; 10 female) (18-24 yrs), participating in daily tennis training and competition on the same tennis team, volunteered as subjects for this study. Two subjects did not perform consistent training during the testing sessions (due to injuries) and two additional subjects were unable to complete the genotyping, and thus were dropped from the study results. Preliminary statistical analyses were performed on the remaining 16 subjects. Subject demographics are provided in Table 6.

It was estimated that 10 of these subjects would be C allele carriers by observing the allele allotment in past literature (15). Permission to recruit subjects from the tennis team was provided by the head coach. Prior to the start of the study, all subjects signed an inform consent form (Appendix I) and completed a Health Status Questionnaire (Appendix II). In addition, habitual caffeine intake was self-reported by subjects, by completing a second form, Caffeine Habits & Frequency Questionnaire (Appendix III) to determine their average weekly intake of coffee, tea, soda, chocolate, and other caffeinated beverages and/or food. Typical milligram doses were assigned to each subject according to Mayo Clinic (50) and an approximate daily intake was obtained. Based on previous criteria (33), subjects were then characterized as having low (0-150 mg/day), moderate (151-300 mg/day) and high (> 300 mg/day) caffeine intake.
Subjects had no known injuries that would impair tennis performance. The James Madison University Institutional Review Board reviewed and approved all screening materials and experimental design prior to the beginning of data collection.

**Testing Procedures**

**Maximal Exercise Test:** Subjects ran at a self-selected pace between 6.0-7.0 mph on the treadmill at 0% grade for 1 minute. Every subsequent minute, grade was increased until the subject reached volitional fatigue. Oxygen uptake was monitored continuously via a metabolic measurement system (Sensormedics Vmax: Yorba Linda, CA). Heart rate was monitored using a Polar Heart Rate Monitor (Lake Success, NY). Results from this test were used to determine appropriate exercise intensity for testing protocols.

**Exercise Tests:** A familiarization trial for the exercise tests was conducted prior to actual testing to enhance validity. Subjects completed two separate exercise tests consisting of a treadmill test and a tennis skills test (TST). The tests were performed in the morning following a 12 hour fast. The participants refrained from consumption of caffeine-containing food and beverages for 24 hours prior to the tests. One hour prior to testing, subjects ingested a capsule containing either 6 mg of anhydrous caffeine per kilogram of body weight or placebo (white flour), administered in double-blind fashion. All tests were separated by a minimum of 48 hours.

Tennis is a repetitive sprint sport, with medium to high aerobic and anaerobic demands (25) and average exercise intensities ranging between approximately 60-70% VO$_{2\text{max}}$ (45). Therefore, intensities above and below this range were used during the treadmill tests to mirror the physiological responses to realistic tennis match play.
The treadmill portion of the exercise test consisted of 15 minutes of a warm-up at a speed corresponding to 50% \( \text{VO}_{2\text{max}} \) followed by 30 minutes of intermittent sprints. Tennis match play involves intermittent exercise bouts that alternate between short periods of high intensity of an average of 4-10 seconds and short recovery periods ranging between 10-20 seconds (25). Therefore, to match the intermittent nature of the sport, subjects completed sprints for 5 seconds at a high intensity (80% \( \text{VO}_{2\text{max}} \)), followed by 15 seconds of low intensity (50% \( \text{VO}_{2\text{max}} \)) jogging between each sprint – a work to rest ratio of about 1:3. Although the duration of play for each single point, game and match for a game of tennis is difficult to approximate due to differences in style of play, sex and level of play (25), the protocol was organized such that each sprint-jog sequence represented 1 point; with 6 sprint-jog sequences representing 1 game of tennis.

According to the International Tennis Federation (ITF), the official time between a change-over is 90 seconds (37) and the first change-over occurs after the first three games have been played. Thereafter, a change-over would occur every two games. As every 6 sprint-jog sequence represented 1 single game, the sprint-jog sequence was repeated eighteen times (3 games) followed by a 90 second rest period. Subsequently, this sprint-jog sequence was repeated twelve times, followed by 90 seconds of rest as it represented two games of tennis. This protocol was repeated for 30 minutes.

