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ACUTE HORMONAL RESPONSES AFTER TWO DIFFERENT RESISTANCE EXERCISE PROTOCOLS IN COLLEGE-AGED MALES

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

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ABSTRACT

PURPOSE: The purpose of the current study was to compare the acute hormonal responses of two resistance training exercise protocols (Traditional and SuperSlow) that had similar exercise volumes but differed in intensity and contraction speeds in college-aged males.

METHODS: Thirteen healthy college-aged male subjects participated in this study. This study was a randomized cross-over design. All participants performed a session of low intensity resistance exercise (50% 1-RM) with slow velocity contractions and a session of traditional high intensity resistance exercise (80% 1-RM) separated by 3weeks. Participants in both exercise conditions performed four upper body (shoulder press, biceps curl, chest press, low row) and four lower body (knee extension, knee flexion, two-leg press, calf raises) exercises. The contraction velocities were either slow speed contraction (10 seconds concentric and 5 seconds eccentric) for low intensity resistance exercise or normal speed contraction (1.5 seconds concentric and 1.5 seconds eccentric) for high intensity resistance exercise. Pre, immediate post exercise, and 15 minutes post exercise blood samples were taken to determine CK, cortisol (COR), and testosterone (TES) concentrations.

RESULTS: Two-way (condition × time) repeated measures analysis of variance (ANOVA) revealed no significant main effects for condition or time and no significant condition × time interaction for CK ($p = 0.121$, $p = 0.286$ and $p = 0.992$, respectively). Repeated measures ANOVA revealed a significant main effect for time for TES ($p = 0.012$) and COR ($p = 0.009$) but no significant main effect for condition and no significant condition × time interaction (TES: $p = 0.614$, $p = 0.509$, COR: $p = 0.452$, $p =$

0.710 respectively). Both significant main effects for time for both TES and COR were no longer significant when hormone values were adjusted to account for changes in plasma volume. There was a significant main effect for time for LA ($p = 0.000$) from pre to post exercise time points.

CONCLUSION: The hormonal responses for the low intensity, slow contraction speed, SS condition and the high intensity, normal contraction speed, TR condition were similar even though the SS condition had fewer sets and repetition compared to the TR condition.

CHAPTER I

INTRODUCTION

Resistance training is important in sport as well as in daily activities. It has been accepted that neural responses and muscular hypertrophy contribute to improvements in muscular strength. Traditional resistance training is an efficient method for increasing muscle strength,[6, 7, 13] hypertrophy,[5, 74] muscular endurance,[17] and bone mineral density.[81] These adaptations are attributed to neural, hormonal, and mechanical demands that have been placed on the various physiological systems by progressive overload used in resistance training.[4, 40] In general, the increases in strength following a strength training protocol are similar between men and women, even though men have higher absolute strength values.[49]

An important factor to consider in order to ensure appropriate adaptations at any age or for either gender is the appropriateness of the resistance training program. Most strength training programs can vary the number of repetitions, sets, and intensity in order to increase different physiological factors depending on the purpose of the program (injury rehabilitation, improve muscle tone, increase muscle mass, improve muscular endurance, or strength improvements). Muscular strength development is primarily dependent on the type of exercise, the amount of training volume, number of repetitions, and exercise intensity.[45]

Although training intensity depends on individual training goals and fitness levels, it is generally accepted that a resistance training intensity of over 60-65% of the one repetition maximum (1-RM) is necessary to achieve substantial muscle hypertrophy.[13, 55] Ratamess et al.[63] suggested that resistance training at high intensity 70-85% of 1 RM (8-

12 reps) is the optimal protocol for beginners and intermediate experienced individuals to promote muscle hypertrophy.

Changes in muscle cross sectional area occur in response to changes in mechanical stress, energy demand, oxygen levels, hormones, and growth factors.

However, recent studies have demonstrated that a low-intensity (20-50% of 1-RM) resistance training program can also increase muscle hypertrophy and strength, similar to high-intensity (80-90% of 1-RM) programs.[36, 79] This type of training is known as KAATSU training and involves blood flow restriction in combination with resistance training. One advantage of this type of low intensity training is the reduction in mechanical stress that is placed on the joints of the body.[67] It has been hypothesized that the mechanism responsible for muscle hypertrophy with this type of training is the reduction in oxygen content and the resultant increases in lactate which stimulate growth hormone release and the early recruitment of high threshold muscle fibers.

Another training technique that has been recently introduced as an alternative resistance training method is the "SuperSlow" technique.[32] Westcott et al.[85] utilized this slow-speed of contraction program with low-intensity (50% of 1-RM) and reported significant improvements in muscular strength. Tanimoto et al.[78] suggested that both low-intensity resistance training (55-60% of 1-RM) with slow movement (3 sec concentric and eccentric phases) and traditional high-intensity resistance training (85-90% of 1-RM) at normal speed protocols were effective interventions to not only increase muscular hypertrophy and strength, but also increase peripheral blood flow and vascular conductance as an additional benefit. Keeler et al.[37] also investigated both traditional resistance training and slow-speed resistance training protocols and reported significant

strength gains for both groups, although the traditional group showed greater strength improvements compared to the slow-speed resistance training group. These authors hypothesized that the reason for the adaptations seen during the slow contraction speed resistance training programs was due in part to the increased metabolic demands placed on the muscle because of the exaggerated contraction times.

As mentioned earlier, the volume and intensity of a resistance training program can be manipulated by varying the repetitions, workloads, contraction speed, and number of sets completed. Traditional resistance training typically involves high loads (80% 1RM) and low repetitions (6-8 reps) that produces improvements in muscular strength; while muscular endurance training typically involves lower intensities and longer training durations. Higher intensity exercise is often associated with a greater incidence of cardiovascular and orthopedic injuries, although habitual vigorous exertion exercise can attenuate the risk of sudden death.[2, 24]

Superslow resistance training programs are characterized by low resistance workloads and slow repetitions compared to traditional resistance training programs. This Superslow training, at least theoretically, could provide an effective way to increase both muscular strength and aerobic endurance simultaneously. Improvements in VO_2 max are dependent on the duration, intensity, frequency of training, the mode of exercise, and the individual's initial fitness level.[24] This implies that by altering the training stimulus, one could achieve differing adaptations to the exercise program. Keeler et al.[37] studied untrained sedentary women during a 10 week, high intensity resistance training program, that utilized different contraction speeds. The slow-speed contraction group performed 10 second concentric and 5 second eccentric contractions, while the traditional

resistance trained group performed 2 second concentric and 4 second eccentric contractions. Each group completed one set of 8 to 12 repetitions on 8 different Nautilus machines 3 times per week. They found that both the traditional and Superslow resistance training groups improved their strength but neither group improved aerobic capacity.

These different studies that involve the manipulation of resistance training programs by the addition of blood flow restriction or by altering contraction speed, provide insight to the different possible underlying mechanisms of adaptation and possible applications not only for improved athletic performance but also, potentially, applications for muscle-related pathologies. The capacity of skeletal muscle to adapt to shifts in metabolic and functional requirements can be readily observed through resistance or endurance training. Endurance training results in increased oxidative capacity of muscle, evidenced by increases in the apparent proportions of oxidative fibers, increased levels of oxidative enzymes, improved capillary blood supply, and increased numbers of mitochondria. In contrast, resistance training leads to greater muscular strength due to neural adaptation, increased motor-unit activation, and muscle fiber hypertrophy.

Resistance training appears to be an effective method of improving muscular hypertrophy, however, it is not clear what specific exercise variable is the best for optimizing this adaptation.

Purpose of the Study

The purpose of this study was to compare the acute hormonal responses of two different resistance exercise protocols (traditional and Superslow) that have similar exercise volumes but differ in intensity and contraction speed in college-aged men.

Previous studies investigating Superslow resistance exercise have not evaluated the endocrine responses to this type of exercise and have only been able to speculate as to why muscle hypertrophy occurs.

Research Question

1. Will an exercise protocol (Superslow) based on low intensity and high volume produce similar endocrine responses as a traditional high intensity, low volume resistance training protocol.

Sub Questions

1. Will the different resistance training protocols result in different amounts of muscle damage?

Research Hypotheses

1. Both exercise groups will experience significant changes in hormonal responses conducive to muscle hypertrophy, but greater changes will occur for the traditional resistance exercise protocol.
2. The traditional high intensity resistance training protocol will result in greater muscle damage than the Superslow protocol.

Significance of the Study

Previous research has suggested that low intensity resistance training with blood flow restriction results in similar beneficial effects on muscular strength and mass compared to a traditional high intensity resistance training programs. Additionally, Superslow resistance training has been found to increase muscular strength similar to traditional forms of resistance training, as well as improving aerobic capacity. Therefore, low intensity Superslow resistance exercise may be better for individuals not capable of

the high orthopedic stresses to the joints associated with traditional resistance exercise, like those recovering from injury or the elderly. This study may also provide some insight into the underlying mechanisms of adaptation for the two different resistance exercise programs which may have implications in clinical settings.

Delimitations

1. This study only included college aged males who had not participated in any structured resistance training or aerobic training program for a minimum of 4 months prior to this study.
2. Only subjects free from any acute or chronic neuromuscular injuries or joint disorders were included.
3. Individuals were not be taking any hormone supplements or medications that could affect muscle or bone (corticosteroids, creatine, etc.).

Limitations

1. Although participants were asked not to change their normal daily activities, daily activities performed outside of the training program were not be controlled.
2. The sample was not random since all participants were volunteers; therefore, they may not represent all college males aged 18-35 years of age.
3. These findings may not apply for women.
4. These finding may not apply for men of other ages.

Assumptions

The Assumptions of the study include:

1. Participants answered all questionnaires honestly.
2. Each participant gave maximal effort during training and testing sessions.

3. All participants understood the testing protocols.
4. All subjects provided accurate information about medical and health history.
5. The subjects gave an honest assessment of exertion and pain after each training set.
6. All participants were in a fasted state for at least 8 hours prior to the blood draws.
7. All devices were calibrated before all testing sessions.

Operational Definitions

The operational definitions for this study include:

1. **1 Repetition Maximum (1-RM) test:** 1-RM is the greatest weight that can be lifted once throughout the complete range of movement, using correct form.[4]
2. **PAR-Q:** PAR-Q (Physical activity readiness questionnaire) is designed to identify the small number of adults for whom physical activity might be inappropriate or those who should have medical advice concerning the type of activity most suitable for them.
3. **Body composition:** It is used to describe the percentages of bone, fat and muscle in human bodies.
4. **Muscular Strength:** The amount of force produced by group of muscles or a muscle.
5. **Cortisol:** The primary glucocorticoid secreted by the adrenal cortex is cortisol. Cortisol increases gluconeogenesis, free fatty acid mobilization, and decreases protein synthesis and glucose uptake by tissue.
6. **Testosterone:** Testosterone secreted from the testes. Testosterone is involved in protein synthesis.
7. **Lactate:** Lactic acid in the muscle occurs only during short bouts of exercise of relatively high intensity and it is usually related to fatigue and muscle soreness.

8. **Creatine Kinase (CK):** An enzyme that is assayed in blood tests as a marker of muscle breakdown.
9. **Hematocrit:** Percentage of the volume of whole blood that is made up of red blood cells. This measurement depends on the number of red blood cells and the size of red blood cells. Normal values are 45% for men and 40% for women.
10. **Uncorrected Hormone Value:** The concentration of the hormone without regard to any changes in plasma volume levels.
11. **Uncorrected Hormone Value:** The concentration of the hormone adjusted for changes in plasma volume levels.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Resistance training is an effective method of improving muscular hypertrophy; however, not all individuals are capable of resistance training for improvements in muscular strength and hypertrophy based on traditional resistance training principles. Therefore, this study was designed to compare the acute hormonal responses of two different resistance exercise protocols (traditional and Superslow) that have similar exercise volumes but differ in intensity and contraction speed in college-aged men. Previous studies investigating Superslow resistance exercise have not evaluated the endocrine responses to this type of exercise and have only been able to speculate as to why muscle hypertrophy occurs.

Resistance Training

The primary aim of resistance exercise is to stress the neuromuscular system to bring about positive neuromuscular adaptation to enhance physical performance. It has been reported that initial muscular strength improvements following training occur from the neural adaptations followed by muscular hypertrophy. Traditional resistance training has become a widely accepted method for improving muscular power, muscular strength, and muscle hypertrophy.[45] It is generally accepted that resistance training programs with high intensity (70-80% of 1-RM) are the best design for optimizing muscle hypertrophy for untrained individuals.[3] The prescription of a resistance training program requires the consideration of several factors, including the intensity, frequency, and volume of exercise.[62] Another resistance training variable is

contraction speed which may influence the effectiveness of strength training programs. An effective resistance exercise program requires a combination of these various factors to stimulate muscular adaptation. Resistance exercise induces increased synthesis of myofibrillar proteins, which in turn results in increased muscular hypertrophy especially in type II fibers.[72,73] The fundamental objective of resistance exercise is to obtain increases in muscular strength and muscular cross sectional size. A report by Chelsey et al.[14] mentioned a single bout of resistance exercise to be sufficient stimulus to produce elevated production of contractile proteins for the following 24 hours. Mechanical stress is believed to play a critical role for muscular hypertrophy.[55] Previous researches have demonstrated the benefits of resistance exercise for a variety of populations.[11, 15] Regular systemic resistance exercise will result in increases in muscular strength due to neural factors and muscle morphology.[19, 80] Neural adapation plays a critical role for increases in muscle strength in early phases of resistance exercise training; however, subsequent muscular strength increases are due to muscle hypertrophy.[45]

Hormonal Responses to a Single Bout of Resistance Exercise

Resistance exercise elicits acute physiological responses, including the neuroendocrine system, that play critical roles in increasing muscular strength, power, and hypertrophy. Many individuals who take part in resistance exercise want to maximize these responses, thus will responsible for select an exercise protocol that they can maintain while optimizing the neuroendocrine responses will be responsible for improved muscular function.[46] Resistance exercise of adequate intensity is a potent stimulus for endocrine secretion.

Acute exercise endocrine responses to resistance training protocols have been examined. The acute hormonal response to an exercise session is a crucial indicator of muscle remodeling after exercise. Testosterone is a steroid hormone secreted from testicular Leydig cells of the testes. A well-established body of evidence exists regarding the acute testosterone responses to a bout of resistance exercise in men,[26, 43] particularly when high volumes are implemented.[8, 24, 25, 64] Acutely, resistance exercise has been shown to increase testosterone concentrations in men post exercise, but the evidence in women is equivocal.[46] Studies implementing an acute resistance bout with greater volume [24, 25, 64] and exercises that utilize large muscle mass[27] have been most efficacious in eliciting significant testosterone responses. Fry and Lohnes[18] also reported similar findings following a single bout of high power resistance exercise; however, some studies have failed to demonstrate significant increases in testosterone concentrations following a single bout of resistance exercise.[2, 16]

The adrenal gland secretes cortisol (glucocorticoid), aldosterone, estrogens, and androgens. Cortisol has a catabolic effect on tissue and is associated with a decrease in anabolic (muscle growth) hormones. Cortisol and its catabolic functions seem to affect type II muscle fibers to a greater extent than type I muscle fibers. In the past, cortisol response to acute exercise has been looked at as a negative response; however, many researchers now believe that these acute elevations are an important part of the remodeling and repair process in muscle. In general acute resistance exercise has been shown to increase circulating levels of cortisol.[21, 50, 53, 54] Fry and Lohnes[18] reported no cortisol responses after a single bout of high power resistance

exercise. Kraemer and Ratamess[46] indicated that the acute hormonal response to a single bout of resistance exercise is dependent on several exercise variables (volume, intensity, nutritional intake, and training experience). In a study of McCaulley et al. [54] they reported a significant decrease in resting cortisol concentration in trained men. The control group also had a similar decrease in resting cortisol; however, the authors mentioned these cortisol level changes could not be attributed to the resistance training program. However, another study by Staron et al. [72] indicated that there were no changes in resting cortisol levels in the control group while the trained men had decreased resting cortisol levels.

Weiss et al. [84] designed a study to determine the comparison of serum testosterone and androstenedione in 20 males and 20 females following three sets of four-heavy resistance exercises. The finding from this study is also in agreement with previous studies that report a significant increase in absolute testosterone response after a single bout of resistance exercise that can facilitate protein synthesis,[72] especially if high amounts of muscle mass are utilized with relatively high intensity.[20, 28]

McCaulley et al. [54] determined the acute hormonal responses to resistance exercise in young men (21.8 ± 1.9). This study manipulated exercise volume in order to make the different intensity exercise protocols equal. The exercise protocols included hypertrophy type (4 sets × 10 repetitions × 75% 1-RM), strength type (11 sets × 3 repetitions × 90% 1-RM), and power type (8 sets × 6 repetitions of jump squats × 0%1-RM) exercises. The authors indicated that the hypertrophy type exercise protocol elicited the greatest hormone response following exercise even

though the exercise volumes were equal among the different exercise protocols.

Goto et al. [23] attempted to examine acute hormone and recovery responses to resistance exercise with slow movement. Six healthy young men (24.3 ± 0.4 years) performed three different types of exercise protocols (high intensity with normal contraction speed, low intensity with slow contraction speed, and low intensity with normal contraction speed). The results of this study showed that the low intensity resistance with slow contraction speed exercise protocol had the greatest increase in growth hormone and free testosterone concentrations and the greatest decrease in cortisol concentration compared to the other two protocols. In addition, there were no significant differences in creatine kinase activity between the exercise protocols. The authors concluded that the slow movement during the resistance exercise was an effective protocol for the increase of hormone secretion.

Linnamo et al. [50] investigated acute hormonal responses to three different exercise types in men and women. Both submaximal and maximal explosive resistance exercise groups had the same protocol but with different weights. The maximal heavy resistance exercise group performed each exercise with a 10-RM (5 sets × 10-RM), the submaximal heavy resistance exercise group performed 10 repetitions with 70% 10-RM (5 sets × 70% × 10-RM), and the maximal explosive resistance exercise group performed at 40% 10-RM (5 sets × 40% 10-RM). The results indicated that the greatest growth hormone, testosterone, and blood lactate responses occurred during maximal heavy resistance exercise. These results suggested that the maximal heavy resistance exercise protocol was a more effective exercise mode for muscular strength development than other exercise modes.

Goto et al. [22] investigated the effects of combinations of high and low intensity resistance exercise training on muscle function, muscle size, and hormonal responses. All subjects performed a period of hypertrophy training first and then the subjects were assigned to one of two groups. One group performed 5 sets of a high-intensity (90% of 1-RM) to failure (strength type) and the other group performed the same training, but the last set was followed by an added set at 50% of 1-RM to failure (combi-type). The authors indicated that the combi-type protocol that added the moderate intensity set after the high intensity sets' regimens had greater hormonal responses (Growth hormone), increased muscular strength, and muscular hypertrophy; however, the potential mechanisms for these changes were not discussed.

Low Intensity Resistance Exercise

High intensity (70-80% 1-RM) for 8-12 repetitions resistance exercise is believed to be the best exercise mode for maximizing training-induced muscular hypertrophy.[3] It has been proposed that high amounts of mechanical stress to the body leads to an increase in muscular strength and size compared to lower intensity resistance exercise. However, two types of resistance exercise using lower exercise intensities have been developed by researchers. Several studies that have combined blood flow restriction with low intensity resistance training have also documented increases in strength and/or muscle hypertrophy.[1, 57, 76, 77] Additionally, low intensity resistance exercise with slow contraction velocity has been reported to improve muscular strength.[38, 79, 85] A study that evaluated muscle biopsies reported a single bout of low intensity resistance exercise (30%1-RM) was equally as effective in

stimulating myofibrillar protein synthesis rates as a single bout of high intensity (90% 1-RM) resistance exercise.[12]

Holm et al. [30] investigated the effects of high and low intensity training on neuromuscular responses in healthy men. Eleven men (24.7 ± 1.1 yr) participated three times per week in a 12-week training program. They applied a protocol in which the same individual trained one leg at 70% 1-RM (heavy loading; HL) while training the other leg at 15.5% 1-RM (light loading; LL). Quadriceps muscle exercises were performed by randomizing half of the participants to train their dominant leg with HL and the contralateral leg with LL, while training was reversed for other half of the subjects. Quadriceps muscle cross-sectional area increased 8% and 3% in HL and LL legs, respectively and 1RM strength increased in both legs (HL: 36 %, LL: 19%). Isokinetic strength performed at 60°/s improved by 13% in HL but remained unchanged in LL (4%, not significant). They concluded that light loading had minimal effects on muscle strength and hypertrophy compared to traditional high loading training in men.

Abe et al. [1] investigated the effects of twice daily sessions of low-intensity resistance training (LIT, 20% of 1-RM) with (LIT-BFR) or without (LIT) blood flow restriction for two weeks on skeletal muscle size and circulating insulin-like growth factor-1 (IGF-1) in young males (mean ± SD age, 23.6 ± 6.5 years). Exercises were performed with loads of 20% 1-RM, 15 repetitions for 3 sets of each exercise for 12 consecutive days (excluding one Sunday) twice per day. Following 2 weeks of resistance training, 1-RM strength of squat (17%) and leg curl (23%) increased in the LIT-BFR group which was significantly higher ($p<0.05$) than in LIT alone (squat:

9% and leg curl: 2%). Muscle volume increased in quadriceps, biceps femoris and gluteus maximus (7.7%, 10.1% and 9.1% for LIT-BFR ($p<0.01$) and 1.4%, 1.9% and -0.6% for LIT ($p>0.05$) respectively).

Another study by Moore et al. [57] examined the neuromuscular adaptations in muscle following low-intensity resistance training with blood flow restriction in untrained males. Eight subjects trained the elbow flexors of both arms three times per week for 8 weeks at 50% 1-RM. One arm was randomly assigned to perform the exercise protocol with blood flow restriction (OCC), and the other arm was not occluded (CON). Following exercise training, isometric maximal voluntary contraction strength only increased in OCC (8.3%). However, both groups had increases in maximal voluntary dynamic strength (OCC: 22%, CON: 23%). Post-activation potentiation (PAP) significantly increased by 51% in OCC whereas PAP was not changed in CON. They concluded that low-intensity resistance training produced an effective stimulus for increasing muscle strength with blood flow restriction by changing indices of neuromuscular function, such as an enhanced PAP. One advantage of this type of low intensity training is the reduction in mechanical stress that is placed on the joints of the body.[67] It has been hypothesized that the mechanism responsible for muscle hypertrophy with this type of training is the reduction in oxygen content and the resultant increases in lactate which stimulate growth hormone release and the early recruitment of high threshold muscle fibers.

Burd et al. [12] also demonstrated that a single bout of low intensity (30% 1-RM) with high volume resistance exercise stimulates greater myofibrillar protein synthesis compared with high intensity (90% 1-RM) with low volume resistance

exercise in young males. The authors also reported that the low intensity resistance exercise had more prolonged muscle protein synthesis rates compared to the high intensity exercise at 24 hours after exercise.

Resistance Exercise Volume

Exercise volume is one of the important variables when constructing a resistance exercise training protocol to maximize muscular strength. Resistance exercise volume is quantifiable by the number of repetitions and sets. A variety of resistance exercise protocols can improve muscular strength and it is well documented that multiple sets per exercise produce greater muscular strength gains. Kraemer et al. [47] investigated the effects of a single set of resistance training exercise to failure and 2 multiple-set protocols on the 1RM squat and indicated multiple sets not performed to failure produced greater muscular improvement than a single set in the 1-RM squat even though 1-RM squat improved significantly in all groups.

Ostrowski et al. [61] investigated the influence of exercise volume on muscular hypertrophy, strength and power in average trained participants over a 10-week training period. Thirty five male subjects were assigned to one of three groups: low volume group (3 sets), moderate volume group (6 sets), and high volume group (12 sets). Subjects were measured for muscular hypertrophy, strength, peak power, and hormonal changes (testosterone and cortisol). The authors reported that all three training groups had similar improvements in muscular strength and power and the trained subjects did not significantly change in testosterone or cortisol levels in any of three groups.

Another study by Hass et al. [29] investigated the effect of 13 weeks of

increasing training volume from one to three sets on muscular strength, body composition and muscular endurance. Forty two recreational weight lifters were assigned to one of two groups: a one set exercise group or a three set exercise group three times per week for 13 weeks. The authors reported that both groups had significant improvement in muscular strength, muscular endurance and lean body mass.

McBride et al.[52] assessed the effects of single versus multiple sets of resistance training on muscular strength, EMG, and body composition in 28 untrained men and women following 12 weeks of resistance training. Participants were randomized to either performing one set, two times per week or performing six sets, two times per week. The participants performed leg press and bicep curl exercises. There were no significant changes in lean body mass for either group, however, both groups increased muscular strength in both the leg press and bicep curl exercises. The authors also reported that the multiple set resistance exercise training group had greater muscular strength gains compared to the one set of resistance exercise training group.

Marx et al.[51] designed a study to determine the association between exercise volume and muscular adaptations in 34 females following 24 weeks of resistance training. The females subjects were assigned to one of three groups: low volume group (single-set circuit), high volume group (periodized high-volume multi-set), and control group. Subjects were measured pre-training, after 12 weeks, and 24 weeks of training for muscular hypertrophy, strength, power, endurance, and hormonal changes. The authors reported that the high-volume group had greater improvements in

muscular strength, muscular power, and muscular endurance compared with the low-volume training group. They also reported that the high-volume training group increased lean body mass. The resting serum testosterone and IGF-1 values were higher in the high-volume training group while the resting cortisol concentrations were lower when compared to the low-volume exercise training group.

Muscle Contraction Speed

Young and Bilby [86] conducted a 7 ½ week study examining the effects of contraction speed on muscular strength, power, and hypertrophy in untrained participants. Subjects were assigned to one of two (fast vs. slow group) training groups. Both exercise groups completed 4 sets of 8-12 repetitions half squat exercise using 8-12RM. The fast group was supervised to conduct the concentric phase of the exercise in a fast controlled manner while the slow group was supervised to lift in a slow controlled manner. The authors concluded that both groups improved similarly in muscular hypertrophy while the slow group increased less than the fast group on maximum rate of force development (23.5%: slow group vs. 68.7%; fast group). However, the slow group had greater improvements in absolute isometric strength than the fast group (31.0%: slow group vs. 12.4%; fast group).

In an 8-week study (3days/week), Shepstone et al. [69] investigated the effects of contraction speed muscle on fiber hypertrophy. Twelve young subjects performed maximal isokinetic eccentric exercise, one arm at slow speed and the other (contralateral arm) at fast speed. Before and after 8-weeks of training, muscle biopsies were analyzed to identify muscle fiber types and muscle fiber cross-sectional areas. The authors reported both arms had increased muscle fiber size for type I, type

IIa, and IIx muscle fibers after 8-weeks of training. However, type IIa and type IIx muscle fiber cross-sectional areas were greater in the fast-trained arm. This study also showed there was a significant increase in the percentage of type IIx isoforms and MHCIIx content in the fast-trained arm. The authors concluded that the fast-trained arm leads to greater muscle fiber hypertrophy and a shifting in the MHC expression compared with the slow-trained arm after 8 week eccentric isokinetic training.

Morrissey et al. [58] investigated the influence of training velocity in weight-resistive-dynamic exercise. Twenty four untrained females were assigned to either fast training group (1-sec lift phase, 1-sec lower phase) or slow training group (2-sec lift phase, 2-sec lower phase) three sets 8-RM for 7 weeks. Both fast and slow training groups showed similar improvement in strength tests which did not support the concept of velocity training specificity for weightlifting exercise.

Another study by Munn et al. [59] examined the effect of number of sets (1 set vs. 3 sets) and contraction speed on muscular strength. One hundred fifteen untrained subjects were assigned to one of five groups (control group, one set slow group, one set fast group, three sets slow group, or three sets fast group). All subjects in the four training groups exercised unilateral elbow flexion exercises with 6-8 repetitions, 3 times per week, over 6 weeks. The authors reported that the exercise groups which utilized multiple sets of exercises improved significantly more in muscular strength than one set exercise groups, and the fast speed training groups showed greater muscular strength gains compared to the slow speed training groups. The authors also mentioned that the influence of contraction speed on muscular strength was less

than the influence of the number of sets completed (three set vs. one set: 23% increase in strength, fast vs. slow contraction speed: 11% increase in strength).

