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The effects of coffee ingestion on the acute testosterone response to exercise

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The Effects of Coffee Ingestion on the Acute Testosterone Response to Exercise

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ABSTRACT

Abstract

This study investigated the effects of coffee ingestion (COF) on serum testosterone responses to exercise in recreationally weight-trained males. Subjects ingested either 12 ounces of 6mg/kg caffeinated coffee (COF), decaffeinated coffee (DEC), or water (PLA) one hour prior to exercise in a randomized, within-subject, crossover design. The exercise session consisted of 21 minutes of high intensity interval cycling (alternating intensities corresponding to two minutes at power outputs associated with 2.0 mmol/L lactate and 4.0 mmol/L lactate) followed by resistance exercise (7 exercises, 3 sets of 10 repetitions, 65% 1RM, 1-minute rest periods). Subjects also completed repetitions to fatigue tests and soreness scales to determine muscle recovery 24 hours following the exercise. T was elevated immediately and 30-minutes post-exercise by 20.5% and 14.3% respectively ($p<0.05$). There was no main effect for treatment and no exercise x treatment interaction. There were no differences in repetitions to fatigue or soreness between treatments ($p>0.05$). No relationships were observed between T and any proxy of recovery, suggesting that adopting high testosterone strategies may not always improve quality of subsequent exercise bouts. The duration of T elevation indicates that this protocol is beneficial to creating a long-lasting anabolic environment. While past literature suggests caffeine may enhance T post-exercise, data from the current study suggest that augmented T response is not evident following caffeine supplementation via coffee.

KEY WORDS: caffeine, recovery, anabolic

Chapter I

Introduction

Testosterone has been widely investigated for its role in anabolic processes. Its supplementation has been shown to directly correlate with increased strength (8, 26, 33) and muscle size (1, 8, 11). Furthermore, inhibition of testosterone blunts strength-training induced muscle adaptations (33). It is for these reasons that the post-exercise testosterone response is often investigated and manipulated to enhance muscle adaptation. During the 30-60 minutes post-exercise that testosterone is elevated, responses are triggered, which contribute to muscle adaptation and remodeling. Some of these responses include increased protein synthesis (51), neurotransmitter synthesis (31, 39), satellite cell number (49), and myonuclei concentration (49), all resulting in enhanced recovery and/or adaptation. These enhanced responses may be due to increases in the concentration and half-life of androgen receptors (50). Increased androgen receptor density facilitates anabolic effects by increasing androgen binding (29, 34).

Overall, there are clear trends showing supplementation of testosterone yields positive muscle adaptations. It is hypothesized that maximizing the acute testosterone response to exercise would be beneficial to muscle adaptation, but the magnitude of these effects are unclear. Studies are limited because it is difficult to minimize outside factors and accurately identify possible effects. Serum testosterone is affected by diet (8, 9, 14, 28, 41, 52), sleep (8), exercise (15, 32, 45, 46, 48), gender (53), age (7), stress (20), and time of day (47).

Resistance training protocols involving multi joint, full body exercises performed using a moderate range of repetitions and shorter rest period maximize testosterone response (15, 32, 45, 46). Furthermore, investigators have observed that brief high intensity interval training before resistance exercise augments testosterone responses (12, 48). However, the mechanism of these responses is not entirely clear. It is hypothesized that these protocols yield higher adrenergic responses and lactate concentrations, which may increase testosterone production from Leydig cells through stimulation of luteinizing hormone (36–38). However, Raastad et. al. (45) noted that exercises eliciting a high testosterone response have no effect on luteinizing hormone, which may suggest that these responses are due to decreased clearance.

Some studies indicate that training programs involving multi-joint exercises performed at a moderate range of repetitions with short rest yield greater hypertrophy and strength gains because of a testosterone tesponse (1, 2, 8, 13, 26). It is noteworthy that others have contradicted these findings, observing no relationship between exercises eliciting high levels of testosterone and muscle adaptations (55, 56). However, Bhasin et. al. (11) illustrated that even small supplemental doses of testosterone (125mg) can lead to hypertrophy, despite only increasing serum levels 10%. Studies show that serum testosterone can increase 10-58% above baseline in response to different exercise protocols, which would therefore be well above this proposed value (7, 14, 15, 32, 45, 46, 48, 53).

Many studies have shown augmented testosterone responses after 3-6mg/kg of anhydrous caffeine consumption (9, 17, 28, 41, 60). The specific mechanism through which caffeine affects testosterone responses to exercise is not clear. It is possible that the caffeine-induced increase in catecholamines (23) during exercise triggers a larger testosterone response (36–38). Even without exercise, caffeine ingestion has resulted in greater testosterone (44), muscle size, and muscle recovery (24) in rats and mice. It has been hypothesized that caffeine reduces hepatic blood flow, which would decrease testosterone clearance (10, 19). It is noteworthy that there is contrary evidence indicating caffeine has no effects on testosterone (59). The equivocal findings indicate that more investigation is required to determine the effect that caffeine has on testosterone and the testosterone response to exercise. Limitations of past studies include: inadequate doses of caffeine, small sample sizes, and unreliable analyses of serum testosterone levels (ie. salivary samples). In addition, certain proxies of performance do not account for possible ergogenic effects of caffeine which lead to differences in workload. For example, during fixed-time exercise tests, supplementation of caffeine may improve work completed (22, 27, 28, 41). Thus, it is then difficult to tell whether an augmented testosterone response is due to the increased work performed or to caffeine supplementation per se.

Literature on the testosterone response to specific modes of caffeine consumption, such as coffee, is very limited. Wedick et. al. (52) observed the effects of prolonged caffeinated coffee consumption on testosterone. Despite increases in testosterone after 4 weeks of coffee consumption no differences were observed in testosterone levels after week 8 (52). Although coffee is one of the most common modes of caffeine consumption, the observed ergogenic properties of coffee are equivocal as some studies suggest similar ergogenic properties compared to pure caffeine (27, 58), while other studies have observed blunted ergogenic effects with coffee consumption (22). The reasons for the possible blunted ergogenic effects are not fully established, but the chlorogenic acid content may be an influence (42). Chlorogenic acids inhibit adenosine transporter activity, increasing extracellular adenosine, which may negate caffeine’s effects (42). Coffee brands vary in their chlorogenic acids, which may explain why some coffees blunt caffeine more than others. Also, unlike coffee, pure caffeine has been shown to lead to greater lactate concentrations at submaximal intensities (5, 16, 21, 40). This would theoretically lead to less lactate-induced stimulation of luteinizing hormone and therefore less testosterone production

The purpose of this study is to examine the effects of coffee ingestion on testosterone levels before and after exercise in college-aged males in a double-blind crossover study. This study also aims to determine if increased acute testosterone responses facilitate muscle recovery after a damaging bout of exercise through the use of soreness scales and repetitions to fatigue tests.

