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The effect of combining a Wingate sprint with circuit weight training on growth hormone in response to exercise.

Hung-Sheng Hsu

Hung-Sheng Hsu
Candidate

Health, Exercise & Sports Science
Department

This dissertation is approved, and it is acceptable in quality and form for publication:

Approved by the Dissertation Committee:

Len Kravitz, Ph.D., Chairperson

Christine Mermier, Ph.D., Co-Chairperson

Suzanne Schneider, Ph.D.

Karol Dokladny, Ph.D.

THE EFFECT OF COMBINING A WINGATE SPRINT WITH CIRCUIT WEIGHT TRAINING ON GROWTH HORMONE IN RESPONSE TO EXERCISE

by

HUNG-SHENG HSU

B.A., Child and Youth Welfare, Providence University (靜宜大學),
Taiwan, 2002
M.S., Exercise Science, Louisiana Tech University, 2008

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The Effect of Combining A Wingate Sprint with Circuit Weight Training On Growth Hormone In Response To Exercise.

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Hung-Sheng Hsu

B.A., Child and Youth Welfare, Providence University (靜宜大學), Taiwan, 2002

M.S., Exercise Science, Louisiana Tech University, 2008

Ph.D., Physical Education, Sports and Exercise Science, University of New Mexico, 2015

ABSTRACT

Human growth hormone (HGH) in response to resistance training has been suggested to be correlated with the muscle hypertrophy adapted to resistance training. The purpose of this study was to investigate whether or not adding a Wingate sprint to a circuit weight training session would optimize the acute HGH response to exercise. **Methods.** Seven recreational resistance-trained male subjects (n=8) performed 4 exercise trials in a balanced order: 1) a Wingate sprint only(W), 2) a circuit weight training session (C), 3) a Wingate sprint prior to circuit weight training session (WC), 4) a Wingate sprint following circuit weight training session (CW). Blood samples were collected at rest prior to each exercise trial, and at 5, 10, 20, 30, 45, and 60 min post cool-down for all trials. The Wingate sprint was against a resistance of 7% of the subjects' body mass (0.7Nm/kg) on a cycle ergometer. The circuit weight training session was a

full body workout (40%-50% 1RM for circuit 1 and 70% 1RM for circuit 2 and 3) consisting of 6 exercises in the following exercise order – bench press, bent-over barbell row, smith machine squat, seated dumbbell shoulder press without back support, Romanian deadlift, and latissiumus dorsi pull-down. HGH was measured at the respective time for blood collections. Blood lactate concentration [La^-] and rate of perceived exertion (RPE) were measured immediately after exercise and prior to cool-down in each trial. **Results.** Both the WC and CW trials resulted in significant increases in serum HGH concentrations up to 30 min post cool-down. The C trial resulted in a significant increase in serum HGH concentration up to 20 min post cool-down. No significant increase in serum HGH concentration post cool-down was found after the W trial. The CW trial resulted in: 1) significantly higher [La^-] and RPE compared to other trials ($p<.05$), 2) a significantly higher serum HGH concentration compared to the W and the C trials at 20 min post cool-down ($p<.05$), 3) a significantly higher HGH concentration compared to the C and the WC trials at 30 min post cool-down ($p<.05$), 4) a significantly higher HGH AUC compared to the C and the W trials ($p<.05$), 5) a significant higher peak HGH concentration compared to the C trial ($p<.05$). No significant difference was found in peak power, mean power, fatigue ratio, and peak cadence between all trials. No correlation was found between [La^-] and HGH AUC, [La^-] and peak HGH, and age and HGH AUC. **Conclusion.** The CW might produce greater muscle adaptation from exercise compared to other trials.

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SYMBOLS/ABBREVIATIONS

>: greater than or equal to

>: greater than

<: less than

±: plus or minus

~: approximately

C°: degrees Celsius

%: percentage

vs.: versus

ml: milliliter

g: gram

kg: kilogram

ng/ml: nanogram per milliliter

µg/ml: migrogram per milliliter

µg/L: migrogram per liter

mM: millimolar

min: minute

sec: second

yr.: years

ml/kg/min: milliliter per kilogram per minute

mmol/L: millimole per liter

ANOVA: analysis of variance

ATP: adenosine triphosphate

bpm: beats per minute

BM: body mass

BFR: blood flow restriction

mmHg: millimeter per Mercury

mU/l: milliuits per liter

ELISA: enzyme-linked immunosorbent assay

CO2: carbon dioxide

HR: heart rate

n: number of subjects

PV: plasma volume

SD: standard deviation

VO2 max: maximum oxygen uptake

HGH: human growth hormone

IGF-1: insulin-like growth factor-1

GHRH: growth hormone releasing hormone

AUC: area under curve

RM: repetition maximum

rep: repetition

PPO: peak power output

NSCA: national strength and conditioning association

CSCS: certified strength and conditioning specialist

CHAPTER 1: Introduction

What is human growth hormone (HGH)?

Human growth hormone (HGH), a peptide hormone synthesized, stored, and secreted by the anterior pituitary gland, and it is one of the most important anabolic hormones. HGH secretion follows a diurnal pattern which interacts with meal time (Birketvedt et al., 2012).The largest and most predictable peak (13 to 72 ng/ml) of HGH occurs within an hour after the onset of sleep. During waking hours, HGH is typically less than 5 ng/mL under non-exercise conditions (Takahashi, Kipnis, & Daughaday, 1968). The highest daily HGH secretion rate (1-1.8mg/) is seen in late puberty phase, and the lower secretion rates (15-100µg) have been observed in middle aged, elderly, lean persons, and obese subjects (Giustina & Veldhuis, 1998). In growth hormone deficient adults, quality of life can be impaired due to reduced maximal oxygen consumption and increased muscle fatigue, but this can be reversed by growth hormone replacement therapy leading to increases in lean body mass (Gibney, Healy, & Sonksen, 2007). In college-age men, there is a significant correlation between mean absolute exercise-induced HGH increase in response to resistance training and the relative degree of muscle hypertrophy in both type I and type II fibers (McCall, Byrnes, Fleck, Dickinson, & Kraemer, 1999). The anabolic function of human growth hormone has been shown to improve performance in exercise and various sports, leading to the problem of HGH abuse in recent years (Hoffman et al., 2009).

HGH response to anaerobic exercise

Many studies have shown the pattern of HGH secretion in response to resistance training (Hoffman et al., 2003; Kraemer et al., 1993; Rahimi, Qaderi, Faraji, & Boroujerdi, 2010; Smilios, Pilianidis, Karamouzis, Parlavantzas, & Tokmakidis, 2007) or to anaerobic sprinting exercises, such as the Wingate anaerobic test (Gilbert, Stokes, Hall, & Thompson, 2008; K. A. Stokes, Gilbert, Hall, Andrews, & Thompson, 2013; Wahl et al., 2013a). In traditional resistance training, a moderate exercise intensity of 10 repetition-maximum (RM) with short rest periods (1 minute) between sets is suggested to stimulate higher post-exercise circulating HGH when compared to higher intensity (5RM) with relatively longer rest periods (3 minutes) between sets (Kraemer et al., 1993). Studies also have reported HGH secretion significantly increases compared to rest after resistance training to exhaustion at an exercise intensity as low as 60% of 1RM (Hoffman et al., 2003; Smilios et al., 2007). When considering the rest period between sets, results have suggested a reduction in rest interval (from 120 seconds down to 90 seconds) significantly increased post-training circulating HGH concentrations (Rahimi et al., 2010). At the same training intensity, the length of the rest interval has been shown to be inversely related to the duration the HGH was increased (Bottaro, Martins, Gentil, & Wagner, 2009).

Circuit training is a type of resistance training which has short rest intervals between exercises. In circuit training, different exercises engage various muscle groups in each circuit. This design allows participants to maintain a greater work-to-rest ratio as compared to traditional resistance training, potentially leading to a greater total production of metabolites, such as lactate and protons. The HGH response to exercise was suggested as a concept of a certain level of metabolic disturbance, such as exercise-

induced increases in blood lactate concentrations and/or decreases in pH levels deviating from its resting level (Wahl et al., 2013b). In college age men, the greater work-to-rest ratio (due to reduced rest intervals) in circuit weight training, when compared to a traditional weight training session with a higher exercise intensity, has led to a significantly greater excess post-exercise oxygen consumption up to 20 min post exercise (Murphy & Schwarzkopf, 1992), leading to an assumption of greater metabolic disturbance. A significant increase in circulating HGH value has been reported after a circuit weight training session at intensities as low as 35% of 1RM with no rest interval between exercises and a 1-minute rest between circuits (Ghanbari-Niaki, Nabatchian, & Hedayati, 2007).

HGH responses have been investigated using all-out sprinting exercises (Goto, Ishii, Kurokawa, & Takamatsu, 2007; K. A. Stokes et al., 2013; K. A. Stokes, Nevill, Hall, & Lakomy, 2002b; Wahl et al., 2013a; Wahl, Zinner, Achtzehn, Bloch, & Mester, 2010) and flywheel resistance exercise (K. A. Stokes, Nevill, Hall, & Lakomy, 2002a; K. A. Stokes, Sykes, Gilbert, Chen, & Frystyk, 2010). Reproducibility of the HGH response to an all-out 30 second cycling sprint exercise may have large inter-individual and within individual differences (K. A. Stokes, Nevill, Lakomy, & Hall, 2003). A 30 second Wingate anaerobic test at 7.5% of body mass resulted in significant increases in circulating HGH values compared to rest (Gilbert et al., 2008; K. Stokes, Nevill, Frystyk, Lakomy, & Hall, 2005; K. Stokes, Nevill, & Hall, 2006; K. A. Stokes, Tyler, & Gilbert, 2008). A Study by Gilbert et al. (2008) reported that HGH increased from 0.09±0.03 µg/l at rest to 6.48±6.01 µg/l after sprinting in young men and from 0.1±0.09 µg/l to 2.6±1.80 µg/l after sprinting in middle age men (Gilbert et al., 2008). A sprint interval of 8 sets of

5-second all-out sprints with a 30-second rest interval between sprints against 5% of body mass resulted in significantly higher HGH value of ~6.5 ng/ml when compared to the resting value at less than 1 ng/ml (Goto, Ishii, Kurokawa, et al., 2007). However, a single sprint of 6 seconds duration at 7.5% body mass didn't cause any significant change in HGH secretion (K. A. Stokes et al., 2002b). An increase in cycling cadence due to a decrease in flywheel resistance (7-7.5% of body mass) also resulted in a significant increase in the HGH secretion response to a 30 second sprint when compared to the sprint at lower cadence with a higher flywheel resistance (9-10% of body mass) (K. A. Stokes et al., 2002a; K. A. Stokes et al., 2010). No significant difference in blood pH level or blood lactate concentration was reported between the high and low cadence trials. The authors (Stokes et al, 2002a) suggested that a proprioceptive mechanism of muscle-pituitary axis pathway (McCall, Grindeland, Roy, & Edgerton, 2000) may play a role in the greater HGH secretion in the higher cadence trial.

HGH response to endurance exercise

Endurance exercise also has been reported to induce significant circulating HGH values (Craig, Lucas, Pohlrman, & Stelling, 1991; Gilbert et al., 2008; Rubin et al., 2003; Sartorio et al., 2008; K. A. Stokes et al., 2013). The magnitude of HGH responses to endurance exercise followed the positive dose-response relationship with exercise intensity in both men and women (Pritzlaff-Roy et al., 2002; Pritzlaff et al., 1999). A minimal exercise intensity at or above lactate threshold, and an exercise duration greater than 10 minutes were suggested to increase HGH secretion (Felsing, Brasel, & Cooper, 1992). In addition, studies have shown the significant increase in HGH secretion with endurance exercise was reported as low as 50% of VO_{2max} for an hour (Goto,

Higashiyama, Ishii, & Takamatsu, 2005), but not after 40% of VO_{2max} for 20 minutes duration (Bussau, Ferreira, Jones, & Fournier, 2006, 2007).

HGH response to single session of concurrent training

Concurrent training is an exercise design that combines both endurance and resistance training for an optimal gain in sports performance. In the concurrent training design, the resistance training and endurance training sessions are often conducted in one session (Ferrari et al., 2013), or on separate days or sessions. The first concurrent training study on HGH responses reported a significantly greater HGH response to endurance exercise alone (30 minutes running at 75% HRmax) than to resistance exercise alone (7 resistance exercises for 3 sets of 10 repetitions at 75% of 1RM), with the endurance exercise performed prior to resistance exercise (Craig et al., 1991). Later, the concurrent training design with the order of a 60-minute endurance training at 50%VO_{2max} prior to strength training was found to suppress circulating HGH values post resistance training (Goto, Higashiyama, et al., 2005). If the endurance exercise duration was reduced to 5 minutes, the post resistance training circulating HGH value was reported to be significantly higher than the pre-concurrent training value at rest. Prior endurance exercise suppression of the HGH response to resistance exercise was also reported by Taipale and Hakkinen (2013). Other studies reported that the acute circulating HGH concentration attenuations during concurrent training were simply due to the different magnitudes of HGH secretion rates between endurance and resistance training (Schumann et al., 2013; Schumann et al., 2014; Taipale & Hakkinen, 2013). The inconsistency of HGH responses to endurance and resistance exercise and the order effect when combining both is shown in the literature. Taipale and Hakkinen (2013) reported no

significant differences in circulating HGH values between endurance and resistance exercise, while Schummann et al. (2013, 2014) showed significant differences in HGH responses to both endurance and resistance exercise. Therefore, it is possible that the understanding of the acute HGH response to exercise is still limited and the evidence remains equivocal.

Though the literature cited above has discussed the HGH response to exercise and its combination order effect, no study has looked at the order effect of a 30 second all-out sprint (Wingate anaerobic test) combined with resistance training. The Wingate anaerobic test has been shown to consistently induce a significant increase in post-exercise circulating HGH values (K. Stokes, 2003). The HGH response to an exercise session consisting of both a Wingate test and resistance exercise is unknown.

Problem statement

Among all the combinations of exercise types and order effects mentioned above, no study has looked the effect of the combination of Wingate and circuit weight training. During a Wingate anaerobic test, muscle fatigue is presented as the decrease in power output, but also as the decrease in cadence (muscle contraction velocity). However, no literature was found on the combination of these two exercise models together, and there is no published study using a similar type of exercise combination to study HGH responses to exercise. If combining a Wingate sprint with circuit weight training results in a greater HGH response, there is a possibility that implementation of this type of exercise combination to a long-term training program may further promote muscle hypertrophic adaptations to training.

Purpose of the study

The purpose of this study is to determine whether or not a 30-second all-out Wingate prior to or after circuit weight training will result in different post-exercise HGH values when compared to circuit weight training only and Wingate anaerobic test only trials.

Hypothesis

The following hypotheses were tested in this study.

Hypothesis I.

A 30-second all-out sprint either prior to or following circuit weight training exercise will increase the duration and the concentration of the circulating HGH value post-exercise compared to the HGH response to the trials of either circuit weight training alone or a 30 second all-out sprint alone when all trials are performed between 6-8 am in the mornings on non-consecutive days.

Rationale

Studies have shown the HGH response to weight training exercise may be promoted by the increase in total training volume and exercise intensity. A single 30 second all-out Wingate sprint exercise has been shown to induce sufficient stress to significantly increase post exercise HGH circulation level. Hypothesis I predicts that adding a short 30-sec Wingate sprint to a weight training circuit will result in greater HGH concentration which would be elevated for a longer duration after exercise due to

an overall greater exercise volume and stress in one training session when compared to the trials of either a Wingate sprint or circuit weight training alone.

Hypothesis II.

A Wingate sprint prior to circuit weight training exercise may result in the highest peak HGH value and longest duration of a significantly elevation in circulating HGH values after an exercise session among all trials.

Rationale

The HGH response to exercise training has been suggested to be caused by a the metabolic disturbance of exercise-induced increases in blood lactate concentrations and/or decreases in pH levels (Wahl et al., 2013b). A mouse model (a study using mice) also showed GH secretion from the anterior pituitary gland was promoted by fast-twitch muscle afferent input which could overwrite the suppression effect by slow-twitch muscle fiber afferent input (McCall et al., 2001). Based on this concept, a 30-second all-out sprint immediately prior to resistance training will cause the weightlifting exercise to be performed at a non-fully-recovered state, which may contribute to a greater metabolic disturbance and greater fast-twitch muscle fiber activation (Sahlin, Sorensen, Gladden, Rossiter, & Pedersen, 2005), leading to the greatest HGH secretion pattern in this study.

Scope of the study

We studied an all-out sprint exercise and a circuit training session with short rest intervals between exercises, therefore, we recruited subjects who have been engaged in recreational resistance training (at least lifting 2 times/week) for more than 6 months,

assessed using a physical activity questionnaire. All subjects were screened for endocrine disorders, and cardiovascular and musculoskeletal disease using a medical history questionnaire. Subjects were excluded from the study if they had any of the following conditions: 1) taking any ergogenic supplements that could impact exercise performance; 2) current smokers;3) were not able to perform every exercise with correct technique, as determined by a NSCA Certified Strength and Conditioning Specialist (C.S.C.S.) In addition to these conditions, subjects had to be able to finish the trial of a 30-second all-out Wingate prior to circuit weight training) by completing all repetitions in each exercise in the first two circuits (a warm-up set and the first working set) without any help.

Assumption

The following assumptions are made:

1. All subjects will follow the pre-test guidelines, including no strenuous exercise during the testing period and overnight fasting prior to all visits. This eliminates meal effects on HGH secretion.

2. All subjects are honest in reporting their health history and physical activity, including any supplements they have been taken in the past 3 months.

Limitations

The hypotheses of this study are based on the rationales from the results of other studies which used a combination of resistance exercise and endurance exercise models. However, since no study used a combination of anaerobic exercise and resistance

exercise models, the results may or may not support the hypotheses. There is only one publication to investigate the acute human growth hormone response to exercise between populations with different training status, such as sprinters and endurance runners (Nevill et al., 1996). This study will recruit the volunteers who meet our recruitment criteria. However, their training regimens still could vary in regards to their training volumes of both endurance and resistance exercise.

Significance of the study

This will be the first study on the order effect of the combination of two types of anaerobic exercise models in one session on HGH secretion. The results of this study could provide a practical application for exercise training design in regards to HGH responses after exercise training. As HGH secretion after resistance training has been suggested to be associated with muscle hypertrophy, the combinations of exercise modes in this study could be implemented on the hard work-out days in the muscle hypertrophy phase of a periodization design for optimal training outcome.

Definition of terms

Human Growth hormone (HGH). HGH is a polypeptide hormone produced by the adenohypophysis (anterior pituitary gland) that stimulates growth and cell reproduction. GH is a potent anabolic agent that facilitates the transport of amino acids into cells. The increased rate of amino acid transport into muscle cells is associated with muscle hypertrophy. The GH secretion in response to exercise training has been suggested to influence muscle hypertrophy (McCall, Byrnes, et al., 1999).

Repetition maximum (RM). The greatest amount of weight that can be lifted with proper technique for a specified number of repetitions. For example, one repetition maximum (1RM) is the greatest amount of weight that can be lifted with proper technique for only one repetition, and 10RM is the weight that can be lifted for no more than 10 repetitions.

Anabolic. Refers to constructive metabolism, the synthesis of large molecules from smaller molecules, where there is an increase in muscle hypertrophy by increasing muscle fiber size (synthesis of larger molecules) from amino acids (smaller molecules). Anabolism is a process of metabolism.

Growth hormone replacement therapy. Growth hormone replacement therapy, also called growth hormone treatment, refers to the use of growth hormone as a prescription medication for growth hormone deficiency (Fisher & Acerini, 2013; Kargi & Merriam, 2013).