Kaneshisa and Miyashita[35] reported that slow speed training was an effective exercise protocol to increase power output of the knee extensor muscles. Twenty-one men aged 23-25 years were assigned to one of three experimental groups and trained on an isokinetic dynamometer for 8 weeks at three specific speeds (1.05 $rad \cdot s^{-1}$, 3.14 $rad \cdot s^{-1}$, and 5.24 $rad \cdot s^{-1}$). Subjects were tested pre and post training on an isokinetic dynamometer for measuring maximal knee extensor power at five different velocities (1.05 $rad \cdot s^{-1}$, 2.09 $rad \cdot s^{-1}$, 3.14 $rad \cdot s^{-1}$, 4.19 $rad \cdot s^{-1}$, and 5.24 $rad \cdot s^{-1}$). The authors indicated that the slow speed training group had significant gains in power output at all five different speeds. However, the fast speed training group had significant gains in power output at the fast speeds.

SuperSlow Resistance Exercise

Resistance exercise at fast contraction velocity and high intensity would not be adequate for some individuals. Thus, it is necessary to develop appropriate exercise resistance regimens with lower mechanical stresses. Keeler et al.[37] investigated the effects of traditional resistance (TR) training versus SuperSlow resistance (SS) training on muscle strength and aerobic capacity. Fourteen sedentary women aged 19-45 years (mean 32.7±8.9 years) volunteered as subjects and performed 8 Nautilus exercises 3 times per week for 10 weeks. The subjects performed each exercise with one set of 8-12 repetitions to muscular failure. The intensity for the TR group was 80% 1-RM while the SS group used 50% 1-RM. Both TR and SS groups increased their strength significantly on all 8 resistance exercises,

however, the TR group had significantly greater increases than the SS group on 5 of the 8 exercises (torso arm (27% vs. 12%), leg extension (56% vs. 24%), leg press (33% vs. 7%), bench press (34% vs. 11%), and leg curl (40% vs. 15%)). This study did not find any significant changes in aerobic capacity, body fat, lean body mass, and body weight for either group following training. The ineffectiveness of SuperSlow training by Keeler et al.[37] may be the result of lower training volume in compared to the traditional resistance exercise group.

Hunter et al. [31] examined cardiovascular responses and metabolism during both traditional and SuperSlow training. Resting energy expenditure was measured in a 12-hour fasted state before exercise and 22 hours after both SuperSlow and traditional strength training exercises. The traditional exercise group performed 2 sets of 8 repetitions for 2 minutes (25% 1RM) whereas, the SuperSlow exercise group performed one set of 8 repetitions for approximately for 30 sec (65% 1RM). The heart rate was lower in the SuperSlow exercise group during exercises and total net energy expenditure for the SuperSlow exercise group was lower than for the traditional exercise group (155 ± 28 kcal vs. 107 ± 20 kcal). Thus, the authors indicated traditional strength exercise had greater increase in energy expenditure compared to SuperSlow exercise training.

Neils et al.[60] investigated muscular adaptations to traditional and SuperSlow resistance training. After 8 weeks of training, the SuperSlow training group had significant strength gains for the squat (3.6%) and bench press (9.1%). Traditional training group (80% 1RM) also showed a significant improvement for the squat and bench press (6.8%, 8.6% respectively). Although this research study indicated there

was no difference in strength improvement between two groups, the traditional training group had more peak power gains than the SuperSlow training group.

A recent study by Kim et al.[38] investigated the effectiveness of SuperSlow compared to traditional resistance training in muscular strength, flexibility, and aerobic capacity after four weeks of training. The original hypothesis was that the SuperSlow training group would improve more in aerobic capacity and flexibility than the traditional training group even if there would be no significant difference in muscular strength between two groups. Both groups experienced muscular strength improvements following five exercises (shoulder press, chest press, leg press, low row, and lat pull down) after four weeks of training; however, only the traditional training group showed significant improvement in muscular strength. Neither group had significant improvements in aerobic capacity and flexibility.

Summary

Traditional resistance exercise results in similar or greater muscular strength gains compared to SuperSlow resistance exercise but not all people can train with high loads. While there are no published studies on the hormonal changes to SuperSlow exercise, it is possible that SuperSlow exercise may enhance the hormonal responses compared to traditional resistance training. Research designed to elucidate the hormonal responses to SuperSlow resistance exercise would contribute to an understanding of the potential mechanism underlying the muscle hypertrophy.

CHAPTER III

METHODOLOGY

A well-established body of evidence exists regarding the acute endocrine response to a bout of traditional resistance exercise in men and women; however, SuperSlow resistance exercise studies have not investigated the endocrine responses and have been confounded by differences in SuperSlow exercise protocols and experimental designs. The acute response of hormones to a single bout of resistance exercise might help plan future resistance exercise interventions for muscular strength. The aim of this study was to investigate the acute hormonal responses of two different resistance exercise protocols that have similar exercise volumes but differ in contraction speed and intensity in college aged men. To our knowledge, this is the first study that has investigated the endocrine response following a bout of SuperSlow resistance exercise.

Subjects

Thirteen healthy college-aged males (18-35 years old) participated in this study. The participants were physically active, but they have not participated in a regular structured resistance or aerobic training program for at least 4 months prior to this study. Participants were instructed to refrain from any exercise and subjects were provided examples of the type of meal to eat the day before each exercise session. Prior to participation, all subjects were informed of the risks associated with this research study. After obtaining informed consent, subjects completed questionnaires and pre-testing prior to participation in the study and were familiarized with the study procedures during the week prior to implementation of the training program. The study was approved by the University of Oklahoma Institutional Review Board for Human Subjects. A non-

probability sampling technique was used because subject recruitment involved voluntary participation. Subjects were recruited from the University of Oklahoman and surrounding area through word of mouth, e-mail, fliers. Based on a power analyses, the number of subjects needed ranged from 5 – 15 to achieve a power > 0.80 with an alpha level of $p \leq 0.05$.

Inclusion Criteria

1. Subjects were male between the ages of 18-35 years.
2. Subjects were free of chronic back or any joint problems.
3. Subjects were free from hypertension and cardiovascular diseases.
4. Subjects were not taking any nutritional supplements or exogenous hormones.

Exclusion Criteria

1. Subjects outside the 18-35 age range
2. Subjects not currently participating in exercise such as resistance training or any moderate to high-intensity aerobic exercises within the last 4 months prior to study.
3. Subjects not able to perform the physical efforts.

Experimental Design

This research was a randomized crossover design in which participants completed two exercise protocols. Thirteen males aged 18-35 years from the University of Oklahoma, Norman, and Oklahoma City and its surrounding area were consented, screened, and randomly assigned to one of exercises in the separate day. Subjects performed a 5-minute warm-up at moderate intensity on a stationary bike. During the study intervention, subjects were instructed to continue their normal life.

Experimental Protocol

A familiarization session took place during the first week. This session was used to familiarize subjects to each exercise and testing procedures and involved subjects performing maximal muscular contractions. An additional goal of the familiarization session was to introduce subjects to the SuperSlow exercise procedures. Participants were instructed how to perform each resistance exercise in a safe method. Although this study was not a true random sample of participants, the participants were randomly assigned to either a traditional high intensity exercise protocol or a SuperSlow resistance exercise protocol each test day. Before starting the initial testing, the participants read and signed an informed consent form and complete a Health Status and Physical Activity Readiness Questionnaire. Subjects in the two resistance exercise protocols performed the same four lower and upper body exercises (upper body exercise: shoulder press, biceps curl, chest press, low row; lower body exercises: knee extension, knee flexion, two-leg press, and calf raises), however the exercise intensities, contraction speeds, and protocols were different.

The traditional resistance exercise protocol utilized three sets of eight repetitions at 80% 1-RM for each exercise. The contraction speed for this resistance exercise protocol was 1.5 seconds concentric and 1.5 seconds eccentric. The SuperSlow resistance exercise protocol utilized 1 set of each exercise until failure at 50% 1-RM. The contraction speed for this resistance exercise was ten seconds concentric and five seconds eccentric. There was one minute rest period between all sets and a one minute rest between different exercises. Subjects were instructed and encouraged by researchers

to complete the prescribed number of repetitions for each exercise and all exercises completed were recorded.

The Borg Rating of Perceived Exertion (RPE) and Pain scale was used to assess effort and discomfort after each set of exercises during the training sessions.

Questionnaires

All subjects filled out and signed an informed consent and completed a health status, Physical Activity Readiness Questionnaire (PAR-Q). The health status questionnaire and PAR-Q was used to determine any additional exclusion criteria.

RPE (Rating of Perceived Exertion) and Pain Scales

The RPE and Pain scales are methods for determining exercise intensity and pain levels. The scale of perceived exertion and pain are subjective measurements. The RPE and Pain scale measurements were asked while the subject was undertaking the exercise. Subjects were given instructions about how to interpret the RPE and pain scales prior to beginning exercise. The rating of perceived exertion was measured using the Borg's RPE scale. The range of RPE scale was from 6 to 20, with 6 indicating no exertion at all and 20 indicating maximal exertion. The rating of pain and discomfort was measured using the pain scale. This perceptual pain scale ranged from 1 to 10, with 1 indicating no distress and 10 indicating unbearable distress.

One Repetition Maximum (1-RM) Testing

1-RM testing was performed to measure maximum strength for lower body (knee extension, knee flexion, two leg press, and calf raises) and upper body exercises (biceps curl, chest press, shoulder press, and low row). A series of sub-maximal warm-up trials for each exercise at 50% of their perceived maximal effort was performed by subjects

before actual 1-RM testing. Weight on the Cybex isotonic weight machines was incrementally increased until subjects reached the maximum weight that could be successfully lifted in one repetition. One minute rest periods were given between each attempt. 1-RM was measured within four to six trials.

Blood Samples

Blood samples (approximately 6 ml) were collected at the beginning of each exercise session day, immediately after completion of exercise and 15 minutes later. After blood samples were obtained, two capillary tubes were used to measure hematocrit in duplicate and a drop of blood was used to assess lactate before the blood was centrifuged (Centra CL3R Refrigerated Centrifuge, Thermo Electron Corporation, Waltham, MA) and the serum was transferred into microtubes. All blood samples were obtained following an 8 hour overnight fast. Blood samples were stored at -80°C freezer in the Bone Density Laboratory. Hematocrits were used to determine changes in plasma volume. The blood tests performed were non-diagnostic tests, which were only used to compare the effects of the two different resistance exercise protocols. Whole blood was analyzed for lactate concentrations before exercises and immediately following exercises.

Hormone and Skeletal Muscle Damage Marker Analyses

1. Testosterone

The serum testosterone was measured in duplicate using an enzyme linked immunosorbent assay (ELISA) technique, based on the principle of competitive binding. Enzyme activity was determined testosterone concentrations are then calculated from a calibration curve fit with a quadratic equation. Assay protocol was performed to

manufacturer's procedures. The range of intra-assay and inter-assay coefficient of variation were 0 to 16.7% and 6.9 to 16.9%, respectively.

1) All serum and reagents were allowed to reach room temperature.
2) Dispense 25 µL of each standard into appropriate wells.
3) Dispense 25 µL of controls into appropriate wells.
4) Dispense 25 µL of samples into appropriate wells.
5) Add 200 µL enzyme conjugate into each well.
6) Incubate for 60 minutes.
7) Wash the wells 3 times with diluted wash solution.
8) Add 200 µL of substrate solution to each well.
9) Incubate for 15 minutes.
10) Add 100 µL of stop solution to each well.
11) Tubes was mixed by tapping plate and incubated at room temperature for 10 minutes.
12) Microtiter plate was read in the BioRad 680XR Plate Reader.

2. Cortisol

The serum cortisol was measured in duplicate using an enzyme linked immunosorbant assay technique (ELISA). Enzyme activity was determined serum concentrations are then calculated from a calibration curve fit with a quadratic equation. The assay protocol was accurately performed to manufacturer's procedures. The range of intra-assay and inter-assay coefficient of variation were 0.9 to 7.5% and 9.4 to 13.6%, respectively.

1) All serum and reagents were allowed to reach room temperature.

2) Dispense 20 µL of each standard into appropriate wells.
3) Dispense 20 µL of controls into appropriate wells.
4) Dispense 20 µL of samples into appropriate wells.
5) Add 200 µL enzyme conjugate into each well.
6) Incubate for 60 minutes.
7) Wash the wells 3 times with diluted wash solution.
8) Add 100 µL of substrate solution to each well.
9) Incubate for 15 minutes.
10) Add 100 µL of stop solution to each well.
11) Microtiter plate was read in the BioRad 680XR Plate Reader.

3. Creatine Kinase

The serum concentration of creatine kinase (CK) was assessed by using EnzyChrom creatine kinase assay kit (Bioassay System, Hayward, CA). The assay protocol was performed according to manufacturer's procedures.

1) All serum and reagents were allowed to reach room temperature.
2) Reagent substrate solution was prepared.
3) Add 110 µL deionized water in the first two wells.
4) Add 10 µL calibrator + 100 µL water in the 3rd and 4th wells.
5) Add 100 µL reconstituted reagents into wells with 10 µL unknown into appropriate wells.
6) Microtiter plate was mixed by tapping plate and incubated at room temperature for 10 minutes.

7) Microtiter plate was read at 10 min and again 40 min at OD 340 nm in the BioRad 680XR Plate Reader.

4. Whole Blood Lactate

Three fingertip blood samples (approximately 0.7µL by volume or about 8 drops) were collected by the same investigator prior to the start of the exercise bout, immediately following the exercise bout, and 15 minutes after exercise bout. The subjects' finger was cleaned with alcohol solution prior to testing. Fingertips were pricked with a lancet, and the finger was lightly squeezed to form a drop of blood to be collected for determining lactate. After calibrating the Accusport portable lactate analyzer (Boehringer Manheim Corporation, Indianapolis, IN), a test strip was inserted at the bottom of the analyzer. A drop of blood was then placed on the yellow target area of the test strip, and the lactate values were determined in about 1 minute.

5. Hematocrits

Hematrocrits were analyzed for each subject at each time point. Two capillary tubes were filled with whole blood obtained from the vacutainer immediately after each blood draw. The blood in the capillary tubes was allowed to clot for five minutes and then centrifuged in a crit-spin micro-centrifuge (Model M961-22, Statspin Inc., Norwood, MA). Capillaries were placed in a digital hematocrit reader (crit-spin Model SI 20-22, Statspin Inc., Norwood, MA) and analyzed for percent (%) hematocrit to estimate plasma volume change between blood draws. Percent change (%) in plasma volume was estimated using the following equation: (100/ (100-Hct pre)) * 100((Hct pre-Hct post)/Hct post).[82]

Statistical Analysis

All values were reported as mean and standard error of the mean. To ensure pre exercise hormonal values were stable across the two difference exercise sessions, a paired sample t-test was calculated to check mean values. A two-way (protocol (2) × time (3)) repeated measure ANOVA was used to compare the effects of the 2 exercise protocols on hormonal responses for both corrected and uncorrected values. If there were any significant time effects then a paired sample t-test was used as a post-hoc procedure. The data was analyzed by SPSS 19.0 (SPSS Inc., Chicago, IL). All statistical analyses used a $p<0.05$ level of significance.

CHAPTER IV

RESULTS AND DISCUSSION

The purpose of this study was to examine the acute effects of low-intensity slow contraction speed resistance exercise (50% 1-RM) compared to traditional high intensity (80% 1-RM) resistance exercise on hormonal responses in college-aged males. Thirteen college-aged male subjects participated in this study.

Subject Characteristics

Table 1 displays the baseline physical characteristics of the subjects for the following variables: age, height, and weight.

Table 1. Baseline Physical Characteristics (n =13)

Variable	Mean ± SE
Age (years)	21.69 ± 0.94
Height (cm)	181.53 ± 1.70
Weight (kg)	79.55 ± 3.19

Muscle Strength

Table 2 displays the 1-RM muscular strength values (kg) for shoulder press, chest press, low row, biceps curl, knee extension, knee flexion, leg press, and calf raises.

Table 2. Muscle strength (1-RM) for Each Muscle Group

	Variable	1 RM (kg)
Upper Body	Shoulder Press	61.84 ± 3.92
	Chest Press	61.36 ± 4.39
	Low Row	65.99 ± 2.77
	Biceps Curl	43.49 ± 2.94
Lower Body	Knee Extesion	81.51 ± 3.62
	Knee Flexion	85.45 ± 3.62
	Leg Press	176.92 ± 7.54
	Calf Raises	79.51 ± 2.57

Values are expressed as Mean ± SE.

Lower body 1 RM's were approximately 35% higher, on average, compared to upper body values. The biceps had the lowest 1 RM values (43.49 ± 2.94 kg) and the leg press had the highest 1 RM value (176.92 ± 7.54 kg).

Training Volume

In Table 3 the results from a paired sample t-test are shown which compared the mean values of training volumes for each muscle group between exercise conditions. Paired t-tests detected significant higher exercise volumes for shoulder press ($p=0.026$), low row ($p=0.015$), bicep curl ($p=0.006$), knee extension ($p=0.003$), knee flexion ($p=0.001$) in upper body muscle groups and leg press ($p=0.011$) in lower body muscle groups between two exercise conditions. Paired t-test also detected no significant differences in exercise volumes for chest press ($p=0.157$) and calf raises ($p=0.767$). There were no significant differences in exercise volumes between conditions for total upper body values but the SS condition had a significantly higher ($p = 0.007$) total lower

body volume compared to TR condition (7354 ± 703 kg versus 6096 ± 356 kg, respectively).

Table 3. Comparison of Exercise Volume Measures across Both Conditions for Each Muscle Groups

Muscle Group		SS	TR	t value	P
Upper Body	SP	3050 ± 316	3562 ± 225*	-2.54	0.026
	CP	3227 ± 291	3534 ± 252	-1.51	0.157
	LR	4792 ± 385*	3801 ± 159	2.85	0.015
	BC	2194 ± 215**	1565 ± 105	3.35	0.006
	TOTAL	3316 ± 198	3115 ± 157	1.36	0.181
Lower Body	KE	3513 ± 305	4695 ± 208**	-3.67	0.003
	KF	7418 ± 643**	4921 ± 208	4.61	0.001
	LP	14008 ± 1464*	10190 ± 434	3.02	0.011
	CR	4476 ± 395	4579 ± 148	-0.30	0.767
	TOTAL	7354 ± 703**	6096 ± 356	2.797	0.007

Values are expressed as Mean ± SE.
SP: Shoulder Press, CP: Chest Press, LR: Low Row, BC: Biceps Curl, KE: Knee Extension, KF: Knee Flexion, LP: Leg Press, CR: Calf Raises, SS: SuperSlow, TR: Traditional.
Exercise volume for SS calculated as: 50% of 1-RM (kg) × Repetitions × 1 set × Contraction time (15 seconds). Exercise volume for TR calculated as: 80% of 1-RM (kg) × Repetitions × 3 sets × Contraction time (3 seconds). *Statistically significant difference between TR and SS ($p < 0.05$). **Statistically significant difference between TR and SS ($p < 0.01$).

Hormone and Creatine Kinase Responses

Table 4 shows the results from baseline (PRE) stability measures for each variable. There were no significant mean differences for pre values of CK, TES, COR, LA, and Hct from the two different exercise conditions (SuperSlow and Traditional).

Table 4. Baseline Stability Measures for CK, TES, COR, LA, and Hct from Paired Sample t-test

	n	t value	p
CK (Pre SS vs. Pre TR)	13	- 1.304	0.217
TES (Pre SS vs. Pre TR)	13	1.119	0.285
COR (Pre SS vs. Pre TR)	13	0.799	0.440
LA (Pre SS vs. Pre TR)	13	- 0.281	0.783
HcT (Pre SS vs. Pre TR)	13	1.243	0.238

Exercise condition, SS: SuperSlow, TR: Traditional, PRE: Pre Exercise, CK: Creatine Kinase, TES: Testosterone, COR: Cortisol, LA: Lactate, Hct: Hematocrit.
t value from paired sample t-test

Based on the paired sample t-test analysis, there were no mean differences between baseline (PRE) values for creatine kinase, testosterone, cortisol, lactate, and hematocrits for the two different exercise protocols (SuperSlow and Traditional). All pre exercise outcome variables were within normal ranges for expected concentrations (expected normal resting values for CK: 38 – 120 U/L, TES: 2.0 – 6.9 ng/mL. COR: 43 – 200 ng/mL, LA: 0.5 – 2.0 mmol/L, Hct: 39 - 49 %). Overall, PRE values were considered to be essentially the same for the two conditions.

The uncorrected and corrected values for CK, testosterone, cortisol, Hct (%), and blood lactate across each time point are shown in Table 5. There were small non-significant increases in CK from PRE to IP for both the SS and TR exercise conditions but there was a somewhat greater % increase with the TR exercise condition (SS: 14.74% versus TR: 39.59%). Testosterone increased slightly from PRE to IP exercise with both exercise conditions (8.04 ± 1.07 to 8.25 ± 1.37 for SS and 7.32 ± 0.98 to 8.50 ± 25 for TR) but significantly decreased ($p \leq 0.05$) from IP to 15P exercise for both exercise

conditions (8.25 ± 1.37 to 7.38 ± 1.26 for SS and 8.50 ± 1.25 to 6.94 ± 0.88 for TR). There was a significant time effect for Cortisol with increases from PRE to IP exercise for both SS and TR (about 50 ng/mL each) and smaller decreases from IP to 15P exercise for both exercise protocols. However, when a post-hoc analysis was completed, there were no significant changes between the time points ($p=0.059$ for PRE versus IP). There was a significant time effect for hematocrits ($p\leq0.05$) with the post-hoc analyses indicating a significant increase from PRE to IP exercise ($p\leq0.01$) and there was a significant decrease from IP to 15P exercise ($p\leq0.01$). Blood lactate values significantly increased ($p\leq0.01$) from PRE to IP time points for both conditions (0.79 to 8.91 mmol/L for SS condition and from 0.82 to 10.13 mmol/L for TR respectively).

Table 5. Descriptive Data for Values across Each Time Point

Variable	n	Time Point	Condition	
			SS	TR
CK (U/L)		PRE	41.16 ± 4.09	59.08 ± 14.62
Uncorrected		IP	47.32 ± 4.87	64.62 ± 11.83
	13	15 P	46.24 ± 4.69	64.56 ± 12.17
Corrected		IP	42.16 ± 4.27	53.94 ± 10.20
		15P	45.84 ± 4.18	65.88 ± 12.58
TES (ng/mL)		PRE	8.04 ± 1.07	7.32 ± 0.98
		IP	8.25 ± 1.37	8.50 ± 1.25
Uncorrected	13	15 P	7.38 ± 1.26*	6.94 ± 0.88*
Corrected		IP	7.34 ± 1.17	7.17 ± 1.09
		15P	7.35 ± 1.21	7.13 ± 0.93
COR (ng/mL)		PRE	166.67 ± 15.13	157.56 ± 8.78
		IP	216.08 ± 18.16	201.03 ± 19.34
Uncorrected	13	15 P	196.92 ± 20.36	198.08 ± 17.71
Corrected		IP	196.16 ± 19.34	170.61 ± 18.17
		15P	198.63 ± 21.70	202.44 ± 18.62
Hct (%)	13	PRE	45.81 ± 0.72	45.25 ± 0.57
		IP	48.65 ± 0.82*	49.69 ± 0.47*
		15 P	45.79 ± 0.88*	44.79 ± 0.63*
LA (mmol/L)	13	PRE	0.79 ± 0.17	0.82 ± 0.09
		IP	8.91 ± 0.63**	10.13 ± 0.60**

Values are Means ± SE. CK: Creatine Kinase, TES: Testosterone, COR: Cortisol, LA: Lactate, Hct: Hematocrit. SS: SuperSlow, TR: Traditional, PRE: Pre Exercise, IP: Immediate Post Exercise, 15P: 15 minutes Post Exercise. * $p \leq 0.05$ IP versus15P; [a]$p \leq 0.05$ PRE versus IP; *$p \leq 0.05$ IP versus 15P; and **$p \leq 0.01$ PRE versus IP.

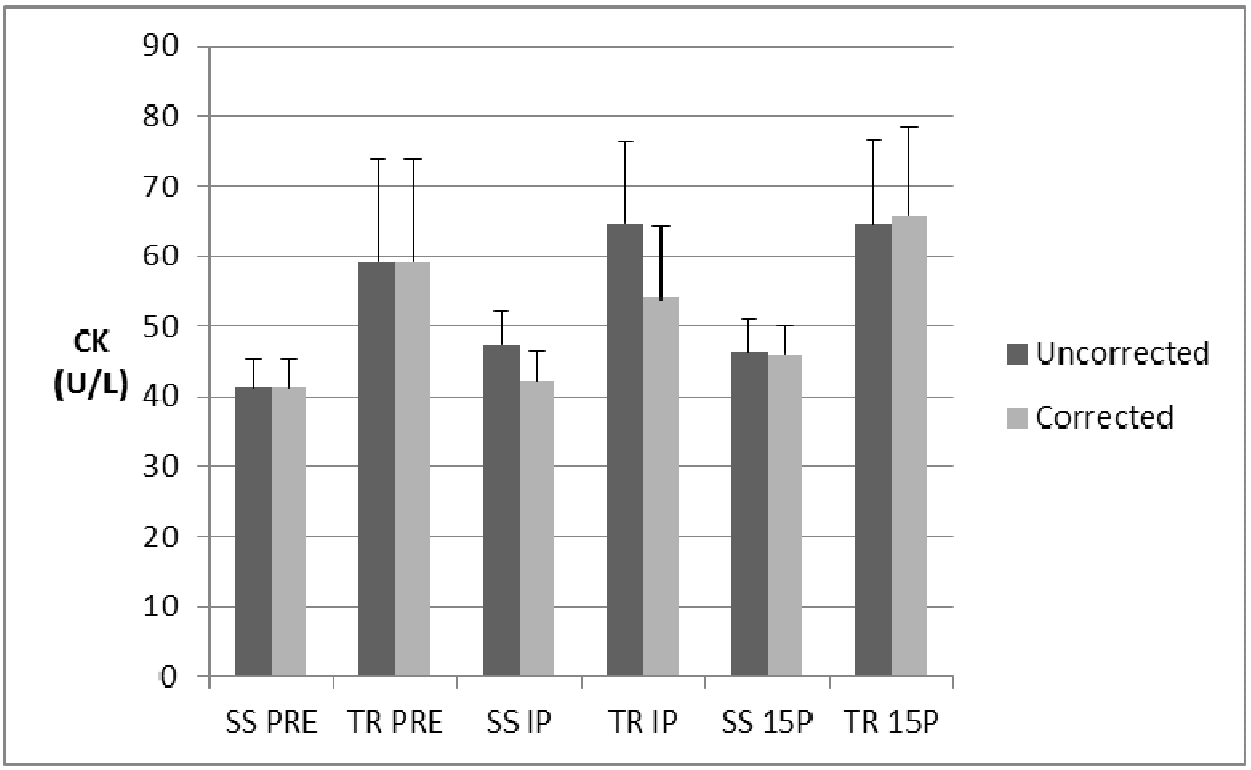


Figure 1. Uncorrected and Corrected CK Values from Pre to IP and 15P (15-min) after Exercise Conditions. SS, SuperSlow; TR, Traditional; CK, Creatine Kinase.

