The Effects of Training with Free Weights or Machines on Muscle Mass, Strength, and Testosterone and Cortisol Levels

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Shane Ronald Schwanbeck

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ABSTRACT

Free weights are generally preferred over machines by practitioners of strength training because they involve incorporation of greater muscle mass because of the greater stabilization that is required. Using free weights may therefore allow one to gain more muscle mass and strength with chronic training; however, this has not been thoroughly addressed. The purpose of this study was to compare the effect of training with free weights or machines on muscle mass, testosterone and cortisol concentrations, and strength. Fifteen males and twenty-one females aged 22 ± 3 y with previous weight training experience trained using only free weights or only machines for eight weeks. Hormone concentrations were assessed via saliva samples pre and post workout at the beginning, mid-way, and end of the study. Muscle thickness, lean tissue mass, and strength were measured at the beginning and the end of the study. Elbow flexor thickness increased significantly by 3.9% and a 5.1% in the free weight group and machine group, respectively ($p<0.01$), with no difference between groups. Knee extensor thickness increased significantly by 4.6% and a 4.9% in the free weight group and machine group, respectively ($p<0.01$), with no difference between groups. No significant changes occurred in the lean tissue mass during the eight week training period. The group x time interaction for machine bench press strength was close to significance ($p=0.054$) with the machine training group experiencing a greater increase in strength compared to the free weight training group (13.9% vs. 8.6%). Free weight bench press, free weight squat, and Smith machine squat strength increased significantly in both groups (11-19%; $p<0.01$) with no difference between groups. The males in the free-weight group had a 21.7% increase in testosterone from before to after acute training sessions ($p<0.01$); however, the acute increase in testosterone to cortisol ratio in males training with free weights did not differ from males training on machines. Results from this study indicate that training with free weights or machines result in similar increases in muscle mass and strength, and testosterone to cortisol ratio. Males training with

free weights may benefit from a greater acute increase in testosterone levels during individual training sessions.

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Chapter 1 - Scientific Framework

Context

Resistance training is an important part of any exercise program. It can be used to maintain or increase muscle hypertrophy, strength, power, and even endurance (McArdle et al., 1999). Avoiding this type of exercise can lead to decreases in lean body mass and sport performance due to losses of speed, strength and power (Fatouros et al., 2005). Resistance training also has positive effects on functional capacity, increases basal metabolic rate, decreases blood pressure, and improves blood lipid profiles, insulin sensitivity and glucose tolerance (Kraemer et al., 2002). The benefits of resistance training can be had by men and women of all ages and can help promote a longer more independent life. In order to achieve one's fitness goals, the appropriate training modality needs to be considered. Resistance training can be done using many different types of equipment such as medicine balls, resistance tubing, thera-balls, and body weight supported movements. One of the controversies is whether the use of a more traditional program consisting of free-weights or machines is better for building muscle mass and strength. Over the years fitness professionals have typically promoted free weights as the best method for strength training. However, the scientific literature is equivocal when it comes to this topic. With the advancement of technology, strength training machines have significantly evolved and are now better suited to perform strength training programs. Free weights utilize the forces of gravity to provide resistance while some machines are now capable of using elastics, hydraulics, and pneumatic resistance. These various forms of resistance may be beneficial as they have a greater chance of matching the various strength curves of typical strength movements.

Objective

The research objective was to compare the effect of training with either free weights or machines on muscle mass, testosterone levels, cortisol levels and strength.

Literature Review

Resistance training is incorporated into many exercise programs. The different components of a resistance training program including sets, repetitions, volume, load, rest and tempo all need to be taken into consideration when training for muscle mass and strength. Perhaps the least studied aspect of resistance training is comparing the use of machines to free weights. The training mode that better increases muscle mass and strength can be determined by measuring hormone levels, strength, and muscle mass before and after an exercise intervention. Training modality needs to be studied to provide information on which apparatus is ideal to reach individuals' fitness goals.

Free Weights vs. Machines: Background Information

Free weights utilize isotonic resistance which provides the same amount of resistance throughout the range of motion. Free weights are a free-form exercise which allow for movement in multiple planes and require balance (Cotterman et al., 2005). Most machines are a fixed-form exercise and are limited to moving through fewer planes and provide a stable environment. However, some machines that utilize pulley's may be more similar to free weights since they can move through more planes of motion compared to most other machines such as a Technogym or Hammer Strength machine. Machines offer different types of resistance depending on the machine being used. Isokinetic resistance can be provided from machines which utilize a constant speed of contraction over the entire range of motion. Machines may also allow for linear variable resistance and compound variable

resistance (Boyer, 1990). Linear variable resistance provides linearly increasing resistance throughout the range of motion to match the resistance to strength at different parts of the range of motion. An example of where this would be beneficial is using a machine leg press and machine bench press. Compound variable resistance provides a load which changes to match the ability of the musculoskeletal lever system to produce force throughout the range of motion. An example of where this would be beneficial is utilizing an arm curl machine. The major difference between training with free weights and machines is that training with most machines provides a very stable environment while training with free weights requires more stabilization and balance.

Free Weights vs. Machines: Advantages & Disadvantages

A review of the literature has revealed both positive and negative aspects of training with free weights or machines. In a round table discussion conducted by Haff (2000), general advantages of free weights included that they require balance and coordination much like actual sporting events, a greater variety of large muscle mass exercises can be performed which can increase energy expenditure, and they can be used for ballistic and explosive exercises. General disadvantages of free weights included that they provide little resistance except in the downward direction, it is sometimes difficult to match the strength curves for some movements, sometimes require a spotter for safety, and they can be psychologically intimidating to some novice trainees. Some advantages of machines included that they can provide resistance in any direction, they can provide resistance through a greater range of motion, and they require much less balance which may be desirable depending on the health status of the trainee. Some disadvantages of machines include that they poorly simulate real world lifting movements, movements are made through only one plane of motion, and ballistic movements such as power cleans are nearly impossible to perform. Stone (2000) suggests that machines have limited

adaptability whereas free weight exercises can be created to fit the activity. Although manufacturers have improved adjustment factors, most machines do not have sufficient adjustments to fit all sizes and populations. Others suggest that free weights are better due to an increased need for motor coordination and balance resulting in greater muscle recruitment. Free weight exercises also incorporate stabilizers to complete the lift whereas machine movements do not require as much activation of muscles required for stabilization (Mayo et al., 1997).

Free Weights vs. Machines: EMG Evidence

There is an increased muscle activity of the lower body, upper body, and truck musculature when training in an unstable environment. McCaw and Friday (1994) compared a free weight bench press to a Universal machine bench press using electromyography (EMG) to measure muscle activity. They measured the triceps brachii, anterior deltoid, medial deltoid, pectoralis major, and biceps brachii. They collected EMG activation for the ascent and descent phases of the lift. Participants performed five trials at 60% 1RM and five trials at 80% 1RM for each mode of bench press. During the descent at 60% 1RM they found the EMG of the triceps brachii, anterior deltoid, and medial deltoid to be higher during the free weight bench press, whereas pectoralis major and biceps brachii EMG were higher during the machine bench press descent. During the ascent phase of the 60% 1RM, all muscles were recruited to a higher extent during the free weight bench press. During the ascent phase of the 80% 1RM all muscles measured were recruited to a higher extent during the free weight press. The only muscles to show higher EMG during machine bench press, were the triceps brachii and biceps brachii during the descent phase of the 80% 1RM lift. Overall, the free weight bench press tended to have higher EMG activity (McCaw et al., 1994). A recent study by Behm and Anderson (2005) looked at EMG activity during squats in a stable and unstable environment. EMG of the soleus, vastus lateralis,

biceps femoris, abdominal stabilizers, upper lumbar erector spinae, and lumbo-sacral erector spinae were measured during different squatting modalities. They had their participants perform light-weight submaximal squats under three levels of stability: relatively unstable, which utilized balance discs under each foot, relatively stable, which utilized a free weight barbell and weights, and very stable, which utilized a Smith machine. A Smith machine consists of an Olympic bar that has each end attached to an upright rail. The bar can only slide up and down this rail in a fixed form manner. Olympic weights are placed on the ends of the bar to add resistance. They found that in the relatively unstable environment the EMG activity of the trunk including the abdominal stabilizers, upper lumbar erector spinae, and lumbo-sacral erector spinae muscles was higher than in the stable environments. The relatively unstable squat also elicited the highest EMG of the soleus. They also found that the vastus lateralis EMG activity was the highest during the stable Smith machine squat and there were no differences for the biceps femoris. Overall, the relatively unstable squats resulted in higher EMG activity for the majority of the muscles measured (Behm et al., 2005). Schwanbeck, Chilibeck and Binsted (In Press) also compared a free weight squat to a Smith machine squat using EMG. Unlike the study by Behm and Anderson (2005), participants performed the exercises at the same relative intensity (i.e. using a weight they could lift for eight repetitions on each machine; 8-RM), rather than the same absolute intensity. This resulted in a higher weight used during the more stable Smith machine exercise. The authors felt this simulated "real-life" weight lifting to a greater extent because one usually aims to complete a desired number of repetitions on a given exercise, rather than using the same absolute load across different exercises. Participants performed one set of heavy squats on each of the free-weight and Smith machine (one week apart) while muscle activity was recorded for the tibialis anterior, gastrocnemius, vastus medialis, vastus lateralis, biceps femoris, lumbar erector spinae, and rectus abdominus. EMG activity was higher over the gastrocnemius, vastus medialis, and biceps

femoris during the heavy free weight squat, compared to the Smith machine squat, with a similar trend for the vastus lateralis ($p=0.06$). There were no differences between training modes for the other muscle groups; however, the EMG activity averaged over all muscles during the free weight squat was 43% higher when compared to the Smith machine squat (Schwanbeck et al., In Press). The increased muscle recruitment seen in free weight acitivities should hypothetically lead to increased muscle mass over time.

Free Weights vs. Machines: Strength and Body Composition Evidence

Both the use of free weights or machines is effective for increasing strength (Cronin et al., 2003; Häkkinen et al., 1998; Häkkinen et al., 2001; Izquierdo et al., 2001; Jowko et al., 2001; Mayhew et al., 1997; Tesch et al., 2004). Studies directly comparing free weights to machines for effectiveness of increasing strength are equivocal. Boyer (1990) utilized three different training modalities including free weights and two different types of machines. Three groups of female participants trained on one of the specified modalities and were later tested on all three of the apparatuses. The three modalities included free weight training, Nautilus training, and Soloflex training. The Nautilus machine uses a cam pulley system in an attempt to match the strength curves of various exercises. The Soloflex machine uses thick rubber bands for resistance which are best suited to match the linear strength curves of movements such as the bench press and leg press. All participants trained three times per week for twelve weeks. Each training session consisted of three sets of two lower body exercises and five upper body exercises (the specific exercises were not listed). Body composition was assessed using skinfold calipers and body density and percent fat was determined based on the skinfold values. Participants were tested for strength using a free weight leg sled, free weight bench press, free weight behind the neck press, Nautilus leg press, Nautilus bench, Nautilus laterals, Soloflex bench, and Soloflex behind

the neck press. It was concluded that although the strength gains were significantly greater when each group was tested on their training modality, the programs produced comparable changes in muscular strength and body composition. Boyer (1990) utilized skinfolds to assess body composition, which is not as precise as other methods. This study only had female participants which limits the generalizability of the study (Boyer, 1990).

A more recent study had older men and women training in a moderate intensity seated resistance training program using machines or a high intensity standing free weight program which also included some machine exercises (Maddalozzo and Snow, 2000). The seated machine program consisted of thirteen exercises including a leg extension, leg press, hamstrings curl, arm curl, triceps press, chest press, pec deck, shoulder press, side lateral raise, lat pull down, seated row, abdominal crunch, and calf raise. The standing free weight program consisted of a back squat, deadlift, biceps curl, triceps extension, and sit ups. The free weight program also included a Hammer Strength machine chest press, incline chest press, shoulder press, high lat pull down, leg curl, gripper (wrist strength), and calf raise. All participants trained three times per week for twenty-four weeks. Strength measurements were taken for quadriceps force, hamstring force, hip abduction force, pectoral force, and latissimus dorsi force. A mean total body strength was derived from these five strength variables. The authors did not state which type of apparatus was used to assess the strength measurements. There was a significant increase in peak force with no differences between groups. Both groups also experienced a significant increase in lean body mass which was measured using dual-energy x-ray absorptiometry (DEXA). Although this study had a machine group and a free weight group, the free weight group also trained using some machine exercises which does not make this a true comparison between training with only machines or only free weights (Maddalozzo and Snow, 2000).

Sanders (1980) compared a group of participants who trained with Nautilus machine chest press and shoulder press to another group who trained with barbell bench press and barbell shoulder press. All exercises were done for three sets of six repetitions, three times per week over five weeks. Participants were tested for muscular strength and endurance of the forearm extensors and shoulder flexors by performing a maximal contraction then repeated contractions for three minutes finished by another maximal contraction. Strength was assessed using a load cell fastened to a special testing table. Significant increases in muscular strength and endurance were experienced in both groups with no differences between groups.

Silvester et al. (1982) conducted two studies where groups in study one were divided into free weight squats and two different types of machine squats, and in study two, the participants did either free weight barbell biceps curls or Nautilus machine biceps curls. In study one, male participants trained using free weight squats, Nautilus Compound Leg Machine, or Universal Variable Resistance Maximum Overload Leg Press Machine three times per week for thirteen weeks. The free weight squats were performed with three sets of six repetitions. The Nautilus group performed a leg extension and leg press movement for three sets of twelve repetitions. The Universal group performed a leg press with the first set for seven to ten repetitions and the second set to failure. All participants also performed the same five upper body exercises which included a barbell bicep curl, barbell bench press, lat pull-down, dips, and sit-ups. Participants were tested pre and post intervention using a static strain gauge measure for hip and knee extension and all three groups experienced strength gains with no significant differences between groups. In study two, male participants were randomly assigned into four groups. Group one performed barbell biceps curls of one set to failure and group two performed barbell biceps curls of three sets of six repetitions. Group three did biceps curls using a Nautilus Omni Bicep Machine for one set to failure and group four used the same machine for three sets of six

repetitions. In addition to these exercises all participants also performed three sets of six repetitions of a bench press, squat, dead lift, triceps extension, upright row, leg curl, and sit-ups. Participants trained three days a week for eight weeks. All four groups experienced strength gains when tested on a strain gauge and there were no significant differences between any of the training modalities (Silvester et al., 1982). These studies demonstrate that substantial strength increases can be made when training with either free weights or machines. However, they do not necessarily demonstrate which training apparatus is most beneficial because they do not measure changes in muscle size and strength tends to increase in a short time frame during any resistance training activity due to neural adaptations (Gabriel et al., 2006). Limited studies have compared the effect of training modality on muscle mass. Based on this literature review, only two studies measured muscle mass when training with free weights or machines. However, Boyer (1990) utilized skinfolds and girths which are not that reliable. Maddalozza and Snow (2000) utilized DEXA to measure muscles mass which is much more reliable; however, their free weight training program also included some machine exercises. The current study utilized air displacement plethysmography and ultrasound to measure muscle mass which is more sensitive than skinfolds and the exercise protocol used only free weights or only machines.

For a summary of Free Weights vs. Machines: Strength and Body Composition Evidence, please see table 1.

Table 1. Free Weights vs. Machines Summary

AUTHOR	GROUPS	PROTOCOL	RESULTS	LIMITATIONS
Boyer (1990)	Free Weights vs. Nautilus Machine vs. Soloflex Machine	Trained 3 x week for 12 weeks. Three sets of two lower body exercises and five upper body exercises	All groups ↑ strength and improved body composition	Only had females Body composition assessed via skinfolds
Maddalozzo and Snow (2000)	Seated resistance training program vs. standing free weight program	Trained 3 x week for 24 weeks 13 exercises including upper and lower body	Both groups ↑ in peak force and lean body mass.	Free weight program also included some machine exercises
Sanders (1980)	Nautilus machine chest press & shoulder press vs. barbell bench press & barbell shoulder press	Trained 3 x week for 5 weeks, 3 sets of 6 repetitions for both exercises	Both groups ↑ in muscular strength and endurance	Do not state what gender participants are
Silvester et al. (1982) Study 1	Free weight squat vs. Nautilus Compound Leg Machine vs. Universal Variable Resistance Maximum Overload Leg Press Machine	Trained 3 x week for 13 weeks. All participants also performed the same 5 upper body exercises	All groups ↑ strength	Only had male participants
Silvester et al. (1982) Study 2	Barbell bicep curls 1 set to failure vs. barbell bicep curls 3 sets of 6 reps vs. Nautilus Omni Bicep Machine 1 set to failure vs. Nautilus Omni Bicep Machine 3 sets of 6 reps	Trained 3 x week for 8 weeks. All participants also performed 3 sets of 6 reps of 7 exercises for the upper and lower body	All groups ↑ strength	Only had male participants

Physiology and Influence of Testosterone

One of the dependent variables assessed in this thesis study was changes in anabolic and catabolic hormone levels because we predicted that an increase in muscle activation during the free weight training would increase testosterone release resulting in a physiological link to increased muscle mass. Testosterone is the main anabolic hormone released during resistance training. Testosterone is a steroid hormone from the androgen group. The release of testosterone in men follows these steps: the hypothalamus releases gonadotropin-releasing hormone which stimulates the pituitary gland to release luteinizing hormone, and this stimulates the testes. Within the testes the Leydig cells, which constitute 20% of the mass of the testes, produce testosterone (McArdle et al., 2007). The amount of secreted testosterone is directly correlated with the amount of luteinizing hormone available. A much lesser amount of testosterone is derived from androgenic steroids formed in the adrenal cortex (Viru et al., 2005). In females, testosterone mainly originates from the adrenal cortex as a by-product of glucocorticoid biosynthesis and is also derived from the ovaries. The secretions from the adrenal cortex, situated along the perimeter of the adrenal glands, can be peripherally converted into testosterone. The production of testosterone in females depends on the rate of glucocorticoid biosynthesis which is stimulated by adrenocorticotropic hormone (ACTH). ACTH is released from the anterior lobe of the pituitary gland. Thus, the influence of luteinizing hormone plays a very minor role, if at all, in controlling the levels of testosterone in women (Viru et al., 2005). Testosterone levels are typically ten times less in females (Viru et al., 2005). The levels of testosterone in both women and men fluctuate in a circadian fashion. Testosterone is important as it induces skeletal muscle hypertrophy which may lead to improved strength and power (Herbst et al., 2004). Research suggests that testosterone induces muscle fiber hypertrophy by acting at multiple steps in the pathways that regulate muscle protein synthesis and breakdown (Ferrando et al., 2003) Testosterone has been shown

to promote the commitment of pluripotent precursor cells into the myogenic lineage and inhibits their differentiation into the adipogenic lineage (Singh et al., 2003). Bhasin et al. (1996) provide support for a link between increased testosterone levels and muscle mass during strength training. They examined the effects of exogenous supraphysiologic doses of testosterone on muscle size and strength. Male participants were divided into the following groups: placebo with no exercise, testosterone (given exogenously) with no exercise, placebo plus exercise, and testosterone plus exercise. Their results showed that both placebo groups did not experience any changes in muscle size, the testosterone with no exercise group had a significant increase in quadriceps and triceps muscle thickness, and the testosterone with exercise group had greater increases in quadriceps and triceps muscle thickness compared to compared to all other groups. In regards to strength, the placebo with no exercise group did not experience a change in bench press and squat strength, the placebo plus exercise and the testosterone with no exercise groups had significant strength increases, and the testosterone plus exercise group experienced increases greater than any other group (Bhasin et al., 1996). This study demonstrates the influential role that testosterone has on building muscle mass and strength.

Testosterone Changes with Resistance Training

Changes in muscle mass may be physiologically linked to changes in hormone responses during resistance training. Studies have shown acute increases in testosterone in males who were resistance training. Ahtiainen et al. (2005) compared a lower intensity short rest period between sets training session to a higher intensity with longer rest between sets training session in trained men. The short rest training session included five sets of leg press and four sets of Smith machine squats with two minutes of rest between sets. The long rest training session included four sets of leg press and three sets of squats with five minutes of rest between sets. All loads were done for a maximum 10RM with the long

rest training session load being approximately 15% higher than the short rest training session. Both groups experienced an acute increase in testosterone regardless of the length of time between sets. A more recent study examined the effects of three loading schemes on acute hormone concentrations (Crewther et al., 2008). Recreationally trained males performed either a power workout which consisted of eight sets of six repetitions at 45% of 1RM with three minute rest periods, a hypertrophy workout which consisted of ten sets of ten repetitions at 75% 1RM with two minute rest periods, or a maximal strength workout which consisted of six sets of four repetitions at 88% 1RM with four minute rest periods. Participants utilizing the hypertrophy protocol experienced a significant increase in testosterone while the participants training using the power or strength protocols experienced little or no change in testosterone levels (Crewther et al., 2008). The study in this thesis therefore incorporated a training program comprised mainly of hypertrophy-type training to optimize the testosterone response.