120 seconds following the treadmill portion of the exercise test, the TST was administered. The 120 second rest represented the official allowable rest time between tennis sets (37). The test is organized by sets of six different shots to emulate a single game within a set of tennis. As the average number of shots during a single point of tennis match play is highly variable, a drill that involves a variety of shots - 4 ground
strokes (the stroke used the most within a tennis match), 1 approach shot and 1 volley closely embodies the skill component of a tennis match. After each set of 6 balls, the subject actively rested at the baseline for 20 seconds, which represents the time allowed for rest between tennis points (37). The 6-ball drill was repeated 6 times, which represents a typical duration and number of balls for 1 game within a set of a realistic tennis match. Every two sessions, the player had a 90 second break, the official time between a change-over during a match (37). The athlete underwent 9 sessions, which represents 1 set of a typical tennis match. The maximum number of successful shots for the TST was 324. A successful shot was defined as each shot that landed in bounds. Each subject was then given a raw score out of a possible 324. The key below simplifies the terminology used to describe the tennis skills test:

- 6 ball drill = 1 point of a typical match
- 6 repetitions of the 6-ball drill = 1 game of a typical match (6 reps of 6-ball drill = 1 session)
- 9 sessions = 1 set of a typical match
- 324 – maximum score

Tennis does not have time limits on matches, leading to high variability between lengths of any match. In order to effectively emulate a realistic tennis match situation, a provisional time of 1.5 hours of exercise testing was used as this is a typical average match length (97). The treadmill portion of the testing lasted 45 minutes, followed by an approximate length of 45 additional minutes of the tennis skills test.

Polar heart rate monitors were worn by all subjects during the exercise tests to obtain individual heart rates. Rating of Perceived Exertion (RPE) was also obtained using the Borg RPE scale (Appendix IV). During the treadmill portion of the test, heart rate and RPE was recorded upon completion each sprinting sequence for a total of five
measurements. A tracking sheet was used to denote each sprint-jog and rest sequence for each subject along with their heart rate and RPE at the end of each resting period. The subjects’ respective running speeds, that were calculated according their VO$_{2\text{max}}$ scores were also recorded (Appendix V).

Heart rate and RPE was recorded after every two sessions throughout the tennis test and a final reading after the final session, for a total of four times during the tennis skills test. Furthermore, a score sheet was used during the tennis tests to quantify successful shots (Appendix VI). A timeline for data collection used for each trial are provided in Table 7. To maintain consistency during testing, subjects were tested indoors to mitigate environmental factors. Furthermore, ball feeding was conducted by a highly skilled tennis coach, for each test to maintain reliability.

Table 7-Data Collection Timeline

<table>
<thead>
<tr>
<th>Testing Day 1</th>
<th>48 h</th>
<th>Testing Day 2</th>
<th>48 h</th>
<th>Testing Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Consent Form</td>
<td>1. Ingest pill (placebo or caffeine); wait 45 minutes</td>
<td>1. Ingest pill (placebo or caffeine); wait 45 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Caffeine Intake Form</td>
<td>3. 1 hour after pill ingestion, commence testing:</td>
<td>3. 1 hour after pill ingestion, commence testing:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. VO$_{2\text{max}}$ testing</td>
<td>• Treadmill Testing - 45 minutes</td>
<td>• Treadmill Testing - 45 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• 120 second transition</td>
<td>• 120 second transition</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Tennis Skills Testing - 45 minutes</td>
<td>• Tennis Skills Testing - 45 minutes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Genotyping: Investigators were blinded to genotype until all subjects completed the study. Furthermore, all genotyping was performed by an investigator not involved with the performance testing. DNA was obtained from whole blood samples via a QiaAmp mini-blood kit (Qiagen Inc.; Valencia, CA). Genotyping was performed using restriction
fragment length polymorphism-polymerase chain reaction (RFLP-PCR), as previously described (reference). Briefly, DNA was PCR amplified using the HotStar DNA Polymerase Kit (Qiagen) with the forward primer (5’-CAACCCCTGCAATCTCAAGCAC-3’) and reverse primer (5’-AGAAGCTCTGTGGCGAGAAGG-3’) to generate a 920 bp fragment of the CYP1A2 gene. PCR conditions consisted of an initial denaturation at 95°C for 5 minutes, followed by 39 cycles at 94°C for 15 seconds, 64.5°C for 1 minute, and 72°C for 1 minute, with a final elongation step of 72°C for 10 minutes. One half of each PCR product was digested using the restriction enzyme ApaI (New England Biolabs, Ipswich, MA) as per manufacturer’s instructions. Digested and undigested PCR products were evaluated in parallel via electrophoresis in a 2% agarose gel stained with ethidium bromide, and DNA bands were visualized by UV light. The presence of a 920 bp fragment following ApaI digestion identified the A/A genotype, while the presence of 709 bp and 211 bp fragments following ApaI digestion identified the C/C genotype. Because of the infrequency of individuals who are homozygous for the C variant, and because of prior research from our laboratory suggests that subjects were grouped as AA homozygotes and C allele carriers.

Statistical Analyses

Statistical analyses were performed using SPSS 16.0 (Thomson Learning, Pacific Grove, CA). Descriptive data (height, weight, age, VO$_{2\text{max}}$) was compared between groups using independent t-tests.