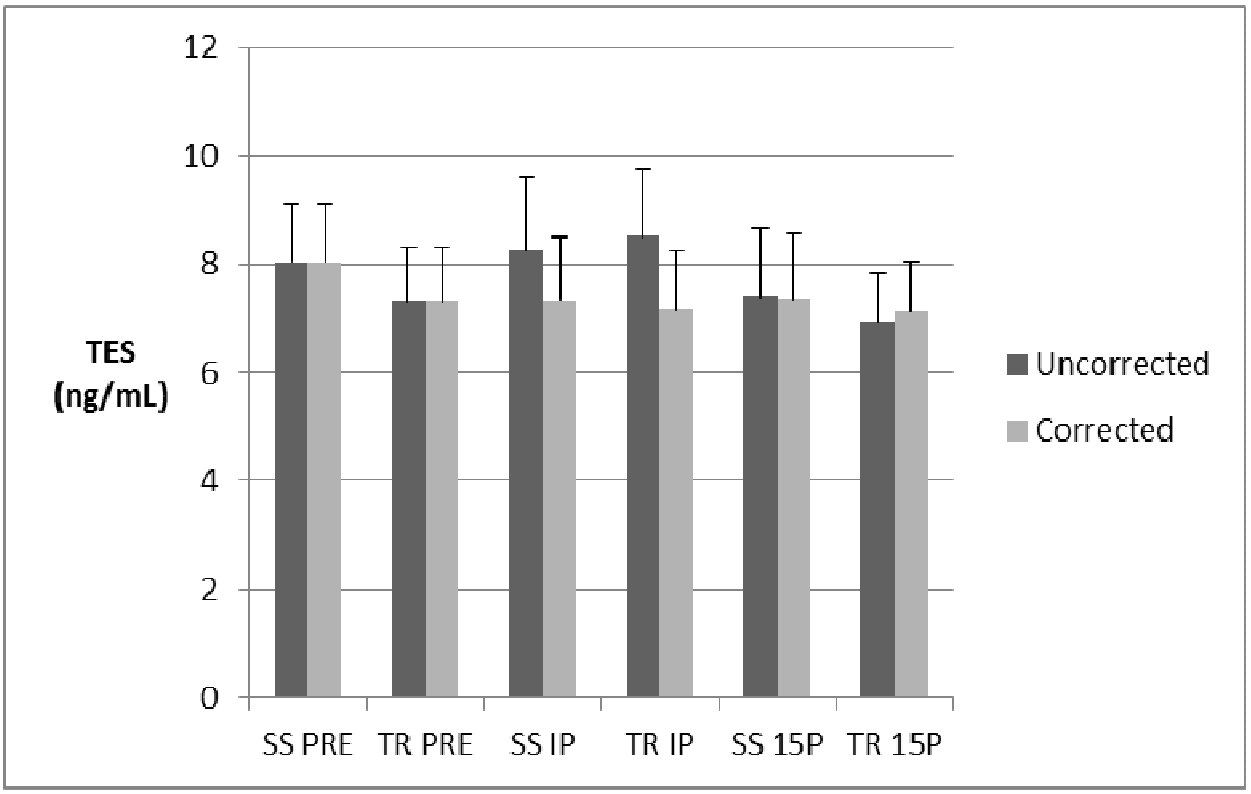


Figure 2. Uncorrected and Corrected TES Values from Pre to IP and 15P (15-min) after Exercise Conditions. SS, SuperSlow; TR, Traditional; TES, Testosterone.

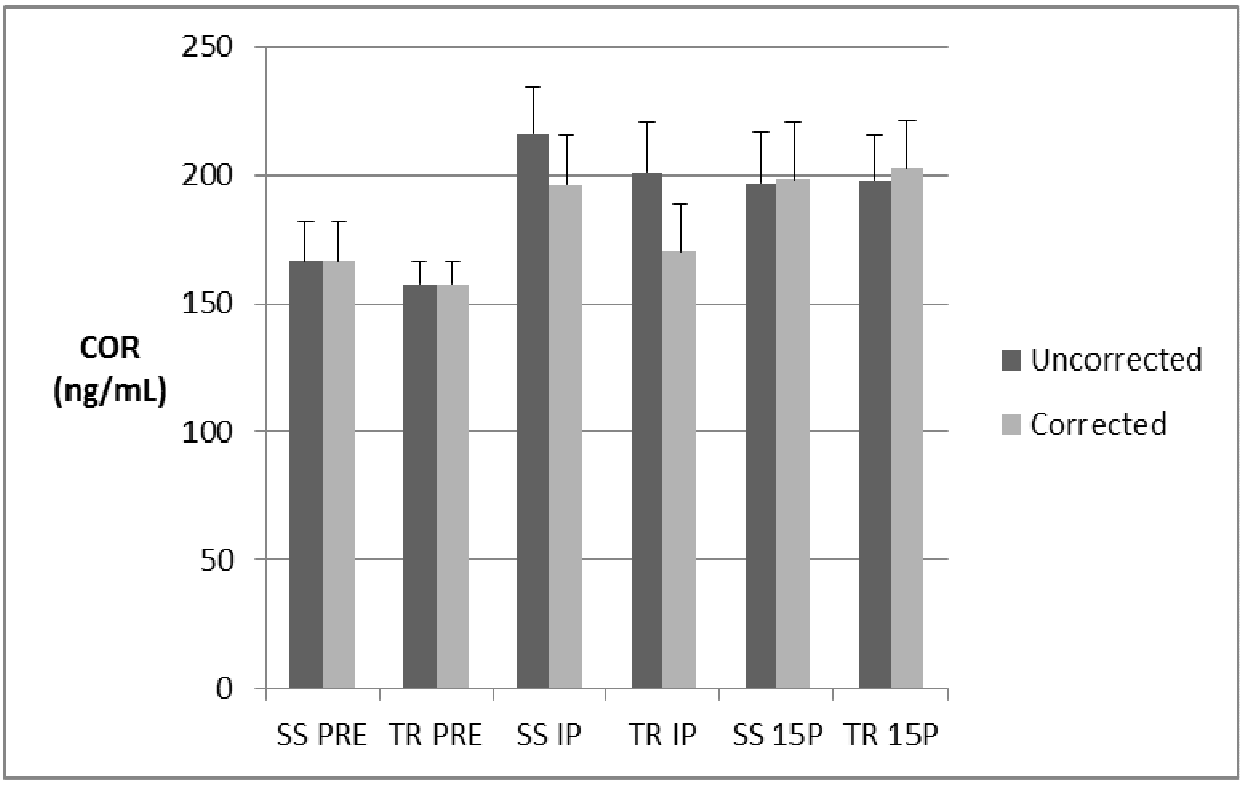


Figure 3. Uncorrected and Corrected COR Values from Pre to IP and 15P (15-min) after Exercise Conditions. SS, SuperSlow; TR, Traditional; COR, Cortisol.

Figure 1, Figure 2, and Figure 3 display the comparison between uncorrected and plasma volume shift corrected CK, TES, and COR levels respectively. Plasma volume decreased approximately 10% for SS condition and 16% for TR condition for immediately after exercise. Two way ANOVA with repeated measures detected no significant condition main effect, time main effect or condition x time interaction for CK, TES, and COR corrected levels.

Table 6. Percent Change for Each Variable

Variable	Comparison	SS	TR	t	P
LA (%)	PRE – IP	1389 ± 204	1266 ± 508	0.91	0.380
Plasma Volume (%)	PRE – IP	-10.35 ± 2.67	-16.0 ± 2.27	2.01	0.067
	PRE – 15P	0.32 ± 1.94	2.18 ± 2.22	-0.57	0.578
CK (%)	PRE – IP	14.74 ± 2.69	39.59 ± 14.39	-1.59	0.138
	IP – 15P	-1.85 ± 1.94	-1.28 ± 3.54	-0.14	0.894
	PRE – 15P	12.26 ± 2.34	37.52 ± 13.04	-1.92	0.080
TES (%)	PRE – IP	0.96 ± 4.39	15.39 ± 7.38	-1.59	0.137
	IP – 15P	-6.97 ± 5.01	-15.72 ± 4.40	1.18	0.261
	PRE – 15P	-5.49 ± 6.70	-5.42 ± 3.79	-0.01	0.994
COR (%)	PRE – IP	55.08 ± 29.56	32.46 ± 15.02	0.71	0.489
	IP – 15P	-9.25 ± 3.70	0.75 ± 4.62	-1.42	0.180
	PRE – 15P	41.29 ± 26.94	31.16 ± 14.83	0.33	0.744

Values are expressed as Mean ± SE.
CK: Creatine Kinase, TES: Testosterone, COR: Cortisol, LA: Lactate, Hct: Hematocrit, PRE: Pre Exercise, IP: Immediate Post Exercise, 15P: 15 minutes Post Exercise. % Change Plasma Volume calculated as (100/ (100-Hct PRE) × 100((Hct PRE–Hct Post)/Hct Post). %Δ calculated as ((IP – PRE)/PRE) ×100, ((15P – IP)/IP) ×100, or ((15P – PRE)/PRE) ×100.

The results from a paired sample t-test are shown (Table 6) which compared the mean values of percent changes between each time points for the SS and TR exercise

conditions in Table 6. CK had a greater % change for the TR exercise condition (~ 40%) versus the SS exercise condition (~ 15%) from PRE to IP but they were not statistically different ($p = 0.138$). There was also a greater % change from PRE to 15P for TR (~38%) than SS (~12%) but once again this difference was non-significant ($p = 0.080$). Testosterone had a higher % change after the TR exercise (PRE to IP) but there was no significant difference between exercise protocols (15.4% versus 1%; $p = 0.137$). There was a larger percent decrease in testosterone from IP to 15P for the TR condition (-15.7%) compared to the SS condition (-7%) but they were not significantly different from each other ($p = 0.261$). There was no significant differences between the two exercise conditions in cortisol response from PRE to IP ($p = 0.489$) but the change was larger the SS condition (55%) compared to the TR condition (32%). Blood lactate had a greater % change for the SS exercise condition (1389%) from PRE to IP versus the TR (1266%) but they were not significantly different from each other ($p = 0.380$). In both exercise conditions, plasma volume decreased from PRE to IP (SS: 10% versus TR: 16%) but there was no significant difference between exercise conditions from PRE to IP ($p = 0.067$).

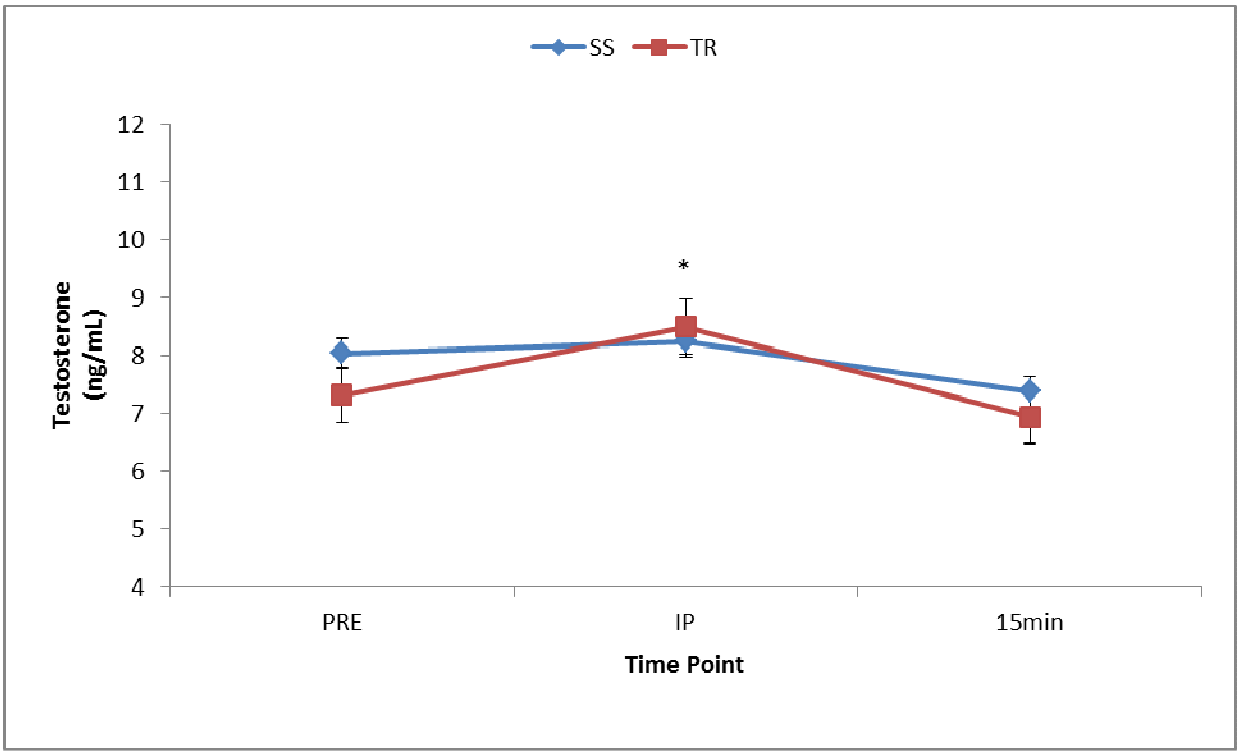


Figure 4. Mean Testosterone Responses across Time for both Exercise Conditions. Significant time effect* $p \leq 0.05$ for IP vs. 15P. Values are Mean ± SE.

Figure 4 shows there was no condition main effect or no condition x time interaction effect for the testosterone response, however there was a significant time effect for IP to 15 min time point ($p = 0.012$). There were no significant main effects for condition or time or no significant interaction when the analysis was run with the plasma corrected testosterone values.

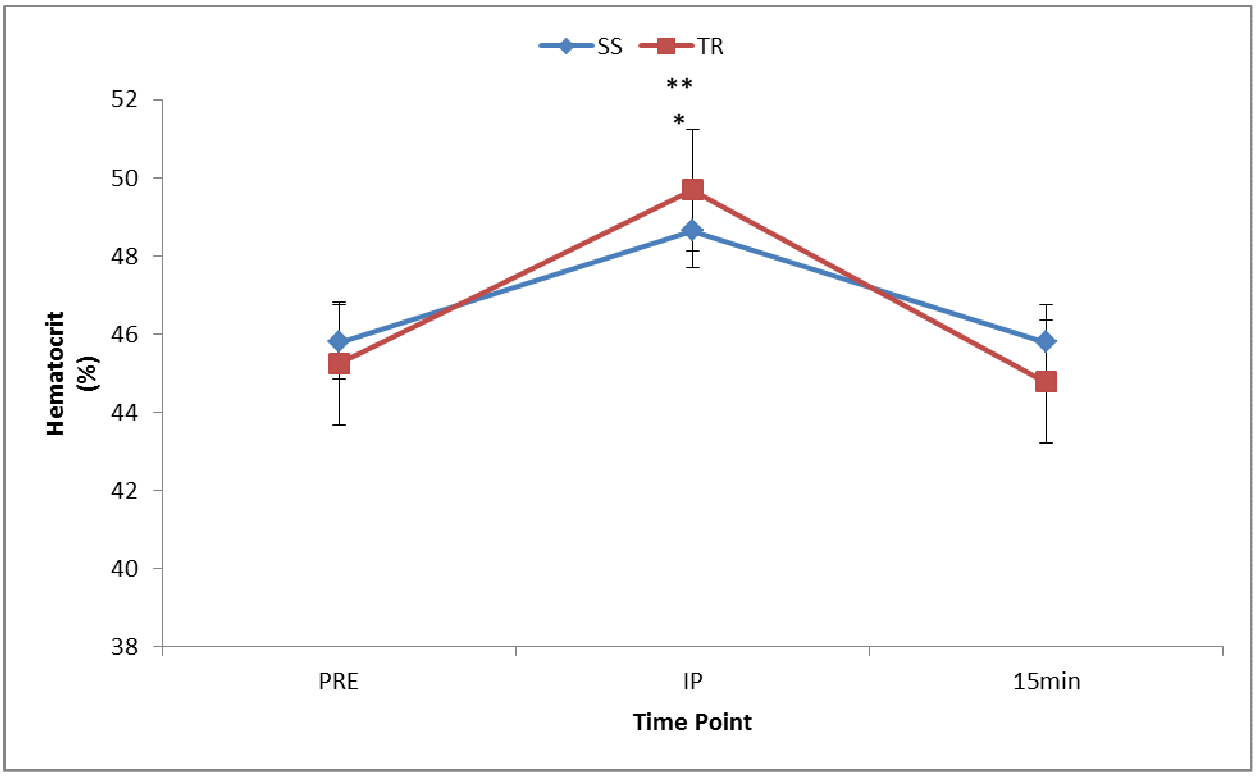


Figure 5. Mean Hematocrit Responses across Time for both Exercise Conditions. Statistically significant time main effect ** $p < 0.01$, statistically significant condition × time interaction * $p < 0.05$. Values are Mean ± SE.

Figure 5 shows there was a significant condition x time interaction effect ($p = 0.021$) and a significant time effect ($p = 0.001$) when looking at the hematocrit change, but no significant main effect for condition. There was a significant increase in hematocrit immediately post exercise compared to pre values ($p=0.000$) and there was also a significant decrease 15 min post exercise compared to immediately post exercise ($p=0.000$). There were no significant main effects for condition or time or no significant interaction when the analysis was run with the plasma corrected hematocrit values.

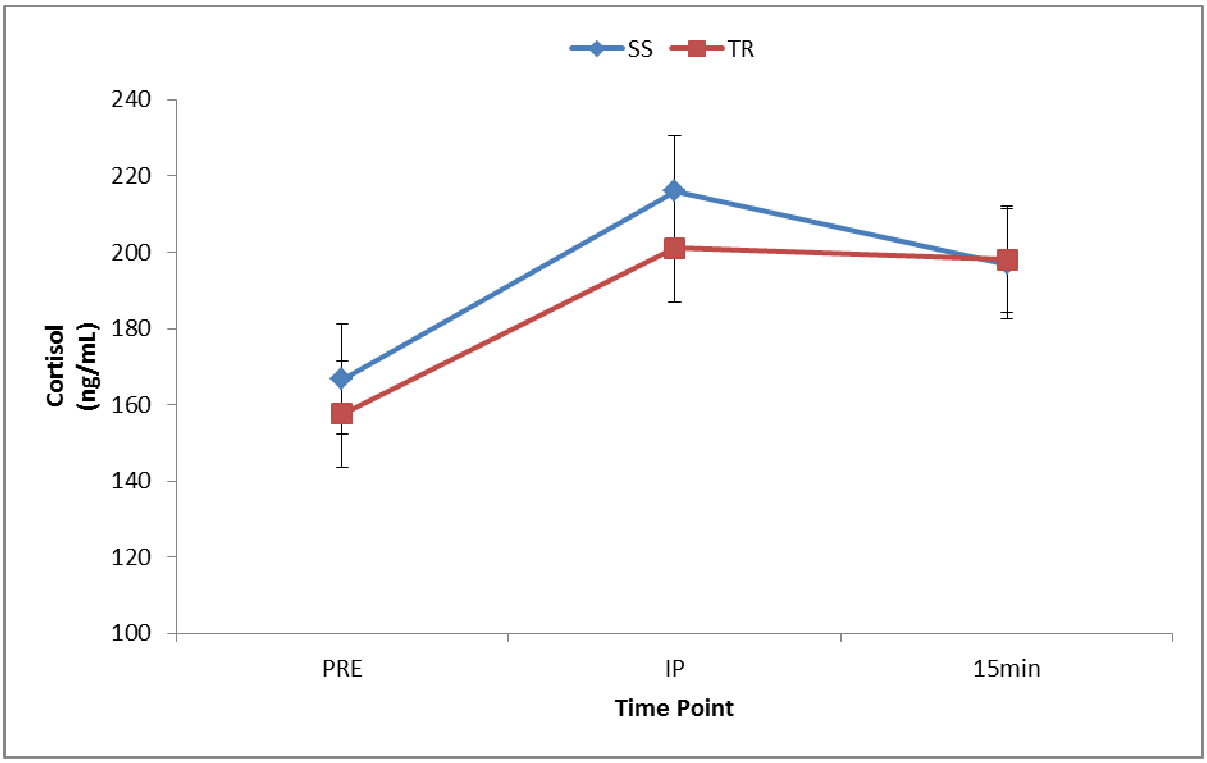


Figure 6. Mean Cortisol Responses across Time for both Exercise Conditions. Values are Mean ± SE.

Figure 6 displays the cortisol values across time for both conditions. There was no significant main effect for time or condition and no significant condition x time interaction for the cortisol response indicating a similar change in cortisol for both exercise conditions, however there was a trend for a significant time effect ($p = 0.059$) from PRE value to IP values. There were no significant main effects for condition or time or no significant interaction when the analysis was run with the plasma corrected cortisol values.

Figure 7 and Figure 8 (Appendix I), both illustrate the TES changes from PRE to IP for each subject individually for the SS and TR resistance exercise conditions. Eight of the 13 subjects in the TR condition had increases in testosterone from PRE to IP whereas only 4 of the 13 subjects in the SS condition had noticeable increases in testosterone.

The number of subjects that demonstrated a decrease in testosterone from IP to 15P was similar for both conditions (8 for SS and 11 for TR) in Figure 9 and 10 (Appendix I). The individual responses for testosterone from PRE to 15P were similar for both exercise conditions (Figure 11 and 12, Appendix I).

In general most subjects had an increase in creatine kinase from PRE to IP exercise although the magnitudes of the response were greater for subjects in the SS condition compared to the TR condition (Figure 13 and 14, Appendix I). It is also interesting to note the large decrease in CK for one subject in the TR condition following the exercise session. The individual responses for CK from IP to 15P for both conditions were similar with about half of subjects demonstrating an increase and about half of the subjects demonstrating a decrease (Figure 15 and 16, Appendix I).

Changes in CK from PRE to 15P were similar to the responses from PRE to IP with the same individuals in the TR condition demonstrating a large decrease (Figure 17 and 18, Appendix I).

Figure 19 and 20 (Appendix I) display the COR changes from PRE to IP for each participant individually for the SS and TR resistance exercise conditions. The number of subjects that demonstrated an increase in COR from PRE to IP was similar for both exercise conditions. The individual changes in COR from IP to 15P were similar between the two exercise conditions (Figure 21 and 22, Appendix I). Figure 23 and Figure 24 (Appendix I) show the COR changes from PRE to 15P for each subject for the SS and TR resistance exercise conditions. Most subjects in both the SS and TR conditions had increases in cortisol values when assessed from PRE to 15P, however, about one third of the subjects in both conditions also had small decreases.

Figure 25 and Figure 26 (Appendix I), both show the individual lactate responses from PRE to IP for both the SS and TR resistance exercise conditions. Individual responses to both conditions were very similar with increases ranging from about 6 to 12 mmol/L from pre- exercise values.

Rating of Perceived Exertion and Pain Scale

Table 7. RPE and Pain Scale from Paired Sample t-test

	n	SS	TR	t value	p
RPE	13	16.25 ± 0.58	15.16 ± 0.28	1.746	0.103
Pain Scale	13	5.57 ± 0.50	4.30 ± 0.49	3.105	0.009**

Values are expressed as Mean ± SE.
Significant difference in RPE and Pain Scale ratings between exercise protocols **$p \leq 0.01$.

Table 7 displays the mean values for RPE and for the Pain Scale for both conditions. Mean values for RPE and Pain Scale values were averaged across all 3 sets and each exercise for the TR condition and across all exercise for 1 set for the SS condition. The Pain Scale was significantly different between exercise conditions with the ratings being higher for the SS protocol after all 8 resistance exercises ($p=0.009$). However, there was no difference in RPE ratings between exercise conditions ($p=0.103$).

DISCUSSION

The purpose of the current study was to compare the acute effects of low-intensity (50% 1-RM) resistance exercise with slow speed contraction and high-intensity (80% 1-RM) traditional resistance exercise on creatine kinase, lactate, and hormonal responses in college-aged men. To our knowledge, this is the first study of a randomized cross-over trial to determine the acute hormone responses to a SS resistance exercise protocol. These current findings will assist in observing what acute hormone concentrations occur as result of exercise contraction speed and exercise intensity manipulation, and may provide hypothetical insight into chronic resistance exercise training adaptations. In general, the results indicated that slow speed contraction resistance exercise and traditional resistance exercise had similar responses on hormonal changes. These data indicate, with this type of slow contraction, lower intensity resistance exercise used in this research study, individuals may be able to train with lower weights and only 1 set but still benefit from muscle hypertrophy based on the endocrine responses in this study.

Testosterone Responses

Acute increases in testosterone after resistance exercise plays a major role in skeletal muscle adaptations. Acute elevations in testosterone appear to be critical for skeletal muscle growth. There were no significant effects for condition or condition × time interaction for testosterone values after exercise, but there was a significant time effect for both exercise conditions with an increase immediately after exercise followed by a decrease 15 minutes into recovery. This was explained by a decrease in plasma volume since there were no significant differences when testosterone values were corrected for changes in plasma volume. Previous studies have indicated TR resistance

exercise leads to acute increases in anabolic hormones such as testosterone.[26, 42, 43] In this present study, the highest peak testosterone concentrations occurred immediately after exercise for both exercise conditions, but these increases may be attributed to plasma volume reductions.[34] Plasma volume changes should be considered in evaluating hormone changes, since hormone effects depend on the plasma volume.[66] The findings of the current study were similar to previous findings in terms of plasma volume changes. In this study, plasma volume had a decrease of -10.35% and -16.0% with SS and TR exercise conditions respectively from PRE to IP exercise.

Goto et al.[23] showed that low-intensity resistance exercise with slow contraction speed (3 second for concentric contraction, 1 second for eccentric contraction) had greater increases in testosterone levels than high-intensity resistance exercise with normal contraction speeds (1 second for concentric contraction, 1 second for eccentric contraction). The magnitude of the elevation of testosterone levels during resistance exercise is dependent on several factors including exercise intensity, exercise volume, and rest between sets.[44, 71] However, we were unable to detect any difference in testosterone levels related to exercise intensity with the two different contraction speeds used in the present study. This is interesting since the SS condition had a significantly higher exercise volume than TR exercise condition, especially for the lower body exercises (7354 kg versus 6096 kg, respectively). In general, modifications of exercise volume and intensity are associated with the hormonal response.[24, 70] Most studies report that high intensity resistance exercise induces acute elevations in anabolic hormone levels such as testosterone and growth hormone. However, it also has been demonstrated that low-intensity resistance exercise with blood flow restriction also resulted in anabolic

hormone increases.[21, 75] In the current study, the exercise volume for the SS condition was calculated as 80% 1RM loads, 3 sets, 8 repetitions, 3 seconds per repetition when compared to the TR condition (50% 1RM loads, 1 set to contraction failure, 15 seconds per repetition). The difference in exercise volumes may have contributed to the larger decrease in plasma volume for the SS condition which negated the significant increases in testosterone over time.

Cortisol Responses

It has been reported that resistance exercise has a significant stimulus on acute cortisol responses. Cortisol response to resistance exercise is related to its catabolic function, which promotes the degradation of proteins from skeletal muscles. Many previous studies have reported that acute exercise leads to an increase in cortisol concentrations dependent on the mode of resistance exercise.[33, 56, 65] However, other studies have demonstrated that cortisol functions as stress hormone, which repairs damage during exercise by increasing protein synthesis.[10] Therefore, resting stress levels, including environmental factors should be considered when measuring the cortisol response to exercise.[83] Our data indicated that there was a non-significant trend for increases in cortisol from PRE to IP although there was substantial variability in the changes for individual subjects. In this study, there was also no significant condition by time interaction, and no significant condition main effects for the cortisol responses. The highest peak cortisol values occurred immediately after exercise for both exercise conditions, and remained elevated at the 15P time point. In the current study, cortisol values increased by 55.08% and 32.46% for the SS and TR exercise conditions, respectively, from PRE to IP exercise. These findings differ from McGuigan et al.[56] who

reported a significant difference in cortisol response between high-intensity (75% 1RM) exercise and low-intensity (30% 1RM) in healthy men and women from PRE to IP exercise. These authors also reported that there was a significant difference in RPE ratings between two exercise protocols (high intensity, 7.1 vs. low intensity, 1.9), however, there was no attempt to equate the total work volume between two exercise groups. Fujita et al.[21] reported low-intensity resistance exercise with blood flow restriction resulted in a significant increase in cortisol concentration from PRE to IP (approximately 39%) in young healthy men. The results of the present study were similar to previous studies in terms of the increased cortisol concentrations immediately following resistance exercise. Additionally, the data from the current study indicates that the SS condition was as effective as the TR condition for eliciting a cortisol response.