Studies that have included females have shown more variation in the testosterone response to exercise with some researchers showing no changes while others have shown an increase. Häkkinen and Pakarinen (1995) examined the acute hormonal responses to heavy resistance exercise. Young women, middle aged women and elderly women all performed a workout consisting of a machine bench press and a leg press machine. Exercises were done for five sets of ten repetitions with three +minutes of rest between sets. The testosterone concentration of the young women and elderly women remained unchanged post exercise session while the middle aged women experienced a significant increase in testosterone concentration (Häkkinen et al., 1995). A more recent study included young females and compared a maximal heavy resistance, submaximal, and explosive training protocol on acute hormonal responses. The maximal heavy resistance protocol consisted of five sets of 10RM for the bench press, machine leg press, and sit-ups. The same protocol but with less weight was used for

the other two protocols. The submaximal protocol utilized five sets of 70% 10RM while the explosive protocol utilized five sets of 40% 10RM. The females included in this study did not experience a significant change from pre to post exercise session for any of the three protocols (Linnamo et al., 2005). Cumming et al. (1987) had females perform three sets of ten repetitions on six pieces of apparatus. Sets were done to muscular failure with one minute of rest between sets. There was a significant increase in testosterone levels from pre to post exercise session (Cumming et al., 1987). A more recent study looked at the effects of an acute resistance exercise test on testosterone response. Female participants performed six sets of 10RM squats with two minutes of rest between sets. Participants experienced a significant acute increase from pre to post exercise session (Nindl et al., 2001). No study has compared a protocol of training with only free weights to training with only machines on increases in testosterone. Based on previous EMG research, which indicated greater activation of muscle mass during free weights (Schwanbeck et al., In Press), one could expect that training with only free weights would promote a more anabolic environment. The acute increase in testosterone seems to be greater and more consistent in males versus females; therefore, gender was included as a factor during the statistical analyses in the current study.

Physiology and Influence of Cortisol

Free-weight training may place a greater stress on the body because of the greater activation of muscle mass. It was therefore anticipated that free-weight training would increase cortisol production in the current thesis study. Cortisol is a catabolic hormone released from the adrenal cortex in response to the stress of exercise. Cortisol stimulates lipolysis in fat cells, increases protein degradation and decreases protein synthesis in muscle cells. This process leads to an increased release of lipids and amino acids into circulation (Kraemer et al., 2005). The degradation of protein into amino acids

stimulates gluconeogenesis which assists in maintaining blood glucose levels and the breakdown of fat into fatty acids for oxidation in the muscle helps provide energy during and after exercise (Brooks et al., 2005). Excessive cortisol release may promote an extremely catabolic environment thus inhibiting increases in muscle mass (Bell et al., 2000).

Cortisol Changes with Resistance Training

Cortisol increases acutely during resistance exercise with similar responses between men and women. A recent study by McGuigan et al. (2005) examined the effect of "psyching-up" on maximal strength and cortisol response. Male and female participants were subjected to two different psyching-up protocols and then performed a Smith machine squat 1RM. Both groups of men and women experienced significant increases in cortisol after their 1RM squat. Another study looked at the effects of different heavy resistance exercise protocols on hormonal concentrations. Female participants randomly performed both a strength protocol consisting of performing five sets of a 5RM with three minutes of rest between sets and a hypertrophy protocol consisting of three sets of a 10RM with one minute of rest between sets. Both protocols included the bench press, double leg extension, military press, bent leg incline sit-ups, seated rows, lat pull down, arm curls, and leg press. Regardless of the exercise protocol the participants experienced significant increases in cortisol post exercise session (Kraemer et al., 1993). Another study by Kraemer et al. (1999b) examined the effects of heavy resistance training on hormonal response in younger (aged thirty) and older men (aged sixty-two). Each participant performed an acute heavy resistance exercise test consisting of four sets of a 10RM squat with ninety seconds of rest between sets. Both the younger and older men experienced significant acute increases in cortisol post exercise session (Kraemer et al., 1993).

No changes in acute cortisol response to resistance training has also been shown in the literature.Kraemer and colleagues (1999a) examined the effects of a single bout of heavy resistance exercise in trained power lifters and untrained men. Participants performed one set of leg press to exhaustion at eighty percent of their 1RM. Regardless of training experience neither group experienced any changes in cortisol level (Kraemer et al., 1999a). Häkkinen et al. (2001) looked at the effects of strength training on hormones in older women. Participants completed a heavy-resistance protocol for the examination of acute hormonal responses which involved doing a bilateral leg press for five sets of a 10RM. The participants did not experience any changes in cortisol levels.

Theoretical Evidence

Testosterone and cortisol play a major role in tissue remodelling and further research is warranted to determine the effects of free weight or machine training on the release of these hormones. Both free weights and machines are shown to be effective at increasing muscle mass and strength but there have been few direct comparisons between the two training modalities. However, research has shown that training in an unstable environment (i.e. Free weights) results in increased muscle activity (McCaw et al., 1994; Behm et al., 2005; Schwanbeck et al., In Press). Theoretically, this increased muscle activation should result in increased testosterone release (Kraemer et al., 2005), and this increase in testosterone should lead to greater increases in muscle mass and strength (Herbst et al., 2004).

Purpose/Hypothesis

The purpose of the present study was to compare the effects of training with only free weights or machines on muscle mass, strength, testosterone levels and cortisol levels. The hypothesis was that

free weight training would result in greater gains in muscle mass and strength, and a more anabolic hormone response as indicated by a greater increase in testosterone during individual workouts and chronically over 8 weeks of training. It was also hypothesized that free-weight training would result in a greater increase in cortisol because of the greater stress involved with a larger muscle activation. These hypotheses are based on the findings that during acute exercise sessions, training with free weights results in greater recruitment of muscle mass, as assessed by EMG, compared to training on machines (Behm et al., 2005; Schwanbeck et al. In Press; McCaw et al., 1994).

Chapter 2 - Methodology

Participants

Ethical approval for this study was obtained by the University of Saskatchewan's biomedical review board for research in human subjects (see Appendix A). Participants were provided with a written and oral overview of the study, were given an opportunity to ask questions about the study, and provided with an informed consent form to read and sign (see Appendix B). Participants also filled out a questionnaire that asked: 1) how long have you been weight training for and 2) do you mostly train with free weights or machines or a combination of both (see Appendix C)? This information allowed for groups to be matched on gender and training experience before randomization.

Using a statistical program (Statistica 7.0, Tulsa Oklahoma) and an alpha = 0.05 with a power of 80%, a participant number of 23 per group was calculated based on an expected change of 5.3% ± 2.7% in lean tissue mass over 8 weeks in the machine group (Chilibeck et al., 2004) versus a 7.6% increase in lean tissue mass in the free weight group. The expected change in the free weight group was estimated to be 43% higher than the machine group. This is based on a 43% higher muscle activation in free-weight compared to machine-based exercise (Schwanbeck et al., in Press). Forty six participants volunteered for this study. The mean age, weight, and height were 22 ± 3 years, 71 ± 13kg, 171 ± 10cm, respectively. For complete participant descriptive please see Table 2. Fifteen males and twenty-one females completed the study. The main reason for dropout was the time commitment needed to complete the workouts. Participants had, on average, just over 2 years strength training experience. Having the previous weight training experience allowed the participants to work out without direct supervision and on their own time. Table 3 outlines a description of participants' training experience.

Participants were recruited by placing advertisements throughout the University of Saskatchewan campus.

Table 2. Participant Descriptives

		Before Training	After Training
Free Weight Group n = 18			
Age (years)	23 ± 4		
Height (cm)	172 ± 10		
Body Weight (kg)		67 ± 8	68 ± 9
Lean Tissue Mass (kg)		54 ± 10	53 ± 10
Body Fat (%)		20 ± 11	22 ± 10
Machine Group n = 18			
Age (years)	22 ± 3		
Height (cm)	171 ± 10		
Body Weight (kg)		74 ± 16	75 ± 17
Lean Tissue Mass (kg)		58 ± 14	58 ± 12
Body Fat (%)		21 ± 7	23 ± 6

Table 3. Participant Training Experience

	Training Experience (Months)	Mostly Free Weights	Mostly Machines	Equal Mix
Free Weight Group	27 ± 25	10	1	8
Machine Group	26 ± 24	8	0	10

Experimental Design

Participants filled out a Physical Activity Readiness – Questionnaire (PAR-Q) to determine health status and were randomly assigned to either the free weight or machine training group after stratifying subjects by gender, months of training experience, and whether they used mostly free weights, mostly machines, or an equal mix of both. The total duration of the exercise study was eight weeks. Hormone levels were assessed via saliva samples pre and post acute hormone collection workout at the beginning, mid-way (4 weeks), and end of the study (8 weeks). Body composition and strength were measured during the week before the training intervention and during the week after the training intervention. Participants were offered familiarization sessions where one of the research assistants was available to provide proper technique for all exercises. Participants were also directed to maintain their current diet and continue to ingest any supplements they were taking. A food record was recorded one day prior to the hormone collection so that the same food could be ingested on each of the days prior to the next two hormone collection days. To minimize the effect of recent exercise, participants were told not to exercise for 2 hours prior to their hormone collection sessions.

Measurements:

Muscle mass

Lean tissue (muscle) mass was measured before and after the exercise program by air displacement plethysmography (BOD POD: Life Measurement Instruments, Concord, CA). Weight to the nearest 0.1 kg and height to the nearest cm was taken before each BOD POD session. Male participants sat in the BOD POD wearing spandex shorts and a swim cap, while females wore spandex shorts and a sports bra or a bathing suit, and the swim cap. The participants were instructed to sit relaxed, breathe normally, and try not to move during the test which took approximately 2-5 minutes to

complete. Body density was calculated using the formula: Density = Mass/Volume. Percent body fat was calculated using the Siri Equation: %Fat = 495/Density – 450 (Siri, 1966). Lean tissue mass was then calculated by the formula: total body mass - (%fat x total body mass). Two consecutive BOD POD measurements were done and the average was used as the individual's result. If there was greater than a 2% difference, a third measurement was taken and the average of the closest two measurements was calculated.

Candow and Chilibeck (2005) demonstrated both BOD POD reliability and validity. They tested participants one week apart and calculated a coefficient of variation of 0.80% for lean tissue mass. They also compared the BOD POD to dual-energy X-ray absorptiometry and reported a correlation coefficient of 0.98 ($p<0.01$) for lean tissue mass.

Muscle Thickness

Muscle thickness was measured before and after the exercise program using B-mode ultrasound (Aloka SSD-500, Tokyo, Japan). The muscle thickness sites included the quadriceps and biceps. The quadriceps site was land marked by having the participant place their hip and knee at a ninety degree angle and measuring the mid-way point from the top of the patella to the crease of the hip. The bicep site was land marked by taking the midpoint between the acromion process and the radial notch. Once the midpoint was established the landmarks were placed down the midline of the anterior part of the arm. All landmarks were mapped using a clear overhead projector sheet to ensure that the sites were measured at the same location during the post-test measurement.

A water-based gel was applied to the transducer head to allow for optimal sound wave transmission. The transducer was held perpendicular to the skin while avoiding compression of the skin and the underlying tissue. An image of the fat/muscle and muscle/bone interface was frozen on the

display screen for measurement. Muscle thickness was measured from the fat/muscle interface to the muscle/bone interface. Measurements were taken to the nearest 0.1 mm. Candow and Chilibeck (2005) determined that the reliability of these ultrasound measurements ranged from a coefficient of variation of 1 to 3%.

Several studies have validated the B-ultrasound by comparing ultrasound to the MRI. Sanada and colleagues (2006) found a correlation of $r = 0.89 – 0.97$ in seventy-two subjects aged 18-61 in the elbow flexors and extensors, knee flexors and extensors, lateral forearm, abdomen, subscapula, and ankle flexors and extensors. Miyatani et al. (2002) used 46 male subjects between the ages of 20 and 70 and found a high correlation ($r = 0.91$) in knee extensors. Miyatani et al. (2000) used 36 healthy adult males (mean age of 25.4) and found a high correlation ($r = 0.96$) in the arm extensors and flexors.

Hormone Collection

A standardized workout was performed at the first, mid-point (4 weeks), and last workout (8 weeks). This workout consisted of performing only the bench press and squat on their designated mode of training. These two exercises consisted of performing 4 sets of 6-10 repetitions with 1.5 minutes of rest between sets. Loads for the first hormone collection workout were calculated as 70% 1RM based on their pre-test strength assessments. Loads for the midway and final workout were based on the weights being used during previous workouts. For example, if a participant was doing sets of squats using 45 kg for four sets of 8-12 during their workouts leading up to their hormone collection then a weight slightly heavier than 45 kg was chosen for their four sets of 6-10 during the hormone collection workout since they only needed to complete 10 repetitions. A slightly lighter weight was chosen if the participant's workouts leading up to the hormone collection workout were too heavy resulting in them only being able to complete sets of 4-6 repetitions during their workouts. Loads were adjusted so that

the appropriate number of repetitions were completed. Salivary hormones were collected prior to the start of these three workouts and fifteen minutes after the workouts. Saliva samples were utilized for hormones collection since they are less invasive compared to blood samples. Salivary hormone levels also reflect the free plasma concentration and bioactive component of steroid hormones (Kraemer et al., 2001). Time of day was recorded for the first workout so that the mid-way workout and final workout were performed at the same time of day. This is important due to the circadian rhythm that affects testosterone levels throughout the day (Kraemer et al., 2001).

Salivary testosterone and cortisol were measured using enzyme immunoassay kits (Salimetrics, State College, PA). Saliva was collected from passive drool through a short straw and into a polypropylene vial. Samples were frozen at minus twenty degrees Celsius until analysis. Once thawed, the saliva samples were pipetted into the appropriate wells, mixed on a plate rotator for 5 minutes at 500rpm and incubated in the dark at room temperature for an additional 25 minutes. The samples were read in a plate reader at 450nm. Three samples were taken and an average value was calculated. Testosterone was calculated to the nearest pg/ml and cortisol to the nearest ug/dL. Details of the testing kit procedures can be found in Appendix D. Our lab had intra-assay coefficients of variation ranging from 4% to 7.2% for cortisol and 4.6% to 8.6% for testosterone.

Strength Measurements

Strength was assessed by performing a one repetition maximum (1RM) on a free weight bench press, 6-10RM free weight squat, 1RM Smith machine bench press and a 6-10RM Smith machine squat. The free weight exercises were performed at least two days apart from the Smith machine exercises. The order in which they performed their bench presses and squats was randomized as well as which mode they were tested on first. A predicted 1RM was determined based on the 6-10RM value for

the squat exercises (Kravitz et al., 2003) for safety reasons. The Life Fitness Smith machine consisted of an Olympic bar that has each end attached to an upright rail. The bar can only slide up and down this rail in a fixed form manner. Olympic weights were placed on the ends of the bar to add resistance. The decreased need for the participant to balance the bar and weights may increase the safety of this mode of training. The free weight bench press was performed using a barbell and flat bench press. The free weight squat utilized a power rack and a barbell. Both free weight exercises were freely isolated from any constraints and the participants needed to incorporate stabilizing and balancing the bar to complete the lift. For all exercises, participants warmed up using a light weight of their choice, and then performed up to five trials for a maximal lift. There was three to five minutes of rest given between each trial as this is the amount needed to fully replenish the creatine phosphate stores after a maximal contraction (Richmond et al., 2004).

During the free weight bench press 1RM hands were placed approximately shoulder width apart, feet on the floor and back against the bench. The participant received help un-racking the bar and they lowered the bar until it contacted their chest at which point they pushed the bar back up to full elbow extension where they received help re-racking the bar. If the participant was unsuccessful, a spotter helped re-rack the bar. For the free weight squat 6-10RM participants' feet were approximately shoulder width apart. The participant received help un-racking the bar and they squatted down until their knees were approximately at 90 degrees where they stood back up until full hip extension was achieved. Once they had reached their 6-10RM they received help re-racking the weight. Depth of each repetition was controlled for by attaching a thera-band between the frames at a height that when the bar touched the band at the bottom range of motion, the participant was at approximately a 90 degree knee angle. Once the bar touched the band the participant received a verbal cue to stand back up. The height

of the thera-band was recorded for the post-test strength assessment. If the participant could not complete the repetition they lowered the bar onto the safety rails.

For the Smith machine bench press 1RM the participant received help un-racking the bar by slightly rotating the safety hooks off of the latches located on the frame of the machine and lowered the bar until it contacted their chest then pushed the bar back up to full elbow extension where they received help re-racking the bar by slightly rotating the safety hooks back onto the latches. If the participant was unsuccessful a spotter helped re-rack the bar. For the Smith machine squat 6-10RM participants' feet were approximately shoulder width apart. The participants received help un-racking the bar (same as the Smith machine bench press) and they squatted down until their knees were approximately at 90 degrees where they stood back up until full hip extension was completed. Once they had completed their 6-10RM they received help re-racking the weight (same as the Smith machine bench press). Depth of each repetition was controlled for by placing a box on the outside of the frame and stacking mats high enough that when the bar touched the mat at the bottom range of motion the participant was at an approximately 90 degree knee flexion angle. Once the bar touched the mat the participant received a verbal cue to stand back up. The height of the box and mats were recorded for post-test strength assessments. If the participant could not complete the repetition they lowered the bar onto the safeties.

Exercise Program

The exercise program lasted for 8 weeks and consisted of a two days on, one day off cycle. Day one trained the chest, back, and triceps muscles. The free weight exercises included the flat barbell bench press, incline barbell bench press, bent over barbell row, chin-ups, supine elbow extension, and kickbacks. The machine exercises were performed on Technogym (Seattle, WA), Hammer Strength

(Cincinnati, OH), Life Fitness (Schiller Park, IL), and APEX (Saanichton, BC) equipment. The machine exercises for the chest, back, and triceps included the Smith machine (Life Fitness) bench press, Smith machine incline bench press, Hammer Strength seated row, Technogym lat pulldown, Technogym machine triceps press-down, and rope press-down (Life Fitness pulley system). Day two trained the legs, shoulders, and biceps. Free weight exercises included the squat, straight leg dead-lift, lunge, single leg calf raise, dumbbell shoulder press, dumbbell lateral raise, camber bar curl, and preacher curl. The machine exercises for the legs, shoulders, and biceps included the Smith machine squat, Technogym quadriceps extension, Technogym seated hamstring curl, APEX machine calf raise, Technogym machine shoulder press, Technogym machine lateral raise, Technogym machine bicep curl, and Hammer Strength machine preacher curl. Technogym machines utilize pulley systems which are potentially better suited for matching the strength curves of the various exercises. Hammer Strength machines utilize iso-lateral movements with divergent and convergent movement arcs to better match the body's natural biomechanical range of motion. For the first three weeks all exercises were done for 4 sets of 8-10 repetitions with 1 minute of rest between sets. For the next three weeks weight was increased and all exercises were done for 4 sets of 6-8 repetitions with 1.5 minutes of rest between sets. For the last two weeks weight was increased again and all exercises were done for 3 sets of 4-5 repetitions with 2 minutes of rest between sets. Intensity was increased throughout the program in order to achieve progressive overload (Baechle et al., 2000). The exercise program also increased load as volume decreased in order to mimic a taper effect which has been shown to promote strength increases (Gibala et al., 1994). If the participant performed a set outside of the desired repetition range they were instructed to adjust the weight for the following sets so that they would complete the appropriate number of repetitions required. Similar training programs have been utilized and have been shown to induce hypertrophy (Pinkoski et al., 2006; Chilibeck et al., 1999).

Workouts were recorded in a detailed activity log book. All workouts took place in the University of Saskatchewan Fit Centre where fully qualified staff was available to provide assistance during the workouts. Fit Centre employees have a minimum of a "Certified Fitness Consultant (CFC)", "Certified Personal Trainer (CPT), "Professional Fitness and Lifestyle Consultant (PFLC)", or "Certified Exercise Physiologist (CEP)", issued by the Canadian Society for Exercise Physiology (CSEP).

Statistical Analysis

A 2 x 2 x 2 mixed design (between-within) ANOVA was conducted with group (free weight group vs. machine group), gender (male vs. female), and time (pre vs. post) as factors to determine the differences between groups for lean tissue mass, muscle thicknesses, and strength over time. The muscle thickness variables included biceps thickness and quadriceps thickness. The strength variables included free weight bench press strength, free weight squat strength, Smith machine bench press strength, and Smith machine bench press strength.

Due to a significant difference in baseline quadriceps thickness between machine and free weight group males an ANCOVA was used to evaluate the difference in post-test means between groups (free weight group vs. machine group) with pre test muscle thickness values as a covariate. This resulted in a 2 x 2 mixed design (between-within) ANOVA with group (free weight group vs. machine group), and time (pre vs. post) being used for female quadriceps thickness.

A 2 x 2 x 2 x 3 (between - within) mixed design ANOVA was conducted with group (free weight group vs. machine group), gender (male vs. female), time during workout (pre vs. post) and time of training program (pre vs. mid vs. post) as factors to determine the difference between groups for hormone levels over time. The hormone variables included testosterone and cortisol.

Tukey's post hoc tests were run when significant interactions were found.

All values are expressed as means ± standard deviation. A p-value less than 0.05 was accepted as significant.

Chapter 3 - Results

Lean Tissue Mass

No significant changes took place in the lean tissue mass during the eight week training period in either the male or female participants. However there was a significant gender main effect for lean tissue mass F (1,32) = 168.721, $p < 0.01$, with males higher than females, as would be expected. There was also a significant mode main effect F (1,32) = 4.83 $p < 0.05$, with the machine group higher than the free weight group. Lean tissue mass values from before to after training are reported in Table 4.