Potential differences in ball consistency, HR and RPE were assessed using Repeated Measures Analysis of Variance (RMANOVA) with treatment (caffeine,
placebo) as the within-subjects factor and genotype (AA homozygotes, C allele carriers) and gender (male, female) as the between-subjects factors. For all RMANOVA procedures, post-hoc tests were performed using independent and dependent t-tests with a Bonferroni correction. Significance was measured at an alpha level of p < 0.05.
Chapter IV

Results

Seven of the 16 subjects were homozygous for the A variant (4 female, 3 male). Descriptive characteristics of the two genotype groups are shown in Table 6. There were no significant differences (P > 0.05) between the two groups for height, weight, age, VO$_{2\text{max}}$, or caffeine intake. In AA homozygotes, all 7 subjects were categorized as having low caffeine intake (0-150 mg/day), 0 subjects as moderate (151-300 mg/day) and 0 as high (> 300 mg/day). Two out of the 9 C allele carriers had moderate intake with seven characterized as having low intake.

Table 6-Mean Subject Demographics

<table>
<thead>
<tr>
<th></th>
<th>A/A (N=7)</th>
<th>C (N=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (N=3)</td>
<td>Female (N=4)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>186.8 ± 2.97</td>
<td>165.4 ± 9.53</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.3 ± 4.69</td>
<td>62.6 ± 13.31</td>
</tr>
<tr>
<td>Age (y)</td>
<td>20.7 ± 3.06</td>
<td>21.0 ± 1.15</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$ (ml/kg/min)</td>
<td>53.2 ± 1.34</td>
<td>48.2 ± 4.71</td>
</tr>
<tr>
<td>Caffeine intake (mg/day)</td>
<td>104.6 ± 34.09</td>
<td>103.9 ± 38.82</td>
</tr>
</tbody>
</table>

Initial analysis revealed no main effect for gender or any Gender x Treatment interaction for the skills test; therefore results for both genders were pooled for statistical analysis. Caffeine significantly (p=0.029) improved performance during the Tennis Skills Test - a 1.77% improvement over the placebo treatment. Figure 1 displays the mean total shot successes during the Tennis Skills Test. There was no genotype effect on tennis performance (p=.454), regardless of gender.
Table 8 displays the mean RPE and heart rate values during the exercise tests. In addition, specific RPE values during the treadmill and Tennis Skills Tests are displayed in Figure 2. There was not a main effect for treatment on RPE during the treadmill portion of the test (RPE in caffeine trial=11.28 ± 1.61; RPE in placebo trial=11.52 ± 1.61). Similarly, there were no significant main effects or interaction effects for RPE following ingestion of caffeine during the Tennis Skills Test (TST) (TST in caffeine trial= 11.36 ± 1.80; TST in placebo trial = 11.13 ± 1.65).
Table 8: mean RPE and HR (mean ± SD) during the treadmill and Tennis Skills tests divided between genotype and gender.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Gender</th>
<th>Caffeine - Treadmill</th>
<th>Placebo - Treadmill</th>
<th>Caffeine - Tennis Skills Test</th>
<th>Placebo - Tennis Skills Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPE</td>
<td>AA</td>
<td>Female</td>
<td>9.7 ± 1.65</td>
<td>9.35 ± 1.54</td>
<td>10.25 ± 2.55</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>Male</td>
<td>12.07 ± 1.21</td>
<td>12.00 ± .87</td>
<td>13.07 ± 2.14</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Female</td>
<td>11.65 ± .84</td>
<td>11.50 ± .74</td>
<td>11.1 ± .66</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Male</td>
<td>11.84 ± 1.90</td>
<td>12.96 ± 1.01</td>
<td>11.96 ± 96</td>
</tr>
<tr>
<td>HR</td>
<td>AA</td>
<td>Female</td>
<td>156.00 ± 12.78</td>
<td>167.1 ± 10.38</td>
<td>160.9 ± 24.37</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>Male</td>
<td>137.87 ± 4.94</td>
<td>139.47 ± 4.08</td>
<td>144.8 ± 9.4</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Female</td>
<td>146.75 ± 24.44</td>
<td>148.65 ± 22.61</td>
<td>147.8 ± 21.11</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Male</td>
<td>152.92 ± 8.01</td>
<td>155.96 ± 9.27</td>
<td>157.48 ± 14.60</td>
</tr>
</tbody>
</table>

Figure 2: Average RPE During Exercise
A significant gender effect existed during both the treadmill portion of the testing (p=0.020) as well as the TST (p=0.027) for RPE, with women exhibiting a lower RPE than men in both situations.