Blood Lactate Responses

Both the SS and TR resistance exercise conditions resulted in significant increases in blood lactate at the IP time point. In the present study, the magnitude of elevation in lactate for both exercise conditions was consistent with previous studies that investigated both high- and low-intensity exercise conditions.[39, 53, 75] The results from this current study revealed a mean increase in blood lactates of 1389 % and 1266 % for SS and TR conditions respectively from PRE to IP time point although there was no significant difference between two different exercise conditions ($p = 0.380$). A previous study reported that the accumulation of lactate stimulates anabolic hormone secretions.[23] This is somewhat similar to the findings of our current study, in which testosterone increased for both exercise conditions immediately after exercise. However, a study by Lagally et al.[48] reported that higher intensity resistance exercises resulted in greater blood lactate

concentrations, similar to other studies.[41, 42, 43] Blood lactate increases were similar for both exercise conditions in the current study, indicating that low intensity SS exercise may be just as effective as high intensity exercise for stimulating a lactate response.

Creatine Kinase Responses

The findings of the present study did not show a significant increase in CK concentrations following exercise although the increases were 14.7% for the SS condition and almost 40% for the TR condition. Increases in CK concentrations are due to several factors, such as the mode of physical activity, amount of muscle mass involved, and gender .[9]

SuperSlow Resistance Exercise Summary

SuperSlow resistance exercise regimens were developed for some individuals who could not exercise at fast contraction velocities or high intensities. The SuperSlow resistance exercise protocol combined lower exercise intensities with slower contraction velocities to achieve very large exercise volumes. In theory, SuperSlow exercise training allows the muscle to spend more time under tension per repetition by slowing the contractions speed. Schuenke et al.[68] compared the muscular adaptations of high and low intensity resistance training with normal muscle contraction velocities to low-intensity resistance training with slow muscle contraction velocities in untrained individuals over a 6 week period. The authors reported that high-intensity training with normal contraction velocities resulted in greater hypertrophic responses than low-intensity with slow contraction velocities. Also, low-intensity training with slow muscle contraction velocities had a significantly greater hypertrophic muscular response and muscular strength gain compared to low-intensity training with normal muscle contraction

velocities. A study by Shepstone et al.[69] examined the effects of two different isokinetic muscle contraction velocities (3.66 rad/s vs. 0.35 rad/s) on muscle fiber hypertrophy. The authors reported that the fast-trained arm had greater muscle fiber hypertrophy compared with the slow-trained arm following 8 weeks of eccentric isokinetic training. Young and Bilby[86] had subject complete 4 sets of 8-12 repetitions at two different training velocities. According to their findings, both slow contraction velocity and fast contraction velocity training groups improved similarly in muscle hypertrophy while the slow training group had greater increases in absolute isometric strength than the fast training group (31.0% vs. 12.4%). Keeler et al. reported that traditional heavy resistance exercise (80% 1-RM) had significantly greater improvements than the SS exercise group on 5 of 8 resistance exercises following 10 weeks of training although both resistance exercise protocols resulted in increased muscle strength for subjects on all 8 resistance exercises. Another SS training study by Neils et al.[60] observed that 8 weeks of SS resistance training led to significant muscular strength improvements for the squat and bench press, although the TR protocol had more peak power gains. Our recently completed study assessed the effects of SS compared to TR resistance training on muscular strength, flexibility, and aerobic capacity following 4 of weeks training in college-aged women. Higher muscular strength gains occurred for the TR group compared to the SS group while both exercise training groups had no significant improvements in flexibility or aerobic capacity. Another SS training study by Hunter et al.[31] reported that traditional high-intensity strength exercise resulted in greater increases in energy expenditure compared to SuperSlow exercise training. Previous studies comparing physiological responses between SS training and TR training protocol have

reported conflicting outcomes. However, the findings of this current study showed that the two different intensity exercise protocols had similar hormone responses (including cortisol, testosterone, and lactate changes), which may play critical roles for improving muscle strength, muscle power, and muscle hypertrophy probably were caused by the increased time under tension during repetitions at slow velocity contractions.

CHAPATER V

CONCLUSIONS

The purpose of this study was to investigate the acute effects of low-intensity slow contraction speed resistance exercise (50% 1-RM) compared to traditional high intensity (80% 1-RM) resistance exercise on hormonal responses in college-aged males.

Research Questions

1. **Will an exercise protocol (SuperSlow) based on low intensity and high volume produce similar endocrine responses as a traditional high intensity, low volume resistance training protocol.**

Yes, the responses of TES and COR were similar between the low-intensity slow contraction speed resistance exercise (50% 1-RM) compared to traditional high intensity (80% 1-RM) resistance exercise in college-aged men, however neither protocol elicited a significant increase in testosterone or cortisol.

2. **Will the different resistance training protocols result in different amounts of muscle damage?**

No, there were similar changes in CK from PRE to IP with both the SS and TR exercise conditions, however perceived pain was significantly higher for the SuperSlow protocol.

The primary finding of this study was that the patterns of response of serum TES, COR, and CK were similar during two different intensity resistance exercise conditions. To our knowledge, no previous study of SS exercise has investigated hormone responses to determine the magnitude of hormonal response patterns in college-aged individuals. When resistance training is adopted as a training modality, it is evident that the exercise

contraction speed and exercise training volume are important factors in the composition of the exercise physiological stimulus that increases hormonal concentrations. The outcomes of the present study make it helpful to understand that low intensity resistance exercise combined with slow speed muscle contractions may prove a viable resistance exercise method compared with traditional resistance exercise.

Clinical Significance

This research study sought to determine the effects of low-intensity and traditional high-intensity resistance exercise on hormone responses in college-aged males. The results of the current study supports the current hypothesis that SS exercise protocols could be an alternative exercise method for individual who are not able to participate in regular recommended high intensity resistance exercise programs.

Suggestions for Future Research

Earlier studies examined the possible beneficial effects of SuperSlow resistance exercise but focused on muscular strength. The current study is the first report of a randomized cross-over design between the traditional heavy resistance exercise and low-intensity with slow speed contractions on acute hormone responses. Future studies should include a chronic SS protocol effects on hormone responses from long-term exercise training as well as examination of possible beneficial effects on muscle hypertrophy and bone health in females. Future research should also incorporate the use of muscle imaging techniques so that changes in muscle size from a long-term training intervention can be accurately determined and potential improvements in cortical or trabecular bone could be differentiated. Additionally, further investigations may be needed to consider the effects of age, nutritional intake, and training status on the

endocrine, muscle, and bone responses to chronic exercise training utilizing the SS method. The current findings support the suggestion that SS exercise could be a beneficial alternative for individuals who cannot perform traditional high-intensity resistance exercise, such as the elderly or those recovering from injury.

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Appendix A

Recruitment Materials

Male Participants Needed!

SuperSlow Exercise (4 visits)

Purpose: **To compare the acute hormonal responses to two different resistance exercise protocols (traditional and SuperSlow) that have similar exercise volumes but differ in intensity and contraction speed.**

Who: **Men between the ages of 18 to 30 years who have not been doing regular (more than twice per week) resistance exercise training or aerobic exercise for the past 4 months.**

Where: **Neuromuscular Laboratory, Department of Health and Exercise Science, University of Oklahoma.**

Total time commitment will be 2 exercise sessions that include 3 blood draws (pre-exercise, post exercise and 15 minutes post exercise) by a nurse with each session requiring about 90 minutes. There will be a familiarization session requiring about 60 minutes and a strength testing session that takes about 60 minutes. If interested, contact Eon Kim at970-978-8591 or eonkim@ou.edu for more information.

IRB number:1116

The University of Oklahoma is an equal opportunity institution.

Eon Kim

Department of Health and Exercise Science
eonkim@ou.edu

The University of Oklahoma is an equal opportunity institution.

Name: Eon Kim
Phone: (970)978-8591
Email: eonkim@ou.edu

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E-mail Message

I am inviting you to participate in a research study we are conducting. The title of the study is "Acute Hormonal Responses after Two Different Resistance Exercise Protocols in College-Aged Males."

We are specifically looking for **males between the ages of 18-30 years**, who are recreationally active but who not have participated in a regular (> 2 days per week) structured resistance or aerobic training program for at least 4 months prior to this study; who have no known orthopedic disorders that would prevent them from participating in the exercise program, and who are not hypertensive.

We are performing this research study to compare the acute hormonal responses of two different resistance exercise protocols (traditional and SupersSlow) that have similar exercise volumes but differ in intensity and contraction speed in college aged men.

This study involves four visits to the laboratory: one visit for consenting and familiarization with weight machines, one visit for strength testing for 8 resistance exercises; and two exercise testing visits. The consenting and familiarization visit will take approximately 1 hour and the strength testing will take about 60 minutes. Each exercise testing visit will take approximately 90 minutes to complete.

For the traditional resistance exercise protocol, participants will perform 3 sets of 8 repetitions of each exercise (knee extension, knee flexion, calf raises, two-leg press, shoulder press, low row, biceps curl, and chest press) at a heavy intensity, which is 80% of maximum strength (1-RM).

For the SuperSlow resistance exercise protocol, participants will perform one set of each exercise at 50% of 1-RM, performing the maximum number of repetitions with correct form for the set.

Blood draws will be taken at three time points during each of these exercise protocols: at the beginning of each exercise session, immediately after completion of the exercise session and 15 minutes later. The blood samples will be analyzed for testosterone, cortisol, creatine kinase, lactate and hematocrit levels.

There will be compensation for participation in the study.

Thank you for considering participation in our study.

If you are interested or have further questions, please contact **Eon Kim (eonkim@ou.edu).**



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Appendix B

Initial Screening Checklist

Pre-Screening Subjects Recruitment Form

University of Oklahoma Neuromuscular Laboratory

"Acute Hormonal Responses after Two Different Resistance Exercise Protocols in College-Aged Males."

NAME: _____ DATE: _____ PHONE NUMBER: _____

Inclusion Criteria- The inclusion criteria for this study requires that each subject:

	YES	NO
Male aged 18-30 yrs	_____	_____
Is recreationally active (not sedentary)	_____	_____
Has no chronic back pain or known orthopedic disorders which would prevent participation in the exercise program	_____	_____
Is not hypertensive (SBP >140 and/or DBP >90)	_____	_____

Exclusion Criteria-The exclusion criteria for this study require that each subject:

Is outside the age range	_____	_____
Is resistance or endurance training more than 2X per week	_____	_____
Has chronic back pain or known orthopedic disorders	_____	_____
Is hypertensive (SBP >140 and/or DBP >90	_____	_____
Is taking any medications such as androgens that are known to affect testosterone and cortisol levels	_____	_____
Qualified for study	_____	_____

PI Signature _____ Date _____



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Appendix C

Health Status Questionnaire

Neuromuscular Research Laboratory
OU Department of Health and Exercise Science
Health Status Questionnaire

Instructions Complete each question accurately. All information provided is confidential.
(NOTE: The following codes are for office use only: RF; MC; SLA; SEP)

Part 1. Information about the individual

1._____
Date

2. _____
Legal name Nickname

3._____
Mailing address

_____
Home phone Business phone

4.Gender (circle one): Female Male (RF)

5. Date of birth: _____ Age _____
Month Day Year

6. Number of hours worked per week: Less than 20 20-40 41-60 Over 60

(SLA) More than 25% of time spent on job (circle all that apply)

Sitting at desk Lifting or carrying loads Standing Walking Driving

Part 2. Medical history

7. (RF) Circle any who died of heart attack before age 50:

Father Mother Brother Sister Grandparent

8.Date of: Last medical physical exam: _____Last physical fitness test: _____
Year Year

9. Circle operations you have had:

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Back (SLA)	Heart (MC)	Kidney (SLA)	Eyes (SLA)	Joint (SLA)	Neck (SLA)
Ears (SLA)	Hernia (SLA)	Lung (SLA)	Other _____		

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

Alcoholism (SEP)	Diabetes (SEP)	Kidney problem (MC)
Anemia, sickle cell (SEP)	Emphysema (SEP)	Mental illness (SEP)
Anemia, other (SEP)	Epilepsy (SEP)	Neck strain (SLA)
Asthma (SEP)	Eye problems (SLA)	Obesity (RF)
Back strain (SLA)	Gout (SLA)	Osteoporosis
Bleeding trait (SEP)	Hearing loss (SLA)	Phlebitis (MC)
Bronchitis, chronic (SEP)	Heart problems (SLA)	Rheumatoid arthritis (SLA)
Cancer (SEP)	High blood pressure (RF)	Stroke (MC)
Cirrhosis, liver (MC)	Hypoglycemia (SEP)	Thyroid problem (SEP)
Concussion (MC)	Hyperlipidemia (RF)	Ulcer (SEP)
Congenital defect (SEP)	Infectious mononucleosis (MC)	Other _____

11. Circle all medicine taken in last 6 months:

Blood thinner (MC)	Epilepsy medication (SEP)	Nitroglycerin (MC)
Diabetic pill (SEP)	Heart-rhythm medication (MC)	Estrogen
Digitalis (MC)	High-blood-pressure medication (MC)	Thyroid
Diuretic (MC)	Insulin (MC)	Corticosteroids
Asthma	Other _____	

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

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

a. Cough up blood (MC) 1 2 3 4 5	d. Leg pain (MC) 1 2 3 4 5	g. Swollen joints (MC) 1 2 3 4 5
b. Abdominal pain (MC) 1 2 3 4 5	e. Arm or shoulder pain (MC) 1 2 3 4 5	h. Feel faint (MC) 1 2 3 4 5
c. Low back pain (SLA) 1 2 3 4 5	f. Chest pain (RF) (MC) 1 2 3 4 5	I. Dizziness (MC) 1 2 3 4 5
j. Breathless with slight exertion (MC) 1 2 3 4 5		

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Part 3. Health-related behavior

14. (RF) Do you now smoke or chew tobacco? Yes No

15. If you are a smoker, indicate number 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	

16. Weight now: _____lb. One year ago: _____lb.. Age 21: _____lb.

17. Thinking about the things you do at work, how would you rate yourself as to the amount of physical activity you get compared with others of your age and sex?

1. Much more active
2. Somewhat more active
3. About the same
4. Somewhat less active
5. Much less active
6. Not applicable

18. Now, thinking about the things you do outside of work, how would you rate yourself as to the amount of physical activity you get compared with others of your age and sex?

1. Much more active
2. Somewhat more active
3. About the same
4. Somewhat less active
5. Much less active
6. Not applicable

19. Do you regularly engage in aerobic (such as running, walking, biking, swimming) exercise?

1. Yes 2. No

20. If you answered "yes" to question #19, how frequently (hours per week) have you engaged in aerobic activities during the past 4 months?

_____

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21. Do you regularly engage in resistance exercise such as lifting weights, using weight-machines?

1. Yes 2. No

22. If you answered "yes" to question #21, how frequently (times per week) have you engaged in strength-training exercise during the past 4 months?

_____

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Appendix D

Informed Consent and Authorization to Use or Disclose Protected Health Information

University of Oklahoma
Institutional Review Board
Informed Consent to Participate in a Research Study

Project Title: Acute Hormonal Responses After Two Different Resistance Exercise Protocols in College-Aged Males
Principal Investigator: Michael G. Bemben
Department: Health and Exercise Science

You are being asked to volunteer for this research study. This study is being conducted at University of Oklahoma in the Department of Health and Exercise Science. You were selected as a possible participant because you meet the study criteria.

Please read this form and ask any questions that you may have before agreeing to take part in this study.

Purpose of the Research Study

The purpose of this study is to compare the acute hormonal responses to two different resistance exercise protocols (traditional and SuperSlow) that have similar exercise volumes but differ in intensity and contraction speed in college aged men, 18-30 years of age.

Number of Participants

About 20 men will take part in this study.

Procedures

If you agree to be in this study, you will be asked to

1. Sign and date an informed consent document (this document) indicating that you understand all procedures and your rights as a research subject. You will then complete a HIPPA form, a Health Status Questionnaire and a Physical Activity Readiness Questionnaire (PAR-Q). You may be excluded from the study if any of your questionnaire responses indicate that you have any criteria that would disqualify you from participating in the study. [Visit 1 about 30 minutes]

2. During the first visit, your height and weight will be measured and then you will undergo a familiarization session where trained personnel will instruct you on correct weight lifting techniques. You will then practice the weight lifting exercises with light to moderate weights. [About 30 minutes]

3. During visit 2, you will have your muscular strength tested for 4 lower body and 4 upper body muscle groups using weight training machines by trained testers. You will warm up for 5-10 minutes by cycling. The following procedure will be used for each exercise. You will perform 8-10 repetitions at a light intensity. After a 2 minute rest, you will complete one repetition at a heavy weight (about 80% of your predicted strength). After each successful lift, the weight will be increased until you can no longer lift the weight. Two minutes rest will be given between lifts, and your maximal strength will be obtained within 5 tries for each exercise. [about 60 minutes]

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4. Approximately one to three weeks after this visit, you will visit the lab again and you will be asked to perform one of two exercise protocols, given in random order, and separated by one week or two weeks. For the traditional resistance exercise protocol, you will perform 3 sets of 8 repetitions of each exercise (knee extension, knee flexion, calf raises, two-leg press, shoulder press, low row, biceps curl, and chest press) at a heavy intensity which is 80% of your maximum strength (1-RM). The contraction speed for this resistance exercise protocol will be 1.5 seconds lifting the weight (concentric) and 1.5 seconds lowering the weight (eccentric). Two minutes rest will be given between each set and between each exercise.

For the SuperSlow resistance exercise protocol, you will perform one set of each exercise at 50% of 1-RM, performing the maximum number of repetitions that you can with correct form for the set. The contraction speed for this resistance exercise protocol will be 10 seconds lifting the weight (concentric) and 5 seconds lowering the weight (eccentric). Two minutes rest will be given between each exercise. Each of these exercise protocols will take up to about 90 minutes, including the exercise bout and the 15 minute recovery period.

5. Venipuncture blood draws (approximately 7.5 ml per sample) will be performed by a registered nurse. Three blood samples will be collected during each exercise testing session: at the beginning of each exercise session, immediately after completion of the exercise session and 15 minutes later. All samples are obtained in the morning, following an overnight fast (approximately 8 hours). These blood samples will be analyzed for lactate, testosterone, cortisol, creatine kinase (CK) and hematocrit concentrations.

The safety of the subject is of utmost importance during the blood draws, therefore standard precautions will be used including cleaning of the venipuncture site with alcohol, use of new sterile disposable needles/syringes and changing of disposable gloves in between subjects by the registered nurse. Note that more than one needle stick may be required to obtain a single blood draw, but no more than 2 needle sticks will be attempted to obtain a single blood draw. Therefore the maximum number of needle sticks that may be attempted is 6 for each exercise testing session.

Since you will be in a fasted condition, you may experience feelings of nausea or light-headedness during the exercise session. However, this can be avoided by following the pre-test dietary instructions given to you by the researchers. Also, there will be breakfast bars and juice available for you after the exercise session or at any time that you feel you need them. In addition, you are free to stop exercising at any time you feel that you cannot continue.

Blood samples will be kept for at least 2 years in case samples have to be reanalyzed. Also, it may be about 1 year before these blood results could be made available to you (upon your request). You will be contacted by a member of the research team if an abnormal value is observed for a blood test result; you will receive a copy of the abnormal result, which you then may bring to your personal physician for consultation.

Length of Participation

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This study involves four visits to the laboratory: one visit for consenting and familiarization, one visit for strength testing; and two exercise testing visits. The consenting and familiarization visit will take approximately 1 hour and the strength testing will take about 60 minutes. Each exercise testing visit will take approximately 90 minutes to complete.

Risks of being in the study are

1. Temporary muscle soreness may occur following each exercise testing session. This soreness should subside within 24-48 hours following testing. Although trained personnel will supervise all exercise sessions, there is a possibility of orthopedic or muscle injury.

2. Blood draws will be performed by qualified personnel, but there may be possible discomfort at the site of venipuncture and possible bruising during and after your blood draws. Also, it is possible that you may feel nauseous or light-headed during the exercise testing or during the blood draws since you will be in a fasted condition.

If the researchers determine at the end of the study that any of your blood values are abnormal, you will be contacted and encouraged to visit your physician, but it should be noted that the blood samples will not be analyzed until all participants have completed the study. Therefore, it may be about 1 year before the blood results can be made available to subjects.

Benefits of being in the study are

There are no direct benefits.

Compensation

You will be reimbursed for your time and participation in this study. You will be paid $30 if you complete the entire study. If you withdraw from the study after only completing one of the exercise training sessions (NOT the muscular strength testing session), you will be paid $15 for your time. You will not be compensated if you withdraw from the study prior to completing one of the resistance exercise training sessions. You will be required to provide the Department of Health and Exercise Science with your name and social security number in order to receive your compensation. This information will be given to the University of Oklahoma Financial Support Services for the purposes of reporting taxable income to the IRS..

Injury

In case of injury or illness resulting from this study, emergency medical treatment is available. However, you or your insurance company will be expected to pay the usual charge from this treatment. The University of Oklahoma Norman Campus has set aside no funds to compensate you in the event of injury.

Confidentiality

In published reports, there will be no information included that will make it possible to identify you. Research records will be stored securely and only approved researchers will have access to the records.

There are organizations that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include the OU Institutional Review Board.

Following collection, your blood samples will not be associated with any information that would identify you as the donor of this sample (i.e., it will be de-identified) and subsequently no

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attempt will be made to make that association. It is possible for your identity to be determined from this sample, but the chances of that occurring are highly unlikely.

Voluntary Nature of the Study

Participation in this study is voluntary. If you withdraw or decline participation, you will not be penalized or lose benefits or services unrelated to the study. If you decide to participate, you may decline to answer any question and may choose to withdraw at any time.

You have the right to access the research data that has been collected about you as a part of this research study. However, you may not have access to this information until the entire research study has completely finished and you consent to this temporary restriction.

Contacts and Questions

If you have concerns or complaints about the research, the researcher(s) conducting this study can be contacted at

Eonho Kim (970)978-8591 eonkim@ou.edu

Michael Bemben (405)325-2717 mgbemben@ou.edu

Contact the researcher(s) if you have questions, or if you have experienced a research-related injury.

If you have any questions about your rights as a research participant, concerns, or complaints about the research and wish to talk to someone other than individuals on the research team or if you cannot reach the research team, you may contact the University of Oklahoma – Norman Campus Institutional Review Board (OU-NC IRB) at 405-325-8110 or irb@ou.edu.

You will be given a copy of this information to keep for your records. If you are not given a copy of this consent form, please request one.

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701-A-1

Statement of Consent

I have read the above information. I have asked questions and have received satisfactory answers. I consent to participate in the study.

Participant Signature	Print Name	Date

Signature of Person Obtaining Consent	Date

Print Name of Person Obtaining Consent

Signature of Witness (if applicable)	Date

Print Name of Witness

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UNIVERSITY OF OKLAHOMA – NORMAN CAMPUS
INSTITUTIONAL REVIEW BOARD

AUTHORIZATION TO USE or DISCLOSE PROTECTED HEALTH INFORMATION FOR RESEARCH

An additional Informed Consent Document for Research Participation may also be required.

Title or Research Project: Acute Hormonal Responses After Two Different Resistance Exercise Protocols in College-Aged Males

Principal Investigator: Michael G. Bemben

IRB Number:

Address: 1401 Asp Ave. 115 HHC, Norman OK 73019

Phone Number: 405-325-2717

If you decide to join this research project, University of Oklahoma (OU) researchers may use or share (disclose) information about you that is considered to be protected health information for their research. Protected health information will be called private information in this Authorization.

Private information To be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your private information. If you give permission, the researchers may use or share with the people identified in this Authorization any private information related to this research from your medical records and from any test results. Information, used or shared, may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form, medical records and charts, name, address, telephone number, date of birth, race, and government-issued identification number.

Purposes for Using or Sharing Private Information. If you give permission, the researchers may use your private information to analyze the data from the project and present the information.

Other Use and Sharing of Private Information. If you give permission, the researchers may also use your private information to develop new procedures or commercial products. They may share your private information with the research

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sponsor, the OU Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Department of Health and Human Services (HHS). The researchers may also share your private information with all researchers collaborating on this project.

Confidentiality. Although the research may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. Any person or organization receiving the information based on this authorization could re-release the information to others and federal law would no longer protect it.

YOU MUST UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING ANY CONDITIONS CONSIDERED AS A COMMUNICABLE OR VENEREAL DISEASE WHICH MAY INCLUDE, BUT ARE NOT LIMITED TO, DISEASES SUCH AS HEPATITIS, SYPHILIS, GONORRHEA, AND HUMAN IMMUNODEFICIENCY VIRUS ALSO KNOWN AS ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS).

Voluntary Choice. The choice to give OU researchers permission to use or share your private information for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OU researchers to use or share your private health information if you want to participate in the research and if you revoke your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care from OU.

Revoking Permission. If you give OU researchers permission to use or share your private information, you have a right to revoke your permission whenever you want. However, revoking your permission will not apply to information that the researchers have already used, relied on, or shared.

End of Permission. Unless you revoke it, permission for OU researchers to use or share your private information for their research will end when all data from the project has been analyzed and all reports have been published. You may revoke your permission at any time by writing to:

Privacy Official
University of Oklahoma
1000 Stanton L. Young Blvd., STE 221,
Oklahoma City, OK 73117
If you have questions, call: (405) 271-2511

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IRB NUMBER: 1116
IRB APPROVAL DATE: 10/08/2012
IRB EXPIRATION DATE: 09/30/2013

Giving Permission. By signing this form, you give OU and OU's researchers led by Michael G. Bemben, PhD., permission to share your private information for the research project called Acute Hormonal Responses After Two Different Resistance Exercise Protocols in College-Aged Males.

Subject Name:

_____ _____

Signature of Subject Date
Or parent if Subject is a Child

Or

_____ _____

Signature of Legal Representative** Date

**If signed by a legal Representative of the Subject, provide a description of the relationship to the Subject and the Authority to Act as Legal Representative:

_____

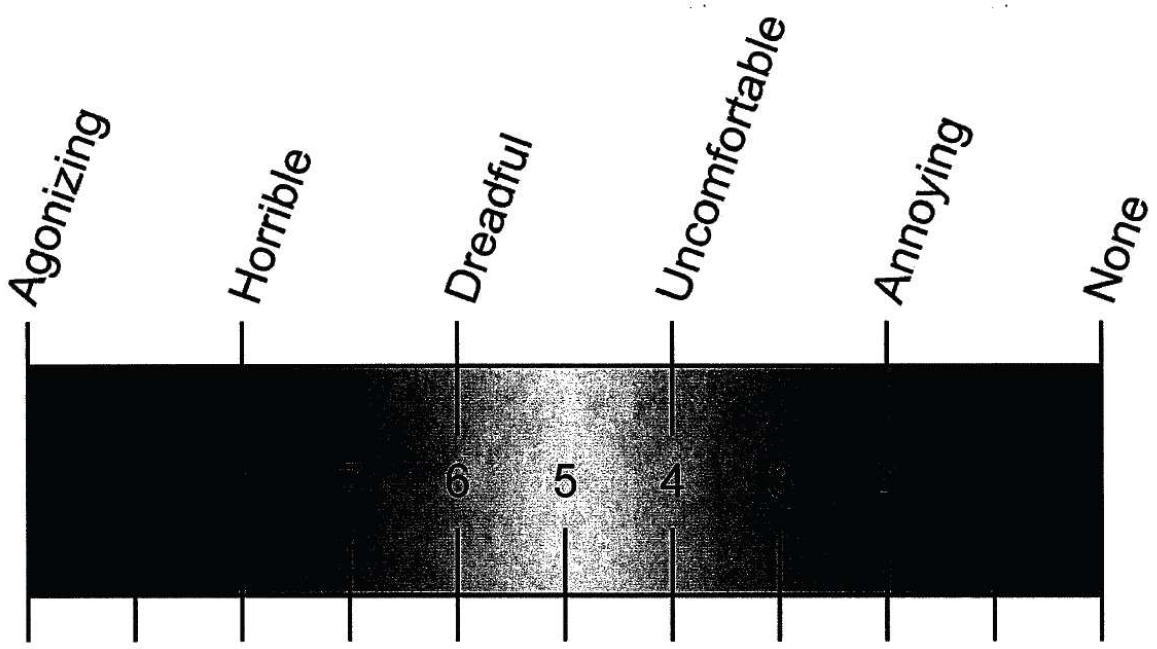
OU may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Subject or the Legal Representative at the time this signed form is provided to the researcher or his representative.