Past literature indicates that some compounds in coffee, perhaps chlorogenic acids, negate the ergogenic acids of caffeine (22, 42). Coffee also seems to have a lesser effect on lactate accumulation than pure caffeine (16, 40). Despite this there are still reports of ergogenic properties in coffee similar to caffeine (27, 58). It is hypothesized that, despite its caffeine content, coffee will have no effect on testosterone response. It is also expected that higher testosterone responses, due to coffee consumption, will enhance recovery. This would be observed through increased reps to fatigue as well as decreased perceived soreness 24 hours later.

1.1. Assumptions-

An important assumption in this study is the subjects' adherence to instructions. It is assumed that they will observe the proper sleep, meal, and exercise protocols to maximize control. Another assumption is that perceived soreness and reps to fatigue tests are valid and reliable methods of determining muscle damage (and therefore recovery). In addition it is assumed that *Enzyme-Linked Immunoabsorbant Assays* (ELISA kits) are valid methods of assessing serum testosterone (3, 30).

1.2. Limitations-

Time and financial constraints provide some limitations to this study. Cortisol, a main catabolic hormone will not be measured in this study. In addition, more subjects and time points at which testosterone can be measured would strengthen this project.

1.3. Delimitations-

Only college-aged, resistance trained males will be examined in this study. Menstrual hormonal fluctuations of women would lead to difficulty identifying the causes of any experimental differences between trials. In addition, subjects must be resistance-trained to prevent a learning effect during the trials.

1.4. Definition of Terms-

Fatigue: Inability to complete a complete muscle contraction with the proper form.

Exhaustion: Inability to continue during a maximal cycling test. (Volitional fatigue or cadence below 50 revolutions per minute)

Testosterone response: The acute post-exercise increase in testosterone

Caffeine: caffeine anhydrous in capsule form

Chapter II

Methodology

2.1. Selection of sample-

The sample size will consist of 10 college-aged men who have participated in, on average, at least 3 days of resistance exercise per week for the last 6 months. Experience with resistance exercise is a criterion to minimize neural adaptations throughout the trials. Subjects must also consume less than 300mg caffeine each day.

2.1.1. Experimental design-

Effect size for caffeine-induced changes in testosterone varies from moderate to very large (0.5 to >2.2) depending on the exercise protocol (9, 41). Beaven et. al. used a similar resistance exercise protocol to the present study and, at a similar dose of caffeine, observed an effect size of 2.19 standard deviations (9). This was calculated using a moderate coefficient of variation of 0.5. If 10 subjects complete the current study and caffeine yields an effect on testosterone levels of only 1.2 standard deviations then the statistical power would still be 0.7.

This study will utilize a double-blind, placebo-controlled, randomized cross-over design. Testing will take place in the Godwin Human Performance Lab and the fitness room in Godwin 218. This study has been submitted and approved of by the James Madison University Institutional Review Board (IRB).

2.2. Recruitment and Benefits-

Subjects will be recruited through mass emails and advertisements around the James Madison University campus and the surrounding Harrisonburg area. They will be informed of the responsibilities, risks, and benefits associated with the study. Benefits include a free DEXA scan, VO_{2max} test, and testosterone analysis.

2.2.1. Preliminary Meetings and Testing-

During the first meeting the informed consent will be discussed and all questions will be answered. The second meeting will be for preliminary testing and familiarization.

2.2.2. VO_{2peak} Test-

Subjects will perform a progressive maximal test on a Velotron cycle ergometer (RacerMate, Inc.) to determine peak oxygen consumption (VO_{2peak}) and workloads corresponding to fixed blood lactate concentrations of 2.0 and 4.0 mmol/L. The intensity will begin at 75 watts and progressively increase by 50 watts every three minutes. The test continues until the subject reaches volitional exhaustion or cannot maintain 50rpm. Heart rate and oxygen consumption will be measured by Suunto heart rate monitor and MOXUS Modular VO_2 Measurement Cart respectively. Blood will be collected before the test and each three minutes via finger stick. Whole blood will be analyzed by a YSI Stat 2300 automated analyzer (Yellow Springs, OH) to determine lactate concentration. This method for determining fixed blood lactate concentrations has been shown to have a reliability of 0.91-0.95 (54).

2.2.3 One Repetition Max Tests-

Immediately following the graded exercise test, subjects will have 1RM determined on Cybex machines for chest press, leg press, shoulder press, leg extensions, rows, leg curls, and lat pull downs to simulate the fatigue experienced after the high intensity interval training that will precede resistance training during the exercise trials. Subjects will perform a warm-up of 10 repetitions at a self-selected weight on the chest press before beginning. Other than the exclusion of warm-up preceding the remaining exercises, protocols to determine 1RM are described in ACSM's Guidelines for Exercise Testing and Prescription (4). If the subject's maximal strength exceeds the capacity of the machine, Wathan's predictive equation will be used (6). In these scenarios subjects will determine their maximum repetitions to fatigue at the machine's capacity. Wathan's equation has been shown to have a very high correlation to actual 1RM (R = 0.97) (35).

2.3. Exercise Trials-

At 7:00am subjects will complete exercise trials on three separate occasions (one intervention per meeting). Each exercise trial will be preceded by ingestion of the designated experimental treatment an hour before (6:00am).

Exercise trials are designed to maximize the acute testosterone response. Short rest, multi-joint, 10 rep, full body resistance exercise preceded by high intensity interval training has been shown to augment the acute testosterone response to exercise (32, 48).

2.3.1. Cycling Phase-

Exercise trials will begin on a Velotron cycle ergometer. Subjects will alternate pedaling at intensities eliciting 2.0 mmol/L for 2 minutes and 4.0 mmol/L for 1 minute for 21 minutes.