Table 4. Lean Tissue Mass

	Before Training	**After Training**
Free Weight Group		
*Males n = 7	65 ± 4 kg	65 ± 5 kg
Females n = 11	47 ± 4 kg	46 ± 4 kg
Genders Combined	56 ± 4 kg	56 ± 5 kg
Machine Group		
*Males n = 8	72 ± 8 kg	70 ± 7 kg
Females n = 10	48 ± 4 kg	48 ± 4 kg
Genders Combined	60 ± 6 kg	59 ± 6 kg

All values are means ± SD
*Significant gender main effect (p<0.01)

Muscle Thickness

There was a significant time main effect, F (1,32) = 13.99, $p < 0.01$ and F (1,33) = 36.24, $p < 0.01$, for the biceps and quadriceps, respectively, with each increasing in muscle thickness over the training program. There was also a gender main effect, F (1,32) = 65.05, $p < 0.01$, for the biceps, with muscle thickness greater in males compared to females, as would be expected. A gender main effect could not be determined for quadriceps muscle thickness because of the separate analyses done for male and females (i.e. ANCOVA for males, ANOVA for females). There were no significant differences between machine and free-weight groups over time. Muscle thickness measurements from before to after training are reported in Tables 5 and 6.

There was one baseline difference between groups for muscle thickness. There was a significant difference in quadriceps thickness at baseline between the free weight training males and machine training males, t (14) = -2.307, $p < 0.05$. We therefore ran an ANCOVA on this one measure with baseline quadriceps thickness as a covariate. There were no significant differences for the muscle thickness measures after running this analysis. The ANCOVA adjusted post-test mean for the free weight training males quadriceps was 6.24 cm and 6.39 cm for the machine training males.

Table 5. Biceps Muscle Thickness

	Before Training	****After Training**
Free Weight Group		
*Males n = 7	4.10 ± .51 cm	4.31 ± .35 cm
Females n = 11	3.38 ± .40 cm	3.46 ± .37 cm
Genders Combined	3.74 ± .46 cm	3.89 ± .36 cm
Machine Group		
*Males n = 8	4.17 ± .33 cm	4.38 ± .27 cm
Females n = 10	3.22 ± .33 cm	3.41 ± .15 cm
Genders Combined	3.70 ± .33 cm	3.90 ± .42 cm

All values are means ± SD
*Significant gender main effect ($p<0.01$)
**Significant time main effect ($p<0.01$)

Table 6. Quadriceps Muscle Thickness

	Before Training	****After Training**
Free Weight Group		
*Males n = 8	5.64 ± .72 cm	5.95 ± .65 cm
Females n = 11	5.65 ± .63 cm	5.88 ± .78 cm
Genders Combined	5.65 ± .68 cm	5.92 ± .72 cm
Machine Group		
*Males n = 8	6.35 ± .49 cm	6.68 ± .50 cm
Females n = 10	5.58 ± .51 cm	5.87 ± .47 cm
Genders Combined	5.97 ± .50 cm	6.28 ± .49 cm

All values are means ± SD
*Significant gender main effect ($p<0.05$)
**Significant time main effect ($p<0.01$)

Strength

There was a strong trend for a group x time interaction, $F(1,31) = 4.006$, $p = 0.054$ for the machine bench press with the machine training group experiencing a greater increase in machine bench press strength compared to the free weight training group (Figure 1). There were no other differences between groups over time for any other strength measure.

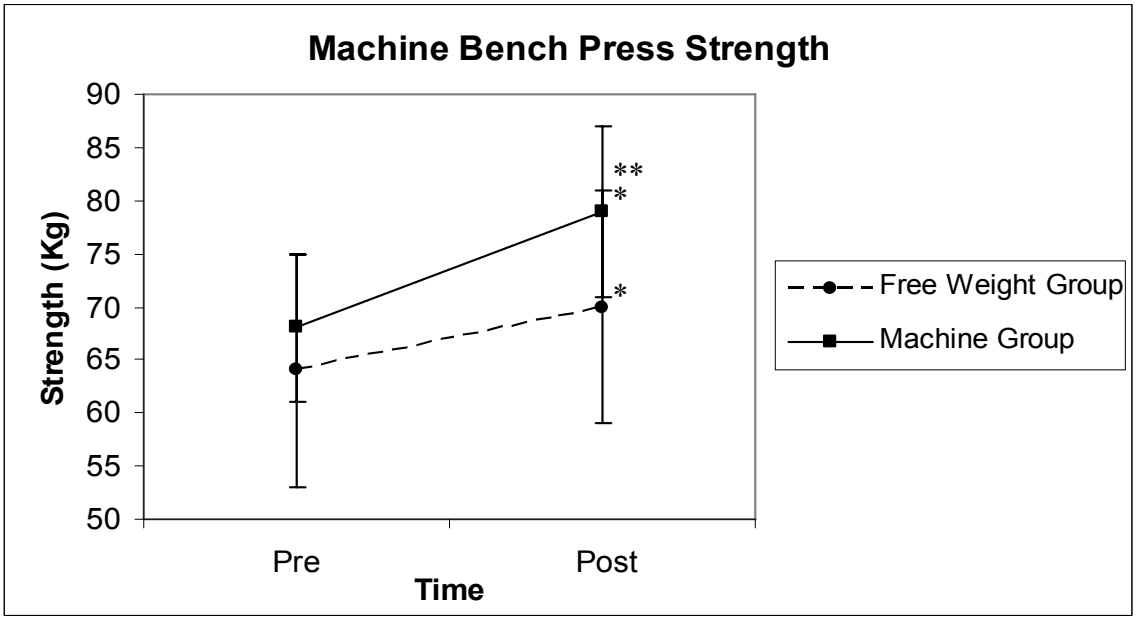


Figure 1. Machine bench press strength from pre-test to post-test. Values are means ± standard error. *Significant increase within group from pre to post. **Group × time interaction (p=0.054) with the post test values significantly higher in the machine training group compared to the free weight training group.

There was a significant gender main effect for bench press strength, F (1,32) = 145.58, $p < 0.01$ and F (1,31) = 132.72, $p < 0.01$, for the free weight bench press and machine bench press, respectively, with males higher than females, as would be expected. There was a significant time main effect for free weight bench press, F (1,32) = 111, $p < 0.01$, with strength increasing from before to after training. There was a significant squat strength gender main effect, F (1,28) = 47.78, $p < 0.01$ and F (1,27) = 39.82, $p < 0.01$, for the free weight squat and machine squat, respectively, again with males higher than females. There was also a significant squat strength time main effect, F (1,28) = 69.57, $p < 0.01$ and F (1,27) = 122.14, $p < 0.01$, for the free weight squat and machine squat, respectively, with strength increasing over time. All strength measurements are presented in Tables 7-10.

Table 7. Free Weight Bench Press Strength

	Before Training	****After Training**
Free Weight Group		
*Males n = 7	81 ± 19 kg	91 ± 19 kg
Females n = 11	36 ± 6 kg	44 ± 7 kg
Genders Combined	59 ± 13 kg	68 ± 13 kg
Machine Group		
*Males n = 8	85 ± 14 kg	95 ± 13 kg
Females n = 10	42 ± 5 kg	48 ± 5 kg
Genders Combined	64 ± 10 kg	72 ± 9 kg

All values are means ± SD
*Significant gender main effect ($p<0.01$)
**Significant time main effect ($p<0.01$)

Table 8. Machine Bench Press Strength

	Before Training	****After Training**
Free Weight Group		
*Males n = 7	86 ± 21 kg	94 ± 21 kg
Females n = 10	41 ± 6 kg	46 ± 7 kg
Genders Combined	64 ± 14 kg	70 ± 14 kg
Machine Group		
*Males n = 8	90 ± 13 kg	101 ± 13 kg
Females n = 10	45 ± 6 kg	56 ± 7 kg
Genders Combined	68 ± 10 kg	79 ± 10 kg

All values are means ± SD
*Significant gender main effect ($p<0.01$)
**Significant time main effect ($p<0.01$)

Table 9. Free Weight Squat Strength

	Before Training	****After Training**
Free Weight Group		
*Males n = 7	146 ± 15 kg	175 ± 22 kg
Females n = 10	98 ± 21 kg	120 ± 24 kg
Genders Combined	122 ± 18 kg	148 ± 23 kg
Machine Group		
*Males n = 6	142 ± 22 kg	157 ± 21 kg
Females n = 9	101 ± 15 kg	118 ± 16 kg
Genders Combined	122 ± 19 kg	138 ± 19 kg

All values are means ± SD
*Significant gender main effect ($p<0.01$)
**Significant time main effect ($p<0.01$)

Table 10. Machine Squat Strength

	Before Training	****After Training**
Free Weight Group		
*Males n = 7	153 ± 11 kg	178 ± 12 kg
Females n = 9	93 ± 23 kg	118 ± 29 kg
Genders Combined	123 ± 17 kg	148 ± 21 kg
Machine Group		
*Males n = 6	141 ± 29 kg	171 ± 33 kg
Females n = 9	103 ± 15 kg	128 ± 23 kg
Genders Combined	122 ± 22 kg	150 ± 28 kg

All values are means ± SD
*Significant gender main effect ($p<0.01$)
**Significant time main effect ($p<0.01$)

Hormones

There was a significant group × gender × time during workout interaction for testosterone, $F(1,56) = 8.1$, $p < 0.05$. Tukey's post-hoc analyses indicated that only the free-weight training males significantly increased testosterone during workouts, increasing from 173 ± 62 pg/ml to 221 ± 98 pg/ml, $p < 0.01$ (Figure 2).

There was no significant change in cortisol at any time point for either gender.

There was a significant acute time × gender interaction for the testosterone to cortisol ratio $F(1,48) = 7.51$, $p < 0.05$. Tukey's post-hoc analyses indicated that only the males had significant increases in the testosterone to cortisol ratio during workouts, increasing from 6.95 ± 3.69 pg/ml to

8.82 ± 5.32 pg/ml $p < 0.01$ (Figure 3). There were no changes over the duration of the eight weeks of training in any hormone measure (i.e. there were no "chronic" changes in any of the hormone measures). All hormone levels are presented in Tables 11-13.

Table 11. Testosterone Levels pg/ml

	Workout 1		Workout 2		Workout 3	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**
Free Weight Group						
Males n = 6	**194 ± 53**	**242 ± 59**	**152 ± 44**	**203 ± 65**	**172 ± 31**	**218 ± 68**
Females n = 10	**66 ± 21**	**69 ± 28**	**78 ± 30**	**80 ± 36**	**89 ± 45**	**78 ± 50**
Machine Group						
Males n = 6	**150 ± 19**	**152 ± 23**	**137 ± 37**	**178 ± 55**	**150 ± 32**	**151 ± 37**
Females n = 10	**70 ± 20**	**95 ± 30**	**80 ± 33**	**87 ± 33**	**76 ± 31**	**87 ± 40**

All values are means ± SD

Table 12. Cortisol Levels ug/dL

	Workout 1		Workout 2		Workout 3	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**
Free Weight Group						
Males n = 6	**.30 ± .14**	**.28 ± .15**	**.26 ± .11**	**.27 ± .14**	**.50 ± .12**	**.26 ± .11**
Females n = 10	**.34 ± .15**	**.35 ± .11**	**.41 ± .11**	**.42 ± .11**	**.55 ± .17**	**.29 ± .15**
Machine Group						
Males n = 6	**.38 ± .15**	**.38 ± .15**	**.38 ± .21**	**.42 ± .19**	**.19 ± .12**	**.17 ± .10**
Females n = 10	**.37 ± .25**	**.40 ± .20**	**.34 ± .18**	**.32 ± .15**	**.40 ± .15**	**.40 ± .13**

All values are means ± SD

Table 13. Testosterone to Cortisol Ratio

	Workout 1		Workout 2		Workout 3	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**
Free Weight Group						
Males n = 6	**8.39 ± 1.63**	**10.04 ± 3.71**	**8.22 ± 3.86**	**9.98 ± 3.77**	**6.65 ± 2.14**	**8.81 ± 5.62**
Females n = 10	**2.73 ± 1.19**	**2.72 ± 1.02**	**2.88 ± 2.25**	**2.94 ± 1.43**	**2.61 ± 1.14**	**3.05 ± 2.00**
Machine Group						
Males n = 6	**5.87 ± 4.52**	**7.48 ± 8.16**	**5.76 ± 3.57**	**6.72 ± 3.39**	**6.82 ± 6.41**	**9.89 ± 7.25**
Females n = 10	**2.67 ± 1.85**	**2.86 ± 1.98**	**2.43 ± 1.06**	**2.85 ± 1.47**	**1.83 ± 0.61**	**2.03 ± 0.66**

All values are means ± SD

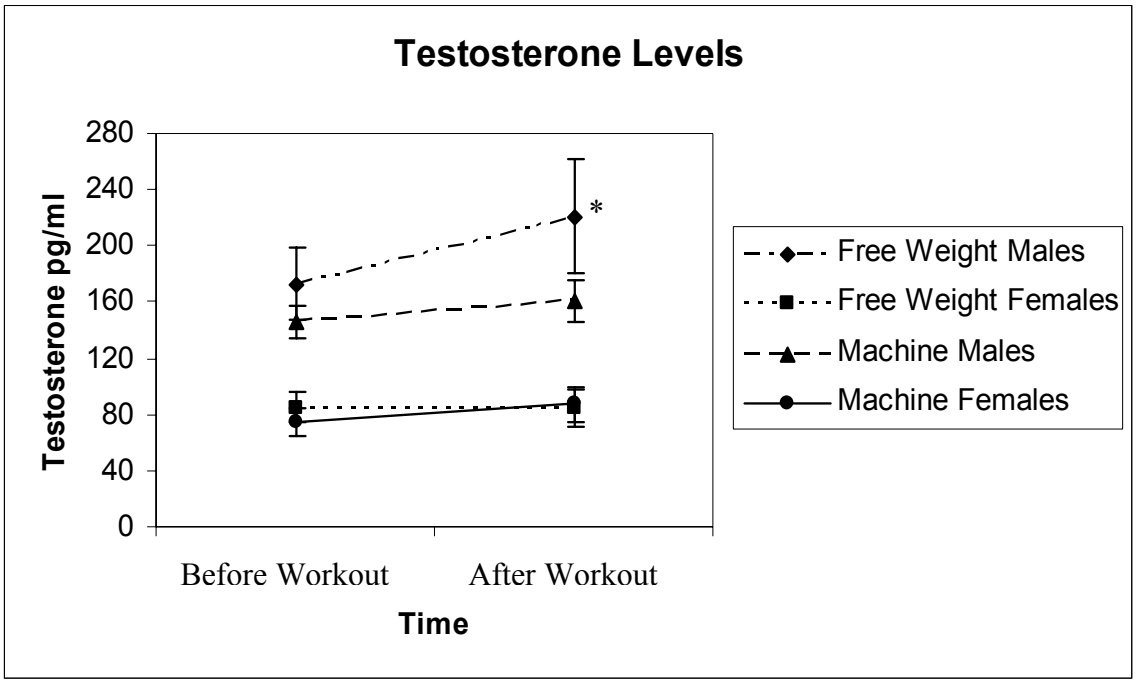


Figure 2. Testosterone before and after workouts (averaged for all three hormone collection workouts). Values are means ± standard error. *Significant increase from pre to post for males training with free weights ($p < 0.01$).

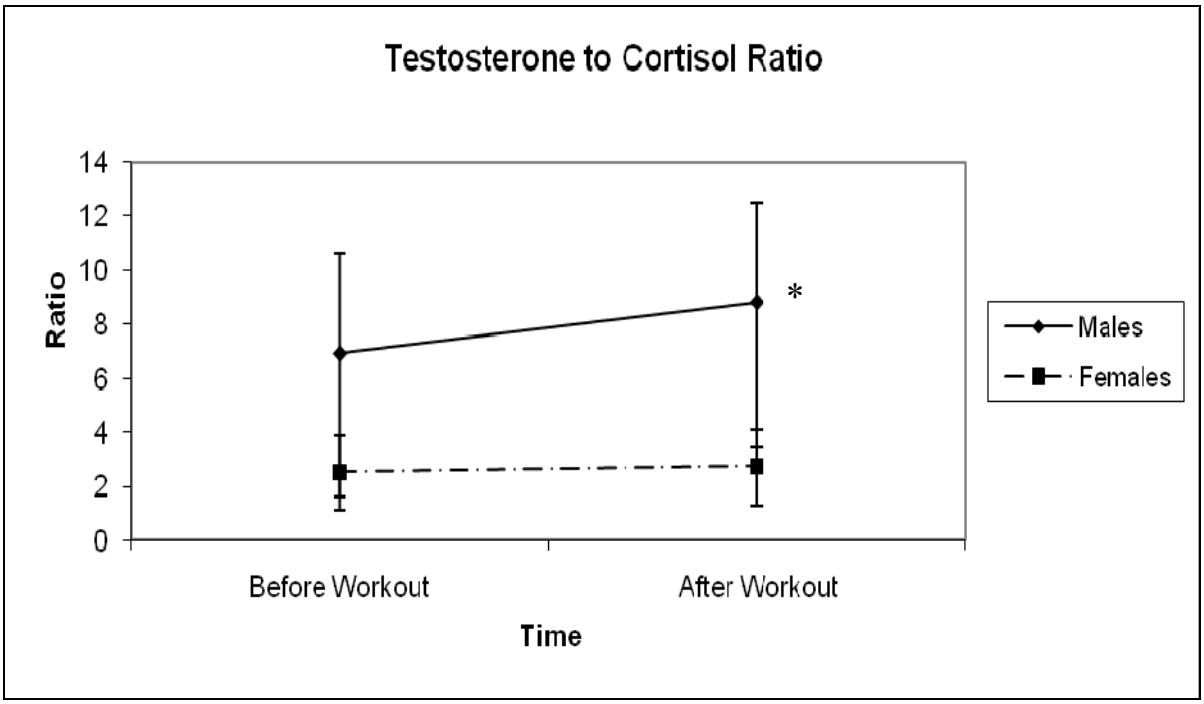


Figure 3. Testosterone to Cortisol Ratio before and after workouts (averaged for all three hormone collection workouts). Values are means ± standard error. *Significant increase from pre to post for males regardless of mode ($p < 0.01$).

Chapter 4 - Discussion

The major finding of this study is that the free weight training group and the machine training group both had significant increases in muscle thickness and strength with no differences between groups. These findings do not support our hypothesis that the group training with free weights would experience greater gains in muscle mass and strength. The second major finding is that the males training with free weights experienced a significant acute increase in testosterone from pre to post workout when averaged over the three acute hormone collection workouts. This finding partially supports our hypothesis that the group training with free weights would have greater increases in testosterone. The hypotheses were based on the evidence that training with free weights activates more muscle mass (McCaw et al., 1994; Behm et al., 2005; Schwanbeck et al., In Press), which should cause a greater increase in testosterone (Kraemer et al., 2005), which should over time cause a greater increase in muscle mass and strength (Herbst et al., 2004). These results suggest that an increased muscle activation may not be directly linked to an increased testosterone release or an increased muscle mass since there was not a corresponding increase in lean tissue mass with increased testosterone release.

The unique aspects of this study were that we assessed not only strength changes, which tend to occur quite quickly during a strength training program, but also lean tissue mass and muscle thickness which increase over a longer term. Many of the previous studies comparing machine and free-weight training did not have muscle size measures as a variable; however, most of them did assess strength changes. Another unique aspect was that we included male and female participants. In previous research comparing free weights to machines, most studies included only one gender, typically males. This is also the case in research looking at testosterone and cortisol levels where the majority of the

time males are the only participants included. By including males and females this study is more generalizable. Our study also was a true comparison of free weights to machines using a "whole body" program with the exercises being matched for similar movements and muscles being used.

Lean Tissue Mass

In contrast to our original hypothesis, there were no significant changes for either group in lean tissue mass assessed by the Bod Pod. One explanation for this finding could be that the participants had previous training experience. Since the participants had previous training experience they might have been close to their ceiling level of lean body mass and eight weeks of resistance training may not have been enough to induce a further increase. The absence of significant increases in overall lean body mass is not consistent with previous research. Maddalozzo and Snow (2000) found that after twenty-four weeks of training with a seated resistance training program or standing free weight program both groups experienced a significant increase in lean body mass which was measured using dual-energy x-ray absorbtiometry (DEXA). The study by Maddalozzo and Snow (2000) incorporated a longer training period (i.e. 24 weeks vs. 8 weeks in our study) and a more precise method for assessing body composition (i.e. DEXA vs. BodPod in our study). However, their free weight standing program did include some machine exercises as well which does not make this a true comparison of free weights to machines. Although our study was a true comparison of free weights to machines, our body composition assessment tool, the Bod Pod, is considered less precise compared to the DEXA which may have contributed to some of the differences in findings for lean body mass. Boyer (1990) found positive changes in body composition after twelve weeks of training. Groups trained on three different modes consisting of two different types of machines or free weights. The difference in findings for lean body mass compared to our study could be attributed to the method for assessing body composition.