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IRB APPROVAL DATE: 10/08/2012
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Appendix E

Pain Scale, PAR-Q, Pre-testing Dietary Instructions, RPE



Task _____

Date _____ *Start* _____ *End* _____

IRB NUMBER: 1116
IRB APPROVAL DATE: 10/08/2012

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of <u>any other reason</u> why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to <u>all</u> PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

→ **DELAY BECOMING MUCH MORE ACTIVE:**

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

<u>Informed Use of the PAR-Q</u>: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

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continued on other side...

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Pre-Testing Dietary Instructions

1. It is very important that you consume high amounts of carbohydrates (400-600 grams) the day before you are scheduled for an exercise testing session. Examples of foods that are good sources of carbohydrate are bagels, whole wheat bread, pasta, rice, low fat milk, fruit juice, and apples.

2. You should eat a good meal for dinner the night before at about 5:30 or 6 pm. Examples of a "good meal" would be pasta or pizza or brown rice with 4-6 oz. of a lean protein source (a half of chicken breast) and a dinner salad. In addition, you should eat a high carbohydrate snack (about 300-400 calories) about 8 or 9 pm the night before the testing session. An example of this would be a small bowl of oatmeal paired with 2 egg whites and a glass of orange juice or low fat frozen yogurt (1.5 cups) with strawberry slices.

3. Be sure to drink lots of water the day before and the morning of the testing session. For example, try to consume a glass of water with every meal and upon waking in the morning.

4. Remember to come to the testing session in a fasted state. That means no food, juice, or coffee after 11 pm, but drink plenty of water. There will be juice and breakfast bars for you at the end of the testing session.

5. Do not drink alcohol the night before the exercise testing.

Practicing good nutrition habits will ensure adequate energy levels during your exercise testing sessions!

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Borg Rating of Perceived Exertion (RPE) Scale

Exertion	RPE
No exertion at all	6
Extremely light	7
	8
Very light	9
	10
Light	11
	12
Somewhat hard	13
	14
Hard (heavy)	15
	16
Very hard	17
	18
Extremely hard	19
Maximal exertion	20

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IRB APPROVAL DATE: 10/08/2012

Appendix F

IRB Approval Letter

The UNIVERSITY of OKLAHOMA

Institutional Review Board for the Protection of Human Subjects

Approval of Initial Submission – Board Review – AP01

Date: October 09, 2012

Principal Investigator: Michael G Bemben, PHD

IRB#: 1116
Study Title: ACUTE HORMONAL RESPONSES AFTER TWO DIFFERENT RESISTANCE EXERCISE PROTOCOLS IN COLLEGE-AGED MALES

IRB Meeting Date: 10/04/2012

IRB Approval Date: 10/08/2012
IRB Expiration Date: 09/30/2013

Collection/Use of PHI: Yes

The review and approval of this submission is based on the determination that the study will be conducted in a manner consistent with the requirements of 45 CFR 46.

To view the approved documents for this submission, open this study from the My Studies option, go to Submission History, go to Completed Submissions tab and then click the Details icon.

You will receive notification approximately 60 days prior to the expiration date noted above. You are responsible for submitting continuing review documents in a timely fashion in order to maintain continued IRB approval.

You are also responsible for:

- Ensuring this research is conducted as approved by the IRB.
- Obtaining consent using the currently approved, stamped consent form and retaining all original, signed consent forms, if applicable.
- Informing the IRB of any/all modifications prior to implementing those changes.
- Reporting any serious, unanticipated harms as per Policy 407 and/or any additional information that may change the risk, benefit, or desire for participants to continue in the study.
- Submitting a final closure report at the completion of the project.
- Keeping and maintaining accurate study records as your study is subject to quality improvement evaluation.

If you have questions about this notification or using iRIS, contact the IRB @ 405-325-8110 or irb@ou.edu.

Cordially,

Aimee Franklin, Ph.D.
Chair, Institutional Review Board

Appendix G

Instructions for Testosterone Elisa Kit and Cortisol Elisa Kit by DRG International Inc.

Instructions for Creatine Kinase Assay Kit (ECPK-100) by BioAssay Systems

DRG

DRG® Testosterone ELISA (EIA-1559)

CE

Revised 22 July 2009 (Vers. 8.1)

IVD

INTRODUCTION

Intended Use

The **DRG Testosterone ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of Testosterone in serum and plasma.

Summary and Explanation

Testosterone (17β-hydroxy-4-androstene-3-one) is a C19 steroid with an unsaturated bond between C-4 and C-5, a ketone group in C-3 and a hydroxyl group in the β position at C-17.
This steroid hormone has a molecular weight of 288.47.
Testosterone is the most important androgen secreted into the blood. In males, testosterone is secreted primarily by the Leydig cells of the testes; in females ca. 50% of circulating testosterone is derived from peripheral conversion of androstenedione, ca. 25% from the ovary and ca. 25% from the adrenal glands.
Testosterone is responsible for the development of secondary male sex characteristics and its measurements are helpful in evaluating the hypogonadal states.
In women, high levels of testosterone are generally found in hirsutism and virilization, polycystic ovaries, ovarian tumors, adrenal tumors and adrenal hyperplasia.
In men, high levels of testosterone are associated to the hypothalamic pituitary unit diseases, testicular tumors, congenital adrenal hyperplasia and prostate cancer.
Low levels of testosterone can be found in patients with the following diseases: Hypopituitarism, Klinefelter's syndrome, Testicular feminization, Orchidectomy and Cryptorchidism, enzymatic defects and some autoimmune diseases.

PRINCIPLE OF THE TEST

The DRG Testosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.
The microtiter wells are coated with a monoclonal [mouse] antibody directed towards an unique antigenic site on the Testosterone molecule. Endogenous Testosterone of a patient sample competes with a Testosterone horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.
The amount of bound peroxidase conjugate is reverse proportional to the concentration of Testosterone in the sample.
After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of Testosterone in the patient sample.

WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.

4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from DRG.

DRG

DRG® Testosterone ELISA (EIA-1559)

CE

Revised 22 July 2009 (Vers. 8.1)

IVD

REAGENTS

Reagents provided

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with a mouse monoclonal anti-Testosterone antibody.
2. **Standard (Standard 0-6),** 7 vials, 1 ml, ready to use
   Concentrations: 0 - 0.2 - 0.5 – 1 – 2 – 6 - 16 ng/mL
   Conversion: 1 ng/mL = 3.467 nmol/L
   contain 0.03% Proclin 300 + 0.005% gentamicin sulfate as a preservative.
3. **Enzyme Conjugate**, 1 vial, 25 mL, ready to use
   Testosterone conjugated to horseradish peroxidase
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as a preservative.
4. ***Substrate Solution***, 1 vial, 25 mL, ready to use;
   Tetramethylbenzidine (TMB).
5. ***Stop Solution***, 1 vial, 14 mL, ready to use;
   contains 0.5M H_2SO_4.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
6. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
   see „Preparation of Reagents".

* BND = 5-bromo-5-nitro-1,3-dioxane
MIT = 2-methyl-2H-isothiazol-3-one

Note: Additional *Standard 0* for sample dilution is available upon request.

a. Materials required but not provided

- A microtiter plate calibrated reader (450±10 nm), (e.g. the DRG Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

1.1 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C - 8 °C. Microtiter wells must be stored at 2 °C - 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (EDTA-, Heparin- or citrate plasma) can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.
(E.g. for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001; for Heparin plasma Sarstedt Monovette – orange cap - # 02.165.001; for Citrate plasma Sarstedt Monovette – green cap - # 02.167.001.)

Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying.
Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) Dilution 1:10: 10 µL Serum + 90 µL *Standard 0* (mix thoroughly)
b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

ASSAY PROCEDURE

General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **25 µL** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3. Dispense **200 µL** ***Enzyme Conjugate*** into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes** at room temperature (without covering the plate).
5. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **200 µL** of ***Substrate Solution*** to each well.
7. Incubate for **15 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
9. Determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader.
   It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 16 Ing/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 ng/mL)	2.1
Standard 1 (0.2 ng/mL)	1.71
Standard 2 (0.5 ng/mL)	1.44
Standard 3 (1 ng/mL)	1.18
Standard 4 (2 ng/mL)	0.89
Standard 5 (6 ng/mL)	0.46
Standard 6 (16 ng/mL)	0.24

EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the DRG Testosterone ELISA the following values are observed:

Population	5% Percentile	95% Percentile
Males	2.0 ng/mL	6.9 ng/mL
Females	0.26 ng/mL	1.22 ng/mL

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or DRG directly.

PERFORMANCE CHARACTERISTICS

Assay Dynamic Range

The range of the assay is between 0.083 – 16 ng/mL.

Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Analyte	Cross Reactivity
Testosterone	100.0
5α-Dihydrotestosterone	0.8
Androstenedione	0.9
11β-Hydroxyestosterone	3.3
17α-Methyltestosterone	0.1
19-Nortestosterone	3.3
Epitestosterone	< 0.1
Oestradiol	< 0.1
Progesterone	< 0.1
Cortisol	< 0.1
Oestrone	< 0.1
Danazol	< 0.1

Sensitivity

The analytical sensitivity of the DRG ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Zero Standard (S0) and was found to be 0.083 ng/mL.

Reproducibility

Intra Assay Variation

The within assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	20	0.73	4.16
2	20	4.88	3.28
3	20	11.26	3.34

Inter Assay Variation

The between assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	20	0.82	9.94
2	20	5.20	6.71
3	20	11.38	4.73

Recovery

Samples have been spiked by adding Testosterone solutions with known concentrations in a 1:1 ratio.
The expected values were calculated by addition of half of the values determined for the undiluted samples and half of the values of the known solutions. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

Sample	Added Concentration 1:1 (v/v) (ng/mL)	Measured Conc. (ng/mL)	Expected Conc. (ng/mL)	Recovery (%)
1	0.0	1.10		
	16.0	9.31	8.55	109.0
	6.0	3.93	3.55	110.7
	2.0	1.67	1.55	107.9
	1.0	0.91	1.05	86.9
2	0.0	6.07		
	16.0	11.81	11.03	107.1
	6.0	6.65	6.03	110.1
	2.0	3.73	4.03	92.5
	1.0	3.26	3.53	92.2
3	0.0	11.62		
	16.0	14.76	13.63	108.3
	6.0	9.33	8.63	108.1
	2.0	7.29	6.63	110.0
	1.0	6.75	6.13	110.1

Linearity

Sample	Dilution	Measured Conc (ng/mL)	Expected Conc. (ng/mL)	Recovery (%)
1	None	1.10	1.10	
	1:2	0.51	0.55	93.8
	1:4	0.24	0.27	86.1
	1:8	0.15	0.14	106.6
2	None	6.07	6.07	
	1:2	3.36	3.03	110.6
	1:4	1.66	1.52	109.2
	1:8	0.68	0.76	89.0
	1:16	0.37	0.38	97.0
3	None	11.26	11.26	
	1:2	5.76	5.63	102.4
	1:4	2.76	2.81	97.9
	1:8	1.55	1.41	110.0
	1:16	0.77	0.70	109.5

LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.25 mg/mL) and Triglyceride (up to 7.5 mg/mL) have no influence on the assay results.

Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Testosterone in a sample.

High-Dose-Hook Effect

No hook effect was observed in this test.

LEGAL ASPECTS

Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid.
Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

REFERENCES / LITERATURE

1. Tietz, N.W. Textbook of Clinical Chemistry. Saunders, 1986.

DRG

DRG® Cortisol ELISA (EIA-1887)

CE

REVISED 8 NOV. 2011 RM (VERS. 1.1) **USA: RUO**

INTRODUCTION

The **DRG Cortisol Enzyme Immunoassay Kit** provides materials for the determination of Cortisol in serum and plasma.

This assay is intended for research use only.

PRINCIPLE OF THE TEST

The DRG Cortisol ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.
The amount of bound peroxidase conjugate is inversely proportional to the concentration of Cortisol in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of Cortisol in the donor sample.
The microtiter wells are coated with a monoclonal antibody directed towards an antigenic site on the Cortisol molecule. Endogenous Cortisol of a donor sample competes with a Cortisol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.

PRECAUTIONS

- This kit is for Research Use Only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- Safety Data Sheets for this product are available upon request directly from DRG International, Inc.
  The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

DRG

DRG® Cortisol ELISA (EIA-1887)

CE

REVISED 8 NOV. 2011 RM (VERS. 1.1) **USA: RUO**

KIT COMPONENTS

Contents of the Kit

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with a anti-Cortisol antibody (monoclonal).
2. ***Standard (Standard 0-6)***, 7 vials, 1 mL, ready to use;
   Concentrations: 0, 20, 50, 100, 200, 400, 800 ng/mL,
   thus corresponding to 0, 55.2, 138, 276, 552, 1104, 2208 nmol/L.
   Conversion factor: 1 ng/mL = 2.76 nmol/l.)
   contain 0.3% Proclin as a preservative
3. ***Enzyme Conjugate***, 1 vial, 25 mL, ready to use;
   Cortisol conjugated to horseradish Peroxidase;
   contains 0.3% Proclin as a preservative.
4. ***Substrate Solution***, 1 vial, 14 mL, ready to use;
   Tetramethylbenzidine (TMB).
5. ***Stop Solution***, 1 vial, 14 mL, ready to use;
   contains 0.5M H_2SO_4.
   Avoid contact with the stop solution. It may cause skin irritations and burns.
6. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
   see "Preparation of Reagents".

Note: Additional *Standard 0* for sample dilution is available upon request.

Equipment and material required but not provided

- A microtiter plate calibrated reader (450±10 nm), (e.g. the DRG International Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled water

Storage and stability of the Kit

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.

Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution

Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

Damaged Test Kits

In case of any severe damage of the test kit or components, DRG® have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

SPECIMEN

Serum or plasma (EDTA-, Heparin- or citrate plasma) can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Donors receiving anticoagulant therapy may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.
(E.g. for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001; for Heparin plasma Sarstedt Monovette – orange cap - # 02.165.001; for Citrate plasma Sarstedt Monovette – green cap - # 02.167.001.)

Specimen Storage

Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying.
Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) Dilution 1:10: 10 µL Serum + 90 µL *Standard 0* (mix thoroughly)
b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

TEST PROCEDURE

General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **20 µL** of each *Standard, Control* and samples with new disposable tips into appropriate wells.
3. Dispense **200 µL** *Enzyme Conjugate* into each well.
4. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for **60 minutes** at room temperature (without covering the plate).
6. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted *Wash Solution* (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add **100 µL** of *Substrate Solution* to each well.
8. Incubate for **15 minutes** at room temperature.
9. Stop the enzymatic reaction by adding **100 µL** of *Stop Solution* to each well.
10. Read the OD at **450±10 nm** with a microtiter plate reader **within 10 minutes** after adding the *Stop Solution*.

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and donor samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

Below is listed a typical example of a standard curve with the DRG® Cortisol ELISA.

Standard	Optical Units (450 nm)
Standard 0 (0 ng/mL)	2.30
Standard 1 (20 ng/mL)	1.67
Standard 2 (50 ng/mL)	1.24
Standard 3 (100 ng/mL)	0.87
Standard 4 (200 ng/mL)	0.57
Standard 5 (400 ng/mL)	0.35
Standard 6 (800 ng/mL)	0.23

LIMITATIONS OF USE

Interfering Substances

Any improper handling of samples or modification of this test might influence the results.
Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 7.5 mg/mL) have no influence on the assay results.

Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Cortisol in a sample.

High-Dose-Hook Effect

No hook effect was observed in this test.

REFERENCES

1. L. Thomas, Labor und Diagnose, 4. Auflage, 1992
2. Tietz, N.W., Textbook of Clinical Chemistry, Saunders, 1968

Version 8/2006_rm

EnzyChrom™ Creatine Kinase Assay Kit (ECPK-100)

Colorimetric Determination of Creatine Kinase Activity at 340 nm

DESCRIPTION

CREATINE KINASE (CK), also known as creatine phosphokinase (CPK), is an enzyme (EC 2.7.3.2) expressed predominantly in skeletal muscle, smooth muscle and the brain. The CK enzyme consists of two subunits, which can be either B (brain type) or M (muscle type), and hence three different isoenzymes: CK-MM, CK-BB and CK-MB. CK catalyzes the conversion of creatine to phosphocreatine, consuming adenosine triphosphate (ATP) and generating adenosine diphosphate (ADP) and the reverse reaction. CK is often determined routinely in emergency patients with chest pain and acute renal failure. Elevation of CK is an indication of damage to muscle and has been associated with injury, rhabdomyolysis, myocardial infarction, myositis, myocarditis, malignant hyperthermia and neuroleptic malignant syndrome, etc. Lower levels can be an indication of alcoholic liver disease and rheumatoid arthritis.

Simple, direct and automation-ready procedures for measuring CK activity are very desirable. BioAssay Systems' QuantiChrom™ Creatine Kinase Assay Kit is based on enzyme coupled reactions in which creatine phosphate and ADP is converted to creatine and ATP by CK, the generated ATP is used to phosphorylate glucose by hexokinase to generate glucose-6-phosphate, which is then oxidized by NADP in the presence of glucose-6-phosphate dehydrogenase. The produced NADPH, measured at 340 nm, is proportionate to the CK activity in the sample.

APPLICATIONS

Direct Assays: CK in serum, plasma and other biological samples.
Pharmacology: effects of drugs on CK activity.

KEY FEATURES

Sensitive and accurate. Detection range: 5 to 300 U/L creatine kinase in 96-well plate assay.
Convenient. The procedure involves adding a single working reagent, and reading the optical density at 20 min and 40 min at room temperature or 37°C.
High-throughput. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer: 12 mL	**Substrate Solution: 1.0 mL**
Enzyme Mix: 120 µL	**Calibrator: 150 µL**

Storage conditions. This kit is shipped on ice. Store all reagents at -20°C. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation. Use non-hemolyzed samples. Samples should be assayed within 4 hours of blood collection if they remain at room temperature, or within 12 hours if stored at 4°C. Samples can be stored at -80°C and should be thawed only once. If turbidity is observed, centrifuge sample and use clear supernatant for assay.

Reagent Reconstitution. Bring all components to room temperature. For each reaction well, mix 10 µL Substrate Solution, 100 µL Assay Buffer and 1 µL Enzyme Mix. Fresh reconstitution is recommended. If the assay is to be carried out at 37°C, warm up the Reconstituted Reagent at 37°C.

1. *Calibrator:* transfer 110 µL water and (10 µL Calibrator + 100 µL water) into separate wells of a clear bottom 96-well plate.

   Samples: transfer 10 µL samples into separate wells. Add 100 µL Reconstituted Reagent and tap plate to mix.

2. *Reaction*. Incubate at room temperature or 37°C. CK is fully activated within 20 min by glutathione provided in the Substrate Solution. Read OD340nm at 20 min and again at 40 min.

3. *Calculation*. Calculate sample CK activity using the equation,

$$\text{CK (U/L)} = \frac{OD_{40min} - OD_{20min}}{OD_{CALIBRATOR} - OD_{H2O}} \times 150$$

   OD_{40min} and OD_{20min} are OD340nm values at 40 min and 20 min for the sample. $OD_{CALIBRATOR}$ and OD_{H2O} are OD340nm values of the Calibrator and water blank at 40 min. The value 150 is the equivalent activity (U/L) of the Calibrator under the assay conditions. *Unit definition*: one unit of CK will transfer 1 µmole of phosphate from phosphocreatine to ADP per min at pH 6.0.

Note: If the CK activity is expected to be higher than 300 U/L, read OD340nm at 20 min and again at 25 min. To calculate the CK activity replace ($OD_{40min} - OD_{20min}$) with ($OD_{25min} - OD_{20min}$) and replace the factor 150 with 600 in the above equation. Linear range: 30 to 1,800 U/L CK activity.

MATERIALS REQUIRED, BUT NOT PROVIDED

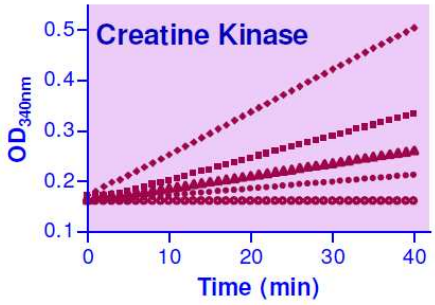
Pipeting (multi-channel) devices. Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

GENERAL CONSIDERATIONS

This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Reconstituted Reagent should be quick and mixing should be brief but thorough. Use of multi-channel pipettor is recommended.

EXAMPLES

Samples were assayed in duplicate using the 96-well protocol. The CK activity (U/L) was 12.0 ± 0.9 for a rat serum sample, 11.0 ± 0.5 for human serum, 28 ± 1 for human plasma, 9.0 ± 0.8 for mouse serum and 49 ± 2 for bovine serum.



Kinetics of CK Reaction at 25 (solid circle), 50 (triangle), 100 (square) and 200 (diamond) U/L. Control: open circle

PUBLICATIONS

1. Itaka K et al (2010). Polyplex nanomicelle promotes hydrodynamic gene introduction to skeletal muscle. J Control Release. 143(1):112-9.
2. Steen C et al (2010). Reduced Creatine Kinase B Activity in Multiple Sclerosis Normal Appearing White Matter. PLoS One 5(5):e10811.
3. Kwon S et al. (2010). ASB9 interacts with ubiquitous mitochondrial creatine kinase and inhibits mitochondrial function. BMC Biol. 8:23.

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Appendix H

Data Collection Sheet

ONE REPETITION MAXIMUM (1-RM)

Subject: _____ **Date:** / /

Height: _____ **(Cm)**

Weight: _____ **(Kg)**

	Exercises	Seat Height/ Sternum Pad/ Shin Pad/ Back Pad etc.	Memo	# of Plates	Actual Weight
Upper Body Ex.	Shoulder Press				
	Chest Press				
	Low Row				
	Biceps Curl				
Lower Body Ex.	Knee Extension				
	Knee Flexion				
	Leg Press				
	Calf Raises				

SuperSlow Study(50%-1RM)

Subject ID:		**Reps.**	**Pain Scale**	**RPE**	**# of plates**	**Seat height**	**Memo**
Upper Body Ex.	**Shoulder Press**						
	Chest Press						
	Low Row						
	Biceps Curl						
Lower Body Ex.	**Knee Extension**						
	Knee Flexion						
	Leg Press						
	Calf Raises						

SuperSlow Study(80%-1RM)

Subject ID:		**Set**	**Reps.**	**Pain Scale**	**RPE**	**# of plates**	**Seat height**	**Memo**
Upper Body Ex.	**Shoulder Press**	1st set						
		2nd set						
		3rd set						
	Chest Press	1st set						
		2nd set						
		3rd set						
	Low Row	1st set						
		2nd set						
		3rd set						
	Biceps Curl	1st set						
		2nd set						
		3rd set						
Lower Body Ex.	**Knee Extension**	1st set						
		2nd set						
		3rd set						
	Knee Flexion	1st set						
		2nd set						
		3rd set						
	Leg Press	1st set						
		2nd set						
		3rd set						
	Calf Raises	1st set						
		2nd set						
		3rd set						

Appendix I

Individual Responses for Each Blood Variable

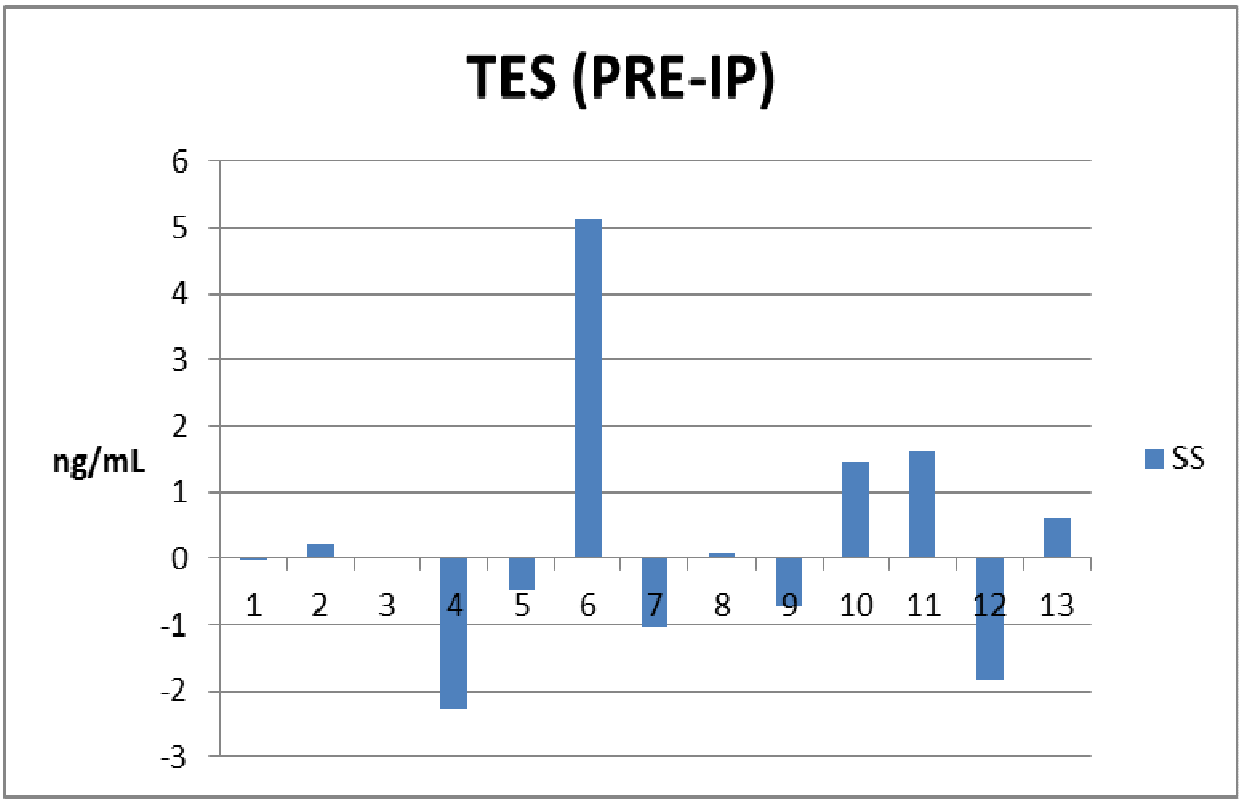


Figure 7. Individual Changes in Testosterone (PRE-IP) for SS Condition.