Circuit training. A form of body conditioning or resistance training that consists of high-intensity aerobic exercise. It targets strength building and muscular endurance. An exercise "circuit" is one completion of all prescribed exercises in the program. When one circuit is complete, one begins the first exercise again for the next circuit. Traditionally, the time between exercises in circuit training is short, often with rapid movement to the next exercise.

Metabolic disturbance. During exercise, the rate of metabolism deviates away from its resting state, called metabolic disturbance. Metabolic disturbance can be determined by measuring metabolites such as blood lactate concentration.

Work-to-rest ratio. Here, work-to-rest ratio represents the amount of time a subject performs a lifting exercise in relation to the amount of time a subject is at rest between exercises in a circuit.

Muscle afferent-pituitary axis pathway. A signal input pathway that works via the skeletal muscle metabo- and mechano-receptors, sensing the chemical and mechanical status within the muscle to signal the information to the brain via group III and IV nerves. This signaling pathway is the input route of the communication circuit between the muscle and brain. Based on the input signals, the brain is able to determine its efferent signals to the heart for heart rate changes, to the muscle for muscle contraction and force production, as well as to other organs, including the brain, for release or suppression of multiple hormones.

Dose-response relationship. The dose–response relationship, or exposure–response relationship, describes the change in effect on an organism caused by differing levels of exposure (or doses) to a stressor (usually a chemical, here we are referring to exercise intensity and duration) after a certain exposure time (Crump, Hoel, Langley, & Peto, 1976).

CHAPTER 2: Review of Literature

The acute human growth hormone secretion in response to exercise.

The review of literature in chapter II includes the following topics: 1) general function of human growth hormone, 2) regulation of human growth hormone secretion, 3) factors that influence human growth hormone secretion, 4) human growth hormone response to aging, 5) human growth hormone response to nutritional status, 6) overview of human growth hormone response to resistance exercise, 7) human growth hormone response to anaerobic exercise, 8) human growth hormone response to sub-maximal intensity (endurance/aerobic oriented) exercise, 9) rest periods greater than 1 hour between two workouts, 10) human growth hormone response to combination exercise – exercise order effect, and 11) conclusion.

Part 1: General function of human growth hormone

Human growth hormone (HGH) is a principal anabolic hormone of the human body. HGH's anabolic effects have attracted attention due to its change in blood concentrations in different phases of maturation among children, individuals undergoing puberty and adolescents, as well as in HGH deficiency patients (Gibney et al., 2007). In HGH-deficient adults, quality of life can be impaired due to reduced maximal oxygen consumption and increased muscle fatigue (low muscular strength) during physical activity. HGH replacement therapy has been shown to increase lean body mass, leading to improvements in VO_{2max}, increased maximal work rate, and increases in muscular strength (Gibney et al., 2007). In addition, the anabolic function of human growth hormone has been confirmed to improved exercise and sports performance, thus leading

to the HGH abuse in recent years (Hoffman et al., 2009). Recently, some evidence indicates that the major factor to increase protein synthesis rate is the intrinsic process within exercised muscle rather than HGH concentration post-exercise (West, Burd, Staples, & Phillips, 2010). However, HGH via its effect on increases in mTOR activation (Hoffman et al., 2009) is still considered one of the major hormones that promote muscle hypertrophy (McCall, Byrnes, et al., 1999) and increase muscular strength (Tavares et al., 2013), especially when combined with a sufficient exercise stimulus (Madarame et al., 2008; Ronnestad, Nygaard, & Raastad, 2011).

Part 2: Regulation of human growth hormone secretion

2.1. Neuro-regulation

HGH is secreted by the anterior pituitary gland through regulation from the hypothalamus. The hypothalamus forms and releases GH-releasing hormone (GHRH) and somatostatin, which act on the anterior pituitary gland. GHRH stimulates GH release while somatostatin inhibits GH release. In humans, HGH also stimulates insulin-like growth factor (IGF-1) secretion from the liver. Both HGH and IGF-1 provide a negative feedback mechanism to somatostatin secretion by the hypothalamus. HGH and IGF-1 provide a negative feedback to inhibit the release of GHRH and a positive feedback to stimulate somatostatin secretion. IGF-1 also has an inhibitory effect on HGH release (Giustina & Veldhuis, 1998).

2.2. Muscle afferent-pituitary axis modulation

The muscle afferent input stimuli have been suggested to affect pituitary growth hormone secretion from the anterior pituitary (McCall et al., 2001). The vibration-

induced activation of muscle afferent inputs have been shown to have a stimulatory effect from fast-twitch muscle fibers and an inhibitory effect from slow-twitch muscle fibers on GH release in the rat model (Gosselink et al., 2000; Gosselink et al., 2004) and in humans (McCall et al., 2000). Exercise-induced GH release from the pituitary was demonstrated by an increase in plasma GH concentration associated with a decrease in pituitary GH concentration by nerve stimulation of muscles in anesthetized (Gosselink et al., 1998) and exercising rats (Bigbee, Gosselink, Roy, Grindeland, & Edgerton, 2000). Another finding concerning the muscle afferent-pituitary axis on GH secretion is noted with a reduced muscle afferent activity after hind limb suspension (Kawano, 2004), and a decrease in exercise-induced GH secretion associated with bed rest (McCall et al., 1997) and spaceflight (McCall, Goulet, et al., 1999). Therefore, the muscle afferent-pituitary axis may be important to GH secretion, especially the decrease in GH response to exercise after exposure to microgravity with a restoration of normal response after 4 days post spaceflight.

Although slow-twitch muscle afferent input has an inhibitory effect on GH secretion, it could be overridden by activation of other muscles, such as fast-twitch muscle afferent input (Gosselink et al., 2000). It is unclear if a threshold of afferent input from fast-twitch muscle activation exists to override the inhibitory input from slow-twitch muscle. Research has shown that during cycling exercise at 75% VO_{2max} (~40% of the maximal dynamic muscle force exerted on the pedals) about 50% of type II fibers were recruited (Altenburg, Degens, van Mechelen, Sargeant, & de Haan, 2007). The individual differences in response to the same type of exercise mode and exercise intensity and the magnitude of fast-twitch muscle recruitment among individuals might

be the explanation of the dissimilar results of GH secretion among the studies using similar exercise intensities.

Part 3: Factors that influence human growth hormone secretion

The pattern of HGH secretion is influenced by several factors, including the following: age, gender, physical activity, exercise, nutrition status, and at various stages during sleep (Ehrnborg & Rosen, 2008). HGH secretion rate follows a diurnal pattern (Birketvedt et al., 2012), where the HGH secretion rate peaks an hour after the onset of sleep with the highest peak observed during sleep at midnight (Janukonyte, Parkner, Lauritzen, Christiansen, & Laursen, 2013). There is no difference in HGH diurnal pattern (lowest at 0600 and highest at 2400) between people who live at sea level and those who live at elevation of 3650 meters. However, high altitude dwellers have significantly higher HGH levels than sea level residents throughout the day (Sawhney, Malhotra, & Singh, 1991).

The peak HGH concentration seems to be more pronounced in normal weight women than over-weight women (Birketvedt et al., 2012). Although secretion of HGH in response to exercise is not influenced by the time of day (Kanaley, Weltman, Pieper, Weltman, & Hartman, 2001), the diurnal effect has always been a consideration and most studies of HGH in response to exercise have been conducted in the morning or at a consistent time during the day.

Part 4: Human growth hormone response to aging

It is unknown whether the decreases in HGH secretion with aging influence human longevity. HGH treatment has been shown to increase lean body mass, immune

function, cardiovascular function, and neuro-cognition (Giordano, Bonelli, Marinazzo, Ghigo, & Arvat, 2008). Longitudinal studies on HGH treatment on growth hormone-deficient patients have indicated significantly greater mortality rates in women treated with GH due to cardiovascular disease (Sattler, 2013).

In a human life span, the daily HGH secretion rate is related to body surface area and is highest in newborns, after which it decreases in childhood. A second increase in HGH daily secretion rate was observed throughout puberty, followed by a downward trend with aging (Giustina & Veldhuis, 1998). Between young (<30 years old) and old (>60 year old) male subjects, 24-hour measures of HGH secretion denote significantly greater HGH levels in the young subjects (Nass, 2013). However in subjects closer in age, Lin, Huang, & Lin (2009) found no significant difference in overnight fasting HGH concentrations between men and women and before and after the age of 50 within the same gender (male: 45.4±4.2 yr. vs. 57.9±5.53 yr.; female: 43.3±4.2 yr. vs. 55.5±4.3 yr.) (Lin, Huang, & Lin, 2009).

No difference in resting HGH concentrations was found in men between age and physical activity level. Resting serum HGH in men over 60 years old has been reported at 2.15±1.32µg/L regardless of training status (Pyka, Taaffe, & Marcus, 1994). Physically active men below 60 years have resting serum HGH concentrations typically below 3µg/L(Gilbert et al., 2008; Hakkinen & Pakarinen, 1993; Linnamo, Pakarinen, Komi, Kraemer, & Hakkinen, 2005; Rahimi et al., 2010; Sartorio et al., 2008; Smilios et al., 2007; Vanhelder, Goode, & Radomski, 1984). In normally menstruating women younger than 30 years, there is no difference in resting HGH concentrations between the luteal phase (1.5±1.6µg/L) and follicular phase (2.2±2.6µg/L) (Sunderland, Tunaley, Horner,

Harmer, & Stokes, 2011). Resting serum HGH concentrations in young women (24.1±4.3 yr.) have also been reported in the range of 4-7 µg/L (Kraemer et al., 1993). Resting HGH secretion appears to decline in middle age, and the difference in resting HGH between physically active and sedentary older men is small.

Part 5: HGH response to nutritional status

HGH level is influenced by nutritional status, such as a decrease in concentration of HGH after a meal (Birketvedt et al., 2012) and an increase in concentration of HGH during fasting (Norrelund, 2005). This effect is due to the blood concentration of glucose, fatty acids, and amino acids (Ehrnborg & Rosen, 2008). In addition, the human gastrointestinal tract is able to synthesize ghrelin, a growth hormone-releasing hormone (Date et al., 2000)**.** Ghrelin secretion provides a positive feedback for HGH secretion by binding to growth hormone secretagogue receptor in the anterior pituitary gland to cause HGH release (Muller et al., 2015). The serum ghrelin level in human has shown to be influenced by nutritional status (Norrelund, 2005) and exhibit a diurnal pattern with meal time (Cummings et al., 2001; Tschop et al., 2001). Ghrelin secretion in response to micronutrient status is suggested to be regulated by the energy balance-induced activation of diverse central nervous system (CNS) sites (Muller et al., 2015). There is a marked increase in GH secretion after exercise with unchanged circulating ghrelin levels (Dall et al., 2002)**.** Therefore, GH secretion at rest and response to exercise may be regulated by different endocrine systems.

Part 6: Overview of human growth hormone response to resistance exercise

The acute and chronic responses of HGH secretion to resistance training have been summarized by Kraemer and Ratamess (2005). Serum GH concentration increases within 30 min of resistance exercise at 10RM with 1 min rest interval in both genders, and women have significantly greater average values than men at rest (~4-7 µg/L vs. <~3µg/L) (Kraemer et al., 1993; Kraemer et al., 1990). However, the HGH response to sprinting showed no sex difference (Nevill et al., 1996). The magnitude of the acute HGH response to resistance exercise appears to vary depending on the muscle mass involved, muscle action, exercise intensity, exercise volume, rest period between sets, and training status. Acute HGH response to exercise may be more pronounced when exercise acts on remodeling tissue. Long-term resistance training does not elevate resting serum GH concentrations regardless of gender (Hakkinen, Pakarinen, Kraemer, Newton, & Alen, 2000), training status(Marx et al., 2001; Pyka, Taaffe, et al., 1994; Pyka, Wiswell, & Marcus, 1992), or age(Kraemer et al., 1999). A significantly greater muscle weight and cross-sectional area training response have been found in an exercise training plus HGH-IGF-1injection group compared to an exercise only group using a rat model (McCall et al., 1998). However, human studies with recombinant HGH administration together with heavy resistance training suggested the increase in lean body tissue was due to non-contractile protein(Yarasheski, Zachwieja, Campbell, & Bier, 1995; Zachwieja, Toffolo, Cobelli, Bier, & Yarasheski, 1996).

6.1. Human growth hormone response to training volume

The acute HGH response to resistance training may be affected by training volumes. Increasing training volume from a single set protocol to a 3-set protocol causes a significant increase in HGH concentration after resistance training in recreational

resistance trained men (Gotshalk et al., 1997). Comparisons of strength-trained male athletes (power lifters, body builders, and weight lifters) under two different fatiguing exercise protocols (20 sets of 1 repetition at 100% of 1RM and 10 sets of 10 repetitions at 70% of 1RM) revealed that the post-exercise concentration of serum HGH was significantly higher after 10 repetitions at 70% of 1RM protocol 1RM (Hakkinen & Pakarinen, 1993). Although the Hakkinen and Pakarinen (1993) study didn't compare the total work volume between sessions, based on the maximal strength data reported in the study, the difference in workout volume in the 1st set and the last set between the protocols were 175±34 kg (100% of 1RM) vs. 1250±240 kg (70% of 1RM), and 158±36 kg (100% of 1RM) vs. 915±190kg (70% of 1RM). Hoffman et al., (2003) had subjects perform 5 sets of 15 repetitions at 60% of 1RM (high volume protocol) and 5 sets of 4 repetitions of 90% of 1RM (low volume protocol). The high volume protocol caused significantly higher peak concentrations of serum HGH than the low volume protocol. The area under the curve (AUC) HGH response was also significantly higher in the high volume protocol. This study confirms the findings of Hakkinen and Pakarinen (1993), showing a positive relationship between HGH response and resistance exercise training volume.

Different from the fatiguing exercise protocols (where the repetitions in each set are performed to voluntary muscle contraction failure) used by Hoffman et al (2003) and Hakkinen and Pakarinen (1993), Leite et al (20) completed a submaximal heavy resistance exercise study to investigate the acute HGH response. The submaximal heavy resistance exercise is designed with attempts to stop the lifter prior to exhaustion. For example, if the subject performs a set of 10 repetitions at 10 RM (roughly about 75% of

1RM), the contracting muscles will generally (barely) make the 11th repetition or fail to complete the 11th repetition. Leite et al. (2011) investigated trained men performing 3 sets of 12 repetitions at 80% of 12RM (high volume) and 3 sets of 6 repetitions at 80% of 6RM (low volume). The high volume protocol induced significantly higher serum HGH concentration than the lower volume protocol.

On the other hand, Raastad, Bjoro & Hallen (2000) reported that training volume had no effect on the acute HGH secretion response. Subjects completed identical sets and repetitions (4-5 sets of 3 reps for squat and front squat, 1 set of 6 reps for leg extension) at different intensities at 100% (high volume) and 70-76% (low volume) of 3-6RM in male power athletes. The researchers found no significant difference in HGH secretion in response to the 2 protocols. The authors suggested the reason there was no significant difference in HGH response was likely due to the extended rest periods (4-6 minutes) between sets when compared to the relatively shorter rest periods (<3 minutes) in the aforementioned studies. The effect of the rest period between sets, as well as the exercise intensity on HGH secretion will be discussed later in this review.

In summary, the above studies have demonstrated the effect of the resistance training volume on the acute HGH response after exercise. A greater training volume can promote acute HGH secretion after exercise while the longer rest periods between sets may prevent or inhibit this effect. Rest periods should probably stay within 3 minutes to allow a pronounced HGH response.

6.2. HGH response to exercise intensity

The effect of exercise intensity on HGH secretion has been studied by Kraemer et al. (1990, 1993). They studied women during the early follicular phase of the menstrual cycle, and compared identical exercise intensities (10RM and 5RM) and rest intervals (1 min and 3 min) between high and low total workout volumes (31,580.3±3,278.0 vs. 24,501.1±2,827.0 Joules, $p<0.05$). There was only a significant increase in HGH secretion post-exercise in the high training volume group, which consisted of moderate intensity (10 repetition-maximum) and a short rest interval (1 min) (Kraemer et al., 1993). No significance difference was found in groups with longer rest intervals (3 min) or lower training volume combinations (5 repetition-maximum). Therefore, the authors suggested the moderate exercise intensity at 10RM was required to induce a HGH response. The impact of intensity of the HGH response to resistance exercise has also been studied using various exercise intensities ranging from 20% of 1RM to 100% of 1RM in both a traditional weight training design (Bottaro et al., 2009; Goto, Higashiyama, et al., 2005; Goto et al., 2009; Goto, Ishii, Kurokawa, et al., 2007; Goto, Takahashi, Yamamoto, & Takamatsu, 2008; Hakkinen & Pakarinen, 1993; Hoffman et al., 2003; Kokalas, Tsalis, Tsigilis, & Mougios, 2004; Kraemer et al., 1993; Kraemer et al., 1990; Leite et al., 2011; Linnamo et al., 2005; Migiano et al., 2010; Ojasto & Hakkinen, 2009; Raastad, Bjoro, & Hallen, 2000; Rahimi et al., 2010; Schumann et al., 2013; Schumann et al., 2014; Smilios et al., 2007) and a circuit weight training design (Ghanbari-Niaki, 2006; Ghanbari-Niaki et al., 2007; Ghanbari-Niaki, Saghebjoo, Rahbarizadeh, Hedayati, & Rajabi, 2008; Ghanbari-Niaki, Saghebjoo, Rashid-Lamir, Fathi, & Kraemer, 2010). The following sections will address the current research findings on HGH response to traditional weight training and circuit weight training.

6.2.1. Traditional weight training

The following section reviews the HGH response to traditional weight exercises in two different protocols: maximal heavy resistance exercise and sub-maximal heavy resistance exercise (Linnamo et al., 2005). A maximal heavy resistance exercise design involves each set of exercise performed to fatigue or with the maximal repetitions closely corresponding to the percent of 1 RM loading based on the NSCA recommendation, such as 10 repetitions at 75% of 1RM (Baechle & Earle, 2000). A sub-maximal resistance exercise design involves repetitions completed in each set before voluntary muscle contraction fatigue. In traditional weight training programs, the rest intervals often range from 1 to 6 min between sets, based on the exercise intensities to allow recovery of the contracting muscle for next set.

6.2.1.1. HGH response to maximal heavy exercise (to fatigue and vary close to voluntary muscle contraction failure)

20% of 1RM (Blood Flow Restriction application)

Resistance exercise stimulates HGH secretion at an exercise intensity as low as 20% (Patterson, Leggate, Nimmo, & Ferguson, 2013; Takano et al., 2005) and 30% (Madarame et al., 2008; Tanimoto, Madarame, & Ishii, 2005) of 1RM. However, some studies reported no significant increase in circulating HGH concentration after resistance exercise at 20% of 1RM (Fujita et al., 2007; Patterson et al., 2013; Takarada et al., 2000; Takarada, Tsuruta, & Ishii, 2004). Among all of low intensity resistance training studies, it appeared that blood flow restriction (BFR) application induces a significantly greater

increase in HGH secretion after low-intensity resistance training when compared to rest or non-BFR application controls.