Although there was not a main effect for Treatment on heart rate during the treadmill portion of the tests, there was a trend toward Genotype x Treatment effect (p=.056). On average, caffeine elevated peak heart rate in AA homozygotes (Caffeine = 154.6 ±17.5 beats/min, Placebo = 148.9 ± 12.9 beats/min) and decreased peak heart rate for C allele carriers (Caffeine = 150 ±16.3 beats/min, Placebo = 152.7 ± 15.8 beats/min).

There was a trend toward Gender x Treatment effect (p=.065) for heart rate during the treadmill portion of the tests. For both treatments, female subjects had a higher mean heart rate as compared to male subjects. More specifically, following caffeine supplementation, female subjects had a mean heart rate of 156.9 ± 5.0 beats/min while male subjects had a mean heart rate of 145.4 ± 5.2 beats/min. Under the placebo treatment, the mean heart rate for female subjects was 152.3 ± 5.0 beats/min while male subjects had a mean heart rate of 147.7 ± 5.2 beats/min. Additionally, there was a trend toward a Genotype x Gender effect (P=0.056) for heart rate during the treadmill portion of the test. There was a noticeable elevation in heart rate (about 11 beats/min) in AA homozygote female subjects.

During the Tennis Skills tests, there was also a trend toward a Genotype x Gender effect (P=0.070) in which heart rate was augmented in AA homozygote female subjects. Mean heart rate response during the treadmill portion of the test and during the tennis skills test following ingestion of either caffeine or placebo are displayed in Figure 3. Although there was a trend toward an increased heart rate following caffeine
supplementation for the exercise tests in AA homozygote female subjects, there was no significant difference in the heart rate response between the ‘caffeine’ and ‘placebo’ trials.

Figure 3: Average HR Response During Exercise
Chapter V

Discussion

Previously published research has clearly established caffeine as an ergogenic aid for aerobic activities (4,5,8,9,14,18,20,27,30,32,39,41,46,47,48,56,58,60,64,66,68), conversely, the effect of caffeine relative to anaerobic activities indicates a variable response (13, 46). The findings on the effect of caffeine on tennis, a sport that combines aspects of aerobic and anaerobic activities has also been equivocal. A study by Hornery et al. (2007) included highly trained male subjects who performed simulated tennis matches against a ball machine. Caffeine was shown to partly attenuate the effects of fatigue and increased serve velocity (35). On the contrary, a study by Ferrauti et al. (1997) included male and female subjects who ingested caffeine during a match. Results of this study indicated that caffeine did not positively affect perception ratings or hitting accuracy (26).

In the current study, caffeine significantly improved performance during the Tennis Skills test. These results support the Hornery (2007) study in that caffeine can enhance tennis performance. However, the current study observed significant ergogenic effects with a variety of shots used throughout a tennis match (groundstrokes; approach shot; volley) versus ergogenic effects concentrated to service speed (35).

To our knowledge, this is the first study to examine the influence of a genetic polymorphism on the ergogenic effect of caffeine supplementation during tennis performance. The major findings of the present study are twofold. There was a significant ergogenic effect of caffeine on tennis performance (p=0.029) for both male and female collegiate tennis players. Furthermore, subjects homozygous for the A
variant of the polymorphism did not experience a more prominent ergogenic effect from caffeine than individuals possessing the C variant during tennis, as originally hypothesized.

A prominent mechanism for enhanced performance detected with caffeine supplementation involves stimulation of the central nervous system. Caffeine has been identified to block adenosine receptors and interact with transmission of dopamine, thereby inhibiting the negative effects adenosine induces on neurotransmission; arousal and pain perception (52,59). One consistent outcome of caffeine ingestion during exercise testing, regardless of mode, intensity, or duration of exercise, is an alteration in the subjects’ perceptual response (24). The hypoalgesic effects of caffeine result in dampened pain perception and reduced perceived exertion during exercise (53). Other potential effects are decreased firing rates of motor units and possibly more sustainable and forceful muscle contractions (19) at a given rating of perceived exertion or effort sense (38,11,54). More typically, the alteration in participants’ perceptual response has manifested itself as a reduced RPE at constant exercise intensities (17,28,10,49,23,24,9).

For both male and female subjects during both the treadmill portion of the test and TST, there were no differences in RPE (p>0.05) among treatments despite the fact that caffeine trials elicited enhanced performance during the Tennis Skills test (p=0.029). Consequently, it would appear that the subjects perceived identical physiological effort despite a higher performance or were able to recruit more motor units at the same perceived effort level following caffeine supplementation (40). This suggests that caffeine may have impacted perception of effort in the present study. Conversely, results indicate that caffeine did not lower RPE during the fixed intensity treadmill trial.
As noted, a significant gender effect existed during both the treadmill portion of the testing \((p=0.020)\) as well as the TST \((p=0.027)\) relative to RPE. The female subjects tended to perceive work as less strenuous as compared to the male subjects. Timing of data collection may have impacted results. Both trials for the female subjects took place during a short respite in their season. Data collection for the male subjects, conversely, was collected during a heavy training period. In other studies, subjects were instructed to refrain from other exhaustive training for 1-2 days prior to testing \((43, 46)\). This protocol could not be followed for this current study as the subjects have specific training guidelines as collegiate athletes, which may have impacted results pertaining to RPE.