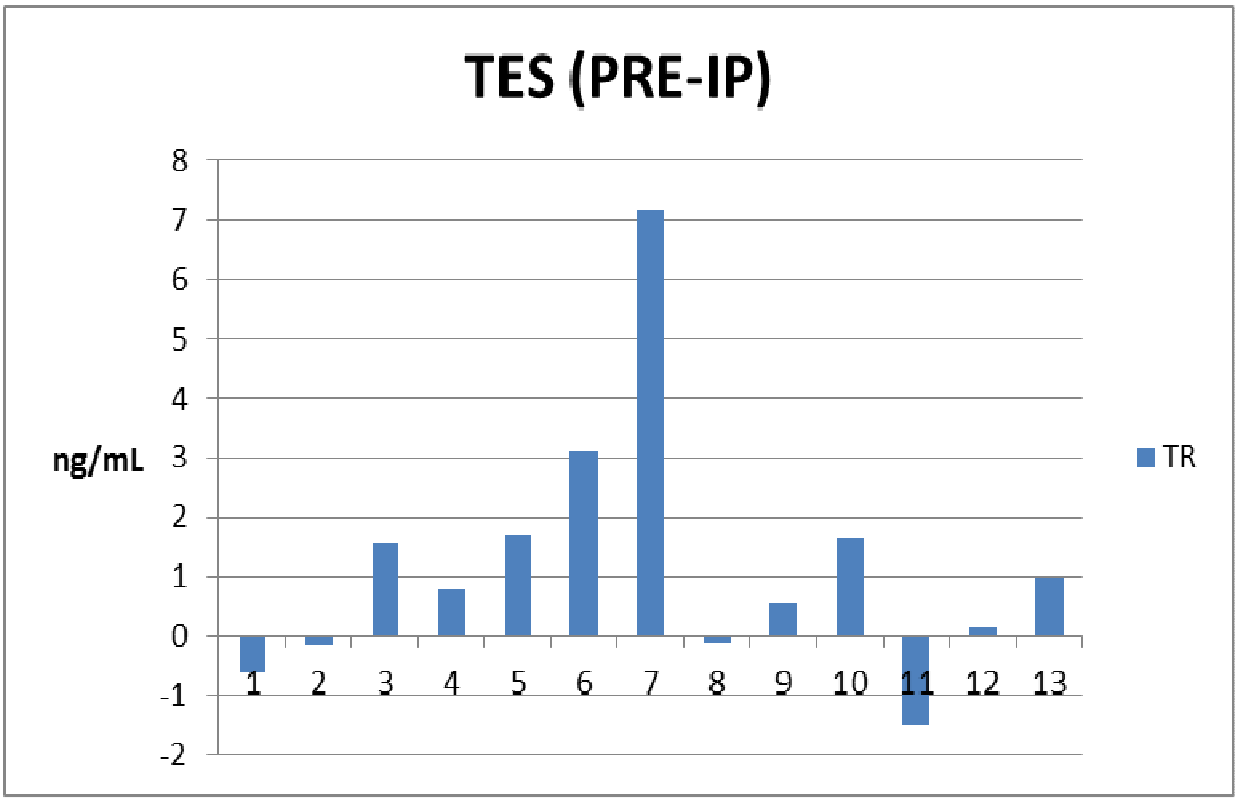


Figure 8. Individual Changes in Testosterone (PRE-IP) for TR Condition.

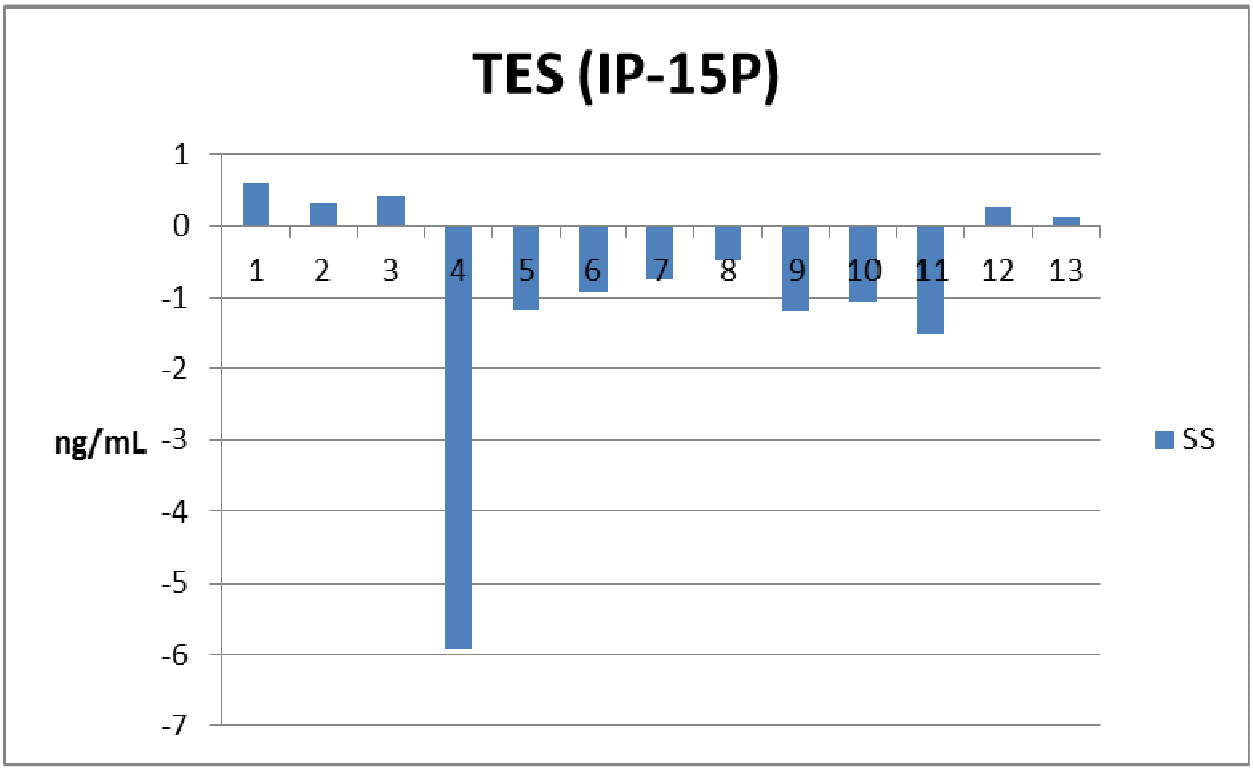


Figure 9. Individual Changes in Testosterone (IP-15P) for SS Condition.

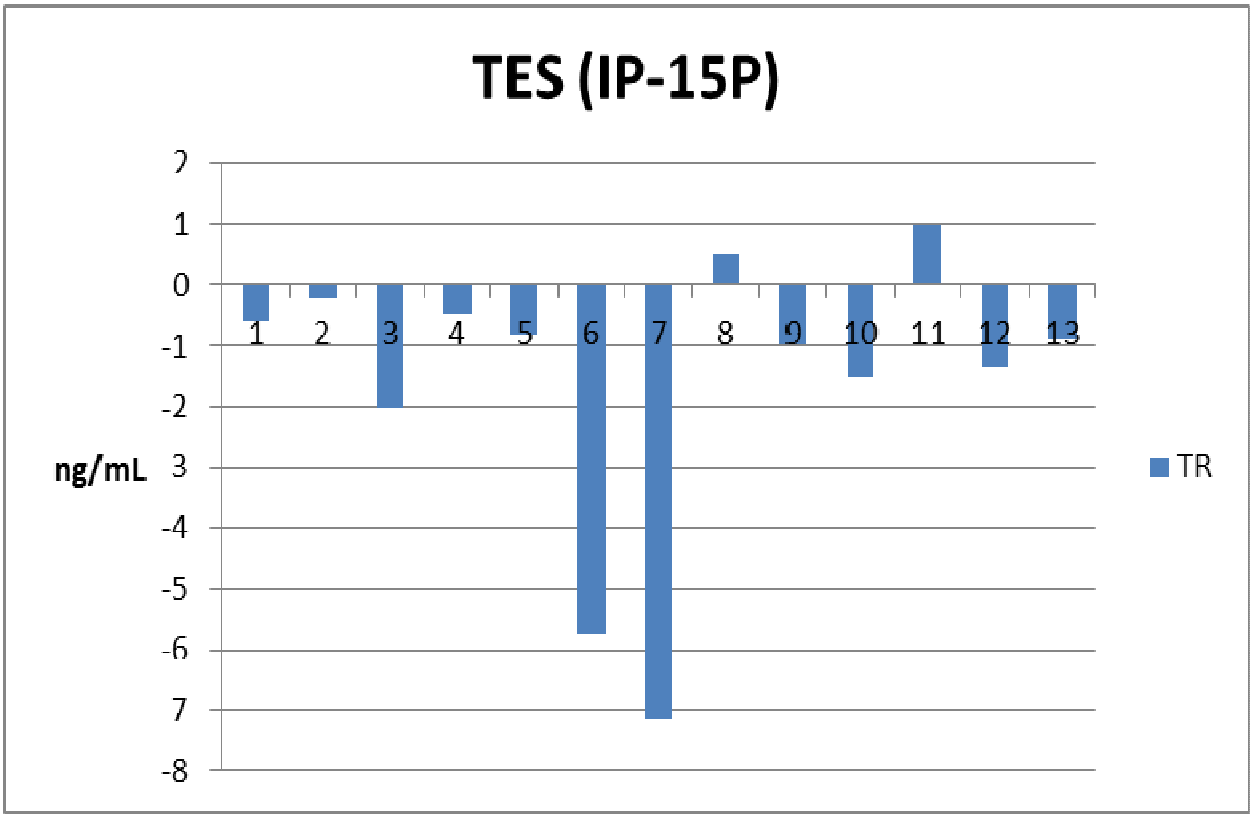


Figure 10. Individual Changes in Testosterone (IP-15P) for TR Condition.

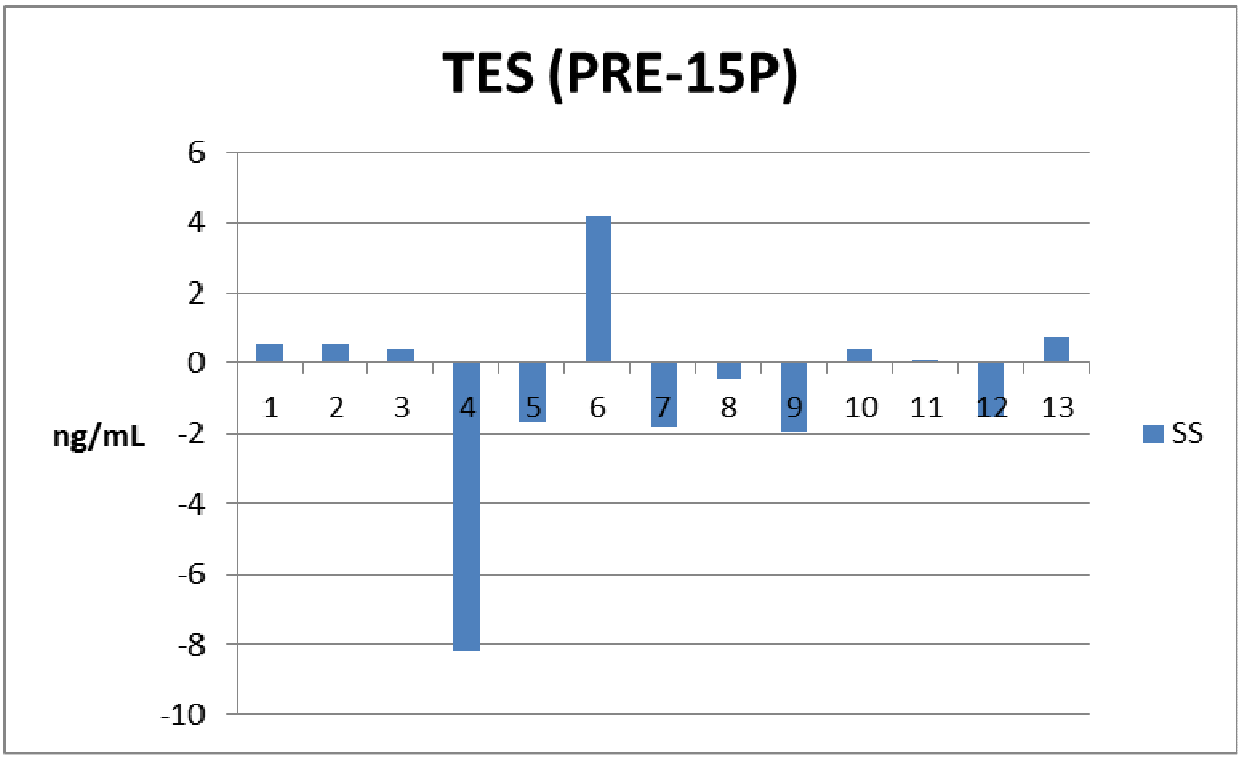


Figure 11. Individual Changes in Testosterone (PRE-15P) for SS Condition.

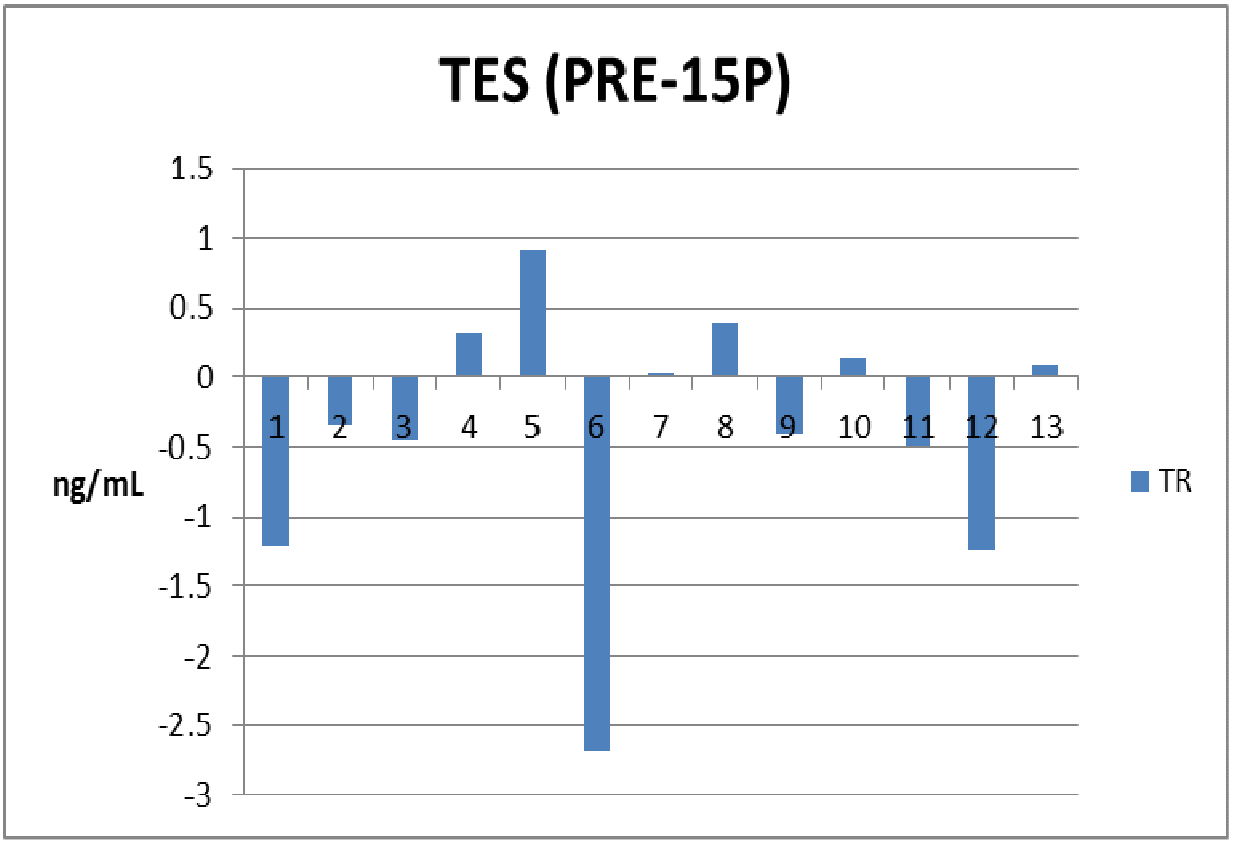


Figure 12. Individual Changes in Testosterone (PRE-15P) for TR Condition.

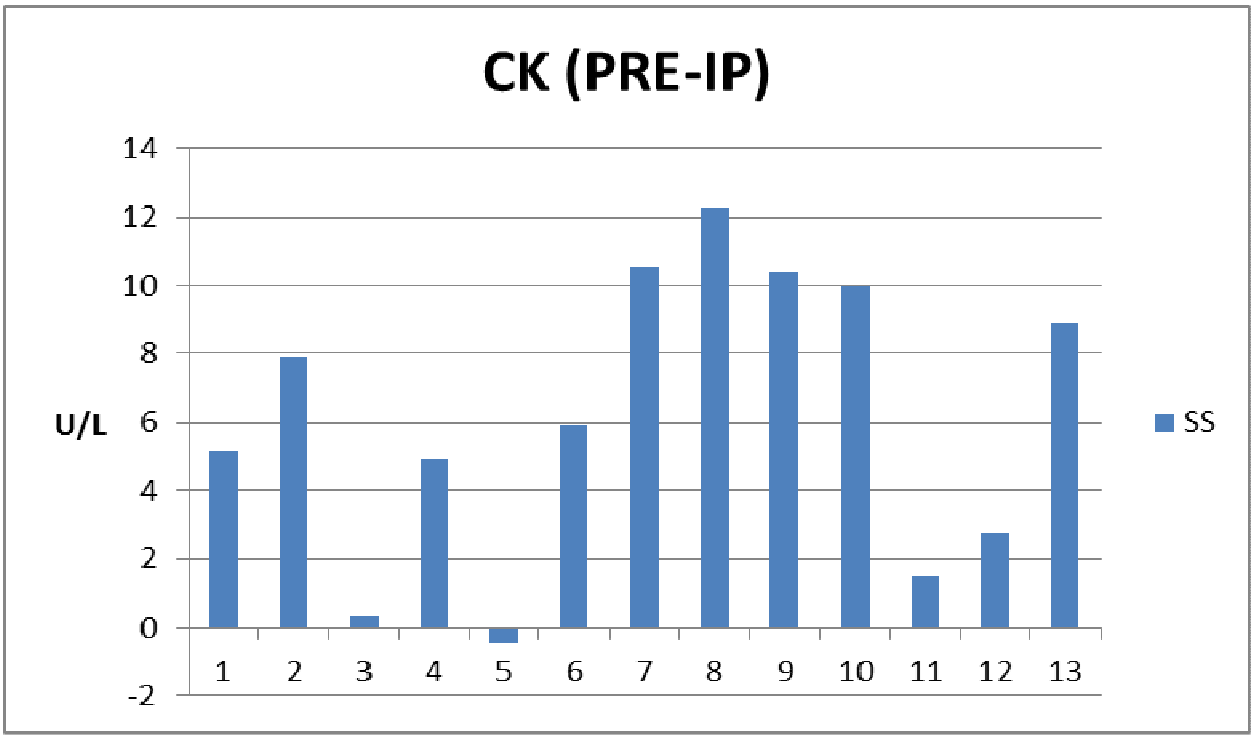


Figure 13. Individual Changes in CK (PRE-IP) for SS Condition.

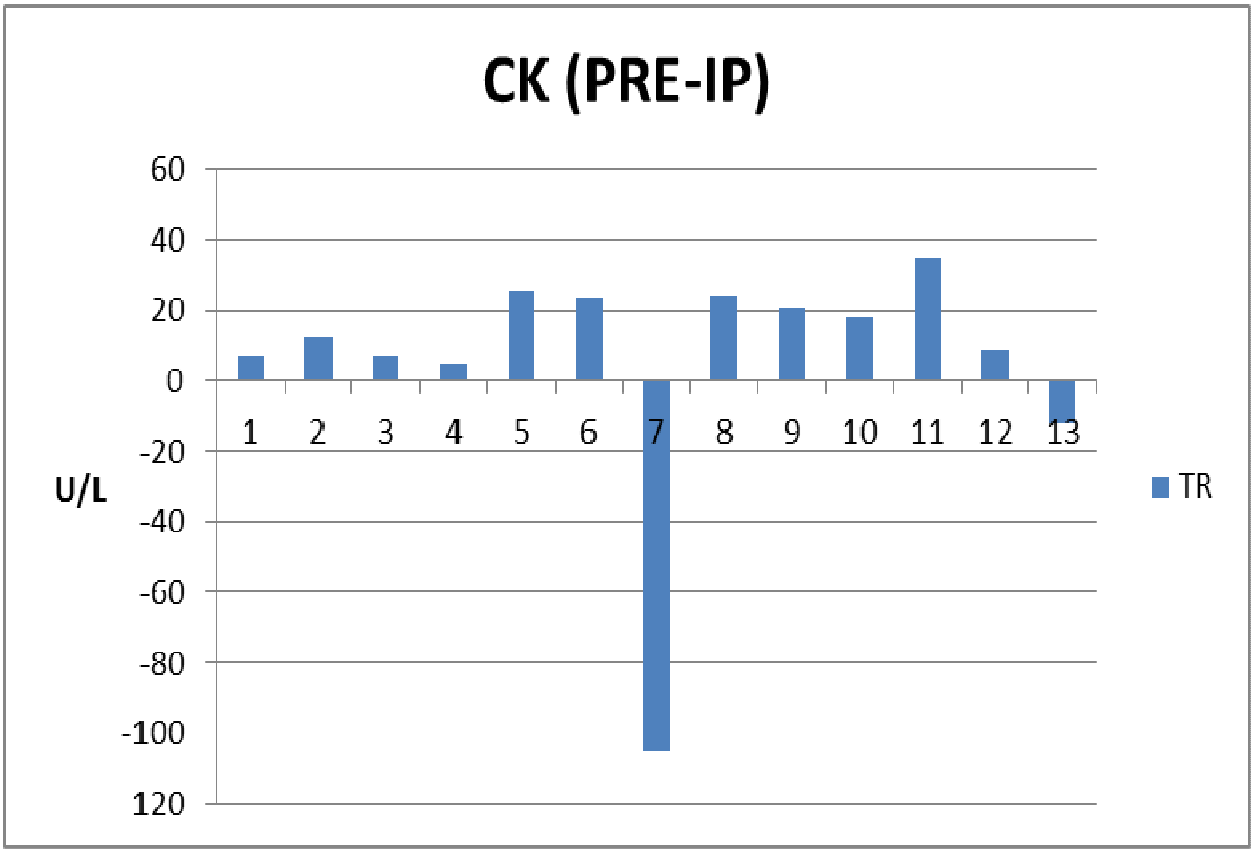


Figure 14. Individual Changes in CK (PRE-IP) for TR Condition.

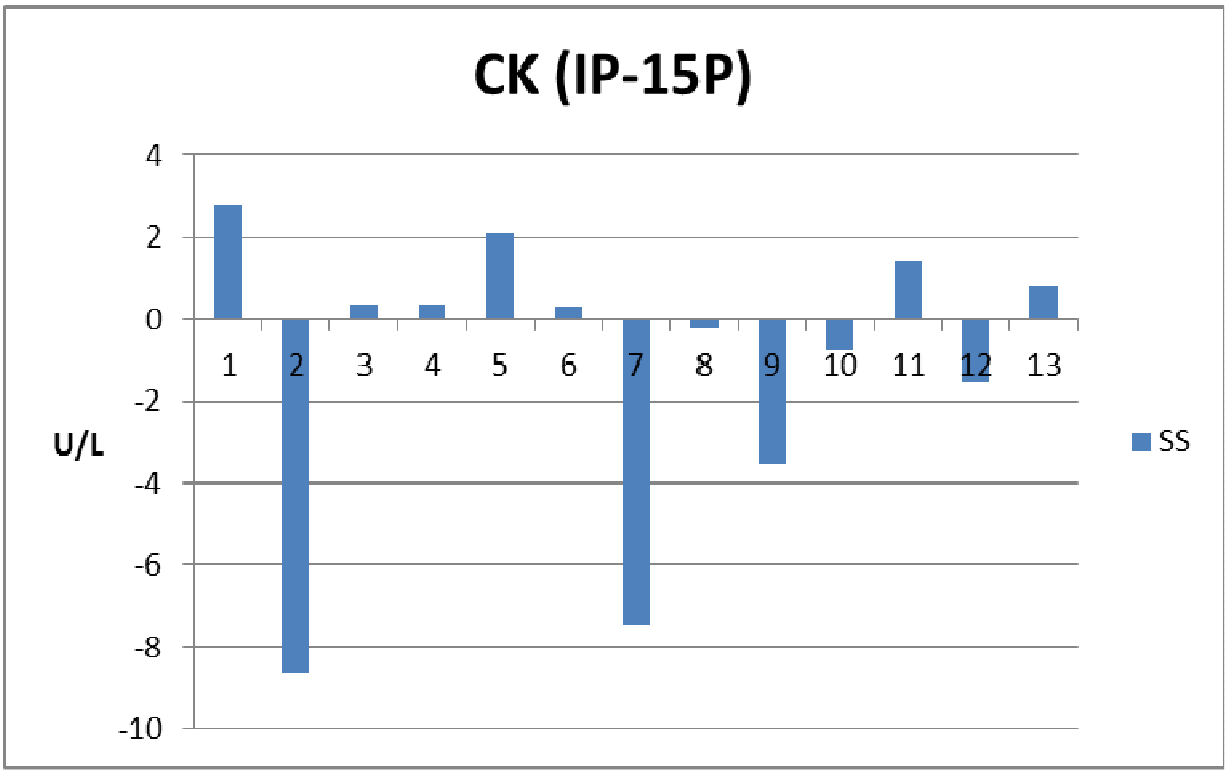


Figure 15. Individual Changes in CK (IP-15P) for SS Condition.

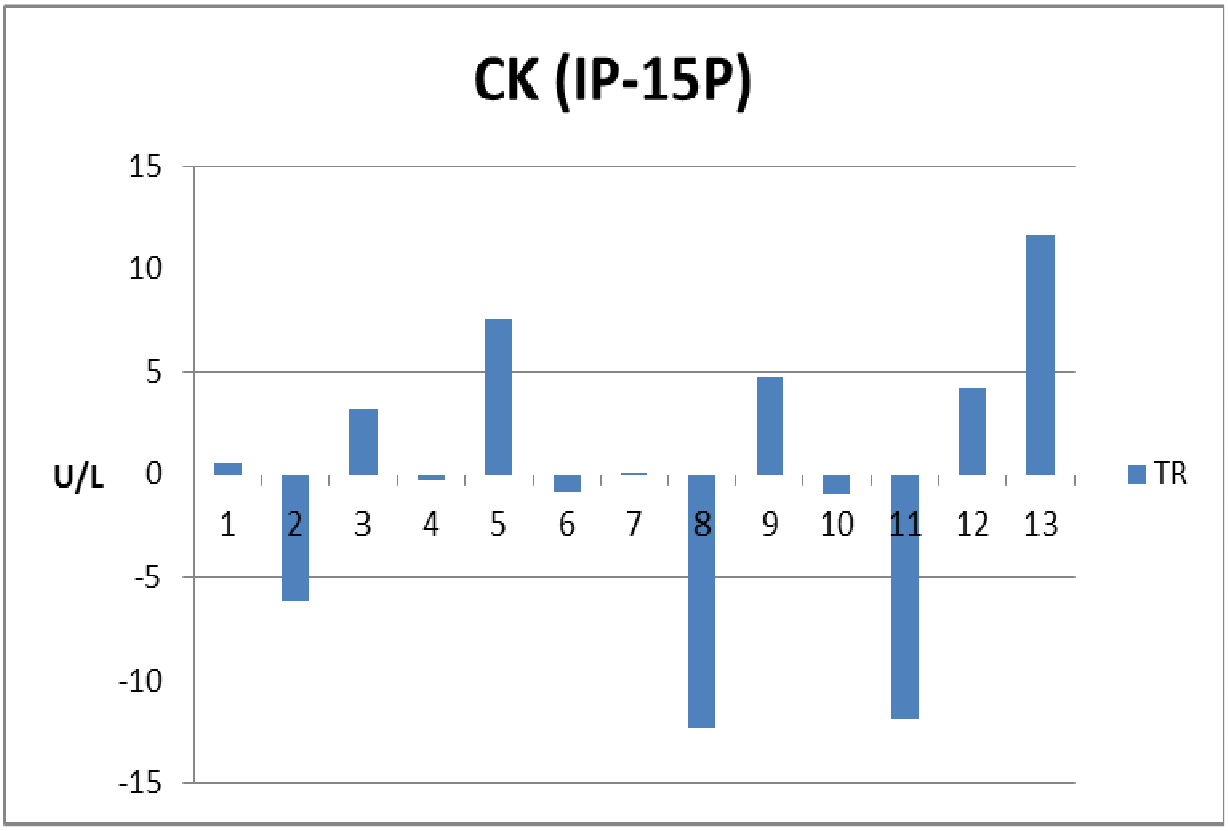


Figure 16. Individual Changes in CK (IP-15P) for TR Condition.