Boyer (1990) utilized skinfolds and girths to calculate body composition while we used the BodPod. The accuracy of skinfold and girth measurements is questionable due to the human error element. Perhaps if our study used DEXA to measure body composition and if the study was longer than eight weeks there may have been significant increases in over-all lean body mass. Another factor to consider is that free weights may involve a longer neural adaptation phase because they involve more co-ordination. This may have put the free weight group at a disadvantage because overall hypertrophy might have been delayed. Again, if the study was longer the free weight group may have had more time to hypertrophy and show a significant increase in overall lean tissue mass.

Muscle Thickness

The original hypothesis was that the free weight group would have greater increases in muscle thickness however, this was not the case. Significant and similar increases in biceps and quadriceps muscle thickness were experienced by the free weight group and machine group. No other study has compared free weight to machine training for increasing muscle thickness; however, our results for adaptation in males and females can be compared to one other study that measured muscle thickness by ultrasound during resistance training in males and females. Similar to our findings, Abe et al. (2000) found significant increases in biceps thickness after eight weeks of progressive heavy-resistance training in males and females. However, they did not find a significant increase in quadriceps thickness even after twelve weeks of training. The different results for quadriceps thickness could be attributed to the intensity and frequency of workouts performed by the participants in the Abe et al. (2000) study. Their participants only trained three times per week and only performed two leg exercises for only one set or three sets. In our study, participants performed four leg exercises and were training four to five times per week. Their workout program might not have included enough leg exercises and was possibly

not intense enough to result in increased quadriceps muscle thickness. Similar to our findings Blazevich and colleagues (2003) found significant increases in quadriceps thickness as soon as five weeks after training. The strength training protocol for this study was similar to our program with participants performing five leg exercises as well as sprint/jump training protocols. This exercise program appears to have been intense enough to illicit increases in quadriceps muscle thickness.

One obvious contradiction in our study is the significant increase in biceps and quadriceps muscle thickness without a significant increase in whole-body lean tissue mass. Thickness of only two muscle groups was assessed; whereas whole-body lean tissue mass is obviously influenced by a larger number of muscle groups. Biceps and quadriceps are muscle groups that often show significant hypertrophy with training and other muscle groups may not have had the same degree of hypertrophy. The Bod Pod may not have been sensitive enough to detect the hypertrophy of the biceps and quadriceps if other muscle groups did not hypertrophy to the same degree.

Strength

Both the free weight training group and the machine training group had significant increases in free weight and Smith machine squat strength and free weight and Smith machine bench press strength. These findings do not support our hypothesis that the free weight group would experience greater gains in strength. The unique finding for our strength data was that the group training with machines experienced greater post-test gains in machine bench press strength compared to the free weight training group. This finding supports the idea of specificity which refers to the concept that the greater the similarity that a training exercise has to the actual physical performance, the greater the probability of transfer (Chandler et al., 2008). Boyer (1990) also had similar results with strength training and specificity. In this study the participants who were training with free weights or using a Nautilus

machine experienced greater gains in strength when tested on their own device. However, when tested on the Soloflex machine the free weight group, the Nautilus machine group, and the Soloflex machine group all had similar increases in strength. Another study conducted by Thorstensson and colleagues (1976) also demonstrated specificity. Their participants trained using free weight barbell squats and were later tested doing a leg press as well as a free weight squat 1RM. Participants had significant increases in free weight squat strength, however, they only had marginal increases in leg press strength. These results that support the idea of specificity may be attributed to an increased kinaesthetic awareness and proprioceptive feedback during performance of an exercise which utilized movement patterns similar to those done while training (Stone et al., 1987). Our free weight bench press, free weight squat, and Smith machine squat results do not support the idea of specificity. For these three strength variables both the free weight training group and the machine training group had significant increases in strength with no differences between the two groups. These findings could be attributed to the fact that the Smith machine does not severely alter the biomechanics of the squat and bench press movement. Similar findings have been reported in previous research. Sanders (1980) found no differences during strength testing after participants trained with either free weights or on a Nautilus machine. Similarily, Silvester and colleagues (1982) ran two studies and had participants training with free weight squats, Nautilus Compound Leg Machine, or Universal Variable Resistance Maximum Overload Leg Press Machine in study one, and in study two the participants trained with free weights or on a Nautilus machine. In both studies, all groups had significant strength increases with no differences between the groups. These findings do not support the concept of specificity, but rather they show that there was good transfer of strength from one mode to the other.

Hormones

Testosterone

The only group that experienced a significant acute increase in testosterone was the males training with free weights. This result partially supports our hypothesis that the free weight group would experience greater increases in testosterone. Similar results have been reported by Crewthers and colleagues (2008) where participants training using a hypertrophy protocol experienced increased testosterone post workout. It appears that a certain level of mechanical stress needs to be placed on the body as well as the recruitment of large amounts of muscle mass is needed to elicit an acute testosterone response (Kraemer et al., 2005). Hypertrophy protocols with higher volume and shorter rest intervals similar to the protocol we used are best suited for eliciting this response. This is also supported by Kraemer et al. (1991) and Häkkinen et al. (1993) who also found that hypertrophy protocols resulted in greater increases in testosterone compared to a strength protocol. The males training with machines experienced only a small non-significant increase in testosterone. Even though the acute workouts for the free weight group and the machine group followed the same hypertrophy protocol, the males training with machines in our study may not have received enough mechanical stress by training in the very stable environment of the Smith machine. Free weight exercise requires more stabalization than Smith machine exercise as evidenced by substantially higher muscle recruitment, as assessed by EMG (Schwanbeck et al., in Press). The added stability and balance needed for the free weight training session may have added the needed stress resulting in an acute testosterone increase. Both groups of males also experienced a significant increase in the testosterone to cortisol ratio. This indicates a similar enhancement in anabolic to catabolic hormone environment in machine and free weight groups. The females, regardless of training mode, did not experience any changes in acute testosterone levels. Similar findings have been reported by Häkkinen et al. (1995) where their

young and elderly female participants did not experience any changes in testosterone levels from pre to post workout. In another study by Linnamo et al., (2005) the female participants did not experience any acute changes in testosterone while performing three different heavy resistance training protocols. Although there was no change in testosterone for females and males training with machines, they still had increases in biceps and quadriceps muscle thickness. This finding indicates that there is not a direct causal relationship between muscle mass and exercise-induced increases in testosterone. This idea is supported by Wilkinson and colleagues (2006) who had participants train a single leg while the other leg served as a control. They found that the control leg did not change size while the trained leg got bigger without any endogenous increases in testosterone or other anabolic hormones.

Cortisol

Regardless of training mode or gender, there were no significant changes in cortisol levels. Our original hypothesis was that the participants training with free weights would experience greater acute increases in cortisol. This idea was based on the theory that training with free weights activates more muscle mass therefore putting a greater physical stress on the body which should have resulted in an increase in the stress hormone cortisol. Similar to our findings, Kraemer and colleagues (1999) and Häkkinen and colleagues (2001) showed no increases in cortisol levels after an exercise session. As noted by Goldfarb et al. (1991) there might be a threshold of exercise intensity above which beta-endorphin concentration is a function of both the duration and intensity of exercise. Cortisol has been shown to follow a similar response to exercise as beta-endorphins (Kraemer et al., 1989, Kraemer et al., 1989, Kraemer et al., 1993) which may signify that cortisol may also have a threshold dependent on duration and intensity of exercise. The workouts during our study may not have surpassed this necessary threshold which resulted in no acute increases. Perhaps if our workout before the acute

hormone collection provided more physical stress (ie. more sets or more exercises), there might have been acute increases in cortisol levels. For example, studies which incorporate high volumes of resistance training combined with aerobic endurance training elicit increases in cortisol concentrations (Bell et al., 2000; Kraemer et al., 1995).

Strengths and Limitations

The main strength of this study was that it provided a true comparison of training with only free weights or only machines. Many of the exercises between the two modes were similar in muscles used and movement through the range of motion. Thus the major difference between the two modes was the stability of training on the machines and balancing and stabilization required while training with the free weights. Another strength of the study, was the use of B-mode ultrasound to detect changes in muscle thickness. B-mode ultrasound is a very sensitive method to measure muscle thickness and has been validated against MRI for assessing the knee extensors (Miyatani et al., 2002) and elbow flexors (Miyatani et al., 2000) which are the two muscle groups that were assessed in our current study. The use of saliva as the biological agent to assess testosterone and cortisol levels was also beneficial. Saliva samples are much less invasive and less stressful to collect compared to drawing blood samples. The ease of collecting saliva may also alleviate any of the anticipatory responses that people may have prior to stressful or uncomfortable situations (Suay et al., 1999) such as during blood collection which might give falsely high numbers during the pre-test sample. Salivary hormone levels also reflect the free plasma concentration and bioactive component of steroid hormones, which is important as it is the biologically active fraction that of testosterone that is available to bind with androgen receptors (Kraemer et al., 2005).

One of the major limitations to our study was the amount of participants that finished the study. We originally recruited forty-six participants which is what our sample size calculation called for, however, only thirty-six people finished the study which leaves our study under powered. Also within these thirty-six participants not all measures were available for post-test analyses. Ideally we would have liked more than forty-six participants at the start of the study leaving room for dropouts and still being above our ideal sample size. Another concern was the accuracy of the BodPod. Although the BodPod was calibrated within normal range we still saw some participants experience extremely large increases or decreases in lean body mass from pre-test to post-test. The use of a DEXA scan would have been more accurate; however, the cost and time involved with using the DEXA did not make it a feasible option.

Another limitation of the study was the influence of variables such as differences in training experience, differences in other physical activities that the participants were doing during the study, and differences in diet. These factors would have been extremely difficult to control for and could have influenced our results. However, the randomization process should have alleviated some of the issues with not controlling for these variables.

The length of the study was also a potential limitation. Our study was only eight weeks long and it has been noted that the effectiveness of one program over another program may take at least eight weeks to manifest itself (Häkkinen, 1985, Kraemer, 1997). Perhaps if our study was longer the acute increases in testosterone experienced by the males training with free weights might have resulted in greater gains in muscle mass and strength.

Future Directions

Future research should assess the effects of training with only machines or only free weights on various functional capacity tests which may be more beneficial for sport specific training and an athletic population. For instance, the power clean would be considered a functional movement and it would be interesting to see if improvements could be made in this movement by only training with free weights or machines. However, including such a complex functional movement creates problems in itself due to the difficulty in performing the task. It may be difficult to find a large enough group of participants that are capable of performing this movement. Another variable that should be assessed when doing mode specific training would be other anabolic hormones such as growth hormone. Mode specific training may elicit different responses on different anabolic hormones. Increasing the length of the training protocol would also be beneficial. By having a study that is longer than eight weeks there may be more time for lean body mass to increase. Another aspect that should be studied is the influence of having a longer workout and the effects that it may have on testosterone and cortisol.

Chapter 5 - Summary and Conclusions

Summary

A comparison of training with only free weights or only machines on muscle mass, strength, and testosterone and cortisol levels has not been researched in the past. Therefore, the purpose of this study was to examine the effect of mode specific training on muscle mass, strength, and hormone levels. Our main hypothesis was that the group training with free weights would have greater gains in lean tissue mass, strength and greater acute increases in testosterone and cortisol. Our hypothesis was based on the theory that training in an unstable environment (i.e. Free weights) results in increased muscle activity (McCaw et al., 1994, Behm et al., 2005; Schwanbeck et al., In Press). Theoretically, this increased muscle activation should result in increased testosterone release (Kraemer et al., 2005), and this increase in testosterone should lead to greater increases in muscle mass and strength (Herbst et al., 2004). Our strength and lean tissue mass results did not support our hypothesis, in that regardless of training modality the participants had significant increase in strength and muscle thickness. Our testosterone results only partially support our hypothesis since only the males training with free weights had a significant increase in testosterone. Finally, our cortisol results did not support our hypothesis since no group had any changes in cortisol levels.

Conclusions

Results of this study show that significant increases in strength, and biceps and quadriceps muscle thickness can be achieved by training with only free weights or only machines. Males training with free weights may also see an added benefit of increased muscle mass over an extended period of time due to acute increases in testosterone. Males, regardless of training mode, may also benefit from a

positive exercise induced increase in the testosterone to cortisol ratio resulting in a more “anabolic environment”.

References

Abe, T., DeHoyas, D., Pollock, M., & Garzarella, L. (2000). Time course for strength and muscle thickness changes following upper and lower body resistance training in men and women. *European Journal of Applied Physiology, 81,* 174-180.

Ahtiainen, J., Pakarinen, A., Kraemer, W., & Häkkinen, K. (2004). Acute hormonal responses to heavy resistance exercise in strength athletes versus nonathletes. *Canadian Journal of Applied Physiology, 29,* 527-543.

Ahtiainen, J., Pakarinen, A., Alen, M., Kraemer, W., & Häkkinen, K. (2005). Short vs. long rest period between the sets in hypertrophic resistance training: influence on muscle strength, size, and hormonal adaptations in trained men. *Journal of Strength and Conditioning Research, 19,* 572-582.

Baechle, T. R. & Earle, R. W. (2000). *Essentials of strength training and conditioning* (2nd ed.) Windsor, Ontario: Human Kinetics.

Behm, D. G., & Anderson, K. G. (2005). Trunk muscle activity increases with unstable squat movements. *Canadian Journal of Applied Physiology, 30,* 33-45.

Bell, G., Syrotuik, D., Martin, T., & Burnham, R. (2000). Effect of concurrent strength and endurance training on skeletal muscle properties and hormone concentrations in humans. *European Journal of Applied Physiology, 81,* 418-427.

Bhasin, S., Storer, T., Berman, N., Callegari, C., Brenda, C., Phillips, J., et al. (1996). The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. *The New England Journal of Medicine, 335,* 1-7.

Blazevich, A., Gill, N., Bronks, R., & Newton, R. (2003). Training-specific muscle architecture adaptation after 5-wk training in athletes. *Medicine & Science in Sports & Exercise, 35,* 2013-2022.

Booth, F., & Thomason, D. (1991). Molecular and cellular adaptation of muscle in response to exercise: perspectives of various models. *Physiological Reviews, 71,* 541-585.

Boyer, B. T. (1990). A comparison of the effects of three strength training programs on women. *Journal of Applied Sport Science Research, 4,* 88-94.

Brooks, G., Fahey, T., & Baldwin, K. (Eds). (2005). *Exercise physiology: Human bioenergetics and its applications 4th edition*. New York: McGraw-Hill.

Candow, D. & Chilibeck, P. (2005). Differences in size, strength, and power of upper and lower body muscle groups in young and older men. *The Journals of Gerontology, 60,* 148-156.

Carolan, B., & Cafarelli, E. (1992). Adaptations after isometric resistance training. *Journal of Applied Physiology,73,* 911-917.

Chandler, J., & Brown, L. (Eds). (2008). *Conditioning for strength and human performance.* Baltimore, MD: Lippincott Williams & Wilkins.

Chilibeck, D., Stride, D., Farthing, J., & Burke, D. (2004). Effect of creatine ingestion after exercise on muscle thickness in males and females. *Medicine & Science in Sports & Exercise, 36,* 1781-1788.

Chilibeck, P. D., Syrotuik, D. G. & Bell, G. J. (1999). The effect of strength training on estimates of mitochondrial density and distribution throughout muscle fibres. *European Journal of Applied Phsyiology, 80,* 604-609.

Claassen, H., Gerber, C., Hoppeler, H., Luthi, J., & Vock, P. (1989). Muscle filament spacing and short-term heavy-resistance exercise in humans. *Journal of Physiology, 409,* 491-495.

Cotterman, M. L., Darby, L. A., & Skelly, W. A. (2005). Comparison of muscle force production using the smith machine and free weights for bench press and squat exercises. *Journal of Strength and Conditioning Research, 19,* 169-176.

Crewther, B., Cronin, J., Keogh, J., & Cook, C. (2008). The salivary testosterone and cortisol response to three loading schemes. *Journal of Strength and Conditioning Research, 22,* 250-255.

Cronin, J., McNair, P. J., & Marshall, R. N. (2003). The effects of bungy weight training on muscle function and functional performance. *Journal of Sports Sciences, 21,* 59-71.

Cumming, D. C., Wall, S. R., Galbraith, M. A., et al. (1987). Reproductive hormone responses to resistance exercise. *Medicine and Science Sports and Exercise, 19,* 234-238.

Fatouros, I.G., Kambas, A., Katrabasas, I., Nikolaidis, K., Chatzinikolaou, A., Leontsini, D., et al. (2005). Strength training and detraining effects on muscular strength, anaerobic power, and mobility of inactive older men are intensity dependent. *British Journal of Sports Medicine, 39,* 776-780.

Ferrando, A., Sheffield-Moore, M., Paddon-Jones D., et al. (2003). Differential anabolic effects of testosterone and amino acid feeding in older men. *Journal of Clinical Endocrinology & Metabolism, 88,* 358-362.

Gabriel, D. A., Kamen, G., & Frost, G. (2006). Neural adaptations to resistive exercise. *Sports Medicine, 36,* 133-149.

Gibala, M., MacDougal, J., & Sale, D. (1994). The effects of tapering on strength performance in trained athletes. *International Journal of Sports Medicine, 15,* 492-497.

Goldfarb, A., Hatfield, H., Armstrong D., & Potts, J. (1991). Plasma beta-endorphin concentration: response to intensity and duration of exercise. *Medicine and Science in Sports and Exercise, 22,* 241-244.

Haff, G. (2000). Roundtable Discussion: machines versus free weights. *Strength and Conditioning Journal, 22, 18-30.*

Häkkinen, K. (1985). Factors influencing trainability of muscular strength during short-term and prolonged training. *Strength and Conditioning Journal, 2,* 32-37.

Häkkinen, K., & Pakarinen, A. (1993). Acute hormonal responses to two different fatiguing heavy-resistance protocols in male athletes. *Journal of Applied Physiology, 74,* 882-887.

Häkkinen, K., Kallinen, M., Izquierdo, M., Jokelainen, K., Lassila, H., Malkia, E., et. al. (1998). Changes in agonist-antagonist EMG, muscle CSA, and force during strength training in middle-aged and older people. *Journal of Applied Physiology, 84,* 1341-1349.

Häkkinen, K., & Pakarinen, A. (1995). Acute hormonal responses to heavy resistance exercise in men and women at different ages. *International Journal of Sports Medicine, 16,* 507-513.

Häkkinen, K., Pakarinen, A., Kramer, W. J., Häkkinen, A., Valkeinen, H., & Alen, M. (2001). Selective muscle hypertrophy, changes in EMG and force, and serum hormones during strength training in older women. *Journal of Applied Physiology, 91,* 569-580.

Herbst, K. L., & Bhasin, S. (2004). Testosterone action on skeletal muscle. *Current Opinion in Clinical Nutrition and Metabolic Care, 7,* 271-277.

Hickson, R. C., Hidaka, K., Foster, C. et al. (1994). Successive time courses of strength development and steroid hormone responses to heavy-resistance training. *Journal of Applied Physiology, 76,* 663-670.

Izquierdo, M., Häkkinen, K., Ibanez, J., Garrues, M., Anton, A., Zuniga, A. et. al. (2001). Effects of strength training on muscle power and serum hormones in middle-aged and older men. *Journal of Applied Physiology, 90,* 1497-1507.

Jowko, E., Ostaszewski, P., Jank, M., Saharuk, J., Zieniewicz, A., Wilczal, J. et. al. (2001). Creatine

and beta-hydroxy-beta-methylbutyrate (HMB) additively increase lean body mass and muscle strength during a weight-training program. *Nutrition, 17,* 558-566.

Kadi, F., Schjerling, P., Andersen, L., Charifi, N., Madsen, J., Christensen, L. et al. (2004). The effects of heavy resistance training and detraining on satellite cells in human skeletal muscles. *Journal of Physiology, 558,* 1005-1012.

Kamen, G., & Knight, C. (2004). Training-related adaptations in motor unit discharge rate in young and older adults. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 59,* 1334-1338.

Kraemer, W. (1997). The physiological basis for strength training in american football: fact over philosophy. *Journal of Strength and Conditioning Research, 11,* 131-142.

Kraemer, W., Fleck, S., Callsiter, R., Shealy, M., Dudley, G., Maresh, C. et al. (1989). Training responses of plasma beta-endorphin, adrenocorticotropin, and cortisol. *Medicine & Science in Sports & Exercise, 21,* 146-153.

Kraemer, W., Patton, J., Knuttgen, H., Marchitelli, L., Cruthirds, C., Damokosh, A. et al., (1989). Hypothalamic-pituitary-adrenal responses to short-term high-intensity cycle exercise. *Journal of Applied Physiology, 66,* 161-166.

Kraemer, W., Gordon, S., & Fleck, S. (1991). Endogenous anabolic hormonal and growth factor responses to heavy resistance exercise in males and females. *International Journal of Sports Medicine, 12,* 228-235.

Kraemer, W., Dziados, J., Marchitelli, L., Gordon, S., Harman, E., Mello, R. et al. (1993). Effects of different heavy-resistance exercise protocols on plasma beta-endorphin concentrations. *Journal of Applied Physiology,74,* 450-459.

Kraemer, W.J., Fleck, S. J., Dziados, J.E., et al. (1993). Changes in hormonal concentrations after

different heavy-resistance exercise protocols in women. *Journal of Applied Physiology, 75,* 594-604.

Kraemer, W. J., Fleck, S. J., Maresh, C. M., Ratamess, N. A., Gordon, S. E., Goetz, K. I., et al. (1999a). Acute hormonal responses to a single bout of heavy resistance exercise in trained powerlifters and untrained men. *Canadian Journal of Applied Physiology, 24,* 524-537.