The application of blood flow restriction (BFR), also called vascular occlusion, causes reduced blood flow to the target muscle at rest and during exercise, leading to regional hypoxia in the contracting muscle. (Abe, Kearns, & Sato, 2006; Fujita et al., 2007; Madarame, Sasaki, & Ishii, 2010; Manini et al., 2012; Pierce, Clark, Ploutz-Snyder, & Kanaley, 2006; Reeves et al., 2006; Takano et al., 2005; Takarada et al., 2000; Takarada et al., 2004; Tanimoto et al., 2005) The typical BFR application restricts blood flow to the extremities by placing tourniquets or a blood pressure cuff on the proximal region of the limbs when the subjects perform leg and arm exercises (Madarame et al., 2010; Takarada et al., 2000; Takarada et al., 2004). The pressure is usually in a range of ~130mmHg (Manini et al., 2012) to ~280mmHg (Pierce et al., 2006) on the thighs and 20mmHg below systolic blood pressure (Reeves et al., 2006) to 130 mmHg (Madarame et al., 2010) on the arms. This stimulates HGH secretion after low intensity (20% of 1RM) resistance training (Takano et al., 2005). To our knowledge, exercise with BFR was first studied in young male athletes by Takarada et al. (2000), with findings of a significant increase in GH during low-intensity resistance with BFR, but no significance increase in GH response to low-intensity resistance exercise without BFR or BFR only. Since then, the significantly greater GH response to EBFR than ENOR has been consistently reported in young men (Fujita et al., 2007; Inagaki, Madarame, Neya, & Ishii, 2011; Manini et al., 2012; Patterson et al., 2013; Reeves et al., 2006; Takano et al., 2005; Takarada et al., 2004), where the outcome in the older men remains inconsistent (Manini

et al., 2012; Patterson et al., 2013; Yokokawa, Hongo, Urayama, Nishimura, & Kai, 2008).

BFR with leg extension exercise at 20% of 1RM in young male athletes (20-22 yr.) caused an increase in HGH secretion to a level of ~290 times as high as that of the pre-exercise level at 15 minutes post-exercise. The peak plasma HGH concentration was greater than 30ng/ml (Takarada et al., 2000; Takarada et al., 2004) while the non-BFR trial showed no significant increase (Takarada et al., 2000; Takarada et al., 2004). In untrained healthy males (34±6 yr.) (Takano et al., 2005), physically active young men (32±2 yr.) (Fujita et al., 2007), and healthy older men (71.0±6.5 yr) (Patterson et al., 2013), BFR with leg extension exercise at 20% of 1RM elicited a significant increase in plasma HGH concentration while no significant increase was found in non-BFR trials. When comparing low-intensity resistance exercise with BFR to moderate to high intensity resistance exercise without BFR application, exercise with BFR has caused either a similar (Tanimoto et al., 2005) or a significantly greater HGH secretion after exercise (Reeves et al., 2006). In addition, an increase in muscle mass recruited (Madarame et al., 2010) or a young age compared to an older age (Manini et al., 2012) also have positive effects on the HGH response to BFR application and low-intensity resistance training.

40%-50% of 1RM (slow muscle action speed)

When exercise intensity increases to 40% and 50% of 1RM, the BFR application becomes unnecessary but the slow muscle contraction velocity becomes the key to increasing HGH secretion (Goto et al., 2009; Goto et al., 2008). Goto et al. (2008) compared the effect of various muscle action speeds in both concentric and eccentric

phases on HGH secretion post- exercise in physically active men (age 24.3±0.4 yr). When the subjects performed a knee extension exercise for 5 sets of maximal repetitions until fatigue at 40% of 1RM with 1 min rest between sets, only slow muscle action speed (3 sec in concentric phase, 3 sec in eccentric phase) significantly increased the post-exercise serum HGH concentration more than that in normal muscle action speed (1 sec in concentric phase, 1 sec in eccentric phase) (Goto et al., 2008). However, in order to match the total training volume, the normal muscle action speed session had to match equal repetitions performed in slow muscle action speed sessions and might not have reached voluntary muscle contraction failure. Later, Goto et al. (2009) conducted another study and confirmed the effect of the slow muscle action speeds on HGH secretion. Nine physically active men (age of 24±0.2 yr.) went through a knee extension exercise for 4 sets of maximal repetitions until fatigue at slow muscle action speed (50% of 1RM) and normal muscle action speed (80% of 1RM). Among three different slow muscle action speeds with the combination of 1 sec & 5 sec, 5 sec & 1 sec, and 3 sec & 3 sec in concentric phase and eccentric phase, serum HGH secretion significantly increased from rest without a significant difference between the 3 trials. The normal muscle action speed (1 sec and 1 sec in concentric phase and eccentric phase, respectively) trial showed no significant difference in HGH secretion compared to rest.

60%-70% of 1RM

HGH response to resistance exercise has shown to increase when the exercise intensity reaches 60% of 1RM (Hoffman et al., 2003; Smilios et al., 2007). At this exercise intensity, neither application of BFR nor slow muscle action speed seemed to be necessary. Single exercise of parallel squat at 60% of 1RM for 5 sets of 15 repetitions

with 3 min rest between sets significantly elevated serum HGH concentration up to 40 min post-exercise in resistance-trained adults (Hoffman et al., 2003). Similarly, HGH response to weight training at 60% of 1RM was also reported in elderly men who had at least nine months of recreational weight lifting experience (Smilios et al., 2007). Smilios et al. (2007) reported a significant increase in serum HGH concentrations up to 15 min after 6 exercises (seated chest press, pectoralis major fly, lateral pulldowns, biceps curls, leg extension, and leg flexion) for 3 sets of 15 repetitions at 60% of 1RM with 90 sec rest between sets in both young (23±1 yr.) and elderly men (69±5 yr.). In addition, the young subjects did show significantly higher HGH value compared to the elderly subjects.

70%-85% of 1RM

As previously mentioned, 10 sets of 10 repetitions at 70% of 1RM using only squat-lifting exercise significantly increased HGH serum value in strength trained athletes (29.7±8.0 yr.) (Hakkinen & Pakarinen, 1993). Multiple exercises (sit-ups, bench press, and leg extension) at 100% of 10RM also showed significant elevation in post-exercise HGH serum concentration in physically fit young men and women (23.3±0.5 yr.). Male subjects (27.1±0.7 yr.) showed higher values compared to females (Linnamo et al., 2005). Goto et al (2005) also reported significantly increased post-exercise HGH serum concentrations at 10RM load for 3-5 sets of 10 repetitions in recreational resistance trained men (22.7±0.5 yr.) when different upper body exercises were chosen (lats pull-down, shoulder press, and leg extension).

HGH response to resistance training may be positively related to the muscle mass recruited (Migiano et al., 2010). In recreational resistance-trained men (20.4±1.2 yr.), 5

dumbbell exercises resulted in a significantly increased post-exercise serum HGH concentrations, while the bilateral arm trial showed significantly greater HGH level than the dominant-sided unilateral arm only trial (Migiano et al., 2010).

Goto et al. (2008, 2009) studied HGH responses to resistance training at normal muscle action speed of 1 sec concentric phase and 1 sec eccentric phase during knee extension exercise at 80% of 1RM with maximal repetitions performed until fatigue in physically active men (avg. 24 yr). No significant increase in serum HGH concentration post-exercise was observed. The major difference between the Goto et al. studies and others are the type of exercises. In studies by Goto et al (2008,2009), there is only knee extension exercise that involved quadriceps muscle while others either used a multiple joint exercise with majority of muscle recruited (parallel squat), multiple exercises that involved major muscle groups (upper body arm exercises) or multiple muscle groups (both upper body and lower body).

A possible explanation of the non-significance in HGH response to exercise intensity at 70-85% of 1RM between Goto et al. (2008, 2009) and other studies (Goto, Ishii, Kizuka, & Takamatsu, 2005; Hakkinen & Pakarinen, 1993; Linnamo et al., 2005; Migiano et al., 2010) might be due to interaction of low exercise volume (4 workout sets) and the small muscle mass recruited in the knee extension exercise. Therefore, the exercise intensity with the specific range from 60% to 85% may require a certain degree of stimulus as an interaction of the muscle mass involved and the volume of the exercise.

85%-100% of 1RM

It was found that high resistance intensity (> 5RM, 8 exercises involving upper and lower extremities) was not considered the best way to increase post-exercise serum HGH when conducted in recreational resistance training experienced women (24.1±4.3 yr.) (Kraemer et al., 1993) and men (24.7±4.3 yr.) (Kraemer et al., 1990). Women were non-responders in regards to HGH secretion after resistance training at an intensity of 5RM regardless of rest intervals and training volumes between sets (Kraemer et al., 1993). In men, although a single resistance training session at an exercise intensity at 5RM with either 1 or 3 min rest between sets resulted in a significant increase in post training circulation HGH values, another session (5RM with 1 min rest between sets) with a higher training volume failed to cause a significant response in HGH levels (Kraemer et al., 1990). Kokalas et al. (2004) reported no significant change in post- exercise (bench pull, leg press, and rowing from standing position) serum HGH values after 6 sets of 6 repetitions at 85%-90% of 1RM with 3 min rest between sets in elite male rowers (20.0±1.6 yr.). Raastad et al. (2000) reported no significant change in post-exercise (squat, front squat, and leg extension) serum HGH values after 4-5 sets of 3-6 repetitions at 100% of 3-6RM with 4-6 min rest between sets in power athletes (26.9±1.4 yr.).

In contrast, it has been reported that exercise intensities ≥85% increased the serum HGH concentration after exercise (Hakkinen & Pakarinen, 1993; Hoffman et al., 2003; Rahimi et al., 2010). Five sets of parallel squat exercise for 4 repetitions at 90% of 1RM with 3 min rest between sets significantly increased serum HGH values up to 40 min post-exercise (Hoffman et al., 2003). However, the same group of subjects also went through a 60% of 1RM trial with 15 repetitions performed in each set, which induced significantly higher HGH serum concentration than that in 90% of 1RM trial at 20 min

and 40 min post-exercise. The area under curve of HGH was also significantly higher in 60% 1RM trials than in 90% 1RM trial. Additionally, a fatiguing exercise protocol using squat-lifting exercise for 20 sets of 1 repetition at 100% of 1RM with 3 min rest between sets also significantly elevated post-exercise serum HGH concentration in strength trained male athletes (29.7±8.0 yr.) (Hakkinen & Pakarinen, 1993). As a result, it is unclear whether or not an exercise intensity range of greater 85% of 1RM can cause a significant increase in HGH secretion.

In summary, the findings with regard to maximal heavy exercise intensity which result in a muscle fatigue level to voluntary contraction failure, it may be necessary to apply various muscle contraction parameters according to the lifting intensities in order to result in a significant HGH response to exercise training. To apply BFR while lifting at an intensity range of 20% - 30% of 1RM induced the best HGH response. A slow muscle action speed (at least one of concentric or eccentric phases for 3-5 sec) is needed when the exercise intensity is at 40% - 50% of 1RM. When the exercise intensity range is 60% to 85% of 1RM, multiple exercises involving major muscle groups with a rest interval less than 3 min have been shown to increase HGH concentrations post training. At exercise intensities greater than 85% of 1RM, conflicting results were found with a greater chance to induce increase HGH levels in men than in women.

6.2.1.2. HGH response to submaximal heavy exercise (stop lifting before voluntary contraction failure)

Submaximal heavy exercise intensity was originally used in circuit weight training in which subjects typically completed 8-12 exercises in a circuit with short rest

intervals (less than 1 min) between exercises and longer rest intervals (greater than 1 min) between circuits (Gettman, Ayres, Pollock, & Jackson, 1978; Wilmore et al., 1978). A submaximal exercise intensity allows the subjects to complete all exercises in a circuit with subjects recovering better during the longer rest intervals between circuits. During a traditional weight training design, the submaximal heavy intensity is typically used for the fast muscle contraction velocity in explosive strength training (Schumann et al., 2014). To our knowledge, only 4 studies report the HGH response to submaximal heavy exercise training (Goto et al., 2008; Leite et al., 2011; Linnamo et al., 2005; Raastad et al., 2000).

Goto et al (2008) reported no significant change in post-exercise serum HGH concentration in physically active men (age of 24.3±0.4 yr.) after knee extension exercise for 5 sets (stopped before fatigue at 40% of 1RM) with 1 min rest intervals between sets at normal muscle action speed (1 sec in concentric phase, 1 sec in eccentric phase). At 70%-76% of 3-6RM using leg exercise (squat, front squat, and leg extension), 4-5 sets of 3-6 repetitions with 4-6 min rest between sets in power athletes (26.9±1.4 yr.) showed neither elevated serum HGH concentrations nor AUC of HGH after exercise (Raastad et al., 2000). Raastad et al. (2000) suggested the lack of HGH response to leg exercise at 70%-76% of 3-6RM was due to both the insufficient exercise intensity and the prolonged rest interval. Ojasto and Hakkinen (2009) conducted a study utilizing eccentric load as the primary focus and no significant difference in serum HGH concentration was found after bench press for 4 sets of 10 repetitions at exercise intensities ranging from 70% of 1RM to 100% of 1RM in the eccentric phase.

When the exercise intensity was at 40% of 10RM, fast (explosive) muscle contraction velocity has caused significant increase in the HGH response to exercise in

both young men and young women (Linnamo et al., 2005). The explosive movement might play a key role in causing a significant increase in HGH secretion because in the same study another trial at a heavier resistance (70% of 10RM) but a sub-maximal "steady" velocity did not cause a significant change in HGH after exercise. The explosive type of muscle contraction may increase HGH secretion via a greater recruitment of fast twitch muscle fibers and its proprioceptive mechanism activating the muscle-pituitary axis pathway (McCall et al., 2000). Therefore, the results by Linnamo et al., (2000) suggest that the effect of exercise intensity together with the muscle action speed, the amount of muscle activated, and the greater fast twitch muscle fibers recruited may positively impact HGH secretion.

When explosive movements were not involved, Leite et al. (2011) reported that exercise involving upper and lower major muscle groups (barbell bench press, leg press, machine front latissiumus dorsi pull down, leg curl, shoulder abduction and leg extension) for 3 sets of 6 and 12 repetitions at intensities of 80% of 6RM and 12 RM significantly increased serum HGH concentrations in recreationally trained men (24.5±7.6 yr). Without any specific muscle action speed implementation, the major difference between Leite et al. (2011) and Raastad et al. (2000) was total muscle mass involved in the exercise training. Leite et al. (2011) used three upper body exercises and three lower body exercises consisting of both agonist and antagonist muscles compared to only three lower body exercises (squat, front squat, knee extension) used by Raastad et al. (2000). Thus, the amount of muscle mass recruited during exercise sessions may be a critical factor in the HGH response.

Based on the above findings using submaximal exercise intensities, muscle action speed, the rest interval between sets, and the total muscle mass recruited, these factors likely have an influence on exercise induced HGH secretion. However, further investigations on these complex interactions are needed.

6.2.1.3. Human growth hormone response to rest interval between sets

Kraemer et al (1993) suggested that short rest intervals of less than 1 min between sets might be best to increase HGH secretion. Since 1993,several studies have been published which measured HGH responses after exercise training using various rest intervals ranging from 30 sec to greater than 3 min in traditional weight training programs (Bottaro et al., 2009; Rahimi et al., 2010).

Starting with short rest intervals between sets, two studies investigated the effect of the rest interval on the HGH response post-exercise (Bottaro et al., 2009; Rahimi et al., 2010). Bottaro et al (2009) compared rest intervals of 30 sec, 60 sec, and 120 sec between sets in resistance trained women (26.83±3.93 yr). All three rest intervals resulted in significantly higher serum HGH concentrations post-exercise compared to the resting values. In this study, the subjects performed 3 sets of 4 different leg exercises (knee extension, back squat, knee flexion, and leg press) for maximal repetitions until fatigue at a 10RM load with 3 sec/rep rhythm. The effect of the rest interval reflected an inverse relationship between the length of the rest interval and the duration for an elevation in serum HGH levels post-exercise. The 30-sec rest interval trial resulted in a significant increase in serum HGH concentration up to 15 min post exercise compared to a significant serum HGH elevation only 5 min after exercise with the 60-sec rest interval

trial. There was only one significantly higher HGH concentration above the resting value at immediately post-exercise in 120-sec rest trial. Therefore, in addition to the total training volume, rest intervals between sets seem to play a role in HGH secretion and its turnover rate in response to exercise.

Similar to Bottaro et al. (2009), Rahimi et al. (2010) examined rest intervals of 60 sec, 90 sec, and 120 sec between sets and their effects on HGH responses in experienced resistance-trained college men (22±2 yr). After 4 sets of maximal repetitions for squat and bench press at 85% of 1RM to voluntary fatigue, immediately post-exercise serum HGH values were only significantly elevated after the 60-sec and 90-sec trials while a significantly greater HGH concentration was measured after the 60-sec trial than after the 90-sec trial. However, an interesting result was reported in this study. The 60-sec rest trial resulted in a significant increase in serum HGH values up to 30 min post-exercise and 90 sec rest trial did not (as expected). The serum HGH value after the trials with 120 sec rest tended to increase with time and reached a level significantly higher than the resting level at 30 min post-exercise. This HGH response pattern after the 120-sec trial was different from that in Bottaro et al (2009). Rahimi et al. (2009) did not speculate as to why the serum HGH value continued to increase post-exercise in the 120-sec trial, but suggested that 85% of 1RM with a shorter rest interval (60, 90, and 120 sec) resulted in the best HGH response to exercise.

Rest interval might have no effect on the HGH secretion to resistance training under certain conditions. As discussed above, blood flow restriction (BFR) is the primary factor for the increase in HGH at an exercise intensity of 20% of 1 RM, with another factor being slow muscle action speed at exercise intensities of 40-50% of 1RM. As

exercise intensity rises above 75% (10RM), training volume and exercise intensity seem to play a more important role than the rest interval between sets on influencing acute HGH response to exercise (Kraemer et al., 1993; Smilios et al., 2007; Wu & Lin, 2010). Also, an additional 30-second break in the middle of a set of 10 repetitions (between 5th and 6th repetition) at 10RM with 1 min rest intervals between sets has shown to decrease the acute HGH response to exercise (Goto, Higashiyama, et al., 2005). Raastad et al. (2000) suggested that the rest interval should stay within 3 min, especially if the exercise protocol is designed with submaximal exercise intensities.

As a result, the main goal of rest intervals between sets should be to allow full recovery of the target muscle to allow completion of the upcoming work set. While other factors such as training volume, exercise intensity, and muscle mass recruited simultaneously interact in the HGH responses to exercise, the length of the rest intervals either could add potential benefits for boosting HGH secretion or could dampen acute HGH responses.

In summary, the HGH response to the traditional weight training is affected primarily by exercise intensity, total muscle mass involved, and rest intervals. At low exercise intensities below 50%, some techniques such as BFR, slow muscle action speed or fast (explosive) muscle action speed may produce additional stimulus to HGH secretion. The exercise intensity range of 60%-85% with a rest interval less than 3 min seems to be the appropriate stimulus to increase HGH secretion. An increase in muscle mass involved (major muscle groups in both upper and lower body) and a decrease in rest interval (30-90 sec) during exercise sessions may induce a greater magnitude of HGH secretion.

6.2.2. Circuit weight training

Circuit training is a resistance training program that has been shown to increase muscular strength, muscular endurance and cardiorespiratory endurance (Gettman et al., 1978). The first (original) circuit training design is 2-3 circuits of 10-15 stations (exercises) for approximately 30 sec per station for as many repetitions as possible at a submaximal intensity (Gettman et al., 1978; Wilmore et al., 1978). Later, the adjustments in total circuits, the length of the rest interval, the exercise intensity, and the repetitions per exercise were varied by the trainers according to the fitness levels among the trainees. Circuit training is a good resistance training method for people with limited time to exercise. Although circuit training typically uses submaximal exercises, it is still associated with increased muscle fiber size in older men and women (mean age 68.2±1 yr.) (Pyka, Lindenberger, Charette, & Marcus, 1994).