2.3.2. Resistance Training Phase-

Five minutes following the conclusion of the cycling phase, subjects will begin the resistance training phase. Three sets of chest press, leg press, rows, leg extensions, shoulder press, leg curls, and lat pull downs will be performed respectively. Each set will consist of 10 repetitions at 65% of 1RM separated by one minute rest. If the subject cannot complete three sets of ten repetitions during the first exercise session, it will be noted, the weight will be adjusted, and it will be reproduced during the remaining exercise trials. Subjects will be informed and reminded of the importance of reproducing the exact exercise session.

2.3.3. Treatments-

Interventions will include: 1) 12fl. oz. of: Folgers coffee (COF) with a caffeine pill, both totaling 6mg/kg body weight of caffeine; 2) Folgers decaffeinated coffee (DC) with a placebo pill, or 3) water (CON) all ingested 60 minutes prior to the exercise trial. Folgers regular and decaffeinated coffee have been reported to contain 144 and 4 mg caffeine per 12 ounces respectively. Subjects will have 5 minutes to finish their treatment immediately after a baseline venous blood draw.

2.3.4. Blood Collection and Analysis-

Pre-exercise venous blood draws will be performed before treatment and immediately before exercise to measure serum testosterone levels before and an hour after treatment consumption. To limit the number of needle-sticks, the needle will be secured after the first stick and 5ml of saline will be infused. The needle will be removed after the second blood draw. This process will be repeated for blood draws performed immediately post and 30 minutes post-exercise. All samples will be drawn into Vacutainer serum collection tubes (Becton Dickinson, Franklin Lakes NJ). At least 30 minutes post blood draw samples will be spun down for 20 minutes at 1500rpm to obtain serum, which will be stored at -80° C until assayed. Testosterone will be measured using validated Enzyme-Linked Immunoabsorbant Assays (ELISA kits) (3, 18, 30, 61, 62).

2.4. Analysis of Recovery-

Twenty-four hours following each exercise trial, recovery and muscle function will be assessed. Visual analog soreness scales will be completed followed by subjects performing repetitions to fatigue tests (with no warm-up) on the chest and leg press machines at 65% 1RM. Reps to fatigue tests have been shown to have high reliability (>0.9) (43).

2.5. Controls-

All exercise trials will be performed at the same time to control for diurnal variations in testosterone. Testosterone levels are highest in the morning. A standardized breakfast of one Ensure beverage (Abbott Laboratories©) will be consumed 90 minutes before the exercise trial (220 calories, 6g. fat, 32g. carbohydrates, and 9g. protein).

Dietary intake logs will be completed for the 72 hours prior to each recovery analysis so type of food, quantity, time of consumption, and method of preparation can be strictly replicated. Physical activity logs will also be completed for the 72 hours preceding each exercise trial.

Strenuous physical activity will be avoided 72 hours before each exercise trial while alcohol and caffeine will be avoided for the preceding 24 hours. Overall exercise trials will be separated by 3-7 days.

2.6. Statistical Analysis-

A 2-way repeated measured ANOVA will be used to determine any differences in acute testosterone response or muscle recovery among the treatments, with treatment and exercise as the two within-subjects factors. A p-value of <0.05, two-tailed, will be used to determine statistical significance. Post-hoc analysis will be performed using t-tests with a Bonferroni correction. Correlational analyses will also be performed to determine any relationships between testosterone response and recovery.

Chapter III

Manuscript

The Effects of Coffee Ingestion on the Acute Testosterone Response to Exercise in Resistance Trained Males

Abstract

This study investigated the effects of coffee ingestion (COF) on serum testosterone responses to exercise in recreationally weight-trained males. Subjects ingested either 12 ounces of 6mg/kg caffeinated coffee (COF), decaffeinated coffee (DEC), or water (PLA) one hour prior to exercise in a randomized, within-subject, crossover design. The exercise session consisted of 21 minutes of high intensity interval cycling (alternating intensities corresponding to two minutes at power outputs associated with 2.0 mmol/L lactate and 4.0 mmol/L lactate) followed by resistance exercise (7 exercises, 3 sets of 10 repetitions, 65% 1RM, 1-minute rest periods). Subjects also completed repetitions to fatigue tests and soreness scales to determine muscle recovery 24 hours following the exercise. T was elevated immediately and 30-minutes post-exercise by 20.5% and 14.3% respectively ($p<0.05$). There was no main effect for treatment and no exercise x treatment interaction. There were no differences in repetitions to fatigue or soreness between treatments ($p>0.05$). No relationships were observed between T and any proxy of recovery, suggesting that adopting high testosterone strategies may not always improve quality of subsequent exercise bouts. The duration of T elevation indicates that this protocol is beneficial to creating a long-lasting anabolic environment. While past literature suggests caffeine may enhance T post-exercise, data from the current study suggest that augmented T response is not evident following caffeine supplementation via coffee.

KEY WORDS: caffeine, recovery, anabolic

INTRODUCTION

Testosterone has been widely investigated for its role in anabolic processes. Following acute exercise, testosterone levels are elevated, which contributes to muscle adaptation and remodeling. Elevated testosterone levels are associated with increased protein synthesis (51), neurotransmitter synthesis (31, 39), satellite cell number (49), and myonuclei concentration (49), all resulting in enhanced recovery and/or adaptation.

Some studies have shown 3-6mg/kg body weight of anhydrous caffeine consumption augments testosterone responses (9, 17, 28, 41, 60). In contrast, data from Wu, et. al. does not support elevations in testosterone with caffeine ingestion before a resistance training bout in trained men (59). Even without exercise, caffeine ingestion has resulted in greater testosterone (44), muscle size, and muscle recovery (24) in rats and mice. Although the purported mechanisms for caffeine-induced testosterone increases are unclear, it may be due to reduced testosterone clearance through restricted hepatic blood flow (10, 19).

Coffee is one of the most common modes of caffeine consumption. However, the literature on its physiological and ergogenic effects compared to pure caffeine varies (22, 27, 58) Chlorogenic acids in coffee inhibit adenosine transporter activity, increasing extracellular adenosine, which may negate the effects of caffeine (42). Also, unlike coffee, pure caffeine leads to greater lactate concentrations at submaximal intensities (5, 16, 21, 40). It is possible that coffee does not attenuate hepatic blood flow to the same degree as anhydrous caffeine, resulting in decreased testosterone clearance. Despite this, Wedick et. al. (52) observed increased testosterone levels in response to 4 weeks of coffee consumption; although it should be noted levels returned to baseline after the consumption duration reached 8 weeks.