Kraemer, W. J., Häkkinen, K., Newton, R.U., et al. (1999b). Effects of heavy-resistance training on hormonal response patterns in younger vs. older men. *Journal of Applied Physiology, 87,* 982-992.

Kraemer, W.J., Patton, J.F., Gordon ,S.E., Harman, E.A., Deschenes, M.R., Reynolds, K., Newton, R.U., Triplett, N.T., & Dziados, J.E. (1995). Compatibility of high-intensity strength and endurance training on hormonal and skeletal muscle adaptations. *Journal of Applied Physiology, 78,* 976-989.

Kraemer, W., Ratamess, N., & French, D. (2002). Resistance training for health and performance. *Current Sports Medicine Reports, 1*, 165-171.

Kraemer, W., Loebel, C., Volek, J., Ratamess, N., Newton, R., Wickham, R., et al. (2001). The effect of heavy resistance exercise on the circadian rhythm of salivary testosterone in men. *European Journal of Applied Physiology, 84,* 13-18.

Kraemer, W. J., & Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Medicine, 35,* 339-361.

Kravitz, L., Akalan, C., Nowicki, K., & Kinzey, S. (2003). Prediction of 1 repetition maximum in high-school power lifters. *Journal of Strength and Conditioning Research, 17,* 167-172.

Linnamo, V., Pakarinen, A., Komi, P., Kraemer, W., & Häkkinen, K. (2005). Acute hormonal

responses to submaximal and maximal heavy resistance and explosive exercises in men and women. *Journal of Strength and Conditioning Research, 19,* 566-571.

Maddalozzo, C., Snow, C. (2000). High intensity resistance training: effects on bone in older men and women. *Calcified Tissue International, 66,* 399-404.

Mayhew, J. L., Ware, J. S., Johms, R. A., & Bemben, M. G. (1997). Changes in upper body power following heavy-resistance strength training in college men. *International Journal of Sports Medicine, 18,* 516-520.

Mayo, J. J., & Kravitz, L. (1997) Fitness assessment part 3: muscle strength and endurance. *Idea Personal Trainer, 8,* 54-58.

McArdle, W. D., Katch, F. I., Katch, V. L. (Eds.) (1999). *Sport & exercise nutrition.* Baltimore, MD: Lippincott Williams & Wilkins.

McArdle, W., Katch, F. & Katch, V. (Eds.) (2007). *Exercise physiology: energy, nutrition, and human performance sixth edition*. Baltimore, MD: Lippincott Williams & Wilkins.

McCaw, S. T., & Friday, J. J. (1994). A comparison of muscle activity between a free weight and machine bench press. *Journal of Strength and Conditioning Research, 8,* 259-264.

McGuigan, M., Ghiagiarelli, J., & Tod, D. (2005). Maximal strength and cortisol responses to psyching-up during the squat exercise. *Journal of Sports Science, 23,* 687-692.

Miyatani, M., Kanehisa, H., Kuno, S., Nishijima, T., & Fukunaga, T. (2002). Validity of ultrasonograph muscle thickness measurements for estimating volume of knee extensors in human. *European Journal of Applied Physiology, 86*, 203-2008.

Miyatani, M., Kanehisa, H., & Fukunaga, T. (2000). Validity of bioelectrical impedance and ultrasonographic method for estimating the muscle volume of the upper arm. *European Journal of Applied Physiology, 82*, 391-396.

Nindl, B. C., Kraemer, W. J., Gotshalk, L. A., et al. (2001). Testosterone responses after resistance exercise in women: influence of regional fat distribution. *International Journal of Sports Nutrition and Exercise Metabolism, 11,* 451-465.

Patten, C., Kamen, G., & Rowland, D. (2001). Adaptations in maximal motor unit discharge rate to strength training in young and older adults. *Muscle & Nerve, 24,* 542-550.

Pinkoski, C., Chilibeck, P., Candow, D., Esliger, D., Ewaschuk, J., Facci, M. et al., (2006). The effects of conjugated linoleic acid supplementation during resistance training. *Medicine & Science in Sports & Exercise, 38,* 339-348.

Richmond, S. R., Godard, M. P. (2004). The effects of varied rest periods between sets to failure using the bench press in recreationally trained men. *Journal of Strength and Conditioning Research, 18,* 846-849.

Sanada, K., Kearns, C., Midorikawa, T., & Abe, T. (2006). Prediction and validation of total and regional skeletal muscle mass by ultrasound in Japanese adults. *European Journal of Applied Physiology, 1*, 24-31

Sanders, M. T. (1980). A comparison of two methods of training on the development of muscular strength and endurance. *Journal of Orthopedic Sports and Physical Therapy, 1,* 210-213.

Sandri, M. (2008). Signalling in muscle atrophy and hypertrophy. *Physiology, 23,* 160-170.

Schwanbeck, S., Chilibeck, P.D., & Binsted, G (In Press) A comparison of a free weight squat to a smith machine squat using electromyography (emg). *Journal of Strength and Conditioning Research*

Semmler, J., & Nordstrom M. (1998). Motor unit discharge and force tremor in skill- and strength-trained individuals. *Experimental Brain Research, 119,* 27-38.

Silvester, J., Stiggins, C., McGown, C., & Bryce, G. (1982). The effect of variable resistance and free-weight training programs on strength and vertical jump. *Journal of National Strength and Conditioning Association, 3*, 30-33.

Singh, R., Artaza, J., Taylor, W., et al. (2003). Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells through an androgen receptor-mediated pathway. *Endocrinology, 144,* 5081-5088.

Siri. W. (1966) Body composition from fluid spaces and density: analysis of methods. In: Brozek J, Henschel A (eds) Techniques for measuring body composition. National Academy of Sciences National Research Council, Washington, 223–224.

Staron, R., Karapondo, D., Kraemer, W., Fry, A., Gordon, S., Falkel, J., Hagerman, F., & Hikida, R. (1994). Skeletal muscle adaptations during early phase of heavy-resistance training in men and women. *Journal of Applied Physiology, 76,* 1247-1255.

Stone, M., & O'Bryant, H. (Eds) (1987). *Weight training – a scientific approach.* Minneapolis: Burgess International Group Inc.

Stone, M., Collins, D., Plisk, S., Haff, G., & Stone, M. (2000). Training principles: evaluation of modes and methods of resistance training. *Strength and Conditioning, 22,* 65-76.

Suay, F., Salvador, A., Gonzalez-Bono, E., Sanchis, C., Martinez, M., Martinez-Sanchis, S., Simon, V. (1999). Effects of competition and its outcome on serum testosterone, coritsol, and prolactin. *Psychoneuroendocrinology, 24,* 551-566.

Sullivan, D., Roberson, P., Johnson, L., Bishara, O., Evans, W., Smith, E., & Price, A. (2005). Effects of muscle strength training and testosterone in frail elderly males. *Medicine & Science in Sports & Exercise, 37,* 1664-1672.

Tesch, P. A., Ekberg, A., Lindquist, D. M., & Trieschmann, J. T. (2004). Muscle hypertrophy following 5-week resistance training using a non-gravity-dependent exercise system. *Acta Physiology Scandinavia, 180,* 89-98.

Thorstensson, A., Hulten, B., Von Doblen, W., & Karlsson, J. (1976). Effect of strength training on enzyme activities and fibre characteristics in human skeletal muscle. *Acta Physiology Scandinavia, 96,* 392-398.

Viru, A., & Viru, M. (2005). Resistance exercise and testosterone. In W. J. Kraemer, & A. D. Rogal (Eds), *The endocrine system in sports and exercise* (p. 321). Malden, MA: Blackwell Publishing.

Wilkinson, S., Tarnopolsky, M., Grant, E., Correia, C., & Philips, S. (2006). Hypertrophy with unilateral resistance exercise occurs without increases in endogenous anabolic hormone concentration. *European Journal of Applied Physiology, 98,* 546-555.

Appendices

Appendix A: Ethics Form

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board (Bio-REB)

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	Bio #
Philip D. Chilibeck	Kinesiology	07-147

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
College of Kinesiology
105 Gymnasium Place
Saskatoon SK S7N 5C2

STUDENT RESEARCHERS
Shane Schwanbeck

SPONSORING AGENCIES
SASKATCHEWAN ACADEMY OF SPORTS MEDICINE

TITLE: Effects of Free Weight or Machine Weight Resistant Training on Muscular Hypertrophy and Testosterone Release

APPROVAL DATE	EXPIRY DATE	APPROVAL OF
06-Sep-2007	05-Sep-2008	Researcher's Summary (24-Aug-2007) Research Participant Information and Consent Form v. 2 (05-Sep-2007)

Full Board Meeting ☐ Date of Full Board Meeting:

Delegated Review ☒

CERTIFICATION
The study is acceptable on scientific and ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research study, and for ensuring that the authorized research is carried out according to governing law. This approval is valid for the specified period provided there is no change to the approved protocol or consent process.

FIRST TIME REVIEW AND CONTINUING APPROVAL
The University of Saskatchewan Biomedical Research Ethics Board reviews above minimal studies at a full-board (face-to-face) meeting. Any research classified as minimal risk is reviewed through the delegated (subcommittee) review process. The initial Certificate of Approval includes the approval period the REB has assigned to a study. The Status Report form must be submitted within one month prior to the assigned expiry date. The researcher shall indicate to the REB any specific requirements of the sponsoring organizations (e.g. requirement for full-board review and approval) for the continuing review process deemed necessary for that project. For more information visit http://www.usask.ca/research/ethics_review/.

REB ATTESTATION
In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

M Desautels — Sept 6, 2007

Michel Desautels, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board

Signature Date

Please send all correspondence to:
Ethics Office
University of Saskatchewan
Room 305 Kirk Hall, 117 Science Place
Saskatoon SK S7N 5C8
Telephone: (306) 966-4053 Fax: (306) 966-2069

Appendix B: Consent Form

Research Participant Information and Consent Form

Title: Effects of Free Weight or Machine Weight Resistant Training On Muscular Hypertrophy and Testosterone Release

Sponsor: Saskatchewan Academy of Sports Medicine Inc.

Principal Investigator: Philip D. Chilibeck, Ph.D., College of Kinesiology, University of Saskatchewan, phone: 966-1072 or 343-6577,

Sub-Investigator: Shane Schwanbeck, B.Sc. (graduate student researcher), College of Kinesiology, University of Saskatchewan, phone: 966-1123 or 374-0056

Introduction: You are being asked to participate in a research study because we want to see which training apparatus (free weights or machines) is optimal for increasing muscle mass and which apparatus is optimal for stimulating testosterone release, which may be involved in stimulating an increase in muscle mass.

Your participation is entirely voluntary, so it is up to you to decide whether or not you wish to take part. If you decide not to take part, you do not have to provide a reason and it will not affect your relationship with the investigators and will have no effect on your academic standing. If you decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision.

This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand. You may ask as many questions as you need to understand what the study involves. Please feel free to discuss this with your family, friends or family physician.

The sponsor of this study (Saskatchewan Academy of Sports Medicine Inc.) will reimburse the researchers for the costs of undertaking this study. However, neither the institution nor any of the investigators or staff will receive any direct financial benefit from conducting this study.

There will be a total of 60 people participating in this study.

Purpose of the study: The purpose of this experimental study is to determine which training apparatus (i.e. free weights or machines) is optimal for increasing muscle mass and which training apparatus is optimal for stimulating the release of testosterone.

Study Design: Initially you will be given a questionnaire (the Physical Activity Readiness Questionnaire) that asks a series of questions about your health and how safe it is for you to perform exercise. If you answer "yes" to any of the questions, we will require that you get permission from your family physician before participating in the study.
You will be randomly assigned (i.e. assigned by chance by a computer) to one of two groups: A group that will train using machines that provide resistance or a group that will do resistance training with free weights (i.e. barbells and dumbbells). The study will last 8 weeks.

Procedures: There will be a number of tests done before you start the training program, at the mid-point (i.e. 4 weeks), and then after the 8-week training program. The following tests will be done before and after the 8-week training program:

1) On day 1 you will have your lean tissue mass measured by air-displacement plethysmography (by the "Bod Pod"). This device requires that you sit in a chamber for about 3 minutes. Your body volume is assessed by the amount of air you displace from the chamber and from this we can estimate your lean tissue mass. The entire test will take about 5 to 10 minutes.

2) On the same day we will assess the muscle thickness of the front of your upper arms and leg. This is done by ultrasound. It involves placing a gel over your skin and then placing a probe over the gel to assess the thickness of your muscles. This test will take about 20 minutes.

3) On the same day we will assess your strength on either the machines or the free weights for your upper body and lower body (bench press and squat exercises). The bench press test is a test of the maximal amount of weight you can lift once. Your squat strength will be predicted from the amount of weight you can lift 6-10 times. This test will take about 15 minutes. These are tests of your voluntary maximal strength and spotters will be employed for safety. You will be given a warm-up and will be allowed to perform sub-maximal practice repetitions before the actual strength tests.

4) The next day we will assess your strength on the opposite device (i.e. machine or free weights), again for the bench press and squat exercises, following similar procedures outlined above. This will take about 15 minutes.

5) About two days later we will assess your hormone response to a single exercise session. The exercise session will involve performing either free weight or machine bench press and squat exercises (depending on the group you were randomized to). Saliva will be collected onto swabs for assessment of testosterone and cortisol before and after the training session. This will require you to "drool" onto swabs. This training session will take about half an hour.

All of the above tests will be done before and after the 8-week training program, except the hormone response to the single exercise session, which will be done before, at the mid-point (i.e. 4 weeks) and at the end of the 8-week program.

The eight week training program will involve training for 2 consecutive days, followed by a "rest" day, with these three days repeated for 8 weeks. On one training day you will be required to do 6 upper body exercises. On the other training day you will do 4 lower body exercises and 4 upper body exercises. The free weight exercises for the upper body will include flat barbell press (for chest and triceps), incline barbell press (chest and triceps), bent over barbell row (back and biceps), chin-ups (back and biceps), dumbbell shoulder press (shoulders), dumbbell lateral raise (shoulders), supine elbow extension (triceps), kickbacks (triceps), camber bar curl (biceps), and preacher curl (biceps). The machine exercises for the upper body will include Smith machine bench press (chest and triceps), Smith machine incline bench press (chest and triceps), seated row (back), lat pull down (back and biceps), machine shoulder press (shoulders), machine lateral raise (shoulders), machine triceps press down (triceps), rope press down (triceps), machine bicep curl (biceps), and machine preacher curl

(biceps). Free weight exercises for the legs will include the squat, straight leg dead lift, lunge, and single leg calf raise. The machine exercises for the legs will include Smith machine squat, quad extension, seated hamstring curl, and machine calf raise. Each training session will take about an hour to complete.

Possible benefits of the study: You will receive information about your body composition, and strength. You may increase your strength and muscle mass as part of the training program. These benefits are not guaranteed.

Foreseeable risks, side effects or discomfort: You may experience muscle injuries during the exercises, or muscle soreness after completion of each exercise session, but a proper warm-up before and cool-down after the exercise sessions will minimize this risk. You will be instructed in proper technique for all exercises to avoid injuries.

There may be unforeseen and unknown risks during the study, or after the study has been completed.

Alternatives to this study: You do not have to participate in this study to have your body composition, or strength assessed, or to receive an exercise program. Your body composition and strength can be assessed by visiting the University of Saskatchewan or other fitness facilities and receiving a fitness assessment, and there are trainers at most facilities that can set up an exercise program for you.

Costs and Reimbursement
You will not be charged for any research-related procedures. You will not be paid for participating in this study.

Research-Related Injury: In the case of a medical emergency related to the study, you should seek immediate care and, as soon as possible, notify the principal investigator. Necessary medical treatment will be made available at no cost to you. By signing this document, you do not waive any of your legal rights.

Confidentiality: The study investigator and his research staff will do everything possible to keep your personal information confidential. Your name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team. It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be revealed.

Voluntary Withdrawal from the Study
If you do decide to take part in this study, you are still free to withdraw at any time and without giving reasons for your decision. There will be no penalty or loss of benefits to which you are otherwise entitled, and your academic standing will not be affected.
If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during enrolment in the study will be retained for analysis up to the point of your withdrawal.

After Completion of the Study: You may contact one of the investigators to find out your personal results and the overall results of the study.

Contact Information: If you have any questions about this study or desire further information about this study before or during participation, you can contact Phil Chilibeck at 966-1072 or 343-6577.

If you have any questions about your rights as a research subject or concerns about the study, you should contact the Chair of the Biomedical Research Ethics Board, c/o the Ethics Office, University of Saskatchewan, at 306-966-4053.

This study has been reviewed and approved on ethical grounds by the University of Saskatchewan Biomedical Research Ethics Board. The Research Ethics Board reviews human research studies. It protects the rights and welfare of the people taking part in those studies.

CONSENT TO PARTICIPATE

I have read the information in this consent form. I understand the purpose and procedures, the possible risks and benefits of the study. I have been informed of the alternatives to participating in this study. I was given sufficient time to think about it. I had the opportunity to ask questions and have received satisfactory answers to all of my questions.

I am free to withdraw from this study at any time for any reason and the decision to stop taking part will not affect my future academic standing. I agree to follow the study investigators' instructions and will tell the study investigators at once if I feel I have had any injuries.

I voluntarily consent to take part in this research study and give permission to the use and disclosure of my de-identified personal information collected for the research purposes described above.

By signing this document I do not waive any of my legal rights. I will be given a signed copy of this consent form.

My family physician can be informed about my participation in this study, and, if required, consulted regarding my health.

❑ Yes, please contact my primary care physician

❑ No, please don't contact my primary care physician OR I do not have a primary care physician.

Printed Name of Participant: Signature Date

Printed Name of person obtaining consent: Signature Date

Appendix C: Data Collection Questionnaire

NAME:

AGE:

GENDER:

TRAINING EXPERIENCE: Months Resistance Training =

Type of Training (please circle the best answer)

Mostly free weights Mostly Machine weights Equal Mix

MUSCLE THICKNESS: RIGHT SIDE OF BODY

	TRIAL 1	TRIAL 2	TRIAL 3
BICEP			
QUAD			

STRENGTH:

	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4	TRIAL 5
SMITH BENCH (1RM)					
SMITH SQUAT (6-10 RM) Depth:					
PREDICTED 1RM					
FREE BENCH (1RM)					
FREE SQUAT (6-10 RM) Depth:					
PREDICTED 1 RM					

ACUTE HORMONE COLLECTION WORKOUT #1 TIME OF DAY:

Appendix D: Testosterone and Cortisol Assay Procedures

SALIMETRICS LLC
101 Innovation Blvd., Suite 302
State College, PA 16803 USA
(T) 814-234-7748, (F) 814-234-1608
800-790-2258 (USA & Canada only)
www.salimetrics.com
techservices@salimetrics.com

EXPANDED RANGE SALIVARY TESTOSTERONE ENZYME IMMUNOASSAY KIT

Catalog No. 1-2402/1-2412, (Single) 96-Well Kit;
1-2402-5/1-2412-5, (5-Pack) 480 Wells

For *in vitro* Research Use

Intended Use

The Salimetrics™ expanded range (ER) testosterone kit is a competitive immunoassay specifically designed and validated for the quantitative measurement of salivary testosterone. It is not intended for diagnostic use. It is intended only for research use in humans and some animals. Please read the complete kit insert before performing this assay. For further information about this kit, or the application, or the procedures in this insert, contact the technical service team at Salimetrics or your local sales representative.

Introduction

To ensure the most accurate results, this salivary immunoassay is designed using a matrix that matches saliva. The level of testosterone in saliva (pg/mL) is significantly lower than levels in the general circulation (ng/mL). The standard curve range is sensitive enough to capture individual differences in the testosterone levels expected in saliva. The current protocol uses only 25 µL of saliva per test. No separation or extractions are necessary.

Test Principle

A microtitre plate is coated with rabbit antibodies to testosterone. Testosterone in standards and unknowns competes with testosterone linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound testosterone peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction using 2-molar sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of testosterone peroxidase detected is inversely proportional to the amount of testosterone present (1).

pH Indicator

A pH indicator in the assay diluent alerts the user to samples with high or low pH values. Acidic samples will turn the diluent yellow. Alkaline samples will turn the diluent purple. Dark yellow or purple wells indicate that a pH value for that sample should be obtained using pH strips. Testosterone values from samples with a pH $\leq$ 4.0 or $\geq$ 9.0 may be artificially inflated or lowered (2).

Precautions

1. Liquid stop solution is a 2-molar solution of sulfuric acid. This solution is caustic; use with care. Stop solution in powdered form is not sulfuric acid-based and is mildly corrosive.
2. This kit uses break-apart microtitre strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch with desiccant and used in the frame provided.
3. Do not mix components from different lots of kits.
4. When using a multichannel pipette, reagents should be added to duplicate wells at the same time. Follow the same sequence when adding additional reagents so that incubation time with reagents is the same for all wells.
5. See 'Material Safety Data' at the end of procedure.
6. We recommend that samples be screened for possible blood contamination (3,4) using a reliable screening tool such as the Salimetrics Blood Contamination EIA Kit (Cat No: 1-1302/1-1312). Do not use dipsticks, which result in false positive values due to salivary enzymes.
7. Routine calibration of pipettes is critical for the best possible assay performance.
8. Pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate.
9. When running multiple plates, or multiple sets of strips, a standard curve should be run with each individual plate and/or set of strips.
10. The temperature of the laboratory may affect assays. Salimetrics' kits have been validated at 68 - 74ºF (20 - 23.3ºC). Higher or lower temperatures will cause an increase or decrease in OD values, respectively. Salimetrics cannot guarantee test results outside of this temperature range.
11. The quantity of reagent provided with this kit is sufficient for three individual runs. The volume of diluent and conjugate used for assays using less than a full plate should be scaled down accordingly, keeping the same dilution ratio.
12. Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month.