Several studies have investigated the effect of circuit training (3-13 exercises) on both acute and chronic HGH responses in various age groups (Ghanbari-Niaki, 2006; Ghanbari-Niaki et al., 2007; Ghanbari-Niaki et al., 2008; Ghanbari-Niaki et al., 2010; Marx et al., 2001; Moghadasi & Siavashpour, 2013; Pyka et al., 1992; K. A. Stokes et al., 2013). Regarding young men with resistance training experience and/or a physically active lifestyle, significant increases in serum HGH in response to circuit training seems to be consistent using submaximal heavy loads (Ghanbari-Niaki, 2006; Ghanbari-Niaki et al., 2007) and maximal heavy loads (K. A. Stokes et al., 2013). In submaximal heavy exercise, serum HGH concentrations were significantly increased in response to a 10-exercise circuit training at both 35% and 60% of 1RM in 3 non-stop circuits of either as many repetitions as possible in 20 sec per exercise (35% of 1RM) (Ghanbari-Niaki et al.,

2007) or 8-12 repetitions per exercise (60% of 1RM) (Ghanbari-Niaki, 2006). Maximal heavy load with a 3-exercise circuits (5 sets of 10 repetions at 75% 1RM) with a 1-minute rest between exercises and a 3-minute rest between circuits has been shown to significantly increase post-exercise HGH values (Gilbert et al., 2008; K. A. Stokes et al., 2013). Therefore, it seems that adjusting the rest interval between exercises based on the exercise load in a circuit is a sufficient modification to maintain the HGH secretion in response to exercise in young active men.

The HGH response to circuit training seems to be different in young (22±1 yr.) and early middle-age (44±4 yr.) men. At an exercise load of 75% of 1RM for 10 repetitions (close to voluntary muscle contraction failure), serum HGH post exercise didn't significantly increase in the middle-age men (Gilbert et al., 2008). The authors suggested that middle-age men may need a stronger exercise stimulus for HGH secretion. This suggestion was in line with the decrease in HGH response to resistance training in old men compared to young men (Pyka et al., 1992). Pyka et al. (1992) conducted the first study to examine the response of HGH to circuit weight training at different loads between genders in both young (men: 28.8±6.9 yr., women: 26.7±1.9 yr.) and old (men: 70.9±1.0 yr., women: 72.0±1.3 yr.) people. No gender difference was observed. The HGH response to circuit training seemed to be intensity dependent and young subjects showed significant HGH response to circuit training, where no significant differences were found in the old subjects (Pyka et al., 1992). In addition, it seemed that the physical fitness level of participants was not a factor contributing to HGH response to exercise training (Pyka, Taaffe, et al., 1994). A 52-week circuit training program also showed no training effect on the HGH response to circuit training in older people (8 exercisers:

67.9±1.1 yr., 6 control:69.5±1.8 yr.) with a large individual variability among subjects (Pyka, Taaffe, et al., 1994).

Between young men and young women, young women seem to require a greater threshold to initiate a HGH response to circuit exercise (Ghanbari-Niaki, 2006; Ghanbari-Niaki et al., 2008; Ghanbari-Niaki et al., 2010). Between college age men (22.8±2.3 yr.) and women (20-30 yr.), in regards to HGH response to circuit training, a 3-set of 9-exercise circuit (8-12 repetitions) at 80% of 1RM resulted in a significant increase in HGH response in women (Ghanbari-Niaki et al., 2008; Ghanbari-Niaki et al., 2010); compared to a significant increase in HGH response to only a single set of 10-exercise circuit (8-12 repetitions) at 60% of 1RM in men (Ghanbari-Niaki, 2006). Men also showed post-exercise HGH levels that were approximately twice as high as observed with women. However, since the results in male and female subjects were from different studies, a single study that examines the differences between males and females is needed. Whether or not long-term training would influence resting HGH levels is uncertain in women (Marx et al., 2001; Moghadasi & Siavashpour, 2013). A 24-week circuit training program in untrained women (avg. age 22.7 yr.) showed no training effect on resting circulating HGH values (avg. 2-3µg/L) at week 0, week 12 and week 24 (Marx et al., 2001), while 12-weeks of circuit training significantly increased the resting circulating HGH values in sedentary women (1.3±2.3 mU/l vs. ~4.7 mU/l, p=0.007) when compared to the control groups (no exercise, 3.8 ±4 mU/l vs. ~3.9 mU/l, p=0.12) (Moghadasi & Siavashpour, 2013).

In summary, circuit training is able to stimulate a significant increase in HGH secretion under the condition of a rest interval equal to or less than 1 min between

exercises. A shorter rest interval requires less exercise intensity to stimulate HGH secretion. A gender difference has been found, as young men showed a HGH response to circuit training at a lower intensity and less training volume compared to young women. Meanwhile, an age-related decrease in HGH response to circuit training was observed in men.

Part 7: Human growth hormone response to anaerobic exercise

GH response to aerobic exercise has been studied with maximal and submaximal exercise intensities (K. Stokes, 2003). K. Stokes used the exercise intensity corresponding to VO_{2max} as the criteria to distinguish maximal and submaximal exercise. Based on this threshold, the protocols with exercise intensity equal to or greater than VO_{2max}, which should fatigue the subjects and terminate the exercise within 5 minutes, was considered as a maximal exercise protocol. This review follows the same definition in Stokes (2003), where the author distinguishes exercise intensities greater than peak power output of VO_{2max} as anaerobic exercise and exercise intensities below peak power output of VO_{2max} as endurance exercise. Subjects in an endurance exercise protocol can continue for at least 20 minutes to exhaustion. To our knowledge, all studies which investigated the HGH response to anaerobic exercise utilized male triathletes/cyclists as subjects (24.7±3.4 yr. & 26.5±5.6 yr.) (Wahl et al., 2013a; Wahl et al., 2010), physically or recreationally active men (Gilbert et al., 2008; Goto, Ishii, Kurokawa, et al., 2007; K. Stokes et al., 2005; K. Stokes et al., 2006; K. A. Stokes et al., 2013; K. A. Stokes, Nevill, Cherry, Lakomy, & Hall, 2004; K. A. Stokes et al., 2002b; K. A. Stokes et al., 2003; K. A. Stokes et al., 2010; K. A. Stokes et al., 2008), or elite handball players (Meckel et al., 2009; Meckel et al., 2011; Nemet et al., 2009). There is only one study that included

middle aged healthy men (40-50 yr.) (Gilbert et al., 2008). Therefore, understanding of the HGH response to anaerobic exercise in young and middle age females and in older populations in both sexes is unknown.

7.1. HGH response to a 30s sprint at an intensity of 7.5% body weight

The investigations of the acute HGH response to a single Wingate anaerobic test in physically or recreationally active men have mostly been conducted by Stokes et al. (2002, 2003, 2005, 2006, 2008, 2010, & 2013). After a 30-sec Wingate test at 7.5% of the subjects (men, 23±1 yr..) body mass, a significant elevation in HGH concentration reached to peak levels of 18.5±3.1µg/L at 40 min post exercise and the significant elevation remained up to 90-120 min after sprint exercise (K. A. Stokes et al., 2002b). However, while significant increases in HGH values (average peak HGH value range of 14-18µg/L) after a 30-sec Wingate test in young men were reported, the average peak HGH values varied from high values ranges of 14-18µg/L(K. Stokes et al., 2005; K. Stokes et al., 2006) to low values ranges of 5.5-6.5µg/L (Gilbert et al., 2008; K. A. Stokes et al., 2004; K. A. Stokes et al., 2008).

7.2. HGH response to a 30s sprint at 7% and 9% of body weight (difference resistance applied to the fly wheel, and different peak and mean revolution per min (RPM))

A Wingate test with adjusted resistance loads were also used to determine whether or not HGH response would be affected by the resistance (K. A. Stokes et al., 2010). Between a resistance of 7% and 9% of body mass (BM) during a 30-sec Wingate test, no significant difference was observed between peak power outputs and mean power

outputs during sprints and peak HGH values after the sprints; however, the 7%BM trial had a greater maximum change from pre-exercise than the 9% trial (23.5 (24.0) vs. 13.2 (13.7) µg/L, P=0.07) (K. A. Stokes et al., 2010). During sprints, the peak and mean pedal revolutions (rpm), and the area under curve (AUC) of HGH were significantly higher ($p<0.5$) in the 7%BM trial than in the 9%BM trial. In the 7%BM trial, the low resistance and its consequent high rpm may have an effect on the HGH secretion after exercise because the AUC may be a more consistent reproducible measure of the HGH response to sprint exercise in comparison to peak HGH values (K. A. Stokes et al., 2003). However, the authors didn't mention or discuss any possible relationship between cadence and HGH response to exercise.

7.3. HGH response to repeated 30s sprints at 120rpm (different rest intervals)

Comparing four repeated bouts of 30-sec sprint cycling at maximal 120rpm with either a 7.5-min active rest interval at 45% of peak power output or a 7.5-min passive rest interval between sprints, Wahl et al (2013b) found no significant difference in HGH response patterns. Passive vs. active rest didn't have a significant effect on mean power output, peak power output, or the fatigue index between the two trials. However, significantly different effects on the metabolic perturbations in concentrations of blood lactate, pH, protons, partial pressure of O2 and CO2 during recovery periods were shown. Based on this observation, the author suggested that HGH responses to sprint exercise would not be affected once a certain level of metabolic disturbance was exceeded. In comparison to another study with a similar protocol (30-sec sprint at 120rpm) with passive 5-min rest intervals between sprints (Wahl et al., 2010), the post sprint peak HGH value was greater after a 5 min rest trial than after a 7.5 min rest trial (70mLU/ml vs.

~44mLU/ml) observed in a 7.5 min rest trial (Wahl et al., 2013b). Perhaps the rest interval length had a greater influence on HGH pattern than the type of the rest interval.

7.4. HGH response to shortened sprint period (5s and 6s)

Total sprint time also appears to be important. Whereas a single 6-sec sprint didn't significantly elevate peak HGH values (K. A. Stokes et al., 2002b), a protocol of 8 sets of 5-sec sprints with 30 sec rest between sprints was able to induce significant increases in serum HGH values (Goto, Ishii, Kurokawa, et al., 2007). The significant increase in HGH response to repeated sprints might result from greater lactate production (5-sec repeated sprint:~8mmol/L vs. 6-sec single sprint:~4 mmol/L) and pH depression (5-sec repeated sprint:<7.2 vs. 6-sec single sprint:> 7.3), leading to sufficient metabolic disturbance. The effect of the metabolic disturbance on the GH response to exercise may be influenced by the magnitude of the decreases in the blood pH level in response to the sprint (Gordon, Kraemer, Vos, Lynch, & Knuttgen, 1994; Wahl et al., 2010). The HGH response to a high intensity all-out cycling sprint was significantly reduced the sodium bicarbonate consumption trial which was done to reduce exercise-induced acidosis and blood lactate concentration as compared to the placebo trial (Gordon et al., 2004, Whal et al., 2010). Lactate is transported from intramuscular to the circulation via monocarboxylate transporter (MCT) along with hydrogen ions released into the circulation(Gladden, 2008). However, infusion of various lactate concentrations in the circulation didn't seem to have an effect on the HGH response to exercise(Sutton, Jones, & Toews, 1976). Therefore, the exercise-induced increases in blood lactate concentration might be just one of the reasons contributing to the increase in blood hydrogen ion levels. A maximal 30-sec all-out sprint has shown to reduce intramuscular ATP concentration to

as low as ~25% of the resting level in fast-twitch muscle fibers(Karatzaferi, de Haan, Ferguson, van Mechelen, & Sargeant, 2001). Rat models have shown that the muscle group IV receptors were sensitive to primarily the decreases in extracellular pH level and secondarily to the decrease in intramuscular ATP concentration (Hoheisel, Reinohl, Unger, & Mense, 2004). As a result, the HGH response to 30-sec Wingate sprint may be affected by the metabolic disturbance, due to the exercise-induced increases in hydrogen ion concentration and their subsequent effect on the fast-twitch muscle afferent input.

In summary, a single session of 30-sec Wingate sprint causes a significant increase in HGH secretion after exercise. Possible factors that alter during this type of sprint might include pedal cadence, intramuscular ATP concentration, partial pressure of CO2, and the magnitude of disturbances in blood pH levels, resulting from the changes in blood lactate concentration. .

Part 8: human growth hormone response to sub-maximal intensity aerobic exercise (endurance exercise)

GH secretion in response to submaximal aerobic exercise appears to depend on the exercise intensity. A higher exercise intensity induces a greater GH secretion with a linear dose pattern in both sexes (Pritzlaff-Roy et al., 2002; Pritzlaff et al., 1999). Non-obese people seem to show greater GH secretion than obese people (Kanaley, Weatherup-Dentes, Jaynes, & Hartman, 1999). However, similar to anaerobic exercise, most studies investigated the HGH response to exercise in healthy active men and male athletes. An understanding of HGH response to sub-maximal intensity exercise in other populations remains unclear, with only three studies involving elite female athletes

(Friedmann & Kindermann, 1989; Sartorio et al., 2008; Tarnopolsky, MacDougall, Atkinson, Tarnopolsky, & Sutton, 1990) and one study involving middle age males (Gilbert et al., 2008). Part 8 covers the current understanding of HGH responses to submaximal aerobic intensity exercise.

8.1. Exercise intensity by %VO_{2max}

Felsing et al. (1992) was the first to compare the effect of exercise duration on HGH response in healthy adult males (27±5 yr.) at high (VO_2 @ 50% between LT and VO_{2max}) and low intensity (VO_2 @ 50% of LT). The results showed that a significant increase in HGH response to submaximal exercise required not only at an exercise intensity greater than the LT but also a duration greater than 10 minutes. Later, a significant linear dose-response relationship between HGH response to exercise intensity was confirmed in both recreational active men (26±1.1 yr.) and women (24.3±1.3 yr.) by comparing HGH responses to 30 min of treadmill running at various intensities (Pritzlaff et al., 2000; Pritzlaff et al., 1999). However, the authors didn't report at which exercise intensity level HGH secretion significantly increased.

Regardless, the exercise intensity corresponding to VT, men (29.0±9.4 yr.) with a VO_{2max} range of 40-43 ml/kg/min who exercised twice per week did not show any significant changes in plasma HGH after 20 min of cycling (50 rpm) at a 100 W power output (Vanhelder et al., 1984). Additionally, an hour of steady-state cycling at 50% of VO_{2max} did not cause a significant increase in HGH in healthy male subjects (26.5±5.6 yr) (Wahl et al., 2010). In physically active men (29.17±3.4 yr.), an exercise intensity greater than 75% of VO_{2max} seemed to be necessary to stimulate HGH secretion when circulating

plasma GH was determined using two different immunoassays. The linear does-response relationship of HGH response to exercise intensity was more profound by using immunofunctional enzyme-linked immunoabsorbent assay (ELIZA, Diagnostic Systemic Laboratory) compared to using an immunoradiometric assay (IRMA, the Nichols Institute Diagnostics) (Rubin et al., 2003). Others also reported a significant increase in circulating HGH values after steady-state exercise over 30-min at exercise intensities greater than 70% of VO_{2max} in young active men (Gilbert et al., 2008; K. A. Stokes et al., 2013) and elite male athletes (Sartorio et al., 2008). In addition, steady-state cycling exercise at an intensity greater than 55% of peak power output for a duration greater than 30 minutes has shown to induce significant increases in post-exercise circulating HGH in both physically active men (29.2±4.9 yr.) (Schumann et al., 2014) and in triathletes/cyclists (24.7±3.4 yr.) (Wahl et al., 2013a). Although the absolute exercise intensities corresponding to the percentage of VO_{2max} were not reported by Schumann et al (2014) and Wahl et al (2013a), the percentage of the peak power output usually reflects at least equal or higher percentages in comparison to percentage of VO_{2max} (Pedersen, Sorensen, Jensen, Johansen, & Levin, 2002).

8.2. HGH response to endurance exercise in middle age and old men (Gilbert, Hagberg)

There is only one study which investigated the HGH response to endurance exercise in middle aged (40-50 yr., 45±7 ml/kg/min)(Gilbert et al., 2008) men, and one other study recruited both trained (65±4 yr., 50.0±4.8 ml/kg/min) and untrained older men (65±4 yr., 26.8±2.4 ml/kg/min) (Hagberg et al., 1988). In middle aged men, a 30-min session of 60rpm cycling exercise at 70% of VO_{2max} significantly increased HGH

secretion (Gilbert et al., 2008). The same exercise intensity for one-hour treadmill running resulted in a significant HGH response in both trained and untrained older men (Hagberg et al., 1988). When comparing the HGH response to the young controls in both studies, the magnitude of HGH responses to endurance exercise were significantly greater in young controls than the middle age group and both trained and untrained older groups. Therefore, exercise intensity at equal or greater to 70% of VO_{2max} seems to be sufficient to stimulate HGH response in all male age groups, while young males have a greater HGH response to exercise than older males.

8.3. HGH response to endurance exercise in women

The impact of HGH in response to endurance exercise in women has only been examined in 4 studies. When comparing the HGH response to a one hour treadmill running session at an exercise intensity of 60% of VO_{2max} in both sexes with different training status (female controls: 25.7±3.0 yr., 47.3±3.8 ml/kg/min, male controls: 24.7±2.9 yr., 53.7±5.7 ml/kg/min, female runners: 23.9±2.6 yr., 60.7±3.1 ml/kg/min, male runners: 24.9±2.8 yr., 69.6±4.8 ml/kg/min,), the post-exercise HGH secretion significantly increased in all groups (Bunt, Boileau, Bahr, & Nelson, 1986). Gender effect was observed because only trained males but not trained females had an increased HGH response during and 15 min post exercise. Also, only male subjects showed a significantly higher HGH response to endurance exercise during and 15 min post exercise in the trained male group, but not in the untrained male group. Another study reported no significant differences in HGH response to 15.5 km treadmill running at 65% of VO_{2max} in female subjects (21.5±0.8 yr., 57.8±1.8 ml/kg/min) while a significant increase in HGH response was found in equally trained male subjects (20.0±0.6 yr., 63.5±1.1

ml/kg/min) (Tarnopolsky et al., 1990). When the exercise intensity increased to 80% of VO_{2max}, both elite male and female athletes showed a similar HGH response to a 60-90 min cycling session (Sartorio et al., 2008). It seems that females may require a greater exercise intensity than males to stimulate a HGH response to endurance exercise.

8.4. HGH response to interval training

Interval training protocols with exercise intensities equal to or greater than lactate threshold seem to guarantee a significant HGH response (Gray, Telford, & Weidemann, 1993; Kokalas et al., 2004). In elite national level rowers (20.0±1.6 yr.), rowing for both a single 6-minute session at 4mM/L blood lactate concentration and a session consisting of 4 sets of 5 min rowing exercise at 4-6mM/L blood lactate concentrations with 5 min rest periods between sets, significantly increased circulating HGH values post-exercise (Kokalas et al., 2004). A running session that consisted of an interval of 1-min treadmill running at a speed and incline corresponding to VO_{2max} with 1-min active walking rest between runs until the subjects (trained males, 31.5±4.5 yr.) reached exhaustion, also caused significant increases in HGH secretion (Gray et al., 1993). However, since no circulating HGH data was reported during the active rest intervals between runs (Gray et al., 1993), it is unclear whether exercise intensity or the length of duration to reach exhaustion was responsible for the significant HGH response to running interval relationship.

Other studies used running as an exercise model to assess HGH responses to high intensity running. Among elite handball players, varying distance running intervals were chosen to determine the HGH response to running at 80% of the subject's personal

maximal speed measured by a 100-meter sprint. A total of 1000 meters was divided into 4 sets of 250 meter runs (Meckel et al., 2011; Nemet et al., 2009), and 100 to 400 meter intervals in both ascending and descending orders (Meckel et al., 2009). All types of running intervals significantly increased serum HGH values post-exercise. Among these three studies, the significant increase in serum HGH value was observed after the 3rd run in the descending trial, and after 4th run in both the ascending trial and during the 250-meter intervals. Based on these three running studies, it seems that at 80% of the personal maximal running speed, and a threshold of a total accumulative distance of at least 900 meters was necessary to stimulate an HGH response. However, due to the various models and protocols used between these studies, more studies using an identical exercise mode are necessary to understand the effect of exercise intensity and rest intervals for interval training on the HGH response to exercise.