Nevertheless, subjects arrived for each testing day in a similar fashion relative to muscle fatigue as data was collected during the same segment of the tennis season. Specific to RPE relative to the treadmill portion of the test, it is important to note that 3 of the 8 male subjects were novice treadmill users which may have misrepresented the RPE outcome during the treadmill portion of the tests for this group.

Despite the fact that prior studies have shown elevations in heart rate, our study did not observe a main effect for heart rate following caffeine supplementation. There was a strong trend toward gender by treatment effect \((p=0.065)\) for heart rate that was observed during the treadmill portion of the test as well as for the TST \((p=0.070)\). For both treatments, female subjects had a higher mean heart rate as compared to male subjects. Additionally, there was a strong trend toward a Treatment by Genotype effect for heart rate during the treadmill portion of the exercise tests \((P=0.056)\). These trends were observed predominately in female AA homozygote subjects following caffeine ingestion, who elicited a higher mean heart rate of about 11 beats/min as compared to the
mean heart rate for other subjects (see Figure 3). It is plausible that the small sample size of AA female homozygotes (n=4) may have inadvertently influenced both of these trends.

A recent study conducted in our laboratory (67), indicated an association between a CYP1A2 polymorphism (A/C) and the ergogenic effects of caffeine on cycling performance. Performance differences were observed following caffeine supplementation with a significant (p<0.05) improvement in a 40k time trial for AA homozygotes, but not carriers of the C variant (67). During the present study, 7 of the 16 subjects were classified as carriers of the A variant and the other 9 subjects as carriers of the C variant. It was hypothesized that the 7 AA homozygotes would elicit a greater performance enhancement following caffeine supplementation as compared to the carriers of the C variant. Contrary to the study conducted by Womack et al. (2009), our study did not indicate any difference in performance following caffeine supplementation based on subject genotyping (67).

The inability to ascertain a significant genotype by treatment interaction for either the treadmill portion of the test or Tennis Skills test could be related to the variations between protocols. As the skill component during the Tennis Skills test is variable between subjects, this may have resulted in a Type II error. As evidenced in existing literature and supported by the current study, the genetic influence appears to be modeselective. This may indicate that the genetic influence is more pronounced for endurance activities and less so for intermittent activities with a strong skill component.

Data from our study provides evidence that caffeine significantly and practically enhances tennis performance although no genotype effect exists, for the CYP1A2
polymorphism. As the current study analyzed the ergogenic effect of caffeine on tennis performance in terms of average shot successes in a Tennis Skills test, future studies should also analyze optimal timing of caffeine ingestion or a multiple dose effect of caffeine throughout a Tennis Skills test that may elicit additional ergogenic benefits.
Appendix I

James Madison University

School of Kinesiology

Consent for Investigative Procedure

Identification of Investigators & Purpose of Study
You are being asked to participate in a research study conducted by Courtney S. Klein from James Madison University. The purpose of this study is to determine if caffeine enhances tennis performance as well as to determine if genetics explain improvement in tennis skill following caffeine supplementation.

Potential Risks & Benefits
The investigator perceives the following are possible risks arising from your involvement with this study
If you choose to participate in this study, you will perform three separate exercise tests on the treadmill. Two treadmill tests will be followed by a tennis skills test performed on a tennis court. The investigator perceives the following are possible risks arising from your participation in the study: nausea, discomfort, dizziness, and in rare occurrences, heart attack, stroke or death. The selection criteria used to obtain participants and the “Health Status Questionnaire” are intended to mitigate these risks. In healthy individuals, the risk of death during vigorous exercise has been estimated at 1 death per year for every 18,000 individuals.

Potential benefits from participation in this study include:

1) Helping researchers determine the impact of caffeine supplementation on tennis performance.
2) Helping researchers understand the role genetics may play in determining the effectiveness of caffeine supplementation.
3) Knowledge of your maximal aerobic capacity (VO$_{2\text{max}}$)
4) Knowledge of whether you are a responder to caffeine supplementation.