Therefore, the purpose of this study was to examine the effects of coffee ingestion on testosterone levels before and after exercise. This study also aimed to determine if there was an association between acute testosterone responses and muscle recovery after a damaging bout of exercise.

METHODS

Experimental Approach: This study utilized a double-blind, placebo-controlled, randomized cross-over design.

Subjects:

Eleven resistance trained college-aged men agreed to participate in the study. One participant was excluded due to a non-related injury before data collection was complete and another was dropped from the study due to non-compliance with instructions. Mean age, height, weight, and aerobic fitness are displayed in Table 1. All subjects averaged at least three days of resistance exercise per week for the previous six months. Subjects could not habitually consume more than 300mg caffeine each dayTrials took place in the Human Performance Laboratory at James Madison University. This study was submitted and approved of by the James Madison University Institutional Review Board (IRB).

(“Insert Table 1. Here”)

Exercise Tests:

VO_{2peak} Test:

Subjects performed a progressive maximal test on a Velotron cycle ergometer (RacerMate, Inc.) to determine VO_{2peak} and workloads corresponding to fixed blood lactate concentrations of 2.0 and 4.0 mmol/L. The initial workload was 75W and progressively increased by 50W every three minutes. The test continued until the subject reached volitional exhaustion or could not maintain a cadence of 50rpm. Heart rate and VO_2 were measured by a Suunto heart rate monitor (Finland) and MOXUS Modular VO_2 Measurement Cart (AEI Technologies, New Orleans, Louisiana) respectively. Blood was collected before the test and each three minutes via finger stick. Whole blood was immediately analyzed by a YSI Stat 2300 automated analyzer (Yellow Springs, OH) to determine lactate concentration. The power output/blood lactate relationship was mathematically determined and the resulting equation was used to estimate the power output associated with 2.0 and 4.0 mmol/L (130±32 and 208+38 Watts) respectively.

One Repetition Maimum (1RM) Tests:

Immediately following the graded exercise test, one repetition maximum (1RM) was determined on Cybex machines for chest press, leg press, shoulder press, leg extensions, rows, leg curls, and lat pull downs. Subjects performed a warm-up of 10 repetitions at a self-selected weight on the chest press before beginning. Other than the exclusion of warm-up preceding the remaining exercises, protocols to determine 1RM are described in ACSM's Guidelines for Exercise Testing and Prescription (4). If the subject's maximal strength exceeded the capacity of the machine, maximal repetitions at the highest setting on the machine were recorded and Wathan's predictive equation was used to estimate 1-RM (6). This equation has a very high correlation to actual 1RM (R = 0.97) (35).

Performance Trials:

Dietary intake logs were completed for the 72 hours prior to each recovery analysis so type of food, quantity, time of consumption, and method of preparation could be replicated. Physical activity logs were also completed for the 72 hours preceding each exercise trial. Strenuous physical activity was avoided 72 hours before each exercise trial, while alcohol and caffeine was avoided for the preceding 24 hours. All performance trials were separated by 7 days.

The timeline for the trials is illustrated in Figure 1. Subjects completed trials on three separate occasions (one intervention per meeting, all at 7:00 AM). A standardized breakfast of one Ensure beverage (Abbott Laboratories©) was consumed 90 minutes before the exercise trial (220 calories, 32g carbohydrates, and 9g protein).

Each trial was preceded by the ingestion of the designated experimental treatment one hour prior to exercise. Full body resistance exercise of this nature preceded by high intensity interval training has been shown to augment the acute testosterone response to exercise (32, 48).

Exercise trials began on a Velotron cycle ergometer. Subjects alternated pedaling at the power outputs associated with 2.0 mmol/L for two minutes and 4.0 mmol/L for one minute for 21 minutes.

Five minutes following the conclusion of the cycling phase, subjects began the resistance training phase. Three sets of chest press, leg press, rows, leg extensions, shoulder press, leg curls, and lat pull downs were performed respectively. Each set consisted of 10 repetitions at 65% of 1RM separated by one minute rest. If the subject could not complete three sets of ten repetitions during the first exercise session, it was noted, the weight adjusted, and the protocol was reproduced during the remaining exercise trials. Subjects were informed and reminded of the importance of reproducing the exact exercise session.

(“Insert Figure 1 here”)

Treatments:

Interventions included 355ml of: coffee (COF) with a caffeine pill (caffeine content of coffee and pill totaling 6mg/kg body weight), decaffeinated coffee (DC) with a placebo pill, or water (CON) all ingested 60 minutes prior to the exercise trial. The utilized brand of regular and decaffeinated coffee have been reported to contain 144 and 4 mg caffeine per 355ml respectively. Subjects had five minutes to consume their treatment.

Blood Collection and Analysis:

Pre-exercise venous blood draws were performed immediately prior to treatment and immediately before exercise. Post-exercise samples were collected immediately after exercise and 30 minutes after exercise cessation. All samples were drawn into Vacutainer serum collection tubes (Becton Dickinson, Franklin Lakes NJ). At least 30 minutes following the blood draw, samples were spun for 20 minutes at 1500 rpm to obtain serum, which was stored at -80° C until assayed. Testosterone was measured using validated Enzyme-Linked Immunoabsorbant Assays (Eagle Biosciences, Nashua, New Hampshire) (62).

Analysis of Recovery: Recovery and muscle function was determined 24 hours following each exercise trial. Visual analog soreness scales were completed followed by subjects performing repetitions to fatigue tests (with no warm-up) on the chest and leg press machines at 65% 1RM. These tests have been shown to have high reliability (>0.90) (43).

Statistical Analysis:

A 2-way repeated measures of variance (ANOVA) was used to determine any differences in acute testosterone response or proxies of muscle recovery among the treatments, with treatment and exercise as the two within-subjects factors. A one-way repeated measures ANOVA was used to determine differences in the markers of recovery between the treatments. Post-hoc analysis was performed using t-tests. A p-value of <0.05, two-tailed, was used to determine statistical significance. Correlational analyses were performed to determine any relationships between testosterone response and markers of recovery.