Storage

All components of this kit are stable at 2 - 8°C until the kit's expiration date.

Reagents and Reagent Preparation

1. **Anti-Testosterone Coated Plate:** A ready-to-use, 96-well microtitre plate pre-coated with rabbit anti-testosterone antibodies in a resealable foil pouch.
2. **Testosterone Standard:** 0.5 mL of testosterone, in a saliva-like matrix with a non-mercury preservative, at a concentration of 600 pg/mL.
3. **Testosterone Controls:** Two controls representing high and low levels of testosterone in a saliva-like matrix with a non-mercury preservative. Each vial contains 0.5 mL. See vials for target ranges.
4. **Wash Buffer:** 100 mL of a 10X phosphate buffered solution containing detergents and a non-mercury preservative. Dilute only the amount needed for current day's use. Discard any leftover reagent. Dilute the wash buffer concentrate 10-fold with room temperature deionized water (100 mL of 10X wash buffer to 900 mL of deionized H_20). (**Note:** *If precipitate has formed in the concentrated wash buffer, it may be heated to 60°C for 15 minutes. Cool to room temperature before use in assay.)*
5. **Testosterone Assay Diluent:** 60 mL of a phosphate buffered solution containing a pH indicator and a non-mercury preservative.
6. **Enzyme Conjugate:** 40 µL of a solution of testosterone labeled with horseradish peroxidase. Dilute prior to use with assay diluent.
7. **Tetramethylbenzidine (TMB):** 25 mL of a non-toxic, ready-to-use solution.
8. **Stop Solution:** 12.5 mL of a solution of sulfuric acid. Stop solution is provided in powdered form to some customers outside the USA. Reconstitute the powdered stop solution with 12.5 mL of deionized water. Let sit for 10 minutes before using.
9. **Non-specific Binding Wells (NSB):** These wells do not contain anti-testosterone antibody. In order to support multiple use, a strip of NSB wells is included. They are located in the foil pouch. Wells may be broken off and inserted where needed.

Materials Needed But Not Supplied

- Precision pipette to deliver 18 µL, 25 µL, and 150 µL
- Precision multichannel pipette to deliver 50 µL, 150 µL, and 200 µL
- Vortex
- Plate rotator (assay sensitivity may be affected if a rotator is not used)
- Plate reader with a 450 nm filter
- Computer software for data reduction
- Deionized water
- Reagent reservoirs
- One 20 mL disposable tube
- Five small disposable tubes
- Pipette tips
- 25 mL serological pipette

Specimen Collection

Due to the episodic secretion pattern of steroid hormones, we can expect reproducible and reliable results only in cases of multiple sampling. Therefore, we recommend taking a minimum of 3 samples within at least a 2-hour period and pooling the samples before testing (5,6).

Collecting whole saliva samples from adults and children over 6 may be done by using the Salimetrics Oral Swab (SOS), P/N 5001.02, or by unstimulated passive drool. Collection protocols are available on request. **Do not use Salivettes, Sorbettes, cotton, or polyester materials to collect samples.** False readings will result (7,8). Do **not** add sodium azide to saliva samples as a preservative. Samples visibly contaminated with blood should be recollected. Avoid sample collection within 60 minutes after eating a major meal or within 12 hours after consuming alcohol. Acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected. Record the time and date of specimen collection. After collection it is important to keep samples cold, in order to avoid bacterial growth in the specimen. Refrigerate sample within 30 minutes, and freeze at or below -20ºC within 4 hours of collection. (Samples may be stored at -20ºC or lower for long term storage.)

Freezing saliva samples will precipitate the mucins. On day of assay, thaw completely, vortex, and centrifuge at 1500 x g (@3000 rpm) for 15 minutes. It is important to avoid additional freeze-thaw cycles. However, if samples have been refrozen, centrifuge again prior to assaying. Samples should be at room temperature before adding to assay plate. Pipette clear sample into appropriate wells. Particulate matter may interfere with antibody binding, leading to falsely elevated results.

Procedure

Bring all reagents to room temperature. **Note:** It is important to keep the zip-lock pouch with the plate strips closed until warmed to room temperature as humidity may have an effect on the coated wells. Mix all reagents before use.

Step 1: Determine your plate layout (see below).

	1	2	3	4	5	6	7	8	9	10	11	12
A	600 Std	600 Std	C-H	C-H								
B	240 Std	240 Std	C-L	C-L								
C	96 Std	96 Std	Unk-1	Unk-1								
D	38.4 Std	38.4 Std	Unk-2	Unk-2								
E	15.4 Std	15.4 Std	Unk-3	Unk-3								
F	6.1 Std	6.1 Std	Unk-4	Unk-4								
G	Zero	Zero	Unk-5	Unk-5								
H	NSB	NSB	Unk-6	Unk-6								

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. If you choose to place non-specific binding wells in H-1, 2, remove strips 1 and 2 from the strip holder and break off the bottom wells. Place the strips back into the strip holder leaving H-1, 2 blank. Break off 2 NSB wells from the strip of NSBs included in the foil pouch. Place in H-1, 2. Alternatively, NSBs may be placed wherever you choose on the plate. Reseal the foil pouch with unused wells and desiccant. Store at 2 - 8°C.

Caution: Extra NSB wells should not be used for determination of standards, controls or unknowns.

Step 3:

- Label five microcentrifuge tubes or other small tubes 2 through 6.
- Pipette 90 µL of testosterone assay diluent in tubes 2 through 6. Serially dilute the standard 2.5X by adding 60 µL of the 600 pg/mL standard (tube 1) to tube 2. Mix well. After changing pipette tips, remove 60 µL from tube 2 to tube 3. Mix well. Continue for tubes 4, 5, and 6. The final concentrations of standards for tubes 1 through 6, are respectively, 600 pg/mL, 240 pg/mL, 96 pg/mL, 38.4 pg/mL, 15.4 pg/mL, and 6.1 pg/mL. Standard concentrations in pmol/L are 2080.5, 832.2, 332.9, 133.2, 53.3 and 21.3, respectively.
- Pipette 18 mL of testosterone assay diluent into the disposable tube. (Scale down proportionally if not using the entire plate.) Set aside for Step 5.

Step 4:

- Pipette 25 µL of standards, controls, and unknowns into appropriate wells. Standards, controls, and unknowns should be assayed in duplicate.
- Pipette 25 µL of testosterone assay diluent into 2 wells to serve as the zero.
- Pipette 25 µL of testosterone assay diluent into each NSB well.

Step 5: Dilute the enzyme conjugate 1:1000 by adding 18 µL of the conjugate to the 18 mL of testosterone assay diluent prepared in Step 3. (Scale down proportionally if not using the entire plate.) Immediately mix the diluted conjugate solution and add 150 µL to each well using a multichannel pipette.

Step 6: Mix plate on a plate rotator for 5 minutes at 500 rpm (or tap to mix) and incubate at room temperature for an additional 55 minutes.

Step 7: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well and then flipping the liquid into a sink. After each wash, the plate should be thoroughly blotted on paper towels before turning upright. If using a plate washer, blotting is still recommended after the final wash.

Step 8: Add 200 µL of TMB solution to each well with a multichannel pipette.

Step 9: Mix on a plate rotator for 5 minutes at 500 rpm (or tap to mix) and incubate the plate in the dark at room temperature for an additional 25 minutes.

Step 10: Add 50 µL of stop solution with a multichannel pipette.

Step 11:

- Mix on a plate rotator for 3 minutes at 500 rpm (or tap to mix). Be sure all wells have turned yellow. If green color remains, continue mixing until green color turns to yellow. **Caution:** *Do not mix at speeds over 600 rpm.*
- Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.
- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding stop solution. (Correction at 490 to 630 is desirable.)

Calculations

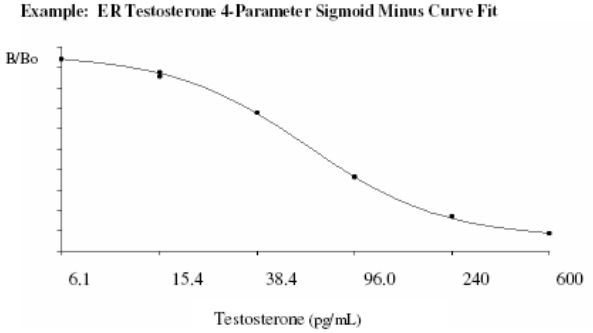
1. Compute the average optical density (OD) for all duplicate wells.
2. Subtract the average OD for the NSB wells from the average OD of the zero, standards, controls, and unknowns (B).
3. Calculate the percent bound (B/Bo) for each standard, control, and unknown by dividing the average OD (B) by the average OD for the zero (Bo).
4. Determine the concentrations of the controls and unknowns by interpolation using software capable of logistics. We recommend using a 4-parameter sigmoid minus curve fit.

Typical Results

The following results are shown for illustration only and should not be used to calculate results from another assay.

Well	Sample	Average OD	B	B/Bo	Testosterone (pg/mL)
A1, A2	S1	0.225	0.203	0.088	600
B1, B2	S2	0.417	0.395	0.170	240
C1, C2	S3	0.863	0.841	0.362	96
D1, D2	S4	1.593	1.571	0.677	38.4
E1, E2	S5	2.026	2.004	0.864	15.4
F1, F2	S6	2.201	2.179	0.939	6.1
G1, G2	Bo	2.342	2.320	NA	NA
H1, H2	NSB	0.022	NA	NA	NA

Example: ER Testosterone 4-Parameter Sigmoid Minus Curve Fit



Material Safety Data*

Hazardous Ingredients

Liquid stop solution is caustic; use with care. ***Note:*** *Stop solution in powdered form is not sulfuric acid-based and is mildly corrosive.*
We recommend the procedures listed below for all kit reagents. Specific kit component MSDS sheets are available from Salimetrics upon request.

Handling

Follow good laboratory procedures when handling kit reagents. Laboratory coats, gloves, and safety goggles are recommended. Wipe up spills using standard absorbent materials while wearing protective clothing. Follow local regulations for disposal.

Emergency Exposure Measures

In case of contact, immediately wash skin or flush eyes with water for 15 minutes. Remove contaminated clothing. If inhaled, remove individual to fresh air. If individual experiences difficulty breathing, give oxygen and call a physician.

*The above information is believed to be accurate but is not all-inclusive. This information should only be used as a guide. Salimetrics shall not be liable for accidents or damage resulting from contact with reagents.

Performance Characteristics

A. Precision:

The intra-assay precision was determined from the mean of 12 replicates each.

Sample	N	Mean (pg/ml)	Standard Deviation (pg/ml)	Coefficient of Variation (%)
H	12	188.83	4.69	2.5
L	12	18.12	1.22	6.7

The inter-assay precision was determined from replicates across 41 lots.

Sample	N	Mean (pg/ml)	Standard Deviation (pg/ml)	Coefficient of Variation (%)
H	64	199.08	11.18	5.6
L	63	19.6	2.69	14.05

B. Recovery:
Saliva samples containing different levels of an endogenous testosterone were spiked with known quantities of testosterone and assayed.

Sample	Endogenous (pg/ml)	Added (pg/ml)	Expected (pg/ml)	Observed (pg/ml)	Recovery (%)
1	29.57	9.60	39.17	37.40	95.5
1	29.57	60.00	89.57	97.91	109.3
1	29.57	400.00	429.57	452.95	105.4
2	76.42	9.60	86.02	90.18	104.8
2	76.42	60.00	136.42	136.23	99.9
3	80.66	200.00	280.66	311.90	111.1

C. Correlation
The correlation between saliva and total serum testosterone was determined by assaying 28 matched samples (15 adult males and 13 females). The saliva-serum correlation was, $r(26) = 0.96$, $p < 0.001$. The saliva-serum correlation was stronger for males, $r = 0.91$, than for females, $r = 0.61$. (9) The relationship between serum and saliva for males as determined by linear regression is y (total serum testosterone in ng/mL) = 0.2421 + 0.0496*x (salivary testosterone in pg/mL). The linear regression equation for females is y (total serum testosterone in ng/mL) = 0.1415 + 0.0055*x (salivary testosterone in pg/mL).

D. Linearity of Dilution:
Four saliva samples were serially diluted with testosterone assay diluent and assayed.

Sample	Dilution Factor	Expected (pg/ml)	Observed (pg/ml)	Recovery (%)
1			71.81	
	1:2	35.91	38.08	106
	1:4	17.95	19.31	107.6
	1:8	8.98	9.69	109
2			404.67	
	1:2	202.34	196.99	97.4
	1:4	101.17	94.12	93
	1:8	50.58	47.19	93.3
	1:16	25.29	24.52	97
3			135.56	
	1:2	67.78	62.34	92
	1:4	33.89	35.86	105.8
	1:8	16.95	18.33	108.1
	1:16	8.47	8.65	102.1
4			553.88	
	1:2	276.94	296.94	107.2
	1:4	138.47	141.01	101.8
	1:8	69.24	72.59	104.8
	1:16	34.62	38.55	111.4

E. Specificity
The following compounds were tested at concentrations up to 1,000 ng/mL for cross-reactivity:

Compound	Spiked Concentration (ng/mL)	% Cross-reactivity
Aldosterone	1,000	ND
Androstenedione	10	1.157
Corticosterone	1,000	ND
Cortisol	1,000	ND
Cortisone	1,000	ND
11-Deoxycortisol	1,000	ND
21-Deoxycortisol	1,000	0.004
DHEA	1,000	ND
Dianabol	10	0.489
Dihydrotestosterone*	500	36.4
Epitestosterone	100	0.165
11-Hydroxytestosterone	10	1.90
19-Nortestosterone†	1000	21.02
Epitestosterone	100	0.165
Estradiol	51	0.025
Estriol	1,000	0.012
Estrone	1,000	0.005
Progesterone	1,000	0.005
17 α-Hydroxyprogesterone	1,000	ND
Transferrin	1,000	ND

ND = None detected (< 0.004)

*Literature states that salivary DHT levels expected in normal healthy adults, presenting no symptoms, is less than 10 pg/ml, well below the levels used to test cross reactivity. (10)

†Literature states that 19-nortestosterone is absent in normal healthy males & females, and that levels for pregnant females peak in the third trimester at 12-60 pg/ml, well below the levels used to test cross reactivity. (11)

F. Sensitivity:
The lower limit of sensitivity was determined by interpolating the mean minus 2 SDs for 10 sets of duplicates at 0 pg/ml standard. The minimal concentration of testosterone that can be distinguished from 0 is < 1.0 pg/ml.

G. Normal Ranges

Gender	N	Mean (pg/ml)	Median (pg/ml)	Range (5-95%)
Female	158	50.55	40.00	7.09-135.14
Male	87	165.50	136.18	59.05-335.12

Note: *Early morning samples may be significantly higher.*

References

1. Chard, T. (1990). *An introduction to radioimmunoassay and related techniques*. Amsterdam: Elsevier.
2. Schwartz, E.B., Granger, D.A., Susman, E.J., Gunnar, M.R., & Laird, B. (1998). Assessing salivary cortisol in studies of child development. *Child Development, 69,* 1503-1513.
3. Kivlighan, K. T., Granger, D. A., Schwartz, E. B., Nelson, V., & Curran, M. (2004). Quantifying blood leakage into the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva. *Hormones and Behavior, 46,* 39-46.
4. Schwartz, E., & Granger, D. A. (2004). Transferrin enzyme immunoassay for quantitative monitoring of blood contamination in saliva. *Clinical Chemistry, 50,* 654-656.
5. West, C.D., Mahajan, D.K., Chavre, V.J., Nabors, C.J. (1973). Simultaneous measurement of multiple plasma steroids by radioimmunoassay demonstrating episodic secretion. *Journal of Clinical Endocrinology & Metabolism, 36(6),* 1230-1236.
6. Brambilla, D.J., O'Donnell, A.B., Matsumotot, A.M., & McKinlay, J.B. *(2007).* Intraindividual variation in levels of serum testosterone and other reproductive and adrenal hormones in men. *Clinical Endocrinology, 67,* 853-862.
7. Kirschbaum, C., Read, G.F., & Hellhammer, D.H. (1992). *Assessment of hormones and drugs in saliva in biobehavioral research.* Kirkland, WA: Hogefe & Huber.
8. Shirtcliff, E.A., Granger, D.A., Schwartz, E., & Curran, M.J. (2001). Use of salivary biomarkers in biobehavioral research: Cotton-based sample collection methods can interfere with salivary immunoassay results. *Psychoneuroendocrinology, 26,* 165-173.
9. Shirtcliff, E. A., Granger, D A., & Likos, A. (2002). Gender differences in the validity of testosterone measured in saliva by immunoassay. *Hormones and Behavior, 42,* 62-69.
10. Wang, C., Wakelin, K., White, J., & Wood, P.J. (1986). Salivary androgens in the hirsutism: Are they of use in routine evaluation? *Annals of Clinical Biochemistry, 23(5),* 590-595.
11. Reznik, Y., Herrou, M., Dehennin, L., Lemaire, M., & Leymaire, P. (1987). Rising levels of 19-nortestosterone throughout pregnancy: Determination by radioimmunoassay and validation by gas chromatography-mass spectrometry. *Journal of Clinical Endocrinology & Metabolism, 64,* 1086-1088.

Seller's Limited Warranty

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It is expressly agreed that this limited warranty shall be in lieu of all warranties of fitness and in lieu of the warranty of merchantability. Seller shall not be liable for any incidental or consequential damages that arise out of the installation, use or operation of Seller's product or out of the breach of any express or implied warranties."

SALIMETRICS LLC
101 Innovation Blvd., Suite 302
State College, PA 16803 USA
(T) 814-234-7748, (F) 814-234-1608
800-790-2258 (USA & Canada only)
www.salimetrics.com
techservices@salimetrics.com

EXPANDED RANGE High Sensitivity SALIVARY CORTISOL ENZYME IMMUNOASSAY KIT

Catalog No. 1-3002/1-3012, (Single) 96-Well Kit;
1-3002-5/1-3012-5, (5-Pack) 480 Wells

For Research Use

Intended Use

The Salimetrics™ cortisol kit is a competitive immunoassay specifically designed and validated for the quantitative measurement of salivary cortisol. It is not intended for diagnostic use. It is intended only for research use in humans and some animals. Please read the complete kit insert before performing this assay. For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics or your local sales representative.

Introduction

Historically, the immunodiagnostic community's approach to the application of immunoassay techniques in the measurement of biomarkers in saliva has been problematic. This assay kit was designed to address those problems. First, prior to the late 1990s the majority of available immunoassays for saliva cortisol were modifications of protocols developed for use with serum/plasma. The standards used in those assay kits were suspended in a human serum matrix. Given that the composition of serum is markedly different from saliva, those standards are likely to produce results that are influenced by matrix differences. To ensure the most accurate results, this salivary immunoassay uses a matrix that matches saliva. Second, the level of cortisol in saliva is significantly lower than levels in the general circulation. The use of a standard curve developed to capture the range of values expected in serum/plasma samples is often not sensitive enough to capture the complete range of individual differences in the level expected in saliva. This assay was designed to capture the full range of salivary cortisol levels (0.003 to 3.0 µg/dL) while using only 25 uL of saliva per test. Third, the pH of saliva is easily lowered or raised by the consumption of food or drink. Performance of immunoassays becomes compromised as the pH of samples to be tested drops below 4 (1). This results in artificially inflated levels. This assay system is designed to be resilient to the effects of interference caused by collection techniques that affect pH. In addition, a built-in pH indicator warns the user of acidic or basic samples.

Test Principle

A microtitre plate is coated with monoclonal antibodies to cortisol. Cortisol in standards and unknowns competes with cortisol linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound cortisol peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of cortisol peroxidase detected is inversely proportional to the amount of cortisol present (2).

pH Indicator

A pH indicator in the assay diluent alerts the user to samples with high or low pH values. Acidic samples will turn the diluent yellow. Alkaline samples will turn the diluent purple. Dark yellow or purple wells indicate that a pH value for that sample should be obtained using pH strips. Cortisol values from samples with a pH $\leq$ 3.5 or $\geq$ 9.0 may be artificially inflated or lowered (1).