Research Procedures
Should you decide to participate in this research study, you will be asked to sign this consent form once all your questions have been answered to your satisfaction. This study consists of three separate exercise tests performed on a treadmill, two of the three treadmill tests to be followed by a tennis skills test performed on a tennis court. Furthermore, you will be asked to recall your consumption of caffeinated beverages for the previous week. All treadmill tests will be separated by at least 48 hours, so that you will be tested three times over a one to two week period, for a total of approximately 5 hours of testing. The first treadmill test will be performed to volitional exhaustion, meaning that you will exercise until you feel you can no longer continue. You will also
be breathing through a mouthpiece during all of the exercise tests so that we can analyze your expired air to determine how much oxygen you are using. Your heart rate will be monitored by a monitor that wraps around your chest. The second two treadmill tests will consist of a 15 minute warm-up at velocities corresponding with 50% of your maximal aerobic capacity (VO\(_{2\text{max}}\)) followed by 45 minutes of intermittent sprints (corresponding with 80% of VO\(_{2\text{max}}\)) and jogging intervals (corresponding with 50% of VO\(_{2\text{max}}\)).

**Cardiovascular Endurance (VO\(_{2\text{max}}\) Test):** During the VO\(_{2\text{max}}\) test, you will begin jogging on the treadmill at a speed that is comfortable for you. Every minute during this test, we will increase the speed and the elevation of the treadmill until you indicate that you can no longer continue. Between test preparation and completion of the exercise test, this test should take approximately one hour.

**Warm-up & Intermittent Sprints:** These tests will be performed in the morning. You will be asked to refrain from food and beverages (except water) for 12 hours prior to these tests. In addition, you will need to refrain from consumption of caffeine-containing beverages (coffee, tea, cola drinks, cocoa) for 24 hours prior to the test. During these two tests you will exercise for 15 minutes at velocities corresponding with 50% VO\(_{2\text{max}}\) followed by 45 minutes of intermittent sprints. The sprints will mirror the intermittent nature of a tennis match such that the subject will sprint for 5 seconds at velocities corresponding with 80% VO\(_{2\text{max}}\) and return to a jog corresponding with the warm-up speed (50% VO\(_{2\text{max}}\)) for 15 seconds. This sequence will be repeated 18 times. After the eighteenth sprint, you will rest for 90 seconds. You will then do 12 sprints, followed by the 15 seconds of jogging between each sprint. After the 12th sprint, you will rest for another 90 seconds. This sequence will be repeated for 45 minutes.

Both tests will be identical in terms of the speed and elevation of the treadmill. One hour prior to the test, you will ingest either caffeine (equivalent to 4-5 six ounce cups of coffee) or a placebo in pill form. Neither the investigator nor you will know which treatment you received at test time. Between test preparation and completion of these exercise tests, each test should take approximately one and a half hours.

**Tennis Skills Test:** The tennis skills tests will immediately follow the Warm-Up and Intermittent Sprint Test. The test will be organized by sets of six different shots to emulate a single game within a set of tennis – 4 ground strokes (the stroke used the most within a tennis match), 1 approach shot and 1 volley. After each set of 6 balls, the subject can actively rest at the baseline for 20 seconds, which represents the time allowed for rest between tennis points. The 6-ball drill will be repeated 6 times, which represents a typical duration and number of balls for 1 game within a set of a realistic tennis match. Every two sessions, the player has a 90 second break – which represents the official time between a change-over during a match. The athlete will undergo 9 sessions, which will represent 1 set of a typical tennis match. The key below simplifies the terminology used to describe the tennis skills test:
- 6 ball drill = 1 point of a typical match
- 6 repetitions of the 6-ball drill = 1 game of a typical match (6 reps of 6-ball drill = 1 session)
- 9 sessions = 1 set of a typical match
- The tennis skills test will be quantified such that each time the ball lands in-bounds, it will count for 1 point. Maximum number of points subject may earn is 324.

**Blood Sampling:** We will obtain a very small (1 drop) sample of blood before and after each exercise test to determine how much lactic acid you accumulate during the test using a finger stick. In addition, we will obtain about 5 ml of blood (about 1 teaspoon) prior to the second two treadmill tests in order to determine the amount of caffeine in your blood. These blood samples will be obtained from an arm vein.

**DNA Sampling:** We will extract a sample of your DNA from your blood sample. The DNA will be stored in our laboratory, but the sample will be coded so that nobody except the primary investigator (Courtney Klein) can detect which sample is yours. The DNA testing will involve determining your sequence of DNA for a specific gene that is related to caffeine metabolism. The results of this genetic testing will only be available to the primary investigator and you. These results will not be made public and will be stored in a locked file cabinet. We will not use the DNA for any purpose other than determining the specific sequence of the CYP1A2 gene. The DNA sample is subject to court subpoena and the participant has the right to request disposal of their DNA sample after data collection is completed.