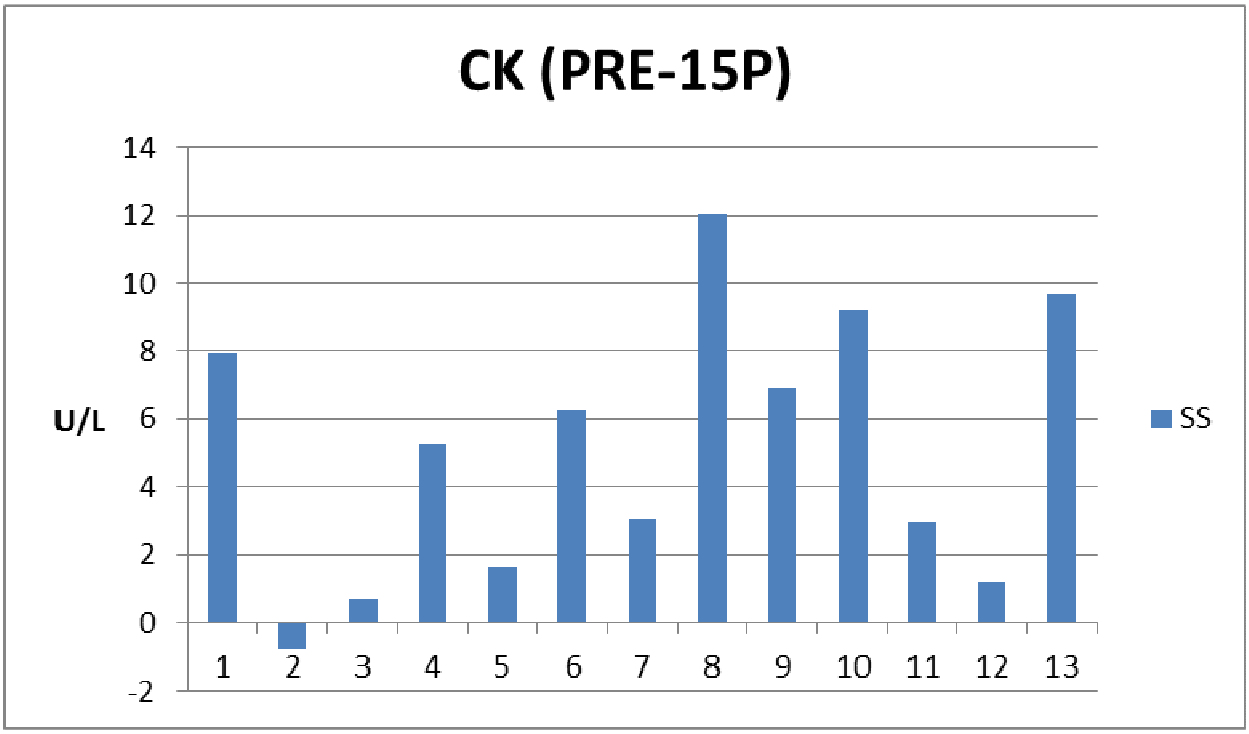


Figure 17. Individual Changes in CK (PRE-15P) for SS Condition.

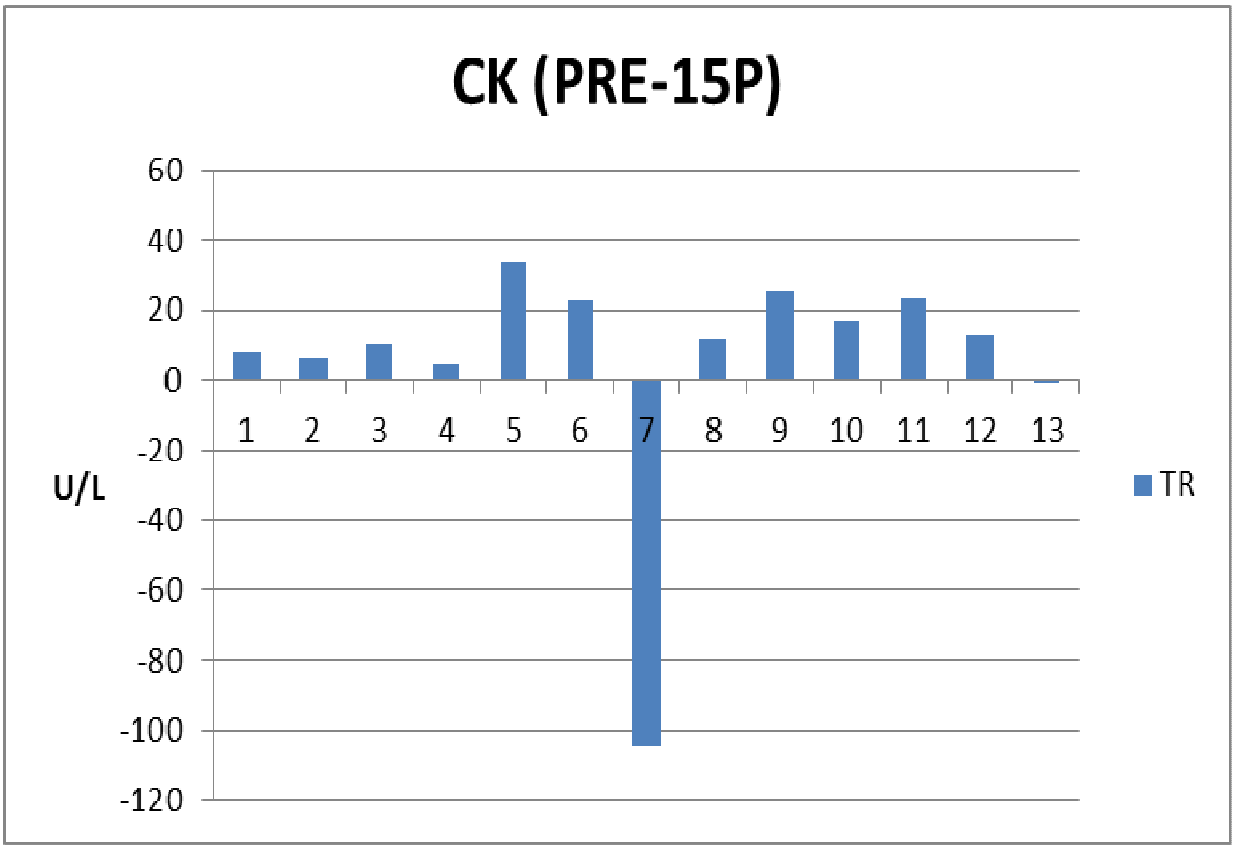


Figure 18. Individual Changes in CK (PRE-15P) for TR Condition.

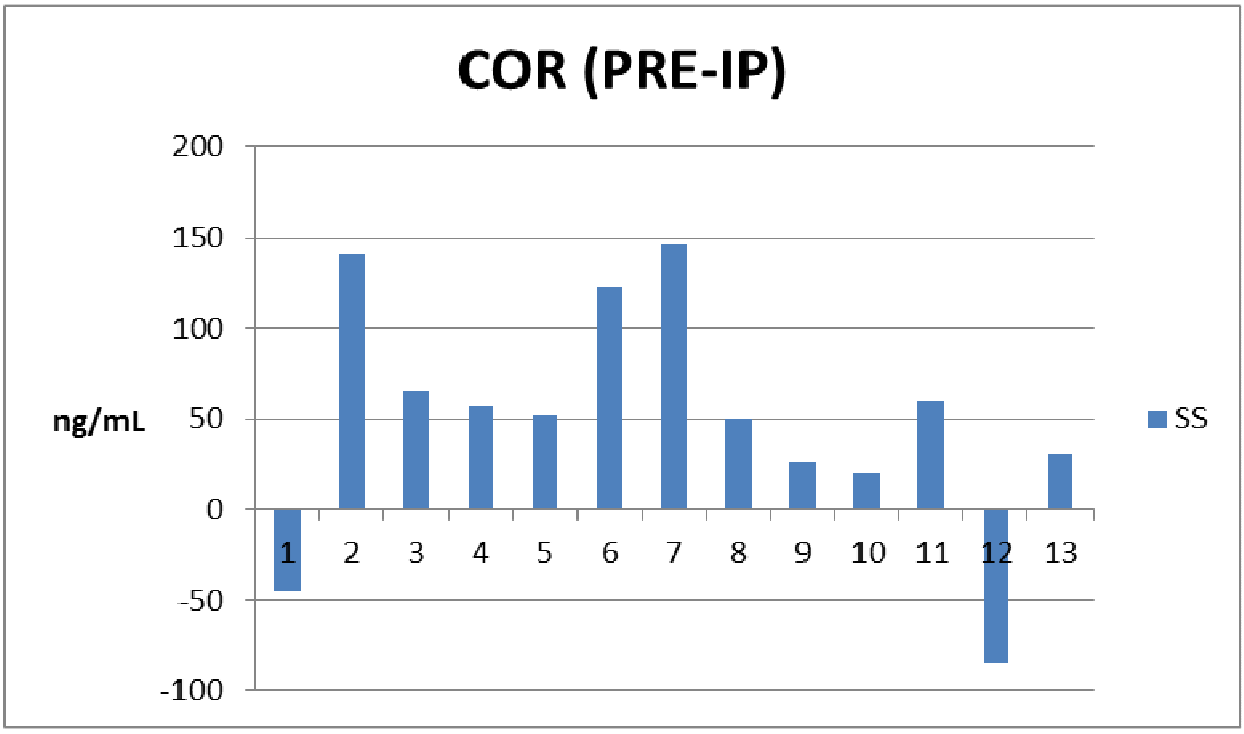


Figure 19. Individual Changes in COR (PRE-IP) for SS Condition.

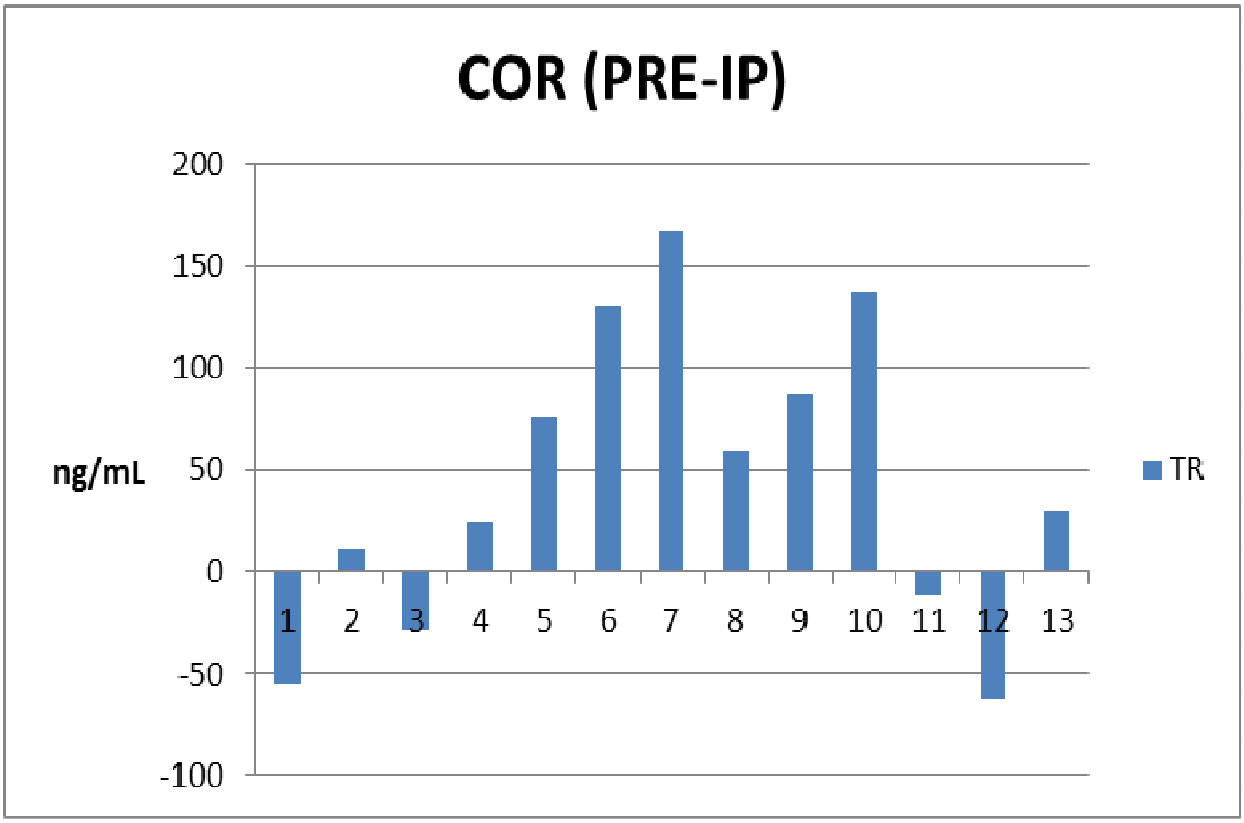


Figure 20. Individual Changes in COR (PRE-IP) for TR Condition.

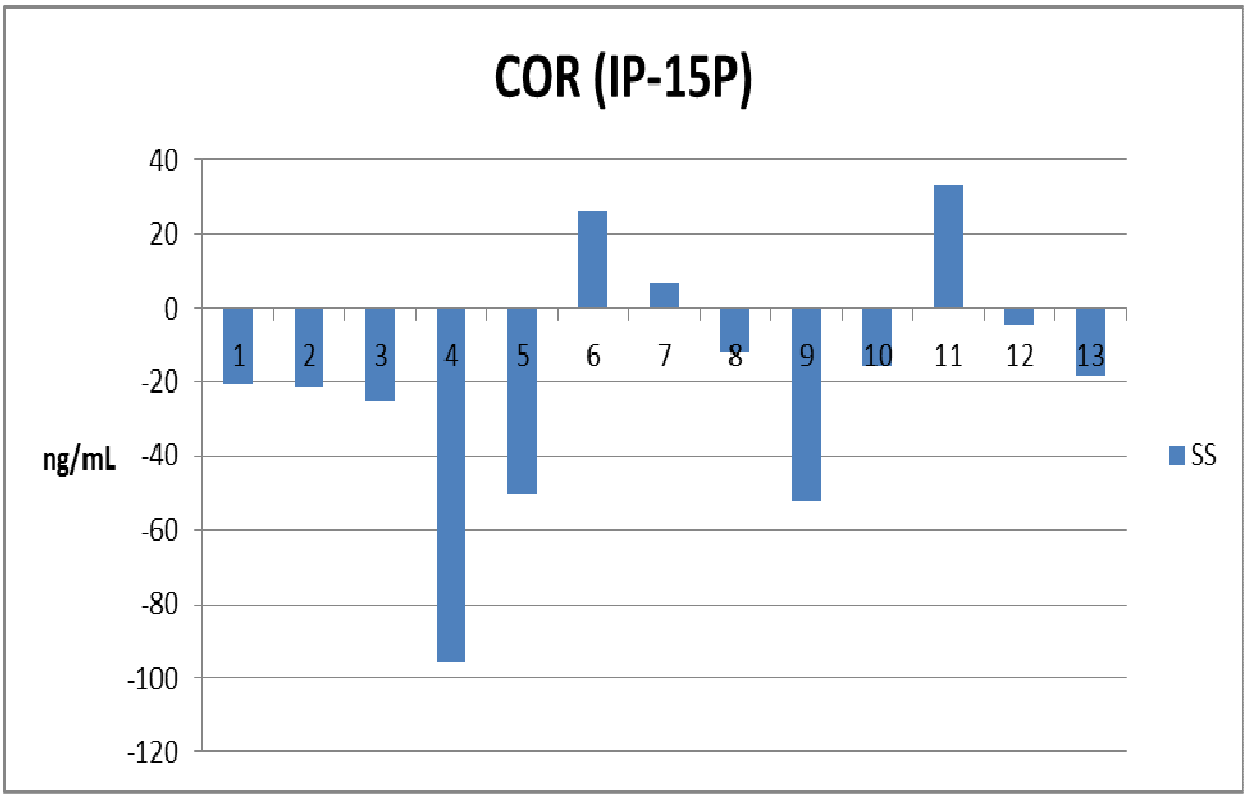


Figure 21. Individual Changes in COR (IP-15P) for SS Condition.

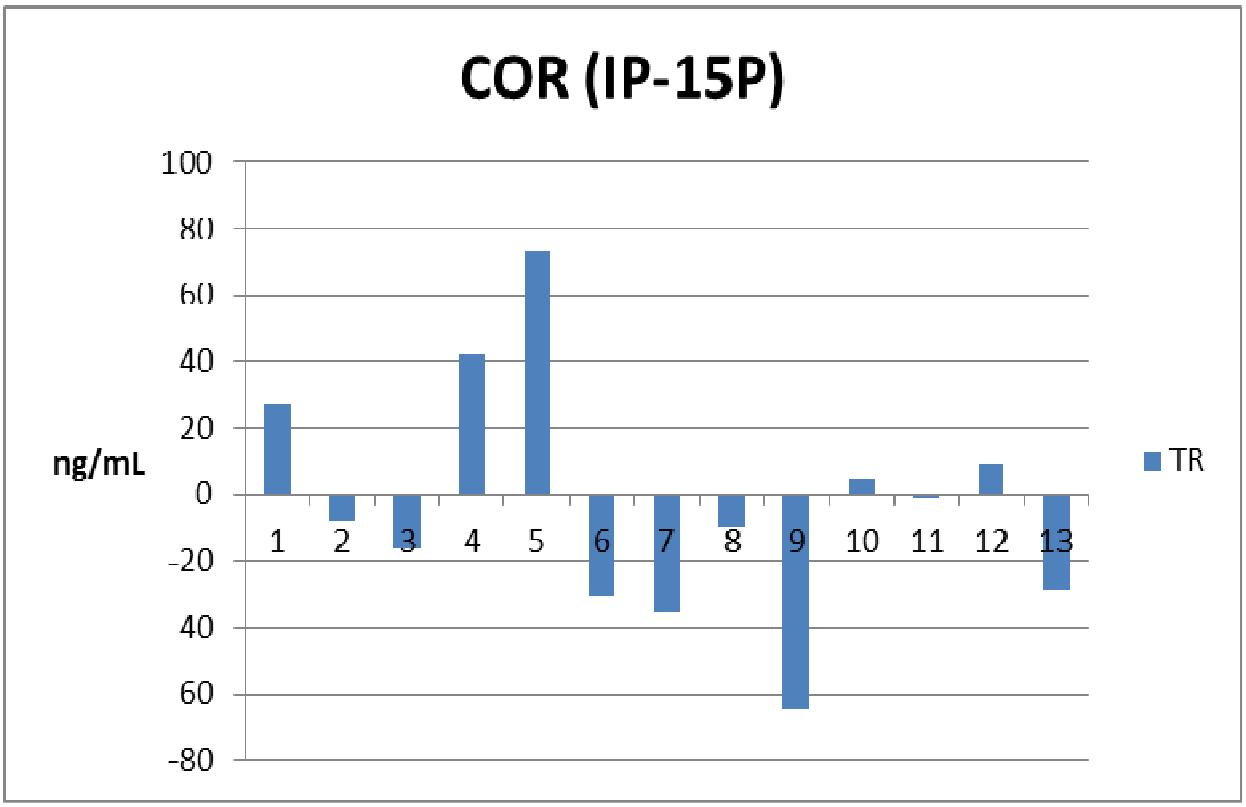


Figure 22. Individual Changes in COR (IP-15P) for TR Condition.

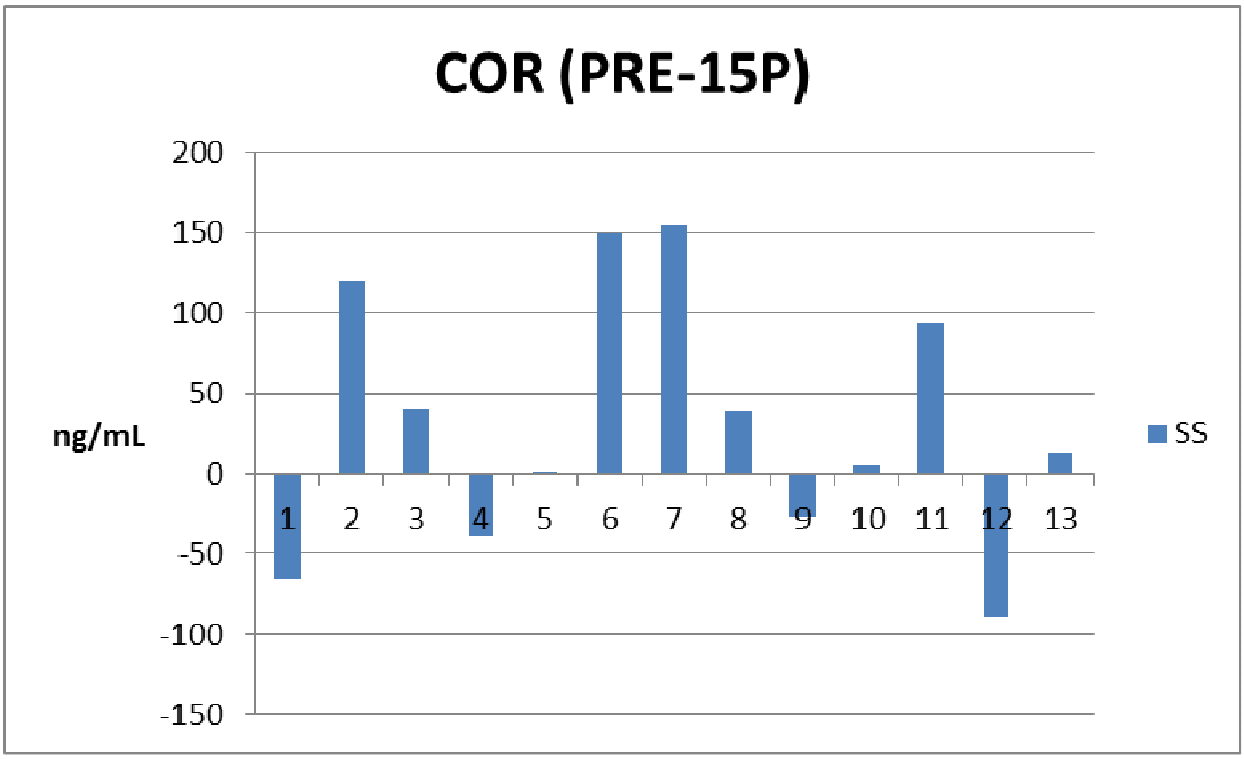


Figure 23. Individual Changes in COR (PRE-15P) for SS Condition.

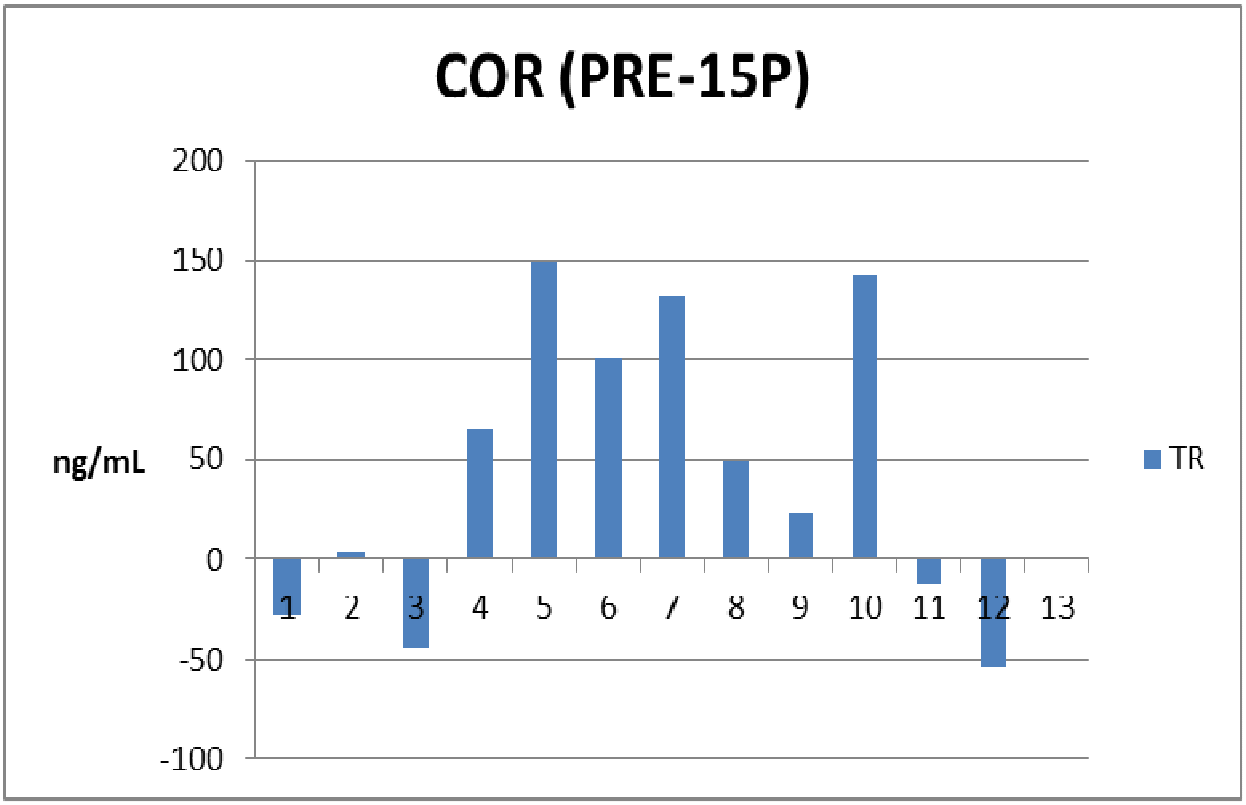


Figure 24. Individual Changes in COR (PRE-15P) for TR Condition.

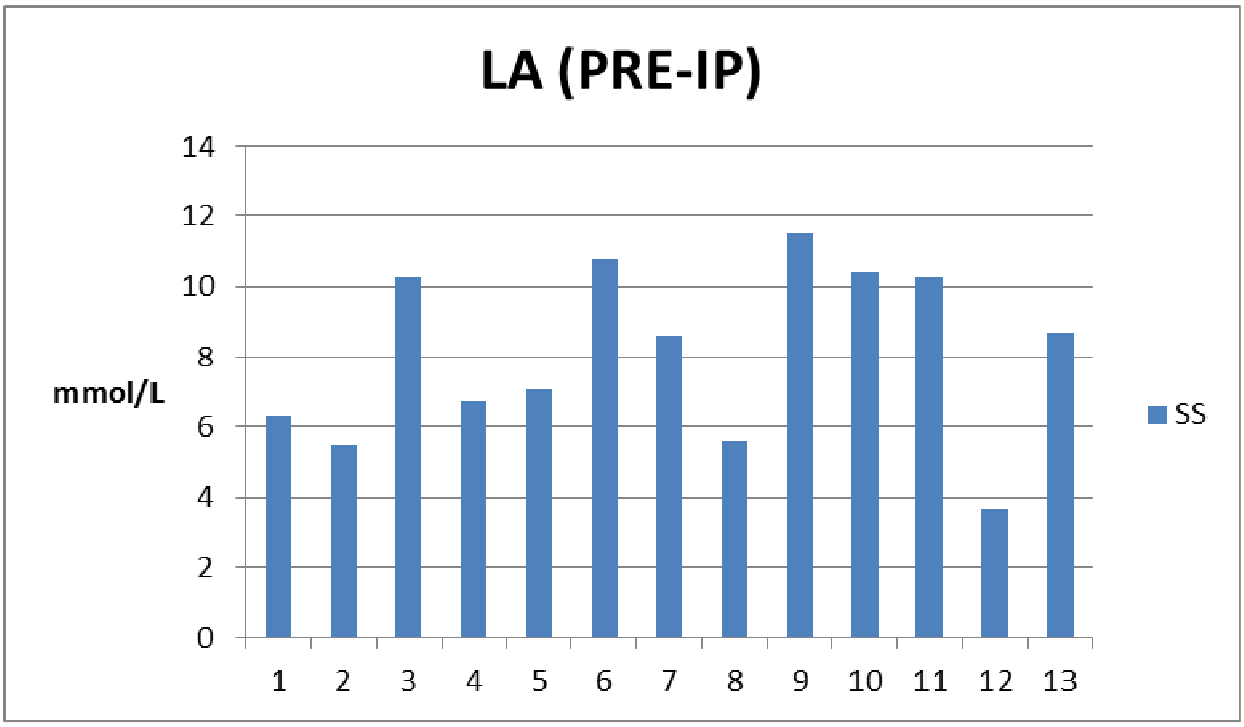


Figure 25. Individual Changes in LA (PRE-IP) for SS Condition.