Precautions

1. Liquid stop solution is a 3-molar solution of sulfuric acid. This solution is caustic; use with care. Stop solution in powdered form is not sulfuric acid-based and is mildly corrosive.
2. This kit uses break-apart microtitre strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch with desiccant and used in the frame provided.
3. Do not mix components from different lots of kits.
4. When using a multichannel pipette, reagents should be added to duplicate wells at the same time. Follow the same sequence when adding additional reagents so that incubation time with reagents is the same for all wells.
5. See 'Material Safety Data' at the end of procedure.
6. As for all quantitative assays for salivary analytes, we recommend that samples be screened for possible blood contamination (3,4). This can be efficiently and economically accomplished using the Salimetrics Blood Contamination EIA Kit (Cat. No.: 1-1302/1-1312). Do not use dipsticks, which result in false positive values due to salivary enzymes.
7. Routine calibration of pipettes is critical for the best possible assay performance.
8. Pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate.
9. When running multiple plates, or multiple sets of strips, a standard curve should be run with each individual plate and/or set of strips.
10. The temperature of the laboratory may affect assays. Salimetrics' kits have been validated at 68 - 74°F (20 - 23.3°C). Higher or lower temperatures will cause an increase or decrease in OD values, respectively. Salimetrics cannot guarantee test results outside of this temperature range.
11. The quantity of reagent provided with this kit is sufficient for three individual runs. The volume of diluent and conjugate used for assays using less than a full plate should be scaled down accordingly, keeping the same dilution ratio.
12. Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month.

Storage

All components of this kit are stable at 2 - 8°C until the kit's expiration date.

Reagents and Reagent Preparation

1. **Anti-Cortisol Coated Plate:** A ready-to-use, 96-well microtitre plate pre-coated with monoclonal anti-cortisol antibodies in a resealable foil pouch.
2. **Cortisol Standards:** Six vials, 500 µL each, labeled A-F, containing cortisol concentrations of 3.000, 1.000, 0.333, 0.111, 0.037, and 0.012 µg/dL, in a synthetic saliva matrix with a non-mercury preservative. (Values in nmol/L are 82.77, 27.59, 9.19, 3.06, 1.02, and 0.33 nmol/L respectively.) Standards are traceable to the NIST standard.
3. **Wash Buffer:** 100 mL of a 10X phosphate buffered solution containing detergents and a non-mercury preservative. Dilute only the amount needed for current day's use. Discard any leftover reagent. Dilute the wash buffer concentrate 10-fold with room temperature deionized water (100 mL of 10X wash buffer to 900 mL of deionized H_2O). (**Note:** *If precipitate has formed in the concentrated wash buffer, it may be heated to 60°C for 15 minutes. Cool to room temperature before use in assay.)*
4. **Assay Diluent:** 60 mL of a phosphate buffered solution containing a pH indicator and a non-mercury preservative.
5. **Enzyme Conjugate:** 50 µL of a solution of cortisol labeled with horseradish peroxidase. Dilute prior to use with assay diluent.
6. **Tetramethylbenzidine (TMB):** 25 mL of a non-toxic, ready-to-use solution.
7. **Stop Solution:** 12.5 mL of a solution of sulfuric acid. Stop solution is provided in powdered form to some customers outside the USA. Reconstitute the powdered stop solution with 12.5 mL of deionized water. Let sit for 10 minutes before use.
8. **Non-specific Binding Wells (NSB):** These wells do not contain anti-cortisol antibody. In order to support multiple use, a strip of NSB wells is included. They are located in the foil pouch. Wells may be broken off and inserted where needed.

Materials Needed But Not Supplied

- Precision pipette to deliver 15 and 25 µL
- Precision multichannel pipette to deliver 50 µL and 200 µl
- Vortex
- Plate rotator (if unavailable, tap to mix)
- Plate reader with a 450 nm filter
- Log-linear graph paper or computer software for data reduction
- Deionized water
- Reagent reservoirs
- One disposable tube capable of holding 24 mL
- Pipette tips
- Serological pipette to deliver up to 24 mL

Specimen Collection

Donors may collect whole saliva by tilting the head forward, allowing the saliva to pool on the floor of the mouth, then passing the saliva through a short straw into a polypropylene vial. Adult samples and samples from children ages 6 and above may also be collected using the Salimetrics Oral Swab (SOS), P/N 5001.02. Infant samples may be collected with the Sorbette, P/N 5029, or cotton ropes, P/N 5016.00. Collection protocols are available on request. For accurate results Sorbettes and cotton collection materials should be completely saturated before removal. Do **not** add sodium azide to saliva samples as a preservative. Samples visibly contaminated with blood should be recollected.

Avoid sample collection within 60 minutes after eating a major meal or within 12 hours after consuming alcohol. Bovine hormones normally present in dairy products can cross-react with anti-cortisol antibodies and cause false results. Acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected. It is important to record the time and date of specimen collection when samples are obtained due to the diurnal variation in cortisol levels. Samples for Cushing's diagnosis should be collected at 11:00 pm. After collection it is important to keep samples cold, in order to avoid bacterial growth in the specimen. Refrigerate samples within 30 minutes, and freeze at or below -20°C within 4 hours after collection. (Samples may be stored at -20°C or lower for long-term storage.)

Freezing saliva samples will precipitate the mucins. On day of assay, thaw completely, vortex, and centrifuge at 1500 x g (@3000 rpm) for 15 minutes. Avoid multiple freeze-thaw cycles. However, if samples have been refrozen, centrifuge again prior to assaying. Samples should be at room temperature before adding to assay plate. Pipette clear sample into appropriate wells. Particulate matter may interfere with antibody binding, leading to falsely elevated results.

Procedure

Bring all reagents to room temperature. **Note**: It is important to keep the zip-lock pouch with the plate strips closed until warmed to room temperature as humidity may have an effect on the coated wells. Mix all reagents before use.

Step 1: Determine your plate layout. Here is a suggested layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.000 Std	3.000 Std	C-H	C-H								
B	1.000 Std.	1.000 Std	C-L	C-L								
C	0.333 Std	0.333 Std	Unk-1	Unk-1								
D	0.111 Std	0.111 Std	Unk-2	Unk-2								
E	0.037 Std	0.037 Std	Unk-3	Unk-3								
F	0.012 Std	0.012 Std	Unk-4	Unk-4								
G	Zero	Zero	Unk-5	Unk-5								
H	NSB	NSB	Unk-6	Unk-6								

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. If you choose to place non-specific binding wells in H-1, 2, remove strips 1 and 2 from the strip holder and break off the bottom wells. Place the strips back into the strip holder leaving H-1, 2 blank. Break off 2 NSB wells from the strip of NSBs included in the foil pouch. Place in H-1, 2. Alternatively, NSBs may be placed wherever you choose on the plate. Reseal the zip-lock pouch with unused wells and desiccant. Store at 2 - 8°C .
Caution: Extra NSB wells should not be used for determination of standards, controls or unknowns.

Step 3:
- Pipette 24 mL of assay diluent into a disposable tube. Set aside for Step 5.

Step 4:
- Pipette 25 µL of standards, controls, and unknowns into appropriate wells. Standards, controls, and unknowns should be assayed in duplicate.
- Pipette 25 µL of assay diluent into 2 wells to serve as the zero.
- Pipette 25 µL of assay diluent into each NSB well.

Step 5: Make a 1:1600 dilution of the conjugate by adding 15 µL of the conjugate to the 24 mL of assay diluent prepared in Step 3. (Scale down proportionally if not using the entire plate.) Immediately mix the diluted conjugate solution and pipette 200 µL into each well using a multichannel pipette.
Step 6: Mix plate on rotator for 5 minutes at 500 rpm (or tap to mix) and incubate at room temperature for an additional 55 minutes.

Step 7: Wash the plate 4 times with 1X wash buffer. A plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300µL of wash buffer into each well, and then discarding the liquid by inverting the plate over a sink. After each wash, the plate should be thoroughly blotted on paper towels before being turned upright. *If using a plate washer, blotting is still recommended after the last wash.*

Step 8: Add 200 µL of TMB solution to each well with a multichannel pipette.

Step 9: Mix on a plate rotator for 5 minutes at 500 rpm (or tap to mix) and incubate the plate in the dark at room temperature for an additional 25 minutes.

Step 10: Add 50 µL of stop solution with a multichannel pipette.

Step 11:
- Mix on a plate rotator for 3 minutes at 500 rpm (or tap to mix). **Caution**: *Do not mix at speeds over 600 rpm.*
- Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.
- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding stop solution. (Correction at 490 to 630 is desirable.)

Calculations

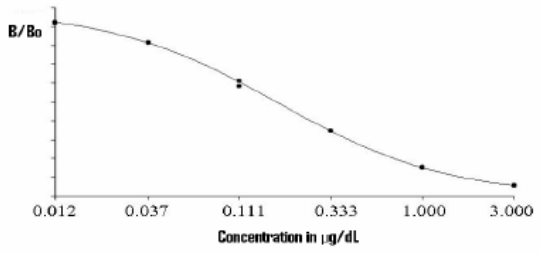
1. Compute the average optical density (OD) for all duplicate wells.
2. Subtract the average OD for the NSB wells from the average OD of the zero, standards, controls, and unknowns.
3. Calculate the percent bound (B/Bo) for each standard, control, and unknown by dividing the average OD (B) by the average OD for the zero (Bo).
4. Determine the concentrations of the controls and unknowns by interpolation using software capable of logistics. We recommend using a 4-parameter sigmoid minus curve fit.

Typical Results

The following charts and graphs are for illustration only and should not be used to calculate results from another assay.

Well	Sample	Average OD	B	B/Bo	Cortisol (µg/dL)
A1,A2	S1	0.094	0.071	0.048	3.000
B1,B2	S2	0.236	0.213	0.145	1.000
C1,C2	S3	0.524	0.501	0.340	0.333
D1,D2	S4	0.897	0.874	0.593	0.111
E1,E2	S5	1.219	1.196	0.812	0.037
F1,F2	S6	1.379	1.356	0.921	0.012
G1,G2	Bo	1.496	1.473	NA	NA
H1,H2	NSB	0.023	NA	NA	NA

Example: Cortisol 4-Parameter Sigmoid Minus Curve Fit



Material Safety Data*

Hazardous Ingredients
Liquid stop solution is caustic; use with care. ***Note:*** *Stop solution in powdered form is not sulfuric acid-based and is mildly corrosive.*
We recommend the procedures listed below for all kit reagents. Specific kit component MSDS sheets are available from Salimetrics upon request.

Handling
Follow good laboratory procedures when handling kit reagents. Laboratory coats, gloves, and safety goggles are recommended. Wipe up spills using standard absorbent materials while wearing protective clothing. Follow local regulations for disposal.

Emergency Exposure Measures
In case of contact, immediately wash skin or flush eyes with water for 15 minutes. Remove contaminated clothing. If inhaled, remove individual to fresh air. If individual experiences difficulty breathing, give oxygen and call a physician.

*The above information is believed to be accurate but is not all-inclusive. This information should only be used as a guide. Salimetrics shall not be liable for accidents or damage resulting from contact with reagents.

HS Cortisol EIA Assay Performance Characteristics

Recovery: Six saliva samples containing different levels of endogenous cortisol were spiked with known quantities of cortisol and assayed.

Sample	Endogenous (µg/dL)	Added (µg/dL)	Expected (µg/dL)	Observed (µg/dL)	Recovery (%)
1	0.088	2.000	2.088	2.176	104.2
2	0.077	0.300	0.377	0.380	100.8
3	0.062	0.011	0.073	0.071	97.3
4	0.066	2.500	2.566	2.723	106.1
5	0.210	0.330	0.510	0.508	99.6
6	0.086	0.011	0.097	0.094	96.9

Precision:

1. The intra-assay precision was determined from the mean of 14 (low) and 18 (high) replicates each.

Sample	N	Mean (µg/dL)	Standard Deviation (µg/dL)	Coefficient of Variation (%)
Level 1	18	0.999	0.033	3.35
Level 2	14	0.097	0.004	3.65

2. The inter-assay precision was determined from the mean of average duplicates for 12 separate runs.

Sample	N	Mean (µg/dL)	Standard Deviation (µg/dL)	Coefficient of Variation (%)
Level 1	12	1.020	0.038	3.75
Level 2	12	0.101	0.006	6.41

Linearity of Dilution: Two saliva samples were diluted with assay diluent and assayed.

Sample	Dilution Factor	Expected (µg/dL)	Observed (µg/dL)	Recovery (%)
1			2.176	
	1:2	1.088	1.065	97.9
	1:4	0.544	0.503	92.5
	1:8	0.272	0.233	85.7
	1:16	0.136	0.109	80.1
2			0.508	
	1:2	0.254	0.247	97.2
	1:4	0.127	0.118	92.9
	1:8	0.064	0.058	90.6
	1:16	0.032	0.031	96.9

Sensitivity: The lower limit of sensitivity was determined by interpolating the mean minus 2 SDs for 10 sets of duplicates at 0 µg/dL standard. The minimal concentration of cortisol that can be distinguished from 0 is < 0.003 µg/dL.

Correlation with Serum: The correlation between serum and saliva cortisol was determined by assaying 49 matched samples using the Diagnostic Systems Laboratories' serum Cortisol EIA and the Salimetrics ER HS Salivary Cortisol EIA.

The correlation between saliva and serum was highly significant, $r(47) = 0.91$, $p < 0.0001$.

Specificity of Antiserum

Compound	Spiked Concentration (ng/mL)	% Cross-reactivity in ER HS Salivary Cortisol EIA
Prednisolone	100	0.568
Prednisone	1000	ND
Cortisone	1000	0.130
11-Deoxycortisol	500	0.156
21-Deoxycortisol	1000	0.041
17 α-Hydroxyprogesterone	1000	ND
Dexamethasone	1000	19.2
Triamincinolone	1000	0.086
Corticosterone	10,000	0.214
Progesterone	1000	0.015
17β - Estradiol	10	ND
DHEA	10,000	ND
Testosterone	10,000	0.006
Transferrin	66,000	ND
Aldosterone	10,000	ND

ND = None detected (<0.004)

Salivary Cortisol Expected Ranges

Each laboratory should establish its own range of expected values. The following values have been reported for salivary cortisol.

Group	Number	Overall Range in µg/dL
Children, neonatal	275	ND - 3.417
Children, age 6 months	165	ND - 2.734

Group	Number	AM Range in µg/dL	PM Range in µg/dL
Children, ages 2.5-5.5	112	0.034 - 0.645	0.053 - 0.607
Children, ages 8-11	285	0.084 - 0.839	ND - 0.215
Adolescents, ages 12-18	403	0.021 - 0.883	ND - 0.259
Adult males, ages 21-30	26	0.112 - 0.743	ND - 0.308
Adult females, ages 21-30	20	0.272 - 1.348	ND - 0.359
Adult males, ages 31-50	67	0.122 - 1.551	ND - 0.359
Adult females, ages 31-50	31	0.094 - 1.515	ND - 0.181
Adult males, ages 51-70	28	0.112 - 0.812	ND - 0.228
Adult females, ages 51-70	23	0.149 - 0.739	0.022 - 0.254
All adults	192	0.094 - 1.551	ND - 0.359

Group	Number	2300h (ug/dL)
Normal subjects	19	0.007 – 0.115
Cushing's subjects	21	0.130 – 2.972

ND = None detected
Expected ranges for neonates to 5.5 years were derived using the Salimetrics Salivary Cortisol Immunoassay Kit.
Expected ranges for 8 to 18 years were reported from an unpublished manuscript, Pennsylvania State University's Behavioral Endocrinology Laboratory. Adult ranges were obtained from published literature (22).

References

1. Schwartz, E.B., Granger, D.A., Susman, E.J., Gunnar, M.R., & Laird, B. (1998). Assessing salivary cortisol in studies of child development. *Child Development, 69,* 1503-1513.
2. Chard, T. (1990). *An introduction to radioimmunoassay and related techniques.* Amsterdam: Elsevier.
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It is expressly agreed that this limited warranty shall be in lieu of all warranties of fitness and in lieu of the warranty of merchantability. Seller shall not be liable for any incidental or consequential damages that arise out of the installation, use or operation of Seller's product or out of the breach of any express or implied warranties."

Appendix E: Statistical Output

Lean Body Mass ANOVA

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time_LBM	Sphericity Assumed	48.475	1	48.475	2.990	.093
	Greenhouse-Geisser	48.475	1.000	48.475	2.990	.093
	Huynh-Feldt	48.475	1.000	48.475	2.990	.093
	Lower-bound	48.475	1.000	48.475	2.990	.093
Time_LBM * gender	Sphericity Assumed	8.712	1	8.712	.537	.469
	Greenhouse-Geisser	8.712	1.000	8.712	.537	.469
	Huynh-Feldt	8.712	1.000	8.712	.537	.469
	Lower-bound	8.712	1.000	8.712	.537	.469
Time_LBM * mode	Sphericity Assumed	4.000	1	4.000	.247	.623
	Greenhouse-Geisser	4.000	1.000	4.000	.247	.623
	Huynh-Feldt	4.000	1.000	4.000	.247	.623
	Lower-bound	4.000	1.000	4.000	.247	.623
Time_LBM * gender * mode	Sphericity Assumed	50.956	1	50.956	3.143	.086
	Greenhouse-Geisser	50.956	1.000	50.956	3.143	.086
	Huynh-Feldt	50.956	1.000	50.956	3.143	.086
	Lower-bound	50.956	1.000	50.956	3.143	.086
Error(Time_LBM)	Sphericity Assumed	518.869	32	16.215		
	Greenhouse-Geisser	518.869	32.000	16.215		
	Huynh-Feldt	518.869	32.000	16.215		
	Lower-bound	518.869	32.000	16.215		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1113563.737	1	1113563.737	5107.441	.000
gender	36785.932	1	36785.932	168.721	.000
mode	1054.004	1	1054.004	4.834	.035
gender * mode	463.717	1	463.717	2.127	.154
Error	6976.888	32	218.028		

Biceps Thickness ANOVA

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time_Bi_Thickness	Sphericity Assumed	.517	1	.517	13.999	.001
	Greenhouse-Geisser	.517	1.000	.517	13.999	.001
	Huynh-Feldt	.517	1.000	.517	13.999	.001
	Lower-bound	.517	1.000	.517	13.999	.001
Time_Bi_Thickness * gender	Sphericity Assumed	.029	1	.029	.795	.379
	Greenhouse-Geisser	.029	1.000	.029	.795	.379
	Huynh-Feldt	.029	1.000	.029	.795	.379
	Lower-bound	.029	1.000	.029	.795	.379
Time_Bi_Thickness * mode	Sphericity Assumed	.014	1	.014	.366	.550
	Greenhouse-Geisser	.014	1.000	.014	.366	.550
	Huynh-Feldt	.014	1.000	.014	.366	.550
	Lower-bound	.014	1.000	.014	.366	.550
Time_Bi_Thickness * gender * mode	Sphericity Assumed	.012	1	.012	.320	.575
	Greenhouse-Geisser	.012	1.000	.012	.320	.575
	Huynh-Feldt	.012	1.000	.012	.320	.575
	Lower-bound	.012	1.000	.012	.320	.575
Error(Time_Bi_ Thickness)	Sphericity Assumed	1.182	32	.037		
	Greenhouse-Geisser	1.182	32.000	.037		
	Huynh-Feldt	1.182	32.000	.037		
	Lower-bound	1.182	32.000	.037		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1009.548	1	1009.548	4923.261	.000
gender	13.340	1	13.340	65.054	.000
mode	.005	1	.005	.025	.876
gender * mode	.129	1	.129	.631	.433
Error	6.562	32	.205		

Quadriceps Thickness ANOVA (Females)

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Pre_Post	Sphericity Assumed	.688	1	.688	19.323	.000
	Greenhouse-Geisser	.688	1.000	.688	19.323	.000
	Huynh-Feldt	.688	1.000	.688	19.323	.000
	Lower-bound	.688	1.000	.688	19.323	.000
Pre_Post * Mode	Sphericity Assumed	.011	1	.011	.314	.582
	Greenhouse-Geisser	.011	1.000	.011	.314	.582
	Huynh-Feldt	.011	1.000	.011	.314	.582
	Lower-bound	.011	1.000	.011	.314	.582
Error(Pre_Post)	Sphericity Assumed	.677	19	.036		
	Greenhouse-Geisser	.677	19.000	.036		
	Huynh-Feldt	.677	19.000	.036		
	Lower-bound	.677	19.000	.036		

Tests of Between-Subjects Effects

Measure:MEASURE_1

Transformed Variable:Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1382.138	1	1382.138	1925.656	.000
Mode	.018	1	.018	.025	.877
Error	13.637	19	.718		

Quadriceps Muscle Thickness ANCOVA (Males)

Tests of Between-Subjects Effects

Dependent Variable: Post_Male_Quad

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.684(a)	2	2.842	30.361	.000
Intercept	.304	1	.304	3.250	.095
Covariate_Pre_Quad	3.538	1	3.538	37.795	.000
Mode	.068	1	.068	.721	.411
Error	1.217	13	.094		
Total	644.969	16			
Corrected Total	6.901	15			

a R Squared = .824 (Adjusted R Squared = .797)