Effect size for caffeine-induced changes in testosterone varies from moderate to very large (0.5-2.0) (9, 41) depending on the exercise protocol. Beaven et. al. used a similar resistance protocol to the present study and, at a similar dose of caffeine, observed a very large effect size (9). Considering the 9 subjects that completed the current study; assuming a moderate coefficient of variation of 0.5, if caffeine yielded a similar effect on testosterone levels as Beaven et. al. (1.6 standard deviations) (9) then the statistical power would be 0.85.

RESULTS

Table 2 displays mean 1RM values for the sample.

("Insert Table 2 here")

Mean serum testosterone changes in response to the treatments are displayed in Figure 2. There was a main effect for exercise, as serum testosterone significantly increased by an average of 20.5% in response to the exercise bout ($p<0.05$). They remained significantly elevated 30 minutes post-exercise by an average of 14.3% ($p<0.05$). There was no main effect for treatment and no exercise x treatment interaction ($p>0.05$).

Mean chest and leg press repetitions to fatigue at 65% 1RM, 24 hours post-exercise are shown in Figure 3. There were no significant differences in chest or leg repetitions to fatigue between treatments ($p>0.05$).

Figure 4 displays the results from the visual analog scale for soreness 24 hours post-exercise. There were no significant differences in soreness between treatments ($p>0.05$).

("Insert Figures 2, 3, and 4 here")

No significant relationships were observed between testosterone responses and any proxy of recovery. The correlation coefficients between testosterone response and chest press repetitions to fatigue, leg press repetitions to fatigue, and soreness score were $R = 0.14$, 0.17, and 0.32 respectively.

DISCUSSION

The purpose of this study was to determine if ingesting caffeinated coffee increased the testosterone response to exercise. Despite the many studies observing 3-6 mg/kg body weight of caffeine ingestion leads to augmented testosterone levels (9, 17, 28, 41, 60) there were no differences in testosterone levels between treatments in the present study. This suggests that either coffee blunts the testosterone increase due to caffeine or that caffeine does not effectively raise testosterone in the reported trial. Although these data do not allow for a definitive conclusion from both possibilities, the large number of previous studies observing a caffeine-induced increase in testosterone may suggest the former, rather than the latter possible cause.

It is possible that caffeine increases endogenous testosterone levels by restricting hepatic blood flow and attenuating clearance (10, 19). Prior studies suggest that anhydrous caffeine ingestion elevates blood lactate, but not muscle lactate concentration (19), suggesting that blood lactate is increased due to decreased clearance, possibly via reduced hepatic blood flow. Furthermore, studies show increased blood lactate levels in exercisers ingesting caffeine (5, 21), but not coffee (16, 40). Therefore, it is possible that coffee attenuates caffeine-induced decreases in hepatic blood flow and thus blunts the increased serum testosterone observed in response to caffeine supplementation in other trials (9, 17, 28, 41, 60). Caffeine has been shown to be an ergogenic aid (22, 27, 28, 41), while results with regards to coffee have been equivocal (22, 27, 58). It has been hypothesized that chlorogenic acid content in coffee interferes with adenosine transporters and increases free adenosine, therefore increasing adenosine binding (42) and negating the typical effects of caffeine. While coffee is a popular vehicle for caffeine ingestion, results from the current study suggest that ingesting supplemental caffeine with caffeinated coffee does not impact the testosterone response to a high-intensity exercise trial.

Testosterone levels, on average, increased by 20.5% during exercise. Rosa et. al. (48) and Cadore et. al. (12) obtained average testosterone levels 58% and 41% greater than baseline respectively, while utilizing similar protocols. This study resulted in a more modest testosterone response, similar to those observed in the majority of literature ranging from 10% to 37% (14, 15, 28, 32, 41, 45, 46, 53, 59). Thus, the testosterone response in the current study could be considered quite typical and while the current findings may not extend to exercise bouts that elicit higher testosterone responses, the current data definitively show that caffeine combined with coffee does not increase resting testosterone or a typical testosterone response to exercise.

It should be noted that a large portion of the prior literature suggests that testosterone returns to pre-exercise levels 15-30 minutes post-exercise (7, 14, 15, 32, 53, 59). Only a few protocols have yielded a testosterone response that persists for 30 minutes or greater (45, 46, 60). This exercise protocol provoked testosterone levels that remained elevated by 14.3% 30 minutes post-exercise. This may suggest that this protocol is beneficial to creating a longer-lasting anabolic environment.

There were no differences in any proxy of muscle recovery 24 hours post-exercise between the treatments. While the treatments were not expected to directly affect recovery, it was hypothesized that there would be a direct relationship between magnitude of testosterone response and facilitated recovery (33, 49, 51). This is due to testosterone's role in increased protein synthesis, satellite cell recruitment, and myonuclei number (33, 49, 51). When analyzing for correlations between testosterone response and 24-hour post-exercise soreness, chest press repetitions to fatigue, and leg press repetitions to fatigue, no relationship was observed (maximum R value was 0.32). However, it should be realized that the combination of a relatively low sample size and a narrow range of testosterone responses may have prevented observation of a significant correlation.

While it is clear that inadequate testosterone levels greatly hinder muscle adaptation (25, 33) and exogenous testosterone enhances adaptations (11, 49), the beneficial impact of maximizing the endogenous testosterone response to exercise is under-researched. Evidence connecting elevated testosterone responses to enhanced muscle adaptation is in the form of correlational (1, 2) or single limb training studies

(25). These studies are limited in their ability to isolate their results as a direct effect of testosterone and have been contradicted by other projects (55, 57).

Limitations of this study include sample size and treatment number. A greater sample size would have increased statistical power, but it is noteworthy that there were no trends indicating possible differences in testosterone responses between treatments. Also, we did not have a clear comparison between equally caffeinated capsules and coffee. Despite this, the results still show that coffee supplemented with caffeine had no effect on testosterone levels post-exercise, while many studies have observed a positive effect in response to caffeine (9, 17, 41, 60). Future studies should also examine cortisol levels in response to coffee ingestion, since cortisol is the primary catabolic hormone. This would allow researchers to the possible effects of coffee on the post-exercise anabolic environment.