In summary, it is difficult to generate an evidence-based interval training model which induces a HGH response after endurance exercise. Both total workout volume (running distance, time) and intensity seems to affect the HGH response to exercise. In contrast, during steady-state exercise, females may require a greater exercise intensity (80% VO_{2max}) than men (65% VO_{2max}) to see a HGH response post-exercise. Similar to resistance training, increasing age seems to have a negative effect on the HGH response to endurance exercise.

Part 9: rest periods greater than 1 hour between two workouts

In certain sports, such as football, the training program includes either "two-a-day" or "three-a-day" design, which means there are two or three training sessions within a

day. This type of protocol raises the question of how HGH responds to the second exercise session within the same day, and how much recovery time before the next training session is sufficient for a normal HGH response pattern between training sessions. The answer to this question is unclear. In physically active men, a 3-hour recovery period between two Wingate anaerobic sprints resulted in a significant increase in circulating HGH values in response to the 2nd sprint (K. Stokes et al., 2005; K. A. Stokes et al., 2008). Stokes et al (2005) reported the HGH response to a 1-hour, 3-hour, and 24-hour recovery period between two Wingate cycling sprints. In the 1-hour recovery trial, the 2nd sprint had no effect on HGH secretion, while the circulating HGH value post 2nd sprint (7.0±4.7µg/L) was even lower than HGH value pre 2nd sprint (~9µg/L). When the recovery period between sprints increased to 3 hours, the pre 2nd sprint HGH value (~0.9µg/L) was restored to resting values (~1.7 µg/L) and the post 2nd sprint HGH value (8.3±7.1 µg/L) was increased significantly. However, the HGH value after the 1st sprint (18.2±12.7µg/L) was still significantly higher than the HGH value after the 2nd sprint (8.3±7.1 µg/L). Therefore, a 24-hour recovery period between sprints seems necessary for a normal HGH secretion (13.6±13.8µg/L) response to a sprint exercise (K. Stokes et al., 2005).

Similar patterns of HGH response to 1-hour and 3-hour recovery periods between resistance training following a repeated sprint interval exercise were reported by Goto et al. (2007). In this study, a 3-hour recovery period after sprint interval exercise allowed circulating HGH values to be significantly increased in response to resistance exercise, whereas no changes in circulating HGH values in response to resistance exercise was found when the recovery period was reduced to one hour.

In regards to endurance exercise, a 6-hour recovery period between exercises seems elicit a normal HGH response to exercise compared to the results with 2-hour and 4-hour period between the endurance training sessions (Sartorio, Agosti, Marinone, Proietti, & Lafortuna, 2005; Sartorio et al., 2008).

In summary, the studies evaluating the effect of rest intervals greater than an hour involved only Wingate sprints and endurance training. At least 3 hours between Wingate sprints and 6 hours between endurance training have been found sufficient to obtain a significant HGH secretion after the second workout session. Further studies are needed to understand the effect of different rest periods between two resistance training sessions on the same day.

Part 10: human growth hormone response to combination exercise – exercise order effect

Concurrent training is a design that focuses on both endurance and resistance training for improved sports performance. In the concurrent training design, the resistance training and endurance training sessions could be on separate days or both sessions could occur in one day. In regards to the concurrent training on the same day, there are only six publications that reported the HGH response to a combination of both resistance and endurance training (Craig et al., 1991; Goto, Higashiyama, et al., 2005; Goto, Ishii, Sugihara, Yoshioka, & Takamatsu, 2007; Schumann et al., 2013; Schumann et al., 2014; Taipale, Mikkola, Vesterinen, Nummela, & Hakkinen, 2013). The first study investigated the combination effect of resistance training at 75% of 1RM for 8-10 repetitions following 30 min running at 75% maximal heart rate (HRmax) on HGH

secretion in untrained male subjects (23.5±1.7 yr.) (Craig et al., 1991). In comparison to running exercise only or resistance training only, running exercise caused significantly higher circulating HGH values immediately post exercise than combination and resistance training only. Another study investigated the effect of different durations of cycling (5 min vs. 30 min @ 50% of VO_{2max}) prior to resistance training on HGH response after resistance exercise (Goto, Higashiyama, et al., 2005). The 30-min duration of cycling exercise at 50% of VO_{2max} prior to resistance training caused a suppression of HGH secretion in response to resistance training (Goto, Higashiyama, et al., 2005). A 30-minute duration of cycling exercise at 50% of VO_{2max} significantly increased circulating HGH values immediately post cycling exercise, but suppressed the HGH secretion in response to resistance training with no significant difference from pre-exercise values. When the duration of cycling exercise prior to resistance was reduced to 5 min, the post resistance training HGH value was significantly higher than at rest and it lasted for a 30-minute period post-exercise. The authors followed up and found that a resistance exercise 20 min prior to endurance exercise suppressed HGH secretion in response to the subsequence endurance exercise when compared to HGH concentration after an endurance only trial (Goto, Ishii, Sugihara, et al., 2007). Even when the break between resistance exercise and the subsequent endurance exercise was increased to 120 min, the HGH response to the subsequent endurance exercise was still suppressed. This result together with Sartorio et al (2005, 2008) suggests that a rest interval of less than 2 hours between two exercise sessions does not allow a normal HGH response to exercise after the second session.

The order effect on the HGH response to endurance and resistance exercise has been recently reported (Schumann et al., 2013; Schumann et al., 2014; Taipale & Hakkinen, 2013). Taipale and Hakkinen (2013) investigated the order effects (ES: endurance + strength, and SE: strength + endurance) of the combination of 60-minute running at lactate threshold and resistance training consisting of both explosive and maximal strength types of muscle actions using 3 leg exercises (bilateral leg press, loaded squat jumps, and calf raises) in both male and female recreational runners (age 21-45 yr., male: 54.5±4.0 ml/kg/min, female: 48.5±4.6 ml/kg/min). In male runners after the first half of the training session, both running and resistance training significantly increased circulating HGH, with no significant difference between running and resistance training. At the end of the exercise session, the ES session had a significantly reduced circulating HGH value at the end of resistance training, while no significant change in circulating HGH value at the end of running exercise was observed in the SE session. In men, a similar HGH pattern response to an ES protocol has been reported (Schumann et al., 2013; Schumann et al., 2014). However, the HGH pattern response to a SE protocol was different from other studies using a combination of cycling (a 30-minute cycling at 65% of maximal aerobic power output) and leg press exercise (10 sets of leg press exercise) in physically active men (Schumann et al., 2013; Schumann et al., 2014). Schumann et al. (2013, 2014) reported a significant increase in HGH value from the first half to the end of the exercise session in SE protocol. Schumann et al. (2013, 2014) also reported a significantly higher HGH value after the first half of an ES training session in than a SE session. In female runners, the only significant difference noted was that the HGH value dropped from the first half to the end of exercise session in ES protocol (Taipale &

Hakkinen, 2013). Therefore, it seems that endurance exercise prior to resistance training suppresses circulating HGH values at the end of exercise training when compared to resistance training alone. However, the order effect of endurance and resistance training on HGH response patterns in female runners has not been re-tested. It may require more studies on HGH response to an ES training session in female subjects.

In summary, it seems that endurance exercise compromises the HGH secretion to subsequent resistance training in both men and women. However, the order effect on HGH response to a training session is a relatively new topic due to total six studies with 3 of them were published after 2013. Meanwhile, the mixed results and the insufficient female data require more studies to be done.

Part 10: Conclusion

In conclusion, the maximization of the circulating level of HGH post-exercise training increases the potential for training-related muscle hypertrophy. In regards to resistance exercise, an increase in training volume increases post-exercise HGH. Application of a special element to exercise intensities of 20% to 50% of 1RM, such as blood flow restriction and slow muscle contraction speed, could certainly be options to increase the rise in HGH after exercise. For people who are comfortable with heavier weights, exercise intensities of 60% to 85% involving major muscle groups may be the best choice. While performing workouts, the rest interval between sets should stay ideally less than 3 min in a traditional resistance exercise design and a rest interval of less than 1 min in a circuit weight training design. In a circuit weight training design, an exercise intensity as low as 35% of 1RM with adjusted rest intervals (no rest between exercises

and circuits) can cause a significant increase in HGH secretion. Therefore, there might be a greater flexibility in the programming of a circuit training design than a traditional weight training design. Studies have shown a single 30-sec Wingate sprint can significantly increase circulating HGH level, which can be altered by the pedaling rate and accumulative sprinting duration. In endurance exercise, at least a10-min duration, at an exercise intensity at the lactate threshold has been suggested to cause a significant increase in circulating HGH levels. Note that the intensity at 80% VO_{2max} for women and 65% of VO_{2max} for men seems to be a threshold intensity to produce a significant HGH response to endurance exercise. A 6-hour rest period between training sessions has been shown to be sufficient to produce a normal HGH response during the 2^{nd} training session of both anaerobic and aerobic training. An exercise order effect where using both resistance and endurance training on the same day has shown mixed results in HGH responses and requires further investigation. Also, further studies on the effect of aging and gender (especially female subjects) on HGH responses after various types of exercise are needed.

CHAPTER 3: Methods

Subjects

Seven men, who engaged in regular resistance exercise at least twice per week for more than 6 months, volunteered for the study. All subjects were recruited by flyers in gyms, the university recreational center and word of mouth. All subjects were screened for cardiovascular and musculoskeletal disease using a medical history questionnaire and exercise habits were recorded using a physical activity questionnaire (Heyward, 1997)(see appendix 5). Subjects were excluded from the study if they any of the following conditions were met: 1) taking any ergogenic supplements that could impact exercise performance, 2) current smokers, 3) were not able to perform every exercise with correct technique, as determined by a NSCA Certified Strength and Conditioning Specialist (C.S.C.S.). In addition to these conditions, subjects had to be able to finish a 30-second all-out Wingate test prior to a circuit weight training protocol consisted of three circuits (a warm-up set and the two working set) without help in the first two sets.

Experimental Design

This repeated measures study investigated one group of 7 male subjects who went through 4 experimental trials conducted in the morning between 6-10 am. The experimental trials were performed in an order to avoid trial order effect on HGH response. Each trial was separated by a minimum of 36 hours and no more than 96 hours to allow adequate recovery. The four experimental trials were as follows: 1) a Wingate sprint prior to circuit weight training, 2) a Wingate sprint after circuit weight training, 3) a Wingate sprint only, and 4) a circuit weight training only. The participants reported to

the same location for each trial. All participants got reminder calls or emails with the following instructions before each visit – 1) 12 hour fast before each visit, 2) no strenuous exercise within 24 hours before visit, 3) no alcohol and caffeine for 24 hours before visit.

Pre-Experimental Procedures

Before the experimental trials, subjects reported to the UNM exercise physiology lab twice for preliminary measures. The first visit included the paper work (consent form and a questionnaire of medical history and physical activity history), anthropometric measurements, and maximal muscular strength tests. The subjects who were willing and qualified to participate in this study continued the assessments in the following order: 1) measurement of height and weight without shoes, 2) body fat estimation via three-site skinfold measurement (Jackson & Pollock, 1978), and 3) maximal muscular strength tests for each exercise.

In the second visit, the subjects performed a familiarization trial of a Wingate sprint prior to a weight training circuit. This trial qualified the subjects to be able to participate in the study. In this trial, following a Wingate sprint, the subject had to be able to complete the warm-up circuit (40% of 1RM) and the first workout circuit (70% of 1RM) on their own without any help from a spotter. If a subject did not have the strength and endurance to complete this first workout circuit, he was excluded from the study.

Experimental Procedures

Table 1. The protocols for each trial.

Circuit weight training only	Wingate + circuit weight training	Circuit weight training + Wingate	Wingate only
Data collection (rest)	Data collection (rest)	Data collection (rest)	Data collection (rest)
Bike warm-up (5 min @ 100W)	Bike warm-up (5 min @ 100W)	Bike warm-up (5 min @ 100W)	Bike warm-up (5 min @ 100W)
Rest (1 min)	Rest (1 min)	Rest (1 min)	Rest (1 min)
Circuit warm up @ 50% 1RM (5 min)	Wingate anaerobic test @ 0.7Nm/kg (30 sec)	Circuit warm up @ 50% 1RM (5 min)	Wingate anaerobic test @ 0.7Nm/kg (30 sec)
Recovery: 3 min	Recovery: 3 min	Recovery: 3 min	Cool-down(5 min bike @ 30W)
Circuit 1 @ 70% 1RM (5 min)	Circuit warm up @ 40% 1RM (5 min)	Circuit 1 @ 70% 1RM (5 min)	Data collection after cool-down (Blood draws & lactate)
Recovery: 3 min	Recovery: 3 min	Recovery: 3 min	
Circuit 2 @70% 1RM (5 min)	Circuit 1 @ 70% 1RM (5 min)	Circuit 2 @70% 1RM (5 min)	
Cool-down(5 min bike @ 30W)	Recovery: 3 min	Recovery: 3 min	
Data collection after cool-down (Blood draws & lactate)	Circuit 2 @ 70% 1RM (5 min)	Wingate anaerobic test @ 0.7Nm/kg (30 sec)	
	Cool-down (5 min bike @ 30W)	Cool-down(5 min bike @ 30W)	
	Data collection after cool-down (Blood draws)	Data collection after cool-down (Blood draws & lactate)	

Pre-exercise

Upon arrival, the subjects' hydration status was checked via a urine sample. Urine specific gravity no greater than 1.2 was considered well-hydrated to start the exercise trial. If a subject was dehydrated, he was asked to re-hydrate and was re-checked for hydration status via urine specific gravity measurements every 15 min until adequately hydrated. Each subject rested on a chair 20 min before the pre-exercise blood draw in order to account for postural changes in plasma volume. Then 5 ml of blood was drawn from an arm vein by an experienced phlebotomist. The subjects then performed one of the exercise trials listed in table 1 (above this paragraph).

Warm-up and cool down

All protocols started with a standardized warm-up session of five-minutes of cycling at 100W on a cycle ergometer (Lode Excaliber Sport 2006, ElectraMed, USA). A

one-minute rest period was given between the warm-up and the Wingate anaerobic sprint session or circuit training session. A three-minute rest period was given between the Wingate anaerobic and circuit training session (See table 1.). A 5 min cool-down on a bike against 30W resistance started soon after the exercise protocol was completed. The subjects were asked to sit down on a chair once they completed the cool-down for the entire 60-min post exercise data collection period to control for posture.

Circuit weight training

The circuit training session consisted of 6 exercises in the following order: bench press, bend-over barbell row, Smith machine parallel squat, dumbbell shoulder press, Romanian deadlift (RDL), and latissiumus dorsi pull-down. There were 3 circuits with a 30-second rest period between exercises and a 3-minute rest period between circuits. The exercise intensity of the first circuit was 50% of the 1RM for all exercises as a warm-up set, followed by 2 working circuits at an intensity of 70% of 1RM. One of the trials required a Wingate anaerobic sprint prior to circuit training, and for this session the warm-up set of the circuit weight training was at 40% of 1RM (to help recovery from a Wingate sprint) and the working sets remained the same at 70% of 1RM. The research team acted as spotters during lifting and helped to complete the necessary repetitions in the third set.

Wingate anaerobic sprint

The Wingate anaerobic sprint was performed on a cycle ergometer (Lode Excaliber Sport 2006, ElectraMed, USA) using the Lode Ergometer Manager computer program (Version 6.0). The seat height and the handlebar position were adjusted based on

the subjects' preference. The subjects were able to see the Wingate time parameter via a computer screen. The Wingate anaerobic sprint was a 30-second all-out cycling sprint at the highest cadence possible against the resistance of 0.7 Nm/kg body mass. Five seconds before the resistance was dropped on the flywheel, the subjects started to pedal as fast as possible to reach maximal cadence. At the end of the 5 seconds, the cycle ergometer program applied resistance to the flywheel for 30-seconds during which the subjects are encouraged to maintain the highest cadence possible. Depending on the trial condition, after the sprint, either a 3-min rest started if followed by the circuit weight training, or the subjects started a 5-min period of cool-down on the bike.

Data Collection

Anthropometric measurements

Physical characteristics collected including the following: height, weight, age, body composition. The body composition was estimated using a three site (chest, abdomen, and thigh) skinfold measurement (Jackson & Pollock, 1978). Skinfolds were measured by the same experienced technician in rotational order at least twice under two values within 1mm were obtained. Body density was converted to %fat using a population-specific equation (Heyward, 1997).

One-repetition maximum (1RM) strength test

The subjects performed 2 warm-up sets at their self-selected 50% (10 repetitions) and 70% (5-7 repetitions) of 1RM with 3 minutes of rest between sets. If the subjects didn't know their 1RM for a particular exercise, the research staff calculated a predicted-

1RM (using NSCA training load chart (Baechle & Earle, 2000) based on the subjects' work-out regimen. On the third set, the weight was adjusted to close to their 1RM load based on their training experience. If the subjects were able to complete the third set for more than 4 repetitions, an additional 5-10% of the existing weight was added to the upper body exercises and 10-20% of the weight was added to the lower body exercises (Baechle & Earle, 2000). A 3-5 minute rest period between sets for 1RM was allowed for a full recovery. A successful 1RM test was defined by a weight which the subject could not complete more than 3 repetitions using correct form with no extra motions such as jerking and/or momentum during the lift. The 1RM was then calculated using the NSCA training load chart (3RM=0.93 x 1RM, 2RM=0.95 x 1RM).

Blood sample collection

Venous blood samples (5 ml/each) were collected before exercise by a needle stick and at six time points after cool-down (5, 10, 20, 30, 45, and 60 minutes) via an indwelling IV catheter placed and secured at antecubital area. For each blood sample, 4 ml of the blood was stored in a serum separate for HGH assay and 1 ml of the blood was stored in an EDTA tube to prevent blood clotting for later hematocrit and hemoglobin mass analysis.

Human growth hormone (HGH) analysis

Human growth hormone was measured using an HGH ELISA Kit (Catalog # EK-310-33, Phoenix Pharmaceuticals, Inc.) from blood serum samples. The blood samples sat at room temperature for ~15 minutes to clot, and then were centrifuged at 3600rpm

for 10 min at 4^0 C (Marathon 21K/BR, Fish Scientific Inc.). The serum was transferred into Eppendorf tubes and stored in a -80^0 C freezer for later analysis.

HGH ELISA assay was used following the manufacturer's procedures, starting by dispensing 50 µl of standard, specimens, and controls into appropriate wells followed by a 100 µl of Enzyme Conjugate Reagent dispensed into each well. A 30 sec mix was then carried out prior to a 45 min period incubation at room temperature. All mixture was removed into a waste container, then washed with distilled water 5 times. After that, a 100 µl TMB reagent solution was dispensed into each well and gently mixed for 10 sec, followed by a second incubation at room temperature in the dark for 20 min. After adding 100 µl of Stop Solution to each well, a final step was to gently mix for 30 sec. Within 15 min after the final step, the plate was read by a microtiter plate reader (Bio-Rad, iMark™ Microplate Absorbance Reader, Hercules, CA) at 450 nm optical density.

Blood lactate, hematocrit, and plasma volume

At 5 min after cool down blood lactate concentrations from finger sticks were measured using a portable lactate analyzer (Lactate Plus, Nova Biomedical Corp., USA).

For each blood collection, 1 mL of blood was stored in a EDTA tube to prevent blood clotting and later transferred into 3 microvascular tubes and spun at 3500rpm in a micro- hematocrit centrifuge (CMH-30 series, Micro Hematocrit Centrifuge, Unico Scientific, USA) for 5 minutes. The serum and red blood cell portions in the microvascular tubes were measured and the hematocrit was then calculated with a correction for 4% plasma trapped with the packed red cells (Dill & Costill, 1974). Hemoglobin mass was assayed using Pointe Hemoglobin standard (Cat No. H7506-STD,

Pointe Scientific Inc.). The changes in total blood volume, red cell volume, and plasma volume were then calculated using hematocrit and hemoglobin mass via a standard equation (Dill & Costill, 1974). The change in plasma volume was used to correct the total HGH concentration post-exercise.