Free Weight Bench Press Strength ANOVA

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time_Free_Bench	Sphericity Assumed	5710.992	1	5710.992	111.130	.000
	Greenhouse-Geisser	5710.992	1.000	5710.992	111.130	.000
	Huynh-Feldt	5710.992	1.000	5710.992	111.130	.000
	Lower-bound	5710.992	1.000	5710.992	111.130	.000
Time_Free_Bench * gender	Sphericity Assumed	107.542	1	107.542	2.093	.158
	Greenhouse-Geisser	107.542	1.000	107.542	2.093	.158
	Huynh-Feldt	107.542	1.000	107.542	2.093	.158
	Lower-bound	107.542	1.000	107.542	2.093	.158
Time_Free_Bench * mode	Sphericity Assumed	2.924	1	2.924	.057	.813
	Greenhouse-Geisser	2.924	1.000	2.924	.057	.813
	Huynh-Feldt	2.924	1.000	2.924	.057	.813
	Lower-bound	2.924	1.000	2.924	.057	.813
Time_Free_Bench * gender * mode	Sphericity Assumed	50.642	1	50.642	.985	.328
	Greenhouse-Geisser	50.642	1.000	50.642	.985	.328
	Huynh-Feldt	50.642	1.000	50.642	.985	.328
	Lower-bound	50.642	1.000	50.642	.985	.328
Error(Time_Free_Bench)	Sphericity Assumed	1644.493	32	51.390		
	Greenhouse-Geisser	1644.493	32.000	51.390		
	Huynh-Feldt	1644.493	32.000	51.390		
	Lower-bound	1644.493	32.000	51.390		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1435857.184	1	1435857.184	1203.744	.000
gender	173652.566	1	173652.566	145.581	.000
mode	1611.254	1	1611.254	1.351	.254
gender * mode	9.254	1	9.254	.008	.930
Error	38170.434	32	1192.826		

Machine Bench Press Strength ANOVA

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time_Machine_Bench	Sphericity Assumed	6809.194	1	6809.194	103.837	.000
	Greenhouse-Geisser	6809.194	1.000	6809.194	103.837	.000
	Huynh-Feldt	6809.194	1.000	6809.194	103.837	.000
	Lower-bound	6809.194	1.000	6809.194	103.837	.000
Time_Machine_Bench * gender	Sphericity Assumed	24.843	1	24.843	.379	.543
	Greenhouse-Geisser	24.843	1.000	24.843	.379	.543
	Huynh-Feldt	24.843	1.000	24.843	.379	.543
	Lower-bound	24.843	1.000	24.843	.379	.543
Time_Machine_Bench * mode	Sphericity Assumed	262.706	1	262.706	4.006	.054
	Greenhouse-Geisser	262.706	1.000	262.706	4.006	.054
	Huynh-Feldt	262.706	1.000	262.706	4.006	.054
	Lower-bound	262.706	1.000	262.706	4.006	.054
Time_Machine_Bench * gender * mode	Sphericity Assumed	30.263	1	30.263	.461	.502
	Greenhouse-Geisser	30.263	1.000	30.263	.461	.502
	Huynh-Feldt	30.263	1.000	30.263	.461	.502
	Lower-bound	30.263	1.000	30.263	.461	.502
Error(Time_Machine_ Bench)	Sphericity Assumed	2032.857	31	65.576		
	Greenhouse-Geisser	2032.857	31.000	65.576		
	Huynh-Feldt	2032.857	31.000	65.576		
	Lower-bound	2032.857	31.000	65.576		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1587491.652	1	1587491.652	1174.041	.000
gender	179461.118	1	179461.118	132.722	.000
mode	3747.225	1	3747.225	2.771	.106
gender * mode	64.782	1	64.782	.048	.828
Error	41916.964	31	1352.160		

Free Weight Squat Strength ANOVA

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time_Free_Squat	Sphericity Assumed	32376.853	1	32376.853	69.573	.000
	Greenhouse-Geisser	32376.853	1.000	32376.853	69.573	.000
	Huynh-Feldt	32376.853	1.000	32376.853	69.573	.000
	Lower-bound	32376.853	1.000	32376.853	69.573	.000
Time_Free_Squat * gender	Sphericity Assumed	84.657	1	84.657	.182	.673
	Greenhouse-Geisser	84.657	1.000	84.657	.182	.673
	Huynh-Feldt	84.657	1.000	84.657	.182	.673
	Lower-bound	84.657	1.000	84.657	.182	.673
Time_Free_Squat * mode	Sphericity Assumed	1415.298	1	1415.298	3.041	.092
	Greenhouse-Geisser	1415.298	1.000	1415.298	3.041	.092
	Huynh-Feldt	1415.298	1.000	1415.298	3.041	.092
	Lower-bound	1415.298	1.000	1415.298	3.041	.092
Time_Free_Squat * gender * mode	Sphericity Assumed	264.993	1	264.993	.569	.457
	Greenhouse-Geisser	264.993	1.000	264.993	.569	.457
	Huynh-Feldt	264.993	1.000	264.993	.569	.457
	Lower-bound	264.993	1.000	264.993	.569	.457
Error(Time_Free_Squat)	Sphericity Assumed	13030.194	28	465.364		
	Greenhouse-Geisser	13030.194	28.000	465.364		
	Huynh-Feldt	13030.194	28.000	465.364		
	Lower-bound	13030.194	28.000	465.364		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	5190264.846	1	5190264.846	1594.454	.000
gender	155538.643	1	155538.643	47.782	.000
mode	1801.108	1	1801.108	.553	.463
gender * mode	2519.259	1	2519.259	.774	.386
Error	91145.585	28	3255.199		

Machine Squat Strength ANOVA

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time_Machine_Squat	Sphericity Assumed	50790.847	1	50790.847	122.135	.000
	Greenhouse-Geisser	50790.847	1.000	50790.847	122.135	.000
	Huynh-Feldt	50790.847	1.000	50790.847	122.135	.000
	Lower-bound	50790.847	1.000	50790.847	122.135	.000
Time_Machine_Squat * gender	Sphericity Assumed	120.100	1	120.100	.289	.595
	Greenhouse-Geisser	120.100	1.000	120.100	.289	.595
	Huynh-Feldt	120.100	1.000	120.100	.289	.595
	Lower-bound	120.100	1.000	120.100	.289	.595
Time_Machine_Squat * mode	Sphericity Assumed	141.195	1	141.195	.340	.565
	Greenhouse-Geisser	141.195	1.000	141.195	.340	.565
	Huynh-Feldt	141.195	1.000	141.195	.340	.565
	Lower-bound	141.195	1.000	141.195	.340	.565
Time_Machine_Squat * gender * mode	Sphericity Assumed	136.120	1	136.120	.327	.572
	Greenhouse-Geisser	136.120	1.000	136.120	.327	.572
	Huynh-Feldt	136.120	1.000	136.120	.327	.572
	Lower-bound	136.120	1.000	136.120	.327	.572
Error(Time_Machine_ Squat)	Sphericity Assumed	11228.206	27	415.859		
	Greenhouse-Geisser	11228.206	27.000	415.859		
	Huynh-Feldt	11228.206	27.000	415.859		
	Lower-bound	11228.206	27.000	415.859		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	5350124.657	1	5350124.657	1166.792	.000
gender	182593.851	1	182593.851	39.821	.000
mode	13.420	1	13.420	.003	.957
gender * mode	7578.852	1	7578.852	1.653	.209
Error	123803.889	27	4585.329		

Testosterone Levels ANOVA

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time_Testosterone	Sphericity Assumed	733.121	2	366.560	.147	.864
	Greenhouse-Geisser	733.121	1.938	378.371	.147	.857
	Huynh-Feldt	733.121	2.000	366.560	.147	.864
	Lower-bound	733.121	1.000	733.121	.147	.704
Time_Testosterone * gender	Sphericity Assumed	5750.095	2	2875.048	1.153	.323
	Greenhouse-Geisser	5750.095	1.938	2967.681	1.153	.322
	Huynh-Feldt	5750.095	2.000	2875.048	1.153	.323
	Lower-bound	5750.095	1.000	5750.095	1.153	.292
Time_Testosterone * mode	Sphericity Assumed	2889.517	2	1444.758	.579	.564
	Greenhouse-Geisser	2889.517	1.938	1491.308	.579	.558
	Huynh-Feldt	2889.517	2.000	1444.758	.579	.564
	Lower-bound	2889.517	1.000	2889.517	.579	.453
Time_Testosterone * gender * mode	Sphericity Assumed	7284.880	2	3642.440	1.461	.241
	Greenhouse-Geisser	7284.880	1.938	3759.798	1.461	.241
	Huynh-Feldt	7284.880	2.000	3642.440	1.461	.241
	Lower-bound	7284.880	1.000	7284.880	1.461	.237
Error(Time_Testosterone)	Sphericity Assumed	139658.552	56	2493.903		
	Greenhouse-Geisser	139658.552	54.252	2574.256		
	Huynh-Feldt	139658.552	56.000	2493.903		
	Lower-bound	139658.552	28.000	4987.805		
Pre_Post	Sphericity Assumed	16539.272	1	16539.272	22.715	.000
	Greenhouse-Geisser	16539.272	1.000	16539.272	22.715	.000
	Huynh-Feldt	16539.272	1.000	16539.272	22.715	.000
	Lower-bound	16539.272	1.000	16539.272	22.715	.000
Pre_Post * gender	Sphericity Assumed	6582.135	1	6582.135	9.040	.006
	Greenhouse-Geisser	6582.135	1.000	6582.135	9.040	.006
	Huynh-Feldt	6582.135	1.000	6582.135	9.040	.006
	Lower-bound	6582.135	1.000	6582.135	9.040	.006
Pre_Post * mode	Sphericity Assumed	1034.208	1	1034.208	1.420	.243
	Greenhouse-Geisser	1034.208	1.000	1034.208	1.420	.243
	Huynh-Feldt	1034.208	1.000	1034.208	1.420	.243
	Lower-bound	1034.208	1.000	1034.208	1.420	.243
Pre_Post * gender * mode	Sphericity Assumed	6549.530	1	6549.530	8.995	.006
	Greenhouse-Geisser	6549.530	1.000	6549.530	8.995	.006
	Huynh-Feldt	6549.530	1.000	6549.530	8.995	.006
	Lower-bound	6549.530	1.000	6549.530	8.995	.006
Error(Pre_Post)	Sphericity Assumed	20387.306	28	728.118		
	Greenhouse-Geisser	20387.306	28.000	728.118		
	Huynh-Feldt	20387.306	28.000	728.118		
	Lower-bound	20387.306	28.000	728.118		
Time_Testosterone * Pre_Post	Sphericity Assumed	1185.850	2	592.925	1.159	.321
	Greenhouse-Geisser	1185.850	1.322	896.843	1.159	.305
	Huynh-Feldt	1185.850	1.511	784.775	1.159	.311
	Lower-bound	1185.850	1.000	1185.850	1.159	.291
Time_Testosterone * Pre_Post * gender	Sphericity Assumed	1859.792	2	929.896	1.817	.172
	Greenhouse-Geisser	1859.792	1.322	1406.538	1.817	.185
	Huynh-Feldt	1859.792	1.511	1230.778	1.817	.182
	Lower-bound	1859.792	1.000	1859.792	1.817	.188
Time_Testosterone * Pre_Post * mode	Sphericity Assumed	252.660	2	126.330	.247	.782
	Greenhouse-Geisser	252.660	1.322	191.084	.247	.689
	Huynh-Feldt	252.660	1.511	167.206	.247	.720
	Lower-bound	252.660	1.000	252.660	.247	.623
Time_Testosterone * Pre_Post * gender * mode	Sphericity Assumed	1518.440	2	759.220	1.484	.236
	Greenhouse-Geisser	1518.440	1.322	1148.377	1.484	.237
	Huynh-Feldt	1518.440	1.511	1004.877	1.484	.238
	Lower-bound	1518.440	1.000	1518.440	1.484	.233
Error(Time_ Testosterone*Pre_Post)	Sphericity Assumed	28651.918	56	511.641		
	Greenhouse-Geisser	28651.918	37.023	773.896		
	Huynh-Feldt	28651.918	42.310	677.191		
	Lower-bound	28651.918	28.000	1023.283		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	3009882.878	1	3009882.878	328.580	.000
gender	373149.861	1	373149.861	40.736	.000
mode	24050.469	1	24050.469	2.626	.116
gender * mode	19120.452	1	19120.452	2.087	.160
Error	256487.881	28	9160.281		

Cortisol Levels ANOVA

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time_Cortisol	Sphericity Assumed	.001	2	.000	.004	.996
	Greenhouse-Geisser	.001	1.725	.000	.004	.991
	Huynh-Feldt	.001	2.000	.000	.004	.996
	Lower-bound	.001	1.000	.001	.004	.947
Time_Cortisol * gender	Sphericity Assumed	.071	2	.036	.396	.675
	Greenhouse-Geisser	.071	1.725	.041	.396	.645
	Huynh-Feldt	.071	2.000	.036	.396	.675
	Lower-bound	.071	1.000	.071	.396	.534
Time_Cortisol * mode	Sphericity Assumed	.202	2	.101	1.123	.333
	Greenhouse-Geisser	.202	1.725	.117	1.123	.327
	Huynh-Feldt	.202	2.000	.101	1.123	.333
	Lower-bound	.202	1.000	.202	1.123	.299
Time_Cortisol * gender * mode	Sphericity Assumed	.239	2	.120	1.330	.273
	Greenhouse-Geisser	.239	1.725	.139	1.330	.272
	Huynh-Feldt	.239	2.000	.120	1.330	.273
	Lower-bound	.239	1.000	.239	1.330	.259
Error(Time_Cortisol)	Sphericity Assumed	4.858	54	.090		
	Greenhouse-Geisser	4.858	46.588	.104		
	Huynh-Feldt	4.858	54.000	.090		
	Lower-bound	4.858	27.000	.180		
Pre_Post	Sphericity Assumed	.052	1	.052	.936	.342
	Greenhouse-Geisser	.052	1.000	.052	.936	.342
	Huynh-Feldt	.052	1.000	.052	.936	.342
	Lower-bound	.052	1.000	.052	.936	.342
Pre_Post * gender	Sphericity Assumed	7.59E-006	1	7.59E-006	.000	.991
	Greenhouse-Geisser	7.59E-006	1.000	7.59E-006	.000	.991
	Huynh-Feldt	7.59E-006	1.000	7.59E-006	.000	.991
	Lower-bound	7.59E-006	1.000	7.59E-006	.000	.991
Pre_Post * mode	Sphericity Assumed	.064	1	.064	1.149	.293
	Greenhouse-Geisser	.064	1.000	.064	1.149	.293
	Huynh-Feldt	.064	1.000	.064	1.149	.293
	Lower-bound	.064	1.000	.064	1.149	.293
Pre_Post * gender * mode	Sphericity Assumed	3.48E-005	1	3.48E-005	.001	.980
	Greenhouse-Geisser	3.48E-005	1.000	3.48E-005	.001	.980
	Huynh-Feldt	3.48E-005	1.000	3.48E-005	.001	.980
	Lower-bound	3.48E-005	1.000	3.48E-005	.001	.980
Error(Pre_Post)	Sphericity Assumed	1.495	27	.055		
	Greenhouse-Geisser	1.495	27.000	.055		
	Huynh-Feldt	1.495	27.000	.055		
	Lower-bound	1.495	27.000	.055		
Time_Cortisol * Pre_Post	Sphericity Assumed	.146	2	.073	1.635	.204
	Greenhouse-Geisser	.146	1.362	.107	1.635	.212
	Huynh-Feldt	.146	1.569	.093	1.635	.210
	Lower-bound	.146	1.000	.146	1.635	.212
Time_Cortisol * Pre_Post * gender	Sphericity Assumed	.004	2	.002	.049	.952
	Greenhouse-Geisser	.004	1.362	.003	.049	.893
	Huynh-Feldt	.004	1.569	.003	.049	.918
	Lower-bound	.004	1.000	.004	.049	.826
Time_Cortisol * Pre_Post * mode	Sphericity Assumed	.102	2	.051	1.143	.326
	Greenhouse-Geisser	.102	1.362	.075	1.143	.311
	Huynh-Feldt	.102	1.569	.065	1.143	.317
	Lower-bound	.102	1.000	.102	1.143	.294
Time_Cortisol * Pre_Post * gender * mode	Sphericity Assumed	.004	2	.002	.050	.951
	Greenhouse-Geisser	.004	1.362	.003	.050	.891
	Huynh-Feldt	.004	1.569	.003	.050	.916
	Lower-bound	.004	1.000	.004	.050	.824
Error(Time_Cortisol*Pre_Post)	Sphericity Assumed	2.412	54	.045		
	Greenhouse-Geisser	2.412	36.775	.066		
	Huynh-Feldt	2.412	42.360	.057		
	Lower-bound	2.412	27.000	.089		

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	17.099	1	17.099	117.059	.000
gender	.152	1	.152	1.043	.316
mode	.001	1	.001	.007	.932
gender * mode	.006	1	.006	.042	.840
Error	3.944	27	.146		

Testosterone to Cortisol Ratio ANOVA

Tests of Within-Subjects Effects

Measure:MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Chronic_Workout	Sphericity Assumed	.602	2	.301	.046	.955
	Greenhouse-Geisser	.602	1.728	.348	.046	.937
	Huynh-Feldt	.602	2.000	.301	.046	.955
	Lower-bound	.602	1.000	.602	.046	.832
Chronic_Workout * Mode	Sphericity Assumed	14.964	2	7.482	1.137	.329
	Greenhouse-Geisser	14.964	1.728	8.657	1.137	.324
	Huynh-Feldt	14.964	2.000	7.482	1.137	.329
	Lower-bound	14.964	1.000	14.964	1.137	.297
Chronic_Workout * Gender	Sphericity Assumed	4.019	2	2.010	.305	.738
	Greenhouse-Geisser	4.019	1.728	2.325	.305	.707
	Huynh-Feldt	4.019	2.000	2.010	.305	.738
	Lower-bound	4.019	1.000	4.019	.305	.586
Chronic_Workout * Mode * Gender	Sphericity Assumed	37.336	2	18.668	2.836	.069
	Greenhouse-Geisser	37.336	1.728	21.601	2.836	.077
	Huynh-Feldt	37.336	2.000	18.668	2.836	.069
	Lower-bound	37.336	1.000	37.336	2.836	.105
Error(Chronic_Workout)	Sphericity Assumed	315.953	48	6.582		
	Greenhouse-Geisser	315.953	41.483	7.616		
	Huynh-Feldt	315.953	48.000	6.582		
	Lower-bound	315.953	24.000	13.165		
Pre_Post	Sphericity Assumed	43.141	1	43.141	11.892	.002
	Greenhouse-Geisser	43.141	1.000	43.141	11.892	.002
	Huynh-Feldt	43.141	1.000	43.141	11.892	.002
	Lower-bound	43.141	1.000	43.141	11.892	.002
Pre_Post * Mode	Sphericity Assumed	.037	1	.037	.010	.920
	Greenhouse-Geisser	.037	1.000	.037	.010	.920
	Huynh-Feldt	.037	1.000	.037	.010	.920

	Lower-bound	.037	1.000	.037	.010	.920
Pre_Post * Gender	Sphericity Assumed	27.257	1	27.257	7.513	.011
	Greenhouse-Geisser	27.257	1.000	27.257	7.513	.011
	Huynh-Feldt	27.257	1.000	27.257	7.513	.011
	Lower-bound	27.257	1.000	27.257	7.513	.011
Pre_Post * Mode * Gender	Sphericity Assumed	.019	1	.019	.005	.942
	Greenhouse-Geisser	.019	1.000	.019	.005	.942
	Huynh-Feldt	.019	1.000	.019	.005	.942
	Lower-bound	.019	1.000	.019	.005	.942
Error(Pre_Post)	Sphericity Assumed	87.066	24	3.628		
	Greenhouse-Geisser	87.066	24.000	3.628		
	Huynh-Feldt	87.066	24.000	3.628		
	Lower-bound	87.066	24.000	3.628		
Chronic_Workout * Pre_Post	Sphericity Assumed	3.617	2	1.809	.665	.519
	Greenhouse-Geisser	3.617	1.795	2.015	.665	.504
	Huynh-Feldt	3.617	2.000	1.809	.665	.519
	Lower-bound	3.617	1.000	3.617	.665	.423
Chronic_Workout * Pre_Post * Mode	Sphericity Assumed	.487	2	.243	.089	.915
	Greenhouse-Geisser	.487	1.795	.271	.089	.896
	Huynh-Feldt	.487	2.000	.243	.089	.915
	Lower-bound	.487	1.000	.487	.089	.767
Chronic_Workout * Pre_Post * Gender	Sphericity Assumed	2.340	2	1.170	.430	.653
	Greenhouse-Geisser	2.340	1.795	1.303	.430	.632
	Huynh-Feldt	2.340	2.000	1.170	.430	.653
	Lower-bound	2.340	1.000	2.340	.430	.518
Chronic_Workout * Pre_Post * Mode * Gender	Sphericity Assumed	2.211	2	1.105	.407	.668
	Greenhouse-Geisser	2.211	1.795	1.231	.407	.647
	Huynh-Feldt	2.211	2.000	1.105	.407	.668
	Lower-bound	2.211	1.000	2.211	.407	.530
Error(Chronic_Workout*Pre_Post)	Sphericity Assumed	130.523	48	2.719		
	Greenhouse-Geisser	130.523	43.086	3.029		
	Huynh-Feldt	130.523	48.000	2.719		

	Lower-bound	130.523	24.000	5.438		

Tests of Between-Subjects Effects

Measure:MEASURE_1

Transformed Variable:Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	4405.851	1	4405.851	104.248	.000
Mode	38.564	1	38.564	.912	.349
Gender	1098.420	1	1098.420	25.990	.000
Mode * Gender	14.788	1	14.788	.350	.560
Error	1014.321	24	42.263		