Overall the results of this study are some of the first to examine the varying effects of different modes of caffeine consumption on the testosterone response to exercise. While coffee is one of the most popular modes of caffeine consumption, it does not appear to augment endogenous testosterone response. More studies should be performed specifically comparing pure caffeine to coffee.

In addition, this is one of the first studies investigating testosterone levels and 24 hour post-exercise muscle function. This study observed no relationship between the two, despite the past literature indicating that increased testosterone levels affect physiological processes associated with recovery.

Practical Applications: This study suggests that caffeine ingestion via coffee does not enhance testosterone response to exercise. In addition, high intensity intervals followed by resistance training seem to elicit, and maintain, elevated testosterone levels for at least 30 minutes post-exercise.

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Table 1. Mean (±SD) age, height, weight, and aerobic fitness of sample (n=9)

Age (years)	Height (cm)	Weight (kg)	VO_{2max} (ml/kg/min)	2mmol/L lactate (W)	4mmol/L lactate (W)
21.8±1.5	176±6.0	84.7±8.2	47.9±4.0	130±32.1	208±37.7

Table 2. Mean (±SD) 1RM of sample (kg.) (n=9)

Chest Press	Leg Press	Shoulder Press	Leg Curl	Row	Leg Extension	Lat Pull Down
115±19.1	195±35.9	90±20.2	89±15.9	114±13.5	140±31.8	116±26.2

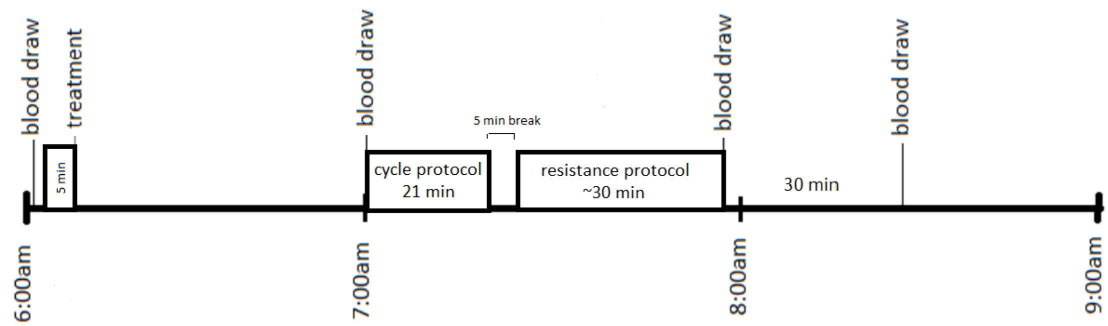

Figure 1. Performance trial timeline

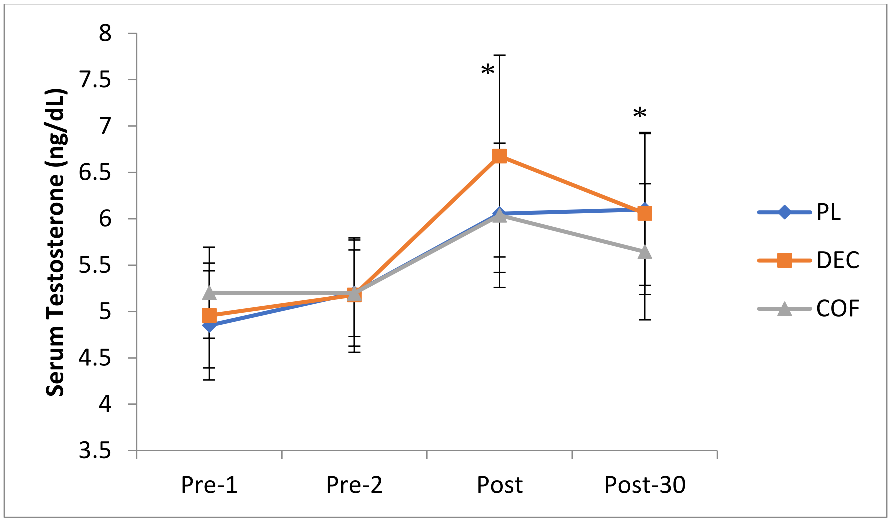

Figure 2. The effects of treatments on the acute testosterone response to exercise (* = significantly higher than Pre-2 levels, p<0.05)

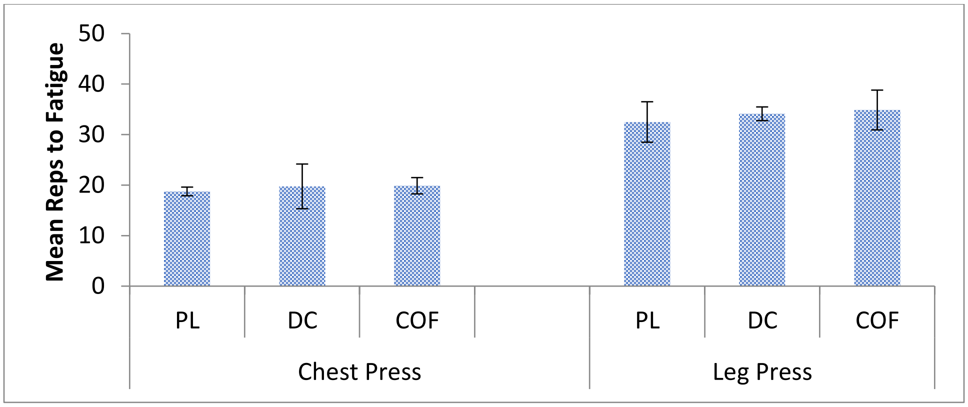

Figure 3. The effects of treatments on chest and leg press reps to fatigue at 65% 1RM 24hr post-exercise