Heart rate (HR) and rating of perceived exertion (RPE)

Heart rate was recorded using a wireless transmitter/ receiver unit (FS1, Polar Electro, Lake Success, NY) before and after the warm-up, Wingate anaerobic sprint, and each circuit training session as well as 5, 10, 20, 30, 45, and 60 minutes post-exercise. Rating of perceived exertion (6-20 scale, 6 represents no effort and 20 is maximal effort) was recorded (Borg, Ljunggren, & Ceci, 1985) when subjects finished each trial to assess physiological stress.

Power analysis

Statistical power needed to find significant differences in HGH responses between exercise trials was calculated using G power (Universitat Kiel, Germany, version 3.1). At least 6 volunteers were needed to reach a power of 0.8, , based on a study of differences in HGH responses after exercise using a similar research design (Goto, Ishii, Kurokawa, et al., 2007).

Statistical analysis

Data are expressed as mean ± SE unless otherwise stated. The areas under the GH concentration-time curve (GH AUC) were calculated using a trapezoidal method for 60 min post exercise. A two-way (time × trial) repeated-measures analysis of variance

(ANOVA) was used, followed by Tukey's LSD *post hoc* test, for HGH concentrations and heart rates. For blood lactate, Wingate parameters, RPE, and GH AUC among trials were analyzed using one-way repeated-measures of variance (ANOVA), followed by Tukey's LSD *post hoc* test. Bivariate correlation was used to test the relationship between blood lactate concentration and HGH AUC, blood lactate concentration and peak HGH, and age and HGH AUC. Differences were accepted as significant if $p < .05$ using SPSS (Version 17.0, Chicago, IL).

CHAPTER 4: Results

Chapter 4 summarizes the collected data and the statistical analysis. All data were presented in tables as mean ± standard deviations. No significant correlations were found for lactate to HGH, age to HGH area under curve, and lactate to peak HGH. Tables 1 and 2 show the subjects' anthropometric characteristics and 1RM for each exercise. Figure 1 represents the individual results of serum HGH concentrations for each trial.

Human Growth hormone response

All serum HGH values were reported (Table 3) with a correction for plasma volume change compared to resting levels using a previous described calculation (Dill and Costill, 1974). There was a significant main time effect for serum HGH response to exercise. The mean serum HGH concentrations significantly increased in three trials (Figure 2): the Wingate sprint prior to a circuit weight training session (the WC trial), the Wingate sprint after a circuit weight training session (the CW trial), and the circuit weight training session (the C trial). The mean serum HGH levels were not significantly elevated after the Wingate sprint (the W trial).

The WC and CW trials resulted in significant elevations in mean serum HGH concentration for up to 30 min post cool down. The C trial resulted in a significant elevation in mean serum HGH concentration for up to 20 min post cool down. Among trials, there were significant differences in serum HGH concentrations at 20 and 30 min post cool down (Figure 3).

At 20 min post cool down, the mean serum HGH concentration was significantly greater after the CW trial versus the W and C trials. At 30 min post cool down, the mean

serum HGH concentration was significantly greater after the CW trial than the C and WC trials. Serum HGH area under the curve (AUC) was significantly greater after the CW trial than the W and C trials (Table 5).

Figure 4 represents the individual HGH concentrations after the W trial, which shows the extensive individual variation similar to the previous reported data (Stokes, 2002). Figure 5 (left panel) presents the individual peak HGH concentrations for the W trial in the present study. Note that the peak HGH concentrations among subjects showed a different response for each trial.

When the peak HGH concentrations are compared to pre-exercise at rest, all trials showed a significant increase in mean peak HGH concentration, and the CW trial resulted in a significantly greater mean peak HGH concentration as compared to the C trial (Figure 6).

Wingate results

No significant differences were observed for the Wingate test for peak power, mean power, peak RPM, and fatigue ratio (Table 4).

Blood lactate concentration and Rating of Perceived Exertion

Physiological and psychological parameters (Tables 5-7) included blood lactate concentrations rating of perceived exertion (RPE), and heart rate (HR). The blood lactate concentration was measured at 5 min post cool down after each trial. Significantly higher [La^-] was measured after the CW trial as compared to the other trials ($P<0.05$) (table 5).

The RPE immediately after exercise was significantly higher after the CW trial versus all other trials ($P<0.05$).

Heart rate results

Heart rate was recorded at rest, during the trials, at the beginning and end of cool down (on a cycle ergometer), and post cool down sitting on a chair. Table 6 depicts the HR response at rest and immediately after each exercise bout (For example, a Wingate sprint is one exercise bout and a circuit weight training session consisted of 3 exercise bouts of circuit 1, circuit 2, and circuit 3. Exercise bouts were separated by 3 min rest intervals.) There were no significant differences for HR at rest among the trials. The HR after Wingate sprint was significantly greater in the CW trial as compared to the W and WC trials. Among the circuits within a trial, both circuit 2 and circuit 3 resulted in significantly greater HR as compared to circuit 1. Among circuits between the trials, only circuit 1 in the WC trial resulted in a significantly greater HR as compared to circuit 1 in trial C and in the CW trial. The HR during cool down and post cool down are presented in Table 7.

CHAPTER 5: Discussion

The purpose of this study was to investigate whether or not adding a Wingate sprint to a circuit weight training session either before (WC) or after (CW) the circuit would result in a greater HGH response than an exercise session consisting of either a circuit weight training (C) session or a single Wingate sprint (W). The WC trial was also hypothesized to result in greater HGH concentrations than the CW trial. The results showed both trial CW and trial WC resulted in an extended duration for serum HGH concentrations to be significantly higher compared to the W and C trials. In addition, the CW trial resulted in a significantly greater serum HGH concentration at 30 min post cool down (P30) compared to that value after the WC trial. Another major finding was the CW trial resulted in a greater mean serum HGH concentration than the C trial at 20 min post cool down (P20) and P30 and the WC trial at P30 (figure 3).

Growth hormone response to exercise

The effect of training elements on HGH response has been studied in regards to training volumes (Kraemer et al. 1993, Hoffman et al, 2003, Leite et al, 2011, Smilios et al. 2003), exercise intensities (Kraemer et al, 1993, Hoffman et al, 2003), rest intervals, types of resistance exercise (Linnamo et al. 2005), and Wingate training (Gilbert et al. 2008, Stokes et al. 2010, 2002, 2008, 2006). The present study is the first to investigate the combined effects of circuit weight training and a Wingate protocol, both before and after the circuit. This study confirmed that circulating HGH levels significantly increased after a resistance training session, but not after a single Wingate sprint at respective time points within 60 min post cool-down. We did expect the W trial would result in a

significant increase in HGH response after exercise. A 30-sec all-out sprint has shown an exercise stress to fast-twitch muscle fibers in which the ATP concentration dropped to ~25% of the pre-exercise concentration compared to 70% of the pre-exercise concentration in slow-twitch muscle fibers (Karatzaferi et al., 2001). The decrease in fast-muscle fiber ATP concentration represented strong fast-twitch muscle fiber activation which has shown to increase anterior pituitary HGH secretion in rat models (McCall et al., 2001). However, when comparing the peak HGH concentrations after the W trial to its resting HGH concentrations, the W trial did result in a significant increase in mean peak serum HGH concentration. This concurs with all previous studies using a 30-second all-out Wingate sprint that have shown significant increases in circulating HGH concentration post-exercise (Goto et al., 2008; K. Stokes et al., 2005; K. Stokes et al., 2006; K. A. Stokes et al., 2002a; K. A. Stokes et al., 2010).

The only similarity among all trials in peak HGH concentrations was that HGH concentration peaked between 5-30 min. It eventually returned to resting values by 60 min post cool down after each trial with exceptions. Two individuals (subjects 2 and 4) showed HGH concentrations that remained well above their resting HGH concentration at 60 min post cool down (figure 1). Results from other studies concur that mean circulating HGH levels peak within 60 min after a single Wingate sprint (Nevill et al., 1996; K. Stokes et al., 2006; K. A. Stokes et al., 2002a, 2002b). Resistance training using various protocols has also resulted in peak mean circulating HGH concentrations within 40 min post-exercise (Bottaro et al., 2009; Gilbert et al., 2008; Hoffman et al., 2003; Kraemer et al., 1990; Rahimi et al., 2010). Therefore, the peak HGH time-frame response

to all combinations of exercise training in this study was similar that reported in the previous literature.

Did an additional a Wingate sprint before circuit weight training optimize serum HGH levels?

The significant elevation in serum HGH response to exercise remained for a longer duration after the CW and the WC trials (up to 30 min post cool-down) than the C trial (up to 20 min post cool-down). This may be due to the additional Wingate sprint adding extra volume to the workout for both the WC and CW trials as compared the C trial. Studies with weight lifting designs have shown that higher training volumes resulted in a greater circulating HGH response post-exercise (Hakkinen & Pakarinen, 1993; Hoffman et al., 2003; Leite et al., 2011). The Wingate sprint is a maximal effort cycling exercise and been shown to be an efficient way to increase circulating HGH levels post-exercise (K. A. Stokes et al., 2013; K. A. Stokes et al., 2010). Although a Wingate sprint is not the type of exercise included in total training volume in weightlifting, the 30-sec duration (volume) of the Wingate sprint has shown to impact the magnitude of HGH response (K. A. Stokes et al., 2002b). For example, a single 30-sec all-out sprint resulted in a significant increase in mean serum HGH of 18.5±3.1 µg/L and a significantly greater HGH AUC compared to a single 6-sec all-out sprint (4.0 ±1.5 µg/L) (K. A. Stokes et al., 2002b). And 8 sets of 5-sec repeated sprint (total duration of 40 sec) with 30-sec rest intervals between sets resulted in significantly greater mean serumH GH concentration of above 6 ng/ml compared to resting HGH concentration of less than 1 ng/ml (Goto et al. 2007). In the present study, the mean peak HGH concentration after a Wingate sprint reached 10.65±10.39 ng/ml compared to the resting level at 0.14±.37 ng/ml.

In weightlifting, HGH response reported by Smilios et al. (2003) showed that the increase in resistance training volume (2 sets vs. 4 sets) resulted in an extended duration for elevated circulating HGH concentration. In addition, Smilios et al. (2003) compared training volume and exercise intensity, investigating a crossover effect on the HGH response. Results suggest that the HGH response is affected by both the tension applied to the muscle in a given time and the activation of anaerobic metabolism. Kraemer et al. (1990, 1993) reported greater resistance training volume at moderate intensity (muscular hypertrophy for 10 repetitions at 10-RM vs. muscular strength for 5 repetitions at 5-RM) with 1 min rest interval between sets optimized serum HGH concentration after exercise in both men and women. Other studies have reported significantly higher peak HGH concentrations after high training volume compared to low training volume sessions (Hoffman et al. 2003, Leite et al. 2011). Hoffman et al. (2003) compared a light-intensity, high-volume (LI) (5 sets parallel squats for 15 repetitions at 60% of 1-RM) and a high-intensity, low-volume (HI) (5 sets parallel squats for 4 repetitions at 90% of 1-RM). Results showed a significantly greater mean HGH concentration response to LI session at 20 and 40 min post exercise as compared to HI session. Leite et al. (2011) reported a significantly higher serum HGH concentration immediately after high-volume (3 sets of 12 repetitions at 80% of 12-RM) than low-volume (3 sets of 6 repetitions at 80% of 6-RM) (~15 µg/L vs. ~10.5 µg/L).

Overall, in the present study the additional Wingate sprint added somewhat to the training volume of the circuit weight training session. However, additional factors other than training volume could have affected the extended duration for HGH elevation up to

90 min, such as extra high velocity of muscle contraction to perform the Wingate sprint and the activation of anaerobic metabolism.

The CW trial resulted in a significantly greater serum HGH concentration at P30 compared to the WC trial (figure 3). This might be due to the fact that the CW trial resulted in a greater blood lactate concentration and rating of perceived exertion (RPE) compared to the WC trial. Hoffman et al. (2003) suggested that greater HGH response to a higher training volume session compared to a lower training volume session was due to a greater blood lactate concentration in higher training volume session. The physiological change in the acid-base balance in muscle has shown to affect the HGH response pattern (Gordon et al., 1994). However, Wahl et al. (2013b) suggested that only once the metabolic disturbance exceeded a certain level, HGH would respond to the exercise stress. Wahl et al. (2013b) compared 2 trials of 4 repeated Wingate sprints with either active recovery (50% of VO_{2max} peak power output) or passive recovery (sitting on the bike) for 7.5 min intervals between sprints. The metabolic disturbance as described by Wahl et al. (2013b) is described as the changes in the levels of blood lactate concentration and blood pH measured before and after sprints, and 10 min after the end of exercise. Between the 2 trials, the passive recovery trial resulted in a significantly greater blood lactate concentrations and a significantly lower blood pH starting both during and after exercise. Although the active recovery resulted in a better buffer to ease the metabolic disturbance, there was no significant difference in serum HGH concentration response at 30 min post exercise between these 2 trials. The results in the present study could not be explained by Wahl's trigger threshold for metabolic disturbance (Wahl et al., 2013b) and no change in acid-base balance was measured in the present study. The present study and Wahl et al.

(2013) measured blood lactate at the same time after end of exercise (10 min post exercise), and serum HGH concentrations only 5 min apart at 30 min vs. 35 min post-exercise. The present study showed significantly greater blood lactate concentration (table 5) as well as a significantly greater serum HGH concentration at P30 (figure 3) after the CW trial than the WC trial. However in the present study, there was no significant correlation between lactate and HGH concentration 5 min post-exercise. As a result, while the recovery types didn't affect the HGH response to exercise in Wahl et al. (2013), the exercise order in the present study might have caused this significant difference in serum HGH concentrations at P30 between the WC and CW trials. Exercise order may only be a partial explanation for the mean serum HGH response to exercise in the present study. The mean serum HGH concentrations at P5 and P10 were not significantly different among all the trials in the present study (table 3). The mean serum HGH concentrations showed no significant differences at respective time points/sampling times (table 3) as well as no significant differences in peak mean serum HGH concentrations (figure 6) among the W, C, and WC trials. Although the mean serum HGH concentrations at respective blood sampling times after the W trial didn't show a significant increase compared to rest, there was a significant increase found between the peak HGH concentrations and the serum HGH concentrations at rest (figure 6). The blood lactate concentrations also were not significantly different among the W, C, and WC trials (Table 5). It seemed that the trigger threshold in metabolic disturbance described by Wahl et al. (2013b) helps explain the relationship between our HGH results and the blood lactate concentrations among the C, W, and WC trials, regardless of the differences in exercise volume.

The WC trial was hypothesized to result in the greatest serum HGH concentration among all trials in the present study. We expected a Wingate sprint to deplete ATP concentration in the fast-twitch muscle fibers (to 25% of resting level), which could require greater fast-twitch muscle fiber activation in the subsequent circuit weight training bout, resulting in a greater HGH response after the WC trial. The blood lactate concentration showed no significant difference between the W and the C trials, supporting the idea that a Wingate sprint results in similar metabolic disturbance compared to a circuit weight training session. However, there was no significant difference in mean serum HGH concentrations among all trials at the 5-min post-exercise (P5) and 10min post-exercise (P10). Only the CW trial resulted in a significantly greater mean serum HGH concentration than the C and the W trials at P20, and a significantly greater mean serum HGH concentration than the C and the WC trials at P30. Perhaps the circuit weight training session in the present study served as an appropriate stimulus to increase fast-twitch muscle fiber recruitment in the following Wingate sprint bout. In the present study, the Wingate sprint prior to circuit exercise was expected to cause a greater level of fast-twitch muscle fiber recruitment (which was not measured) during the circuit exercise session, and to result in a greater HGH secretion and a greater serum HGH concentration after exercise compared to the other trials. Rat model research has shown that the HGH secretion from the anterior pituitary gland is stimulated by the afferent input from fast-twitch muscle activation and inhibited by the afferent input from slow-twitch muscle activation, whereas the afferent input from fast-twitch muscle overrides that from slow-twitch muscle (McCall et al., 2001). A heavy exercise protocol to fatigue, recruiting fast-twitch muscle fibers, has been suggested to decrease subsequent exercise

efficiency in humans (Sahlin et al., 2005). This theory was suggested due to greater blood lactate concentration, lower blood pH, and lower gross efficiency (Watt/O_2 L/min) in the exercising muscle (Sahlin et al., 2005). Muscle biopsy results also showed greater anaerobic metabolism to support the possibility of a greater fast-twitch muscle fiber activation in the pre-fatigued muscle, such as lower levels in creatine phosphate (PCr) and creatine phosphate/creatine (PCr/Cr) ratio, and a greater level in Cr (Sahlin et al. 2005). Meanwhile, the increase in blood lactate concentration during exercise has been suggested to result in a greater anaerobic metabolic environment and to promote fast-twitch muscle fiber activation (Takarada et al., 2000). In the present study, we assumed that the Wingate sprint prior to circuit weight training with a 3 min rest interval between the two exercise sessions would result in elevation in blood lactate concentration. This in turn would create a greater anaerobic metabolic environment while performing the following circuit weight training bout, in which the non-fatigued muscles worked at high anaerobic metabolic environment (upper body exercises) and the previous fatigued thigh muscle would have recovered well enough to be able to complete lower body exercises at a lower exercise efficiency. Therefore, the elevation in blood lactate concentration and a more acidic intramuscular environment (similar to the condition created by vascular occlusion) was expected to increase fast-twitch muscle fiber activation during upper body exercises. Application of vascular occlusion to exercising muscles, inducing greater blood lactate concentration, has shown a greater relative muscle integrated electromyogram signal (1.8 times greater). This results in greater muscle fiber activation than exercise without vascular occlusion under light exercise conditions (Takarada et al., 2000). This phenomenon results from a local hypoxia and a suppression of blood lactate

clearance, so a more acidic intramuscular environment is created to increase intramuscular metaboreceptors and group III and IV afferent input. Unfortunately, due to the nature of the research design in the present study, it would be very difficult to measure blood lactate concentration and/or to apply electromyography (EMG) during the weight training sessions. The lactate concentration during exercise and EMG to predict muscle recruitment pattern are lacking.

A greater fatigue ratio and/or a greater muscle lactate concentration could also support the theory of a greater muscle activation pattern (K. A. Stokes et al., 2002b). Although the CW trial vs. the WC trial did result in a trend for a greater fatigue ratio (no significant difference), the nature of weight lifting (circuit training) does not produce a fatigue ratio as a marker to indicate muscle fatigue. Thus, this study was not able to determine if a previous Wingate sprint would have an effect on the muscle activation pattern in the subsequence circuit weight training session in the WC trial compared to the CW trial when the exercise order was switched. It was also difficult to compare the muscle fatigue status between two different types of the exercises (weightlifting vs. Wingate sprint). We expected a different serum HGH response to the order effect of a Wingate sprint with a circuit weight training session. There was a significantly greater mean serum HGH concentration at P30 after the CW trial as compared to the WC trial. This one significant difference at one time point might not be strong enough. Also, there was no significant difference in the HGH AUC between the CW and the WC trials. Therefore, we don't think there were distinct patterns in HGH in response to exercise between the CW and the WC trials.

In summary, when a Wingate sprint is added either prior to, or after a circuit weight training session, the factors that influence HGH responses to exercise training might involve more complex interactions among the order of exercises, the type of exercise, and the velocity of muscle contraction. Although it is not easy to determine the magnitude of these factors that contribute to the HGH response to exercise training, the suggestion could be made that the CW trial resulted in the optimization of the HGH response among all trials, due to its significantly higher HGH concentrations than other trials, and the extended duration for the increased HGH concentration after exercise.