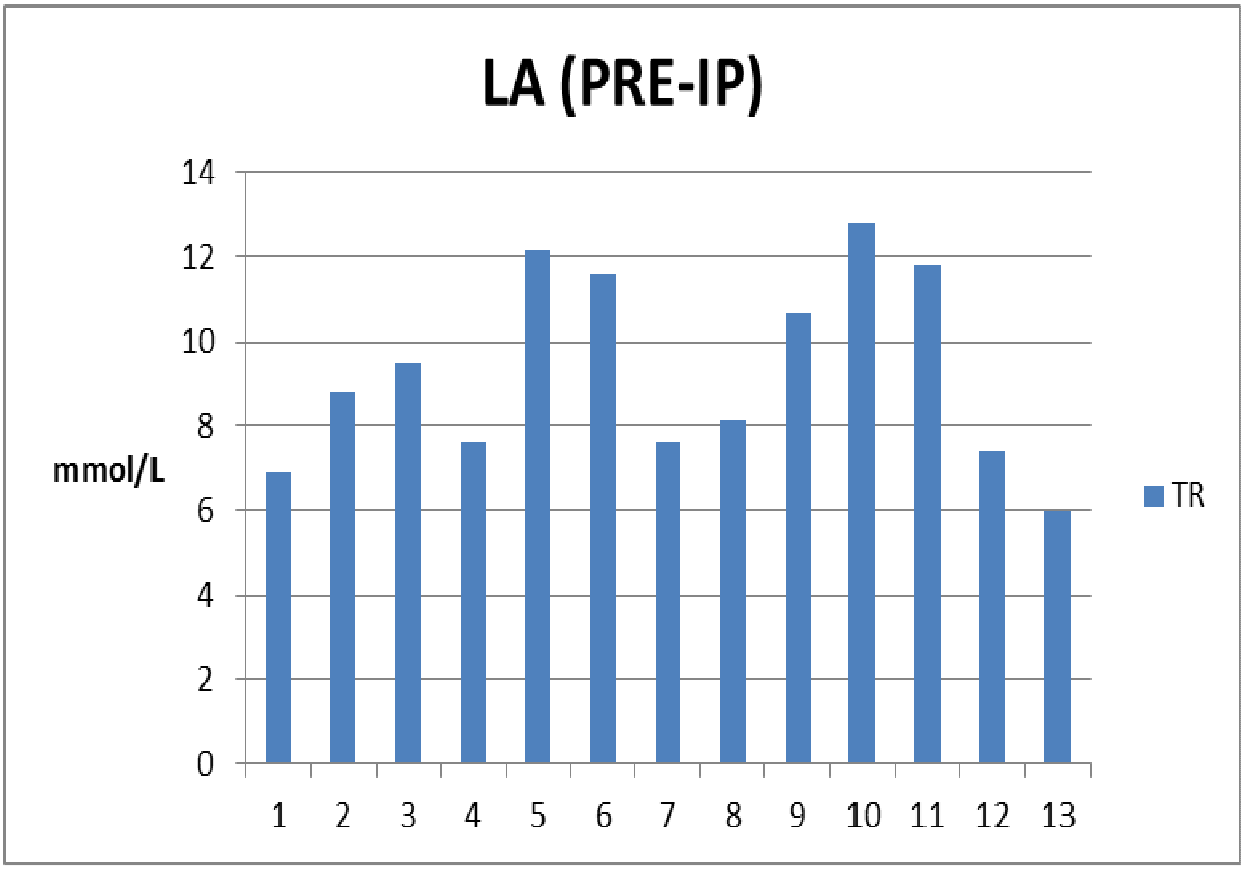


Figure 26. Individual Changes in LA (PRE-IP) for TR Condition.

Appendix J

Raw Data

ID	Age year	Height cm	Weight kg	OneRMSP kg	OneRMCP kg	OneRMLR kg	OneRMBC kg	OneRMKE kg
1	23	178	72	42.613636	45.45455	56.81818	28.40909091	73.86363636
2	19	174	68.2	56.818182	52.27273	56.81818	31.25	68.18181818
3	26	179.4	71.5	45.454545	45.45455	51.13636	25.56818182	53.97727273
4	23	187	103.6	62.5	52.27273	68.18182	56.81818182	107.9545455
5	28	171.5	69.5	85.227273	93.18182	73.86364	51.13636364	79.54545455
6	19	185	84	85.227273	79.54545	90.90909	56.81818182	88.06818182
7	26	183	82	73.863636	59.09091	68.18182	42.61363636	88.06818182
8	18	194	80.6	56.818182	52.27273	68.18182	45.45454545	90.90909091
9	21	178.5	80.5	56.818182	65.90909	62.5	39.77272727	73.86363636
10	23	182	68.1	73.863636	81.81818	73.86364	56.81818182	79.54545455
11	19	176	80.5	62.5	72.72727	62.5	42.61363636	90.90909091
12	19	187	72.8	56.818182	52.27273	62.5	39.77272727	85.22727273
13	18	184.5	100.9	45.454545	45.45455	62.5	48.29545455	79.54545455

OneRMKF kg	OneRMLP kg	OneRMCR kg	Pain SS Scale	Pain TR Scale	RPE SS	RPE TR	CK SS PRE
62.5	136.3636364	79.1	4.5	6.083333333	14.375	14.875	47.60234
68.18181818	172.7272727	56.81818182	2.875	2	16.25	15.792	57.83133
73.86363636	118.1818182	68.18181818	5.5	3.958333333	16.5	16	26.08187
107.9545455	172.7272727	90.90909091	8.75	4.5	18.625	13.458	21.9883
79.54545455	200	79.54545455	4.125	2.416666667	14.75	14.875	32.63965
96.59090909	209.0909091	79.54545455	8.875	7.375	19.375	17	33.29682
102.2727273	200	90.90909091	5.625	5.666666667	13.625	15.381	32.74854
90.90909091	154.5454545	90.90909091	5.125	5.083333333	14	15.292	45.01643
79.54545455	181.8181818	79.54545455	7.125	6	19.75	16.083	54.97076
90.90909091	200	79.54545455	4.75	4.083333333	15.625	15.125	68.01752
85.22727273	200	79.54545455	4.875	1.458333333	15.375	15.167	42.05915
90.90909091	181.8181818	79.54545455	4	2.625	14.875	14.75	20.26287
82.38636364	172.7272727	79.54545455	6.25	4.708333333	18.125	13.25	52.51462

CK SS IP	CK SS 15P	CK TR PRE	CK TR IP	CK TR 15P	TES SS PRE	TES SS IP	TES SS 15P	TES TR PRE
52.74854	55.55556	45.61404	53.09942	53.68421	2.892	2.865	3.466	2.991
65.71742	57.06462	148.9595	161.5553	155.4217	5.063	5.275	5.602	6.911
26.43275	26.78363	19.06433	26.43275	29.59064	4.946	4.957	5.35	4.673
26.90058	27.25146	24.21053	29.23977	28.88889	13.046	10.783	4.86	5.218
32.20153	34.28258	42.49726	68.56517	76.12267	8.921	8.436	7.27	7.849
39.21139	39.53998	21.46769	45.12596	44.24973	16.728	21.853	20.92	16.034
43.27485	35.78947	138.7135	33.68421	33.80117	7.227	6.177	5.412	8.309
57.28368	57.06462	26.72508	50.931	38.55422	6.599	6.686	6.187	5.958
65.38012	61.87135	30.17544	50.87719	55.55556	8.286	7.562	6.361	7.114
77.98467	77.21796	45.23549	62.97919	61.99343	6.556	8.018	6.957	6.865
43.59255	45.01643	30.01095	64.95071	53.01205	12.126	13.734	12.235	12.513
23.0011	21.46769	33.18729	42.16867	46.33078	7.333	5.51	5.779	7.081
61.40351	62.22222	162.2222	150.4094	162.1053	4.839	5.437	5.564	3.707

TES TR IP	TES TR 15P	COR SS PRE	COR SS IP	COR SS 15P	COR TR PRE	COR TR IP	COR TR 15P	LA SS PRE
2.372	1.777	196.156	150.915	130.53	163.286	108.411	135.385	2.8
6.775	6.556	36.623	177.691	156.144	104.849	116.449	108.787	0.6
6.222	4.213	203.282	268.71	243.404	206.62	178.128	161.982	0.6
6.029	5.538	211.596	268.71	173.153	168.111	191.729	233.515	0.7
9.561	8.755	158.813	210.892	160.723	153.031	228.983	302.276	0.4
19.126	13.349	203.497	326.148	352.471	173.583	304.604	273.913	0.4
15.481	8.333	134.197	281.149	288.045	143.276	310.494	275.111	0.6
5.83	6.347	78.077	128.37	116.278	132.6	192.063	182.287	0.8
7.686	6.705	213.56	239.559	187.454	177.829	265.319	200.742	0.6
8.53	7.004	189.542	209.63	193.896	98.753	235.924	240.571	0.5
11.023	12.019	160.174	220.541	253.735	163.797	151.762	151.008	0.5
7.23	5.85	177.787	92.807	88.122	164.592	101.378	110.498	1.1
4.683	3.797	203.282	233.971	215.955	197.9	228.148	198.959	0.7

LA SS IP	LA TR PRE	LA TR IP	HC SS PRE	HC SS IP	HC SS 15P	HC TR PRE	HC TR IP	HC TR 15P
9.1	1.8	8.7	46.5	50.5	47.25	43.5	52	45.5
6.1	1	9.8	43	45	42.5	43.25	49.25	43.25
10.9	1.1	10.6	44.75	47.5	44.25	45	48.25	46.5
7.4	0.7	8.3	49.25	50.5	47.25	47.5	49.75	46.25
7.5	0.7	12.9	44.5	48.5	44.5	42.5	51	43.25
11.2	0.6	12.2	47.25	51.5	48.5	45.75	49.25	42.75
9.2	0.7	8.3	46.5	43.75	42.75	44.75	47.5	45
6.4	0.7	8.8	44	50.25	44	45.75	48	42.75
12.1	0.7	11.4	50.5	50.5	50.75	48.75	51.75	48.5
10.9	0.6	13.4	46	53.5	49	44.5	51	45
10.8	0.7	12.5	42.25	45	41	44	51	45.75
4.8	0.6	8	42.5	46	43.5	44	46.75	40.25
9.4	0.8	6.8	48.5	50	50	49	50.5	47.5

EV SS SP	EV TR SP	EV SS CP	EV TR CP	EV SS LR	EV TR LR	EV SS BC	EV TR BC	EV SS KE
1598.011	2454.545	2386.364	2618.182	3409.091	3272.727	1491.477	1022.727	2215.909
1704.545	3272.727	2352.273	3010.909	2556.818	3272.727	2343.75	1125	2045.455
1704.545	2618.182	2045.455	2618.182	4602.273	2945.455	1150.568	920.4545	4048.295
4687.5	3600	4417.045	3010.909	7670.455	3927.273	2556.818	2045.455	4857.955
3835.227	4909.091	4892.045	5367.273	6093.75	4254.545	2684.659	1840.909	3579.545
3835.227	4909.091	4176.136	4581.818	4772.727	5236.364	2556.818	2045.455	3963.068
4985.795	4254.545	3988.636	3403.636	4602.273	3927.273	2876.42	1534.091	3963.068
2556.818	3272.727	2744.318	3010.909	4602.273	3927.273	1704.545	1636.364	2727.273
2556.818	3272.727	3460.227	3796.364	3281.25	3600	1789.773	1431.818	2769.886
3877.841	4254.545	3068.182	4712.727	6647.727	4254.545	3409.091	2045.455	2982.955

3281.25	3600	4363.636	4189.091	4687.5	3600	1598.011	1534.091	2727.273
2982.955	3272.727	2352.273	3010.909	4218.75	3600	3281.25	1431.818	3835.227
2045.455	2618.182	1704.545	2618.182	5156.25	3600	1086.648	1738.636	5965.909

EV TR KE	EV SS KF	EV TR KF	EV SS LP	EV TR LP	EV SS CR	EV TR CR	%Change PV SS (PRE IP)
4254.545	6562.5	3600	10227.27	7854.545	3559.5	4556.16	-14.80521884
3927.273	5625	3927.273	11659.09	9949.091	2982.955	3272.727	-7.797270955
3109.091	4985.795	4254.545	8863.636	6807.273	3068.182	3927.273	-10.4786854
6218.182	11335.23	6218.182	12954.55	9949.091	5454.545	5236.364	-4.877335024
4581.818	5965.909	4581.818	21000	11520	3579.545	4581.818	-14.86022105
5072.727	5795.455	5563.636	12545.45	12043.64	4772.727	4581.818	-15.64441172
5072.727	11505.68	5890.909	13500	11520	6136.364	5236.364	11.74899866
5236.364	8863.636	5236.364	12750	8901.818	4772.727	5236.364	-22.21037669
4254.545	7159.091	4581.818	17727.27	10472.73	5369.318	4581.818	0
4581.818	8181.818	5236.364	22500	11520	2982.955	4581.818	-25.96053998
5236.364	5113.636	4909.091	22500	11520	3579.545	4581.818	-10.58201058
4909.091	5454.545	5236.364	6818.182	10472.73	4176.136	4581.818	-13.23251418
4581.818	9886.364	4745.455	9068.182	9949.091	7755.682	4581.818	-5.825242718

% Change PV TR (PRE IP)	%Change PV SS (PRE 15P)	%Change PV TR (PRE 15P)	% Chg CK SS (PRE IP)
-28.93124575	-2.966918855	-7.779830789	10.81081081
-21.46738523	2.063983488	0	13.63636364
-12.24682054	2.045146611	-5.865102639	1.34529148
-8.614501077	8.340500951	5.148005148	22.34042553
-28.98550725	0	-3.015833124	-1.342281879
-13.09972163	-4.88591391	12.93556472	17.76315789

-10.4786854	16.39613051	-1.005530417	32.14285714
-8.640552995	0	12.93556472	27.25060827
-11.31141746	-0.995173409	1.005783254	18.93617021
-22.96414061	-11.33786848	-2.002002002	14.65378422
-24.50980392	5.279273572	-6.830601093	3.645833333
-10.50420168	-3.998001	16.63708962	13.51351351
-5.824111823	-5.825242718	6.191950464	16.92650334

% Chg CK TR (PRE IP)	% Chg CK SS (IP 15P)	% Chg CK TR (IP 15P)	% Chg CK SS (PRE 15P)
16.41025641	5.321507761	1.089324619	16.70761671
8.455882353	-13.16666667	-3.946441156	-1.325757576
38.65030675	1.327433628	10.67193676	2.69058296
20.77294686	1.304347826	-1.214574899	23.93617021
61.34020619	6.462585034	9.928057554	5.033557047
110.2040816	0.837988827	-1.98019802	18.75
-75.71669477	-17.2972973	0.346020761	9.285714286
90.57377049	-0.382409178	-32.10227273	26.76399027
68.60465116	-5.366726297	8.421052632	12.55319149
39.2251816	-0.983146067	-1.590106007	13.52657005
116.4233577	3.266331658	-22.52066116	7.03125
27.06270627	-6.666666667	8.983451537	5.945945946
-7.281903389	1.333333333	7.215007215	18.48552339

% Chg CK TR (PRE 15P)	% Chg TES SS (PRE IP)	% Chg TES TR (PRE IP)	% Chg TES SS (IP 15P)
17.69230769	-0.933609959	-20.69541959	20.97731239

4.338235294	4.187240766	-1.967877297	6.199052133
55.21472393	0.222401941	33.14787075	7.928182368
19.3236715	-17.34631305	15.54235339	-54.92905499
79.12371134	-5.436610245	21.81169576	-13.82171645
106.122449	30.6372549	19.28402145	-4.269436691
-75.63237774	-14.52885015	86.31604285	-12.38465274
44.26229508	1.318381573	-2.148371937	-7.463356267
84.10852713	-8.737629737	8.040483554	-15.88204179
37.04600484	22.30018304	24.25345958	-13.23272637
76.64233577	13.260762	-11.90761608	-10.91451871
39.6039604	-24.86022092	2.104222567	4.882032668
-0.072098053	12.35792519	26.32856757	2.335846974

% Chg TES TR (IP 15P)	% Chg TES SS (PRE 15P)	% Chg TES TR (PRE 15P)	% Chg COR SS (PRE IP)
-25.08431703	19.84785615	-40.58843196	-23.06378597
-3.232472325	10.64586214	-5.136738533	385.1896349
-32.28865317	8.168216741	-9.843783437	32.18583052
-8.143970808	-62.74720221	6.132617861	26.99200363
-8.430080536	-18.50689385	11.5428717	32.79265551
-30.2049566	25.05978001	-16.74566546	60.2716502
-46.17272786	-25.11415525	0.288843423	109.5046834
8.867924528	-6.243370208	6.529036589	64.41461634
-12.76346604	-23.23195752	-5.749226877	12.17409627
-17.8898007	6.116534472	2.024763292	10.59817877
9.035652726	0.898894937	-3.94789419	37.68838888
-19.08713693	-21.19187236	-17.3845502	-47.79877044
-18.91949605	14.98243439	2.427839223	15.09676213

% Chg COR TR (PRE IP)	% Chg COR SS (IP 15P)	% Chg COR TR (IP 15P)	% Chg COR SS (PRE 15P)	% Chg COR TR (PRE 15P)
-33.60667785	-13.50760362	24.88123899	-33.4560248	-17.0872
11.06352946	-12.12610656	-6.57970442	326.3550228	3.755878
-13.78956539	-9.417587734	-9.064268391	19.73711396	-21.6039
14.04905092	-35.56138588	21.79430342	-18.16811282	38.90525
49.63177395	-23.78895359	32.008053	1.202672325	97.52599
75.48031777	8.070875799	-10.07570485	73.20697602	57.79944
116.7104051	2.452791936	-11.39571135	114.6433974	92.01471
44.8438914	-9.419646335	-5.089996512	48.92734096	37.47134
49.19894955	-21.750383	-24.33938014	-12.22419929	12.88485
138.9031219	-7.505605114	1.969702107	2.297116206	143.6088
-7.347509417	15.05116962	-0.496830564	58.41210184	-7.80784
-38.40648391	-5.048110595	8.996034643	-50.43394624	-32.8655
15.28448711	-7.700099585	-12.79388818	6.23419683	0.535119

% Chg LA SS (PRE IP)	% Chg LA TR (PRE IP)					
225	383.3333333					
916.6666667	880					
1716.666667	863.6363636					
957.1428571	1085.714286					
1775	1742.857143					
2700	1933.333333					
1433.333333	1085.714286					
700	1157.142857					
1916.666667	1528.571429					
2080	2133.333333					
2060	1685.714286					
336.3636364	1233.333333					
1242.857143	750					

SS Pain/ID													
SS SP	5	2	3	8	4	7	3	4	5	4	3	5	6
SS CP	5	2	4	9	4	8	4	4	6	3	5	3	8
SS LR	3	2	5	8	4	9	5	5	7	3	4	3	4
SS BC	7	5	6	9	4	9	5	8	7	5	4	4	4
SS KE	4	2	5	10	4	10	6	5	7	5	6	4	9
SS KF	2	2	6	8	4	9	5	5	9	5	6	4	4
SS LP	4	4	8	9	4	10	7	4	8	6	5	5	10
SS CR	6	4	7	9	5	9	10	6	8	7	6	4	5

Pain/ID													
TR SP 1	5	2	2	5	2	5	3	4	3	4	0	2	2
TR SP 2	6	2	3	5	2	6	4	4	5	4	0	2	3
TR SP 3	7	3	3	7	2	8	5	6	6	5	1	2	3
TR CP 1	5	2	4	1	2	5		5	6	3	0	2	3
TR CP 2	7	2	5	2	3	8		7	7	4	1	2	3
TR CP 3	7	3	5	4	3	9		8	8	3	1	3	4
TR LR 1	5	1	3	4	2	6	3	3	6	1	1	2	7

TR LR 2	6	1	4	5	2	8	5	4	8	1	1	2	7
TR LR 3	6	1	4	6	2	9	7	4	8	2	2	3	6
TR BC 1	4	2	3	2	2	5	4	8	6	2	1	2	6
TR BC 2	5	2	4	4	3	6	8	8	7	4	2	2	6
TR BC 3	6	2	6	6	4	8	9	8	8	6	4	3	7
TR KE 1	6	1	4	2	2	6	5	4	6	4	2	2	6
TR KE 2	6	1	4	4	2	7	7	6	6	5	2	2	6
TR KE 3	6	1	5	6	3	9	9	6	7	6	3	2	6
TR KF 1	7	4	3	4	2	8	5	2	5	3	1	4	4
TR KF 2	7	4	5	6	2	8	6	2	6	4	1	4	4
TR KF 3	7	4	5	6	3	10	7	3	7	5	1	4	7
TR LP 1	6	1	4	4	2	7	6	4	7	4	1	3	2
TR LP 2	6	1	4	5	2	8	6	6	5	5	2	3	3
TR LP 3	7	2	4	5	2	9	6	7	5	6	2	3	3
TR CR 1	7	2	2	4	3	6	3	4	3	4	1	3	5
TR CR 2	6	2	4	5	3	8	5	5	4	6	3	3	5
TR CR 3	6	2	5	6	3	8	6	4	5	7	2	3	5

SS RPE/ID													
SS SP	12	15	14	19	15	18	10	13	20	15	14	15	20
SS CP	15	16	15	18	15	18	12	13	20	15	15	15	19
SS LR	15	16	16	18	14	19	13	14	20	15	16	14	16
SS BC	16	17	18	18	15	20	13	18	20	15	16	15	17
SS KE	14	15	16	20	15	20	15	14	20	17	15	15	20
SS KF	10	17	17	18	15	20	12	14	20	16	17	15	20
SS LP	16	17	19	19	14	20	16	13	18	16	14	15	15
SS CR	17	17	17	19	15	20	18	13	20	16	16	15	18

RPE/ID													
TR SP 1	10	17	12	13	14	14	14	14	17	15	11	14	8
TR SP 2	15	15	14	14	14	15	15	14	17	17	15	14	11
TR SP 3	19	17	16	16	15	18	19	16	19	18	16	16	11
TR CP 1	13	16	17	9	15	14		15	16	14	13	13	12

TR CP 2	15	17	17	11	15	17		17	17	14	15	15	14
TR CP 3	16	18	19	14	16	19		18	20	15	15	18	17
TR LR 1	12	16	14	12	14	15	12	13	16	12	18	15	15
TR LR 2	12	16	16	14	14	18	14	15	18	13	17	16	17
TR LR 3	15	17	17	16	15	19	17	15	18	13	18	16	19
TR BC 1	10	14	15	10	14	15	14	17	15	12	16	12	15
TR BC 2	12	16	17	13	16	16	18	17	19	13	17	14	19
TR BC 3	15	18	19	16	17	18	19	17	20	16	17	16	19
TR KE 1	14	13	14	12	15	16	13	13	12	15	14	11	12
TR KE 2	15	14	17	13	15	17	15	15	13	16	15	13	14
TR KE 3	15	14	17	17	15	19	18	17	15	18	16	14	17
TR KF 1	17	17	16	11	15	17	14	12	12	14	14	17	7
TR KF 2	17	17	17	13	14	18	15	13	17	16	14	17	10
TR KF 3	19	18	18	16	15	20	15	13	16	17	15	17	10
TR LP 1	15	13	15	13	14	16	16	13	15	15	14	14	14
TR LP 2	16	15	16	14	14	18	17	17	15	16	16	14	15
TR LP 3	18	15	17	16	15	19	17	17	15	18	15	15	15
TR CR 1	14	15	13	12	15	15	12	16	14	13	13	14	6
TR CR 2	16	15	15	13	15	17	14	16	15	16	15	14	9
TR CR 3	17	16	16	15	16	18	15	17	15	17	15	15	12

SS Cor. CK PRE	SS Cor. CK IP	SS Cor. CK 15P	TR Cor. CK PRE	TR Cor. CK IP	TR Cor. CK 15P
47.60233918	44.93900152	53.9072673	45.61403509	37.7370929	49.50766979
57.8313253	60.59325019	58.2424265	148.9594743	126.8736109	155.4216867
26.08187135	23.66294398	27.33139015	19.06432749	23.19557726	27.85512168
21.98830409	25.58855315	29.52437043	24.21052632	26.72090612	30.37609038

32.63964951	27.41631436	34.28258488	42.49726177	48.69120752	73.82693974
33.29682366	33.07699956	37.6080888	21.46768894	39.21458345	49.97367814
32.74853801	48.3592158	41.6575625	138.7134503	30.15454808	33.46128855
45.01642935	44.56075903	57.06462212	26.72508215	46.53027695	43.54142254
54.97076023	65.38011696	61.25561786	30.1754386	45.12226129	56.11432403
68.01752464	57.73942556	68.4630917	45.2354874	48.51655986	60.75231858
42.05914567	38.97958356	47.39296981	30.0109529	49.0314198	49.39100665
20.26286966	19.95747209	20.60941052	33.18729463	37.73919206	54.03887066
52.51461988	57.82660535	58.59762675	162.2222222	141.6493476	172.1427408

SS Cor. TES PRE	SS Cor. TES IP	SS Cor. TES 15P	TR Cor. TES PRE	TR Cor. TES IP	TR Cor. TES 15P
2.892	2.44083048	3.363166592	2.991	1.685750851	1.638752407
5.063	4.863693957	5.717624355	6.911	5.320584651	6.556
4.946	4.437571565	5.459415344	4.673	5.460002826	3.965903226
13.046	10.25707696	5.265348346	5.218	5.50963173	5.823096525
8.921	7.182391753	7.27	7.849	6.789695652	8.49096381
16.728	18.43422671	19.89786681	16.034	16.62054724	15.07576853
7.227	6.902735648	6.299358583	8.309	13.85879471	8.24920915
6.599	5.201014215	6.187	5.958	5.32625576	7.168020293
8.286	7.562	6.297697019	7.114	6.816604454	6.772437767
6.556	5.936483904	6.16822449	6.865	6.571158806	6.86377978
12.126	12.28066667	12.88091912	12.513	8.321284314	11.19803005
7.333	4.780888469	5.547955522	7.081	6.470546218	6.823269743
4.839	5.120281553	5.239883495	3.707	4.410256843	4.032108359

SS Cor. COR PRE	SS Cor. COR IP	SS Cor. COR 15P	TR Cor. COR PRE	TR Cor. COR IP	TR Cor. COR 15P
196.156	128.571704	126.6572808	163.286	77.04634717	124.8522761
36.623	163.8359513	159.3667864	104.849	91.45044458	108.787
203.282	240.5527245	248.3819687	206.62	156.3129835	152.4815894
211.596	255.6041131	187.5948276	168.111	175.2125032	245.5363642

158.813	179.5529826	160.723	153.031	162.6111159	293.1598603
203.497	275.1240641	335.2495704	173.583	264.7017239	309.3451934
134.197	314.1811923	335.2732341	143.276	277.9583106	272.3446752
78.077	99.85853945	116.278	132.6	175.4676947	205.8668529
213.56	239.559	185.5885076	177.829	235.3076603	202.7610294
189.542	155.20892	171.9123265	98.753	181.7460809	235.7547638
160.174	197.203328	267.1303648	163.797	114.5654314	140.6932459
177.787	80.52630057	84.59888156	164.592	90.72905042	128.8816513
203.282	220.3416214	203.3750971	197.9	214.8604054	211.2784427