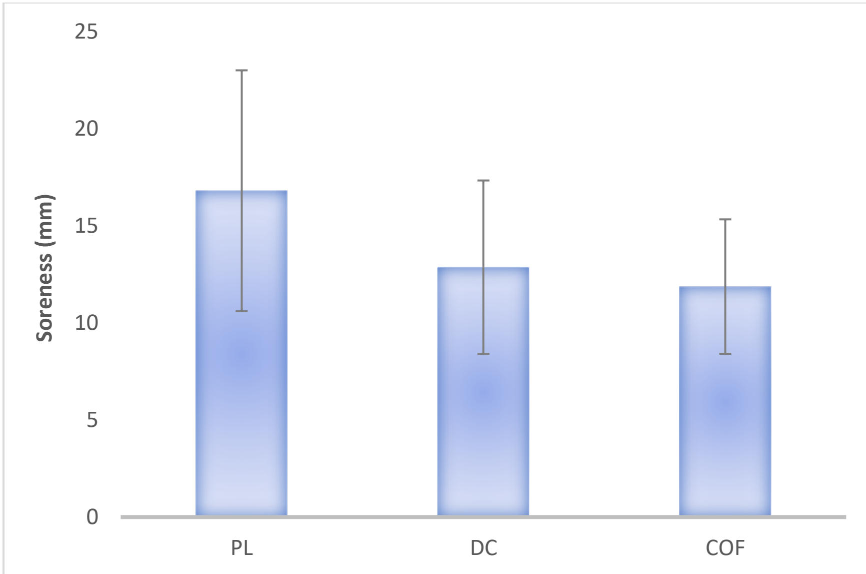

Figure 4. The effects of treatments on soreness 24hr post-exercise (soreness was measured on a scale of 0-100mm)

Appendix A

Informed Consent

Consent to Participate in Research

Identification of Investigators & Purpose of Study

You are being asked to participate in a research study conducted by Taylor Landry from James Madison University. The purpose of this study is to determine the effects of coffee ingestion on the testosterone response to exercise as well as identify any association between testosterone response and muscle recovery. This study will contribute to the researcher's completion of master's thesis.

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 8 visits. You will be asked to complete exercise and dietary logs 72 and 24 hours prior to the exercise trials respectively. You must abstain from strenuous exercise and caffeine 48 hours prior to the exercise trials. Alcohol must be avoided 24 hours before exercise trials. You will be asked to consume a standardized breakfast of one Ensure beverage before each exercise protocol. Each visit is detailed below:

Visit 1

Before any data collection you will be asked to complete some forms. This is to verify that you meet the study criteria and completely understand what the study entails.

Visit 2: Preliminary Testing and Familiarization

This visit is for preliminary testing and familiarization. You will perform a maximal cycle test lasting between eight and twelve minutes. This test starts out very easy and gets harder until you cannot continue. During this time heart rate will be measured and finger sticks will be performed every three minutes to measure lactate levels in your blood. You will also be breathing through a mouthpiece so we can collect your expired air to measure how much oxygen you are consuming. Immediately after the maximal test we will determine your maximal strength on chest press, leg press, shoulder press, leg extension, row, leg curl, and lat pull down respectively. This should take approximately 1.5-2 hours.

Visits 3, 5, and 7: Exercise Trials

These visits are the exercise trials. They will be performed between 6am and 8am and the time will be consistent throughout the study. Each exercise trial will be preceded by ingestion of coffee that either is decaffeinated or contains 6 mg of caffeine per kg of your body weight. For a 175 pound person, this would correspond to 477 mg of caffeine or the equivalent of the amount in 40 fluid ounces of coffee (5 cups). This will be consumed an hour before the exercise trial. You will be permitted to read or access your laptop during the hour between treatment consumption and exercise. We will obtain 4-5mL or 1 teaspoon of blood immediately before the treatment and immediately before exercise to measure testosterone.

Exercise trials begin with stationary cycling. You will alternate pedaling at different intensity levels for 21 minutes. You will then begin the resistance training protocol. Three sets of Chest press, leg press, rows, leg extensions, shoulder press, leg curls, and lat pull downs will be performed respectively. Each set consists of 10 reps at separated by one minute rest. After exercise testing we will obtain a blood sample from an arm vein immediately, and 30-minute, post exercise. Serum testosterone levels will be measured from these blood samples.

Visits 4, 6, and 8: Recovery Analysis

The morning following each exercise trial we will meet to determine recovery and muscle function. You will complete a questionnaire to determine your level of soreness. You will then perform repetitions to fatigue tests on the chest and leg press machines at 65% one repetition maximum.

Optional Body Composition Analysis:

A DEXA scan is used to determine percent body fat and bone density. You will lie completely still on the machine while it slowly scans your entire body. There will be no discomfort during this procedure, but it can take up to 10 minutes. No data collected from this analysis will be used or reported for this research study. This part of the study is not required and is simply something we can provide in appreciation for your completion of the study.

Time Required

Participation in this study will require about 11 hours of your time divided into 8 separate visits.

Risks

The investigator perceives the following are possible risks arising from your involvement with this study: Use of the DEXA scan for body composition assessment exposes one's self to 1-3 millirads of radiation, a similar amount of radiation to what one is exposed to during a transatlantic flight. You may choose to decline to receive a DEXA scan. Blood draws can result in some bruising and/or discomfort. The investigator perceives the following are possible risks arising from your participation in the exercise protocol: nausea, discomfort, dizziness, and in rare occurrences, heart attack, stroke or death. The selection criteria from the health screening 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.

Benefits

Potential benefits from participation in this study include: receiving information regarding one's fitness and health. This includes aerobic fitness assessment (VO_{2max}), body composition, and resting testosterone levels. Coffee is one of the most popular modes of caffeine consumption and participation in this study will help investigate its possible effects on testosterone levels before and after exercise. Therefore, participation in this study helps benefit the academic community as well as resistance trained athletes as this project determines if coffee may augment, hinder, or have no effect on muscle adaptation.

Confidentiality

The results of this research will be presented to a number of sources including, but not limited to, peer reviewed journals, scientific conferences, and to JMU faculty. 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. 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. Upon completion of the study, all information that matches up individual respondents with their answers will be coded and stored in a secure location within Godwin Hall. This information will be destroyed three years after the completion of the study.

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.

Questions about the Study

If you have questions or concerns during the time of your participation in this study, or after its completion or you would like to receive a copy of the final aggregate results of this study, please contact:

Taylor Landry
Kinesiology
James Madison University
landrytm@dukes.jmu.edu

Chris Womack
Kinesiology
James Madison University
womackcx@jmu.edu
540-568-6145

Questions about Your Rights as a Research Subject

Dr. David Cockley
Chair, Institutional Review Board
James Madison University

(540) 568-2834
cocklede@jmu.edu

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 Participant (Signed) Date

______________________________________ ______________
Name of Researcher (Signed) Date

Attachment 2: Sample Recruitment Email

Appendix B

Recruitment Email

Testosterone Research at James Madison University

The Human Performance Lab at James Madison University is investigating the effect of coffee on testosterone levels and recovery from exercise.