A Wingate sprint only trial

In the present study, the blood lactate concentration (W: 8.9±1.9mM/L, C: 7.7±3.4mM/L, WC: 9.2±3.3mM/L) and subjects' RPE (W: 16.6±2.1, C: 14.6±1.2, WC: 15.1±2.1) were not significantly different among the W, C, and WC trials, showing that both physiologically and psychologically the subjects were challenged and similarly stressed among the trials. The W trial only resulted in a significant increase between the mean peak serum HGH concentration and resting HGH. This finding was similar to the findings of Stokes et al. (2010), who reported a significant increase in peak HGH concentrations up to 23.5 ±24.0 µg/L using a Wingate sprint against 7% of body mass and 13.2 ±13.7 µg/L using a Wingate sprint against 9% of body mass above pre-exercise HGH concentrations. Other published data using healthy recreational active young men have shown a significant elevation in peak serum HGH concentrations after a single Wingate sprint (K. Stokes et al., 2005; K. Stokes et al., 2006; K. A. Stokes et al., 2013; K. A. Stokes et al., 2002b; K. A. Stokes et al., 2010; Wahl et al., 2010).

Individual HGH responses to the W trial in the present study had a similar trend to those observed by Stokes et al. (2002). In the present study, HGH responses to a single Wingate sprint showed quite different characteristics among subjects (figure 4). In the 60 min period after the W trial, HGH responses were observed in the patterns of bell shape curve (subject #1 and #3), right skewed curve (subject #5 and #6), and left skewed curve (subject #4), resulting in a large within-subject variation in HGH response patterns. Although the subjects in the present study were all recreational resistance-trained men, their training regimens were quite varied. For example, one subject was training for a marathon event, another was in CrossFit style training, and another was training for an Olympic weight lifting event as well as high intensity skill conditioning for his upcoming rugby season. These variations among the subjects in the present study might have affected their HGH responses to exercise. Individual variations in HGH response to exercise may also be due to age, gender, body composition, fitness status, and prior HGH secretion (rest intervals between exercise bouts) (K. A. Stokes et al., 2003). However, there is only one study which indicated a significantly greater serum HGH response to a 30-second maximal treadmill sprint in sprint-trained athletes compared to endurance-trained athletes (Nevill et al., 1996). Others have reported that individuals' serum HGH concentrations after a single Wingate sprint has been reported to peak at 10 to 60 min post-exercise (Stokes 2003) and remain elevated for 90 to 120 min after exercise (K. A. Stokes et al., 2002b). One study reported individuals' HGH response to a Wingate sprint (K. A. Stokes et al., 2002b) in which the serum HGH concentrations peaked at a range from 2.3 to 39.7 µg/L without any discussion regarding the possible factors for this large individual variation; the time to reach the peak HGH concentrations ranged from 20 min

in 3 participants to 60 min in another. The present study monitored serum HGH level over the 60 min after exercise, therefore, we do not know whether the HGH concentration remained elevated for the 90 min time point (as reported by Stokes et al, 2002b) after the W trial.

To our knowledge, there are only two previous studies which mentioned large individual variation in HGH response after a single Wingate sprint (K. A. Stokes et al., 2002b; K. A. Stokes et al., 2003) and only one study reported each participant's HGH concentrations over the respective times/blood sampling after a Wingate sprint exercise (K. A. Stokes et al., 2002b). Both the present study and Stokes et al. (2002b) demonstrated a similar trend in highly variable HGH response patterns after a Wingate sprint among subjects. In terms of the other exercise combinations in the present study, because this study was the first one to document the HGH responses to the combinations of a Wingate sprint and a circuit weight training session, we cannot compare the combination trials in the present study to other previous published data.

Is there an offset mechanism in subject 1?

Subject #1 had an unexpected serum HGH response to the WC trial compared to the rest of the trials. While subject #1 had increases in serum HGH concentrations in response to the CW (24.1 ng/ml at P5), C (7.44 ng/ml at P5) and W (12.3 ng/ml at P20) trials with no detectable HGH levels detected at rest for all trials, there was almost no HGH detected after the WC trial WC (only 0.13 ng/ml serum HGH concentration at P5). Previously published data shows that under the condition of a rest interval of 1-4 hours between Wingate sprints and a 2nd exercise bout, the HGH response to the 2nd exercise

bout was impacted (Goto, Ishii, Kurokawa, et al., 2007; K. Stokes et al., 2005). We did not expect to see the blunted HGH response occurring in subject #1 because there was only a 3-min rest interval between the Wingate sprint and the subsequent exercise bout in the present study. Therefore, subject #1 might possibly be an individual whose HGH response to the circuit weight training was impacted by the previous Wingate sprint in trial WC.

Conclusion

The results in this study show that trial CW and WC resulted in a longer duration of significant elevation in serum HGH concentration compared to the C and W trials. The results also show that the CW trial resulted in a significantly higher acute HGH response to exercise training at P30 compared to the WC trial, and thus may be a better order choice for optimal HGH release. However, we believe that the optimal HGH response would be to achieve both greater peak concentration as well as longer duration of significant elevation in HGH concentration after an exercise trial. Our results show that combining two anaerobic types of exercise increased the potential for elevations in circulating level of HGH after exercise. One application of these results is to implement this type of exercise session in a long-term training program in order to attempt to further increase muscle hypertrophy and strength. There were large inter-individual differences in HGH secretion in response to the exercise sessions, which could account for the non-significant differences in HGH responses after the W trial. These differences in HGH response patterns might be due to the subjects' personal training regimens. The subjects in the present study included those with a greater endurance training volume relative to strength training volume, as well as those with a greater strength training volume relative

to endurance training volume. To our knowledge, there is no research investigating the effects of strength versus endurance training regimens on HGH responses to an acute resistance exercise bout in humans. Our results showing large individual variation in HGH response to exercise may explain why some people's muscular strength and mass adapted to training is better than others. However to date, the understanding of the individual variation in HGH response to exercise is still limited.

CHAPTER 6: Summary, Conclusions, Additional Findings, Limitations, and Recommendations

Summary

The purpose of this study was to determine whether or not adding a Wingate sprint to a circuit weight training session would optimize acute human growth hormone (HGH) response to exercise. In this study, seven healthy recreational resistance-trained male volunteers performed 4 trials with at least one day rest between trials. The 4 trials were: 1) a Wingate sprint only (the W trial), 2) a circuit weight training session (the C trial), 3) a Wingate sprint prior to circuit weight training session (the WC trial), 4) a Wingate sprint following circuit weight training session (the CW trial). The hypotheses in this study were: 1) the WC and CW trial would result in a greater HGH response (both concentration and duration) after exercise compared to the W and C trials, and 2) the WC trial would result in a greater HGH response (both peak HGH concentration and duration for the significant elevation) to exercise compared to the CW trial.

These 4 trials were assigned to the subjects in a balanced order to avoid trial order effects. The Wingate sprint was a traditional 30-second all-out sprint with a flying start against a resistance of 7% of the subjects' body mass (0.7Nm/kg) on a cycle ergometer (Lode Excaliber Sport 2006, ElectraMed, USA). The circuit weight training session was a full body workout consisting of 6 exercises in the following exercise order – bench press, bent-over barbell row, smith machine squat, seated dumbbell shoulder press without back support, Romanian deadlift, and latissiumus dorsi pull-down. During circuit weight training, all subjects performed 10 repetitions (reps) at 50% (40% if the circuit weight

training was after a Wingate sprint) of 1 repetition-maximum (RM) for each exercise in the 1st circuit, and 10 reps at 70% of 1RM for each exercise in the 2nd and 3rd circuits. There were 30-second rest intervals between resistance exercises and 3-minute rest intervals between circuits. All trials started with a 5-minute warm-up cycling at 100 Watts and ended with a 5-minute cool-down cycling at 30 Watts. In the trials consisting of both a Wingate sprint and the circuit weight training session, a 3-minute rest interval was given between the Wingate sprint and circuit weight training session.

Urine specific gravity was measured to ensure a hydrated status before each trial started. A urine specific gravity over 1.2 would require the subject to drink water and re-check the urine specific gravity every 15 min. Heart rate was monitored using Polar heart rate monitor (FS1, Polar Electro, Lake Success, NY) at rest, immediately after the Wingate sprint, after each circuit, and at the 1st and last minutes during cool down and with each blood sample collection. Blood samples were collected before each trial at rest and at 5, 10, 20, 30, and 45 minutes after cool-down for hematocrit and hemoglobin mass to correct for plasma volume contraction, and for measurement of HGH concentration using HGH ELISA Kit (Catalog # EK-310-33, Phoenix Pharmaceuticals, Inc.). Rating of perceived exertion (RPE) was reported immediately after the end of exercise in each trial. Blood lactate concentration was measured using a portable lactate analyzer (Lactate Plus, Nova Biomedical Corp., USA) at 5 minutes after cool down in each trial.

A two-way (time × trial) repeated-measures analysis of variance (ANOVA) was used, followed by Tukey's LSD *post hoc* test, for HGH concentrations and heart rates. Blood lactate, Wingate parameters, RPE, and GH AUC among trials were analyzed using one-way repeated-measures of variance (ANOVA), followed by Tukey's LSD *post hoc*

test. Bivariate correlation was used to test the relationship between blood lactate concentration and HGH AUC, blood lactate concentration and peak HGH, and age and HGH AUC. Differences were accepted as significant if $p < .05$ using SPSS (Version 17.0, Chicago, IL).

The first hypothesis was not accepted. Both the WC and CW trials compared to the W and C trials resulted in longer durations for significant elevation in mean serum HGH concentration compared to pre-exercise values, but only the CW trial resulted in a significantly higher mean peak HGH concentration than the C trial. Both the WC and CW trials had significant increases in mean serum HGH up to 30 min post cool-down, while the C trial had a significant elevation in mean serum HGH only up to 20 min post cool-down. The W trial showed no significant elevation in mean serum HGH post cool-down, but it resulted in a significant increase in peak HGH concentration compared to the resting value. The second hypothesis was also not accepted. There was no significant difference in peak HGH response between trial WC and trial CW. However, trial CW did result in a significant greater serum HGH concentration compared to trial WC at P20.

Conclusions

Based on the data analysis, the following conclusions were made:

1. There was a statistically significant time-by-trial effect for acute HGH response to exercise. Both the CW and WC trials resulted in an extended duration of statistically significant elevation in circulating HGH concentration after exercise compared to the C and W trials.

2. The CW trial resulted in a significantly greater serum HGH concentration compared to trial WC at P20. Therefore, trial CW might be a better protocol to optimize acute HGH response to exercise.

Additional findings

This primary purpose in this study was to document the acute HGH response after training sessions consisted of different matches of a Wingate sprint and a circuit weight training session. Other physiological and performance parameters were also monitored including post-exercise blood lactate concentrations, heart rate, rating of perceived exertion, and Wingate power and fatigue parameters.

Wingate parameters

Wingate parameters (peak power, mean power, fatigue ratio, and peak revolution per min) showed no significant differences among the trials. A 3-5 minute relief period is generally recommended for a sufficient recovery interval between weightlifting sets for muscular power and strength exercises. A 3-min rest interval between circuits and Wingate sprint was set to buffer the metabolic disturbance (assist the recovery to restore blood pH and lactate concentration towards resting values) so that the subjects were able to continue and complete the following exercise bouts. The recovery time in the present study was actually more than 3 min between the Smith machine squat and Wingate sprint. Between the Wingate sprint and the circuit, the non-squat exercises (bench press and bend-over barbell row and two 30-second recovery intervals) made a total rest interval of 5-min (3 min + 2 min). This gave time for the recovery of the leg muscles for subsequent

performance and to restore the capacity of power generation. Therefore, it was reasonable to see no significant differences between the Wingate sprint parameters for each trial.

Blood lactate concentration, RPE, and HR response

Blood lactate concentrations and RPE after each circuit showed no significant differences between the C and WC trials, meaning there was no significant impact of the previous Wingate sprint on the metabolic disturbance at the end (last) of the subsequent circuit weight training session. However, the HR immediately after circuit 1 was significantly greater in trial WC compared to trial C and trial CW. It showed that a previous Wingate sprint did increase the metabolic disturbance in circuit 1 in the WC trial, but this metabolic disturbance probably was buffered over the entire circuit weight training session. As a result, we assumed that a greater metabolic disturbance only in circuit 1 might not be enough to induce a significantly greater HGH response after trial WC than other trials.

Is there an offset mechanism in subject 1?

We did not expect to encounter the suppression of the HGH response most likely due to the previous Wingate sprint affecting the subsequent exercise bouts because of the short 3-min rest interval between Wingate sprint and circuit weight training session. Subject #1 might possibly be the responder whose HGH response to the circuit weight training was most impacted by the previous Wingate sprint in the WC trial.

The blood collection at P5 after the WC trial was very close to the same time as the blood collection at P30 after the W trial due to the very short exercise session for the W. However, subject #1 had a serum HGH value detected at 9.3 ng/ml for the W, but 0

ng/ml for the WC. The subjects in the present study performed all trials in non-consecutive days. This recovery period between trials should allow the same Wingate performance and reproduce a very similar HGH response to Wingate sprint (Goto, Ishii, Kurokawa, et al., 2007; K. Stokes et al., 2005). Thus, subject #1 should have had a serum HGH concentration somewhere around ~9.3 ng/ml (measured during the W only trial) at P5 after the WC trial because there was more than a day recovery between the trials in this study. It has been shown that more than 24 hr of recovery is adequate to restore the HGH response to a Wingate sprint(K. Stokes et al., 2005).

A circuit weight training following a Wingate sprint might have an effect on the serum HGH turnover rate in subject #1. Repeated Wingate sprints with 60 min between sprints showed no HGH response after 2nd Wingate sprint, and the circulating HGH value detected 5 min after 2nd sprint was even lower than the value prior to 2nd Wingate sprint (K. Stokes et al., 2005). When the rest interval had increased to 4 hours, the 2nd Wingate sprint was able to induce a significant elevation in circulating HGH values with a tendency for a smaller HGH response after 2nd sprint than the 1st sprint. Similar finding in HGH response to the 2nd exercise bout was reported with repeated 5-second Wingate sprints followed by a resistance exercise session with different recovery periods (1 hour and 3 hours) between exercise bouts (Goto, Ishii, Kurokawa, et al., 2007). Both 1 hour and 3 hours recovery periods between 5 sets of 5-second Wingate sprints and a resistance exercise session showed reduced HGH responses to the following resistance exercise. One hour recovery period had a stronger impact on the reduction in HGH secretion after 2nd bout exercise compared to 3 hours recovery period. The study also showed a trial of resistance exercise with no previous Wingate sprint resulted in a significant greater HGH

response to resistance exercise compared to both trials with 1 hour and 3 hour recovery periods after Wingate sprints. Therefore, the only explanation to the different response to trial WC in subject #1 and the rest of the subjects in this study might due the individual differences.

Limitations

Overall, the limitations in this study are listed below:

- Blood lactate concentration was measured only at one time point post-exercise.
- Blood pH levels were not measured.
- Individual differences are unpredictable.
- Individual's training regimens and training status may vary their HGH response to exercise training.
- Muscle fiber activation patterns and metabolic disturbance were not measured during the trials.
- This is the first study to investigate this kind of the exercise order effect on HGH response to exercise, therefore, were no examples or suggestions to consider before the study started.

Recommendations

The results and findings of this study suggest the following recommendations for future research.

- To document the acute HGH response to different types of exercises among athletes and the general population in different types of sports and training regimens.
- To further document whether or not the effect of application of previous muscle fatigue could affect the HGH response by programing an exercise bout that ensures a greater metabolic disturbance throughout the entire training session.
- To investigate if all-out sprint exercise performed prior to resistance training with a rest interval greater than 3 min reduces HGH response to the exercise combination compared to sprint exercise alone or resistance training alone. If so, what is the recommendation for the rest interval?
- To investigate whether an application of blood flow restriction or hypoxia could promote metabolic disturbance and HGH response to high intensity exercise training.
- Another suggestion for future research is to investigate whether endurance and strength training status, subject age, or training volume has an effect on the HGH response to different types of exercise training. Among the above populations, there are no data on the HGH response to a Wingate sprint in women to date. It is important to investigate whether or not a modification of the present study would increase the HGH response to exercise in an older population to prevent aging-related muscle mass loss which could help promote increased quality of life. As a result, more work has to be done to understand the HGH response to exercise in other populations.

- One future proposed research direction is to document exercise training design for optimal HGH response, including: 1) implementation of a longer rest interval for the circuit weight training session in CW trial, 2) manipulation of training volume in either the circuit weight training session and/or Wingate sprint session.

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APPENDIX 1: Figures

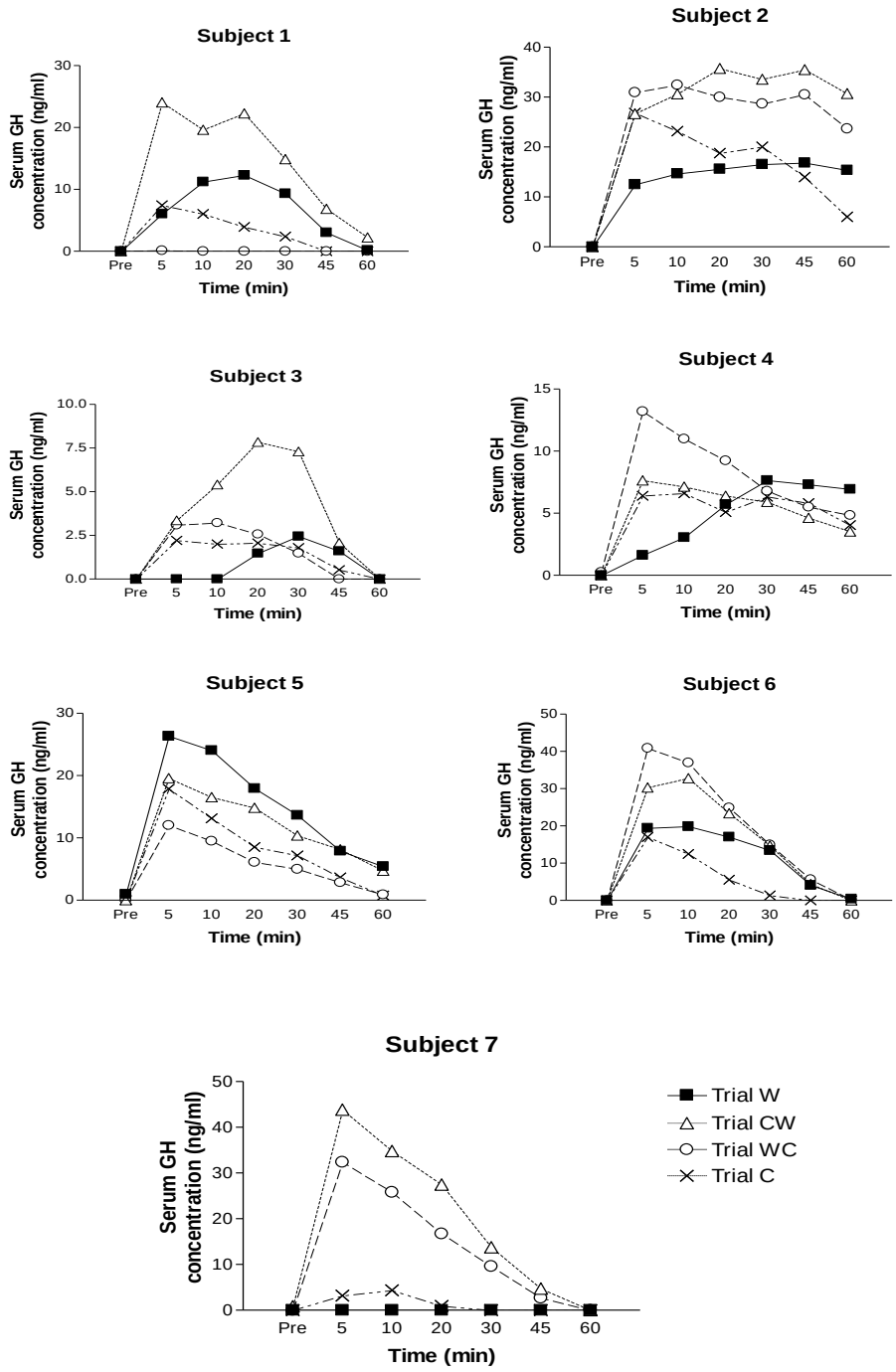

Figure 1. Individual human growth hormone (HGH) response to each trial.