**Confidentiality**
The results of this research will be presented at conferences and published in exercise science journals. The results of this project will be coded in such a way that your identity will not be attached to the final form of this study. The researcher retains the right to use and publish non-identifiable data. However, you can ask that your data be removed from the study at any point prior to presentation and publication. While individual responses are confidential, aggregate data will be presented representing averages or generalizations about the responses as a whole. All data will be stored in a secure location accessible only to the researcher. Final aggregate results will be made available to participants upon request. Final aggregate results will be made available to you upon request.

**Participation & Withdrawal**
Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind. Your right to withdraw includes the right to request that your DNA and blood samples be discarded at any time.
Questions
You may have questions or concerns during the time of your participation in this study, or after its completion. If you have any questions about the study, contact Courtney S. Klein at kleincs@jmu.edu or by phone at 540-476-2439.

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

______________________________  ______________________________
Name of Participant (Printed)    Name of Researcher(s) (Printed)

______________________________  ______________________________
Name of Participant (Signed)     Name of Researcher(s) (Signed)

______________________________  ______________________________
Date                          Date

For questions about your rights as a research subject, you may contact the chair of JMU’s Institutional Review Board (IRB). Dr. David Cockley, (540) 568-2834, cocklede@jmu.edu.
Appendix II  
James Madison University  
School of Kinesiology and Recreation Studies  
Health Status Questionnaire

Instructions: Complete each question accurately. All information provided is confidential.

Part I: General Information

1. Participant Number  Date

2. Gender (circle one)  Male  Female

3. Date of Birth  (Month/Day/Year)

Part II: Medical History

4. Circle any that died of heart attack before age 50:  Father  Mother  Brother  Sister  Grandparent

5. Date of last medical exam: ___________  Last physical fitness test: ___________

6. Circle operations you have had:  Back  Heart  Kidney  Eyes  Joint  Neck  Ears  Hernia

   Lung  Other ________________

7. Please circle any of the following for which you have been diagnosed of treated by a physician or health professional:

   Alcoholism  Diabetes  Kidney Problems
   Anemia (sickle cell)  Emphysema  Mental Illness
   Anemia (other)  Epilepsy  Muscular Injury
   Asthma  Eye Problems  Neck Strain
   Back Strain  Gout  Obesity
   Bleeding trait  Hearing Loss  Orthopedic Injuries
   Bronchitis, chronic  Heart Problem  Phlebitis
   Cancer  High Blood Pressure  Rheumatoid arthritis
   Cirrhosis, liver  Hypoglycemia  Stroke
   Concussion  Hyperglycemia  Thyroid problem
   Congenital defect  Infectious Mononucleosis  Ulcer

Other ______________________
8. Circle all medications taken in the last six months:

- Blood thinner
- Epilepsy medication
- Nitroglycerin
- Diabetic pill
- Heart-rhythm medication
- Other __________________
- Digitalis
- High-blood pressure medication
- Diuretic
- Insulin

9. Any of these health symptoms that occur frequently is the basis for medical attention. Circle the number indicating how often you have each of the following:

- 5 = Very often
- 4 = Fairly often
- 3 = Sometimes
- 2 = Infrequently
- 1 = Practically never

a. cough up blood
b. abdominal pain
c. low back pain
d. leg pain
e. arm or shoulder pain
f. chest pain
g. swollen joints
h. feel faint
i. dizziness
j. breathless on slight exertion

Part III: Health Related Behavior

10. Do you smoke? Yes No

11. If you are a smoker, indicate the number of smoked per day:

Cigarettes:

- 40 or more
- 20-39
- 10-19
- 1-9

Cigars or pipes only:

- 5 or more or any inhaled
- less than 5, none inhaled

12. Do you exercise regularly? Yes No

13. How many times in a week do you spend at least 30 minutes in moderate to strenuous/vigorous
exercise?

1 2 3 4 5 6 7   days per week

14. Can you walk 4 miles briskly without fatigue? Yes No

15. Can you jog 3 miles continuously at a moderate pace without discomfort? Yes No

16. Weight now: _________ lb. One year ago: _________ lb  Age 21: _________ lb
Appendix III

Caffeine Habits & Frequency Questionnaire

This questionnaire asks about your caffeine intake patterns during a typical month. For each item listed, respond by indicating your usual intake of that food per Day, Week or Month. For example: Coffee. If you drink 2 (8 oz.) cups of coffee every day, respond 2 Daily. If you think you average 2 cups of coffee a week over the month, respond 2 Weekly.

For example, if you drink 2 (8 oz.) cups of coffee per week, you would write 2 under the week column.