Benefits:

1) Free testosterone analysis 2) Free DEXA scan analyzing body fat percentage and bone density 3) Free aerobic fitness assessment (VO_{2max} test)

Responsibilities:

About 12 hours of your time including preliminary fitness testing and health screening as well as exercise protocols involving interval and resistance training. Laboratory assessments will also be performed including finger-stick blood samples or venous blood draws (approximately 1 teaspoon) each visit.

Am I a good candidate?

Subjects must be 18-26 year old non-smoking males with no known cardiovascular, respiratory, or metabolic disease. You must participate in, on average, 3 days of weight lifting a week for the last 6 months.

If you are interested contact Taylor Landry at landrytm@dukes.jmu.edu or 6039301004

Appendix C

Dietary Intake Log

Dietary Log & Instructions
DIET RECORD
*Subject number*_____________ *Date*_______________ *Day of Week*_______________

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

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

Instructions For Keeping Your Three-Day Food Record

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

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

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

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

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

   Combination foods:
   List parts of food on separate lines
   Include preparation method, quantity, and brand name of each food
   Example: Sandwich (4 oz healthy choice turkey, 2 slices Sara Lee wheat bread,
   1 tbsp Hellman's light mayo, 2 oz Kraft American cheese, 1 slice of red fresh tomato)

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

6. Record quantity consumed
   Do not record any food not eaten
   Example: made two cups of vegetables but ate half so you would record one cup

   Quantity of food and/or drink
   Example: cups, ounces, liters, grams, each, or other unit of measure
   Example: 1 cup of vegetables, 4 ounces of meat, one medium apple

7. Record brand name
   Example: fast food chain name and/or package name
   Example: Wendy's, Betty Crocker, Lean Cuisine, Gatorade, Thomas Bagel

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

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

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

	Low-sodium or regular? Added fat or sauce?

Helpful Hints with Portion Sizes

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

Appendix D

Physical Activity Log

Physical Activity Log

Physical Activity Records

Subject #_______________ Trial #_________ Date:___________

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

Intensity Scale

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

Appendix E

Subject Prescreening Questionnaire

Prescreening Questionnaire

Subject Prescreening Information

Please Complete the Following:

Gender: Male Female (circle one)

Age:____years

Height_____________Weight_______________

Typical Exercise Habits over the Past 6 Months:

Average number of days of lifting per week____________

Average number of hours of lifting per week___________

Do you have a muscle or joint injury/condition that precludes the completion of the training protocol?

List any allergies you have here:

Appendix F

Visual Analog Scale of Soreness

Soreness Scale

Muscle Soreness Questionnaire

Subject #__________ Trial #_____ Circle: Pre 4-Post 24-Post Date:_______

Muscle Soreness

➔Please place a mark on the line below corresponding to your level of muscle soreness

0 millimeters (left) = complete absence of muscular soreness
100 millimeters (right) = extremely sore with noticeable pain and stiffness at all times

0 mm ____ 100 mm

Score:_______mm (to be completed by researcher)

Appendix G

Health History Questionnaire

AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire

Assess your health status by marking all *true* statements

History

You have had:

________ a heart attack
________ heart surgery
________ cardiac catheterization
________ coronary angioplasty (PTCA)
________ pacemaker/implantable cardiac
________ defibrillator/rhythm disturbance
________ heart valve disease
________ heart failure
________ heart transplantation
________ congenital heart disease

Symptoms

________ You experience chest discomfort with exertion
________ You experience unreasonable breathlessness
________ You experience dizziness, fainting, or blackouts
________ You take heart medications

Other Health Issues

________ You have diabetes
________ You have asthma or other lung disease
________ You have burning or cramping sensation in your lower legs when walking short distances
________ You have musculoskeletal problems that limit your physical activity
________ You have concerns about the safety of exercise
________ You take prescription medication(s)

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

Cardiovascular risk factors

________ You are a man older than 45 years
________ You smoke, or quit smoking within the previous 6 months
________ Your blood pressure is > 140/90 mmHg
________ You do not know your blood pressure
________ You take blood pressure medication
________ Your blood cholesterol level is > 200 mg/dl
________ You do not know your cholesterol level
________ You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)
________ You are physically inactive (i.e. you get < 30 minutes of physical activity on at least 3 days of the week)
________ You are > 20 pounds overweight

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

________ None of the above

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

Appendix H

Preliminary Testing Data Sheet

Preliminary Trial

Subject Number: ______________ Date: _________________

Body Weight: _______________ kg Height: _______________ cm

$VO_{2\ peak}$ Test

Min	Watts	HR	RPE	Lactate
1	75		X	X
2	X		X	X
3	X			
4	125		X	X
5	X		X	X
6	X			
7	175		X	X
8	X		X	X
9	X			
10	225		X	X
11	X		X	X
12	X			
13	275		X	X
14	X		X	X
15	X			

VO_{2max}- ___________________ml/kg/min Resting lactate- __________________mmol/L

HR_{max}- _____________________bpm Finishing lactate-___________________mmol/L

Resistance Training Preliminary Trials

	Seat Back Position	Seat Height	Leg/Knee Position	1RM	65% 1RM
Chest Press					
Leg Press					
Shoulder Press					
Leg Extension					
Row					
Leg Curl					
Lat Pull Down					

Appendix I

Exercise Bout Data Sheet

Treatment A

Pre-1: ___________________________ Pre-2: _____________________________

Post: ____________________________ Post-30: ____________________________

RTF Chest: _____________________ RTF Legs: __________________________

Soreness:_______________

Treatment B

Pre-1: ___________________________ Pre-2: _____________________________

Post: ____________________________ Post-30: ____________________________

RTF Chest: _____________________ RTF Legs: __________________________

Soreness:_______________

Treatment C

Pre-1: ___________________________ Pre-2: _____________________________

Post: ____________________________ Post-30: ____________________________

RTF Chest: _____________________ RTF Legs: __________________________

Soreness:_______________

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