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
<th>Unit</th>
<th>Daily</th>
<th>Weekly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee (1 cup = 8 fl. oz.)</td>
<td>1.00</td>
<td>item</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

**Beverages**

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
<th>Unit</th>
<th>Daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coca Cola (1 can = 12 fl. oz.)</td>
<td>12.00</td>
<td>fl. oz.</td>
<td></td>
</tr>
<tr>
<td>Pepsi (1 can = 12 fl. oz)</td>
<td>12.00</td>
<td>fl. oz.</td>
<td></td>
</tr>
<tr>
<td>7-Up (1 can = 12 fl. oz)</td>
<td>12.00</td>
<td>fl. oz.</td>
<td></td>
</tr>
<tr>
<td>Root Beer (1 can = 12 fl. oz)</td>
<td>12.00</td>
<td>fl. oz.</td>
<td></td>
</tr>
<tr>
<td>Coffee (1 cup = 8 fl. oz)</td>
<td>8.00</td>
<td>fl. oz.</td>
<td></td>
</tr>
<tr>
<td>Tea (Circle: BLACK, GREEN, WHITE) (1 cup = 8 fl. oz.)</td>
<td>8.00</td>
<td>fl. oz.</td>
<td></td>
</tr>
<tr>
<td>Hot chocolate or cocoa (1 cup = 8 fl. oz.)</td>
<td>8.00</td>
<td>fl. oz.</td>
<td></td>
</tr>
<tr>
<td>Chocolate Milk</td>
<td>8.00</td>
<td>fl. oz.</td>
<td></td>
</tr>
<tr>
<td>Red Bull Energy Drink (1 can = 8.3 fl. oz.)</td>
<td>8.00</td>
<td>fl. oz.</td>
<td></td>
</tr>
<tr>
<td>5-Hour Energy (1 bottle = 2 fl. oz.)</td>
<td>2.00</td>
<td>fl. oz.</td>
<td></td>
</tr>
<tr>
<td>Monster Energy Drink (1 can = 8.3 fl. oz.)</td>
<td>8.00</td>
<td>fl. oz.</td>
<td></td>
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<tr>
<td>Propel + Caffeine (1 bottle = 8 fl. oz.)</td>
<td>8.00</td>
<td>fl. oz.</td>
<td></td>
</tr>
</tbody>
</table>

**Other**

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
<th>Unit</th>
<th>Daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine added performance bars (1 serving = 1 bar)</td>
<td>1.00</td>
<td>bar</td>
<td></td>
</tr>
<tr>
<td>Dark Chocolate Bar (1 bar), M&amp;Ms (1 pkg.)</td>
<td>1.00</td>
<td>item</td>
<td></td>
</tr>
<tr>
<td>Milk Chocolate Bar (1 bar), M&amp;Ms (1 pkg.)</td>
<td>1.00</td>
<td>item</td>
<td></td>
</tr>
<tr>
<td>Coffee flavored Ice Cream (1 serving = 1 cup)</td>
<td>1.00</td>
<td>cup</td>
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</tbody>
</table>

Please list your approximate WEEKLY intake of the following:

- Doses of caffeinated pills (No-Doz, Vivarin, etc.):
Appendix IV

Borg Rating of Perceived Exertion Scale

<table>
<thead>
<tr>
<th>Exertion</th>
<th>RPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>no exertion at all</td>
<td>6</td>
</tr>
<tr>
<td>extremely light</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td>very light</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>light</td>
<td>11</td>
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<tr>
<td></td>
<td>12</td>
</tr>
<tr>
<td>somewhat hard</td>
<td>13</td>
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<tr>
<td></td>
<td>14</td>
</tr>
<tr>
<td>hard (heavy)</td>
<td>15</td>
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<tr>
<td></td>
<td>16</td>
</tr>
<tr>
<td>very hard</td>
<td>17</td>
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<tr>
<td></td>
<td>18</td>
</tr>
<tr>
<td>extremely hard</td>
<td>19</td>
</tr>
<tr>
<td>maximal exertion</td>
<td>20</td>
</tr>
</tbody>
</table>
Appendix V
Treadmill Testing

Subject # - MPH at 50% VO2max -
VO2max - MPH at 80% VO2max -

Begin with 15 minute warm up at a speed of 50% VO2max, followed by:

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Duration</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>80%</td>
<td>5 sec</td>
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<td>50%</td>
<td>15 sec</td>
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90 SEC REST

90 SEC REST

90 SEC REST

90 SEC REST

90 SEC REST

90 SEC REST
## Appendix VI

### Tennis Skills Score Sheet

<table>
<thead>
<tr>
<th></th>
<th>6-ball -1</th>
<th>6-ball -2</th>
<th>REST - 90sec</th>
<th>6-ball -3</th>
<th>6-ball -4</th>
<th>REST - 90sec</th>
<th>6-ball -5</th>
<th>6-ball -6</th>
<th>REST - 90sec</th>
<th>6-ball -7</th>
<th>6-ball -8</th>
<th>REST - 90sec</th>
<th>6-ball -9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (rest 20 sec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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