Pre = at rest before exercise started, 5-60 = the time post cool-down
Trial CW: Wingate sprint after circuit weight training
Trial W: Wingate sprint trial,
Trial C: circuit weight training trial
Trial WC: Wingate sprint prior to circuit weight training trial.

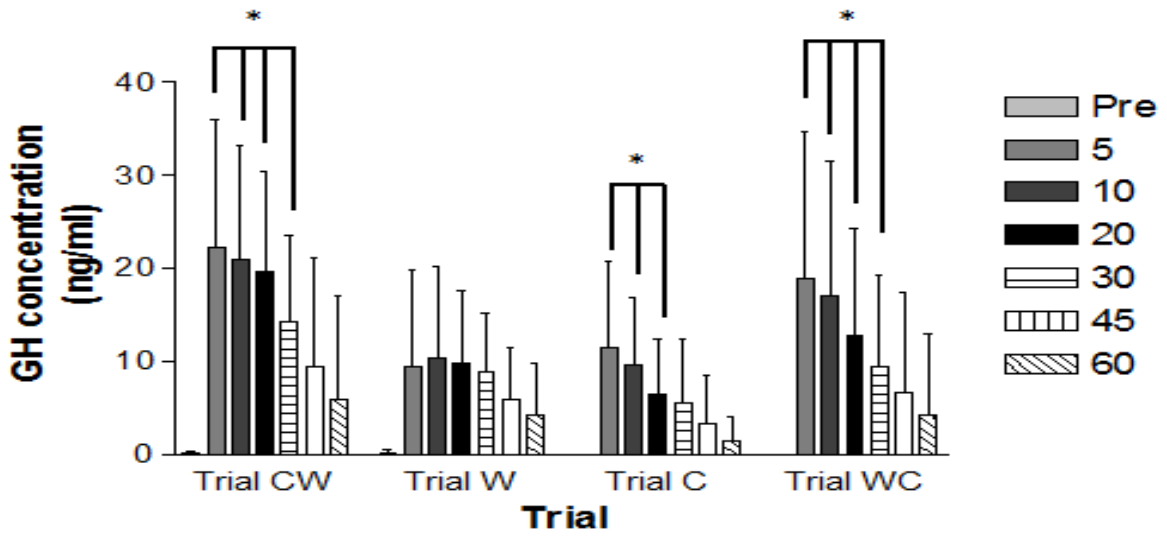

Figure 2. The comparison of serum HGH concentrations at rest and post cool-down within trials.

Values are mean ± SD, N = 7. Pre: pre-exercise, 5: 5 min post cool-down, 10: 10 min post cool-down, etc.. Trial CW: Wingate sprint after circuit weight training, trial W: Wingate sprint trial C: circuit weight training, trial WC: Wingate sprint prior to circuit weight training.
* indicates a significantly greater mean serum HGH concentration than at rest.

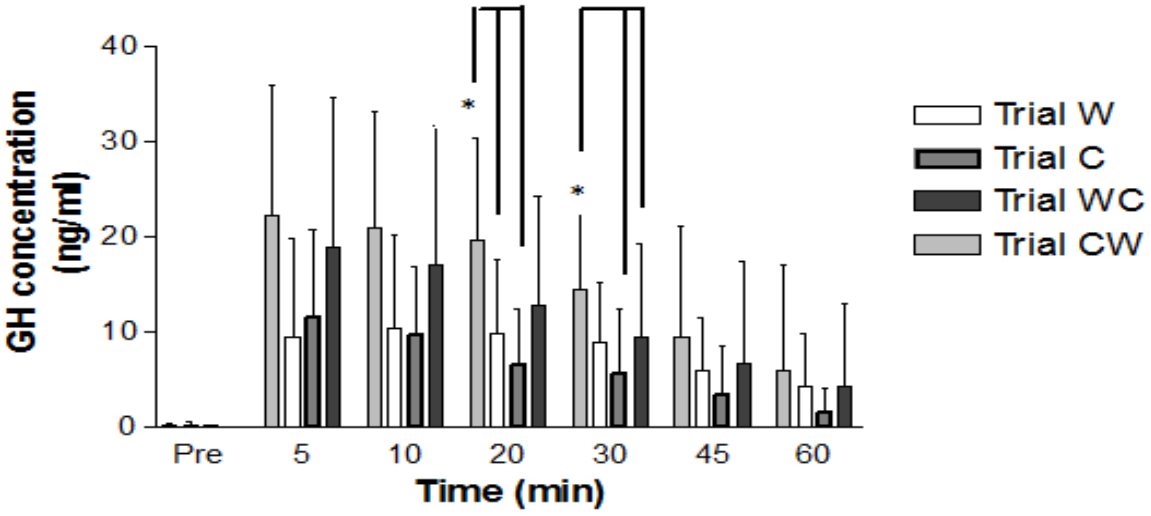

Figure 3. The comparison in serum HGH concentrations among trials.

The x axis represents the time of blood draws, Pre: pre exercise, 5: 5 min post cool-down, 10: 10 min post cool-down, 20: 20 min post cool-down, 30: 30 min post cool-down, 45: 45 min post cool-down, 60: 60 min post cool-down. Trial CW: the Wingate sprint after circuit weight training trial, trial W: the Wingate sprint trial, trial C: the circuit weight training trial, trial WC: the Wingate sprint prior to circuit weight training trial. * indicates significant difference in mean serum HGH concentrations among the trials at the respective time/blood sampling time.

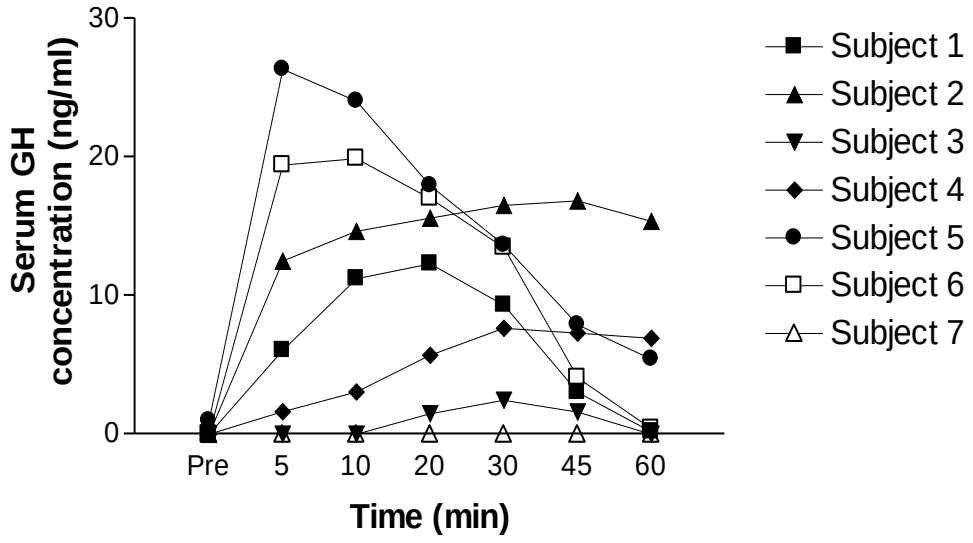

Figure 4. Individual HGH concentrations after the Wingate trial.

Figure 4 represents the serum HGH concentrations for each subject for the W trial in the present study. The x axis represents the time of blood sample collections, pre = at rest before exercise started, 5-60 = time post cool-down.

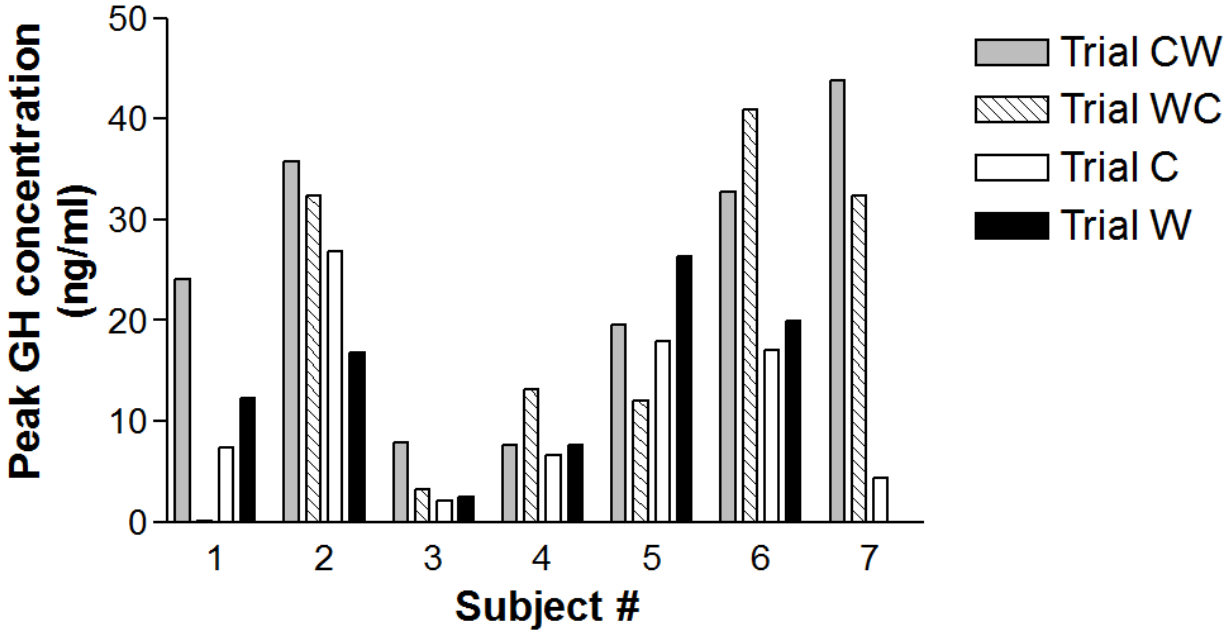

Figure 5. Individual peak HGH concentrations for all trials.

Trial CW: Wingate sprint after circuit weight training, trial W: Wingate sprint, trial C: circuit weight training , trial WC: Wingate sprint prior to circuit weight training. Each bar represents the subject's peak serum HGH concentration for each trial.

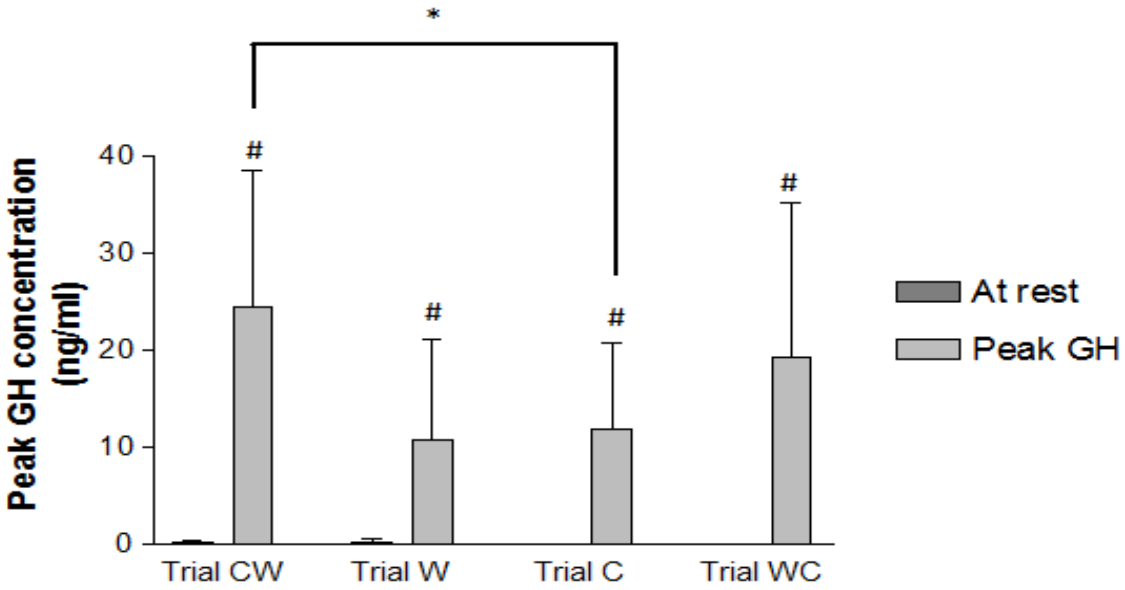

Figure 6. The peak serum HGH concentration (ng/ml) among all trials.

Trial CW: Wingate sprint after circuit weight training, trial W: Wingate sprint, trial C: circuit weight training, trial WC: Wingate sprint prior to circuit weight training.
* indicates significant difference in peak serum HGH concentrations among the trials.
# indicates significant difference in peak serum HGH concentrations compared to rest.

APPENDIX 2: Tables

Table 1. Anthropometric characteristics (N = 7)

Variable	Mean ± SD	Range
Age (yrs)	24.9 ± 4.6	22-35
Height (cm)	175.9 ± 8.0	167.6-188.1
Weight (kg)	78.4 ± 9.7	62.5-94.4
BMI (kg/m^2)	25.3 ± 2.2	23.0-29.7
Body fat (%)	8.5 ± 3.4	5.5-14.8

BMI = body mass index

Table 2. One repetition-maximum tests (N = 7)

Variable (kgs)	Mean ± SD	Range
Bench press	95.8 ±9.3	85.5-107.7
Bent-over barbell row	75.0 ± 11.8	55.9-90
Smith machine squat	107.0 ± 27.5	61.4-138.6
Seated dumbbell shoulder press	25.5 ±4.9	16.8-31.4
Romanian deadlift	121.5 ± 43.5	129-446
Lat pull-down	85 ± 14.3	59.1-101.4

Table 3. Human growth hormone (HGH) concentrations pre and post exercise (N = 7)

	Time (min)						
Variable and trial	Pre	P5	P10	P20	P30	P45	P60
GH concentration (ng/ml)							
CW	0.12 ± 0.33	22.21 ± 13.73*	21.00 ± 12.11*	19.71 ± 10.64*@	14.37 ± 9.19*a	9.49 ± 11.64	5.88 ± 11.10
W	0.14 ± 0.37	9.43 ± 10.38	10.42 ± 9.72	9.80 ± 7.82	8.88 ± 6.30	5.95 ± 5.54	4.28 ± 5.61
C	0.00 ± 0.00	11.56 ± 9.16*	9.68 ± 7.21*	6.42 ± 5.99*	5.59 ± 6.89	3.43 ± 5.18	1.55 ± 2.46
WC	0.00 ± 0.00	18.95 ± 15.78*	16.99 ± 14.64*	12.80 ± 11.43*	9.50 ± 9.83*	6.74 ± 10.73	4.23 ± 8.75

All results are corrected for plasma volume changes.
CW: Wingate sprint after circuit weight training, W: Wingate sprint, C: circuit weight training, WC: Wingate sprint prior to circuit weight training.
P5, 10 etc. represents the time (min) post-exercise
* significantly higher than pre-exercise value.
@ significantly higher than the W trial.
a significantly higher than the C and WC trials.

Table 4. Peak power, mean power, fatigue ratio, and peak revolutions per min.

Variable and Trial	Peak Power (Watts)	Mean Power (Watts)	Fatigue Ratio (%)	Peak RPM
CW	816.9 ± 163.9	570.3 ± 99.8	60.8 ± 17.4	164.1 ± 31.7
W	814.7 ± 178.0	596.1 ± 96.7	52.3 ± 15.7	167.4 ± 62.6
WC	805.9 ± 170.7	603.8 ± 104.5	47.9 ± 10.3	162.4 ± 25.3

RPM = revolutions per min
CW: Wingate sprint after circuit weight training, W: Wingate sprint, WC: Wingate sprint prior to circuit weight training.

Table 5. Lactate, rating of perceived exertion, and human growth hormone area under curve.

Variable and Trial	Lactate (mM/L)	RPE	AUC (PVC)
CW	12.3 ± 3.7[@]	18.6 ± 0.8[@]	738.9 ± 452.2[#]
W	8.9 ± 1.9	16.6 ± 2.1	431.0 ± 434.4
C	7.7 ± 3.4	14.6 ± 1.2	286.9 ± 280.7
WC	9.2 ± 3.3	15.1 ± 2.1	381.0 ± 308.5

RPE = rating of perceived exertion, AUC = area under the curve, PVC = plasma volume corrected, CW: Wingate sprint prior to circuit weight training, W: the Wingate sprint trial, C: the circuit weight training trial, WC: the Wingate sprint after circuit weight training trial,
[@] significantly higher than the W, C, and WC trials.
significantly higher than the W and C trials.

Table 6. Heart rate immediately after each circuit and Wingate sprint.

Variable and Trial	At rest	Wingate	Circuit 1	Circuit 2	Circuit 3
Heart rate (bpm)					
CW	59.6 ± 8.0	171.4 ± 13.2[@]	116.1 ± 23.5	145.3 ± 26.4[a]	157.0 ± 29.9[*ab]
W	56.0 ± 7.3	160.7 ± 14.9	N/A	N/A	N/A
C	56.7 ± 5.4	N/A	117.6 ± 23.8	146.9 ± 29.3[a]	151.1 ± 29.1[a]
WC	57.0 ± 6.0	162.7 ± 11.7	130.5 ± 29.8[*]	153.3 ± 32.1[a]	157.7 ± 33.0[a]

bpm: beats per min,
CW: Wingate sprint after circuit weight training, W: Wingate sprint, C: circuit weight training t, WC: Wingate sprint prior to circuit weight training.
[@]significantly higher than other trials.
[*]significantly higher than the C trial.
[a]significantly higher than circuit 1
[b]significantly higher than circuit 2

Table 7. Heart rate during cool-down and post cool-down.

	Time post cool-down (min)						
Variable and Trial	1st min CD	End of CD	P5	P10	P20	P30	P45
Heart rate (bpm)							
CW	149.7 ± 22.0[@]	114.9 ± 22.0[a]	99.8 ± 18.7[*]	95.9 ± 16.6	88.4 ± 13.2	87.7 ± 16.2[#]	77.3 ± 10.2
W	137.0 ± 23.1[#]	103.9 ± 23.1	83.4 ± 20.2	80.7 ± 18.6	77.7 ± 14.2	74.9 ± 14.0	71.1 ± 11.4
C	125.6 ± 23.5	104.6 ± 15.4	89.1 ± 15.4	86.4 ± 15.2	83.7 ± 14.6	76.3 ± 11.3	71.3 ± 9.8
WC	133.3 ± 25.3	113.7 ± 21.3[#]	96.6 ± 19.4[*]	78.4 ± 33.8	74.3 ± 33.4	80.1 ± 12.7	73.9 ± 11.6

CD = cool down, P = post cool down, P5 = 5 min post cool-down, P10: 10 min post cool-down, P20: 20 min post cool-down, P30: 30 min post cool-down, P45: 45 min post cool-down.
CW: Wingate sprint after circuit weight training, W: Wingate sprint, C: circuit weight training t, WC: Wingate sprint prior to circuit weight training.
[@]significantly higher than other trials.
[*]significantly higher than the W trial.
[#]significantly higher than the C trial.
[a]significantly higher than the W and the C trials.

APPENDIX 3: Informed Consent

The University of New Mexico Health Science Center
Combined Consent / HIPAA Authorization to Participate in Research

10/29/2014

The effect of the combination training of a Wingate sprint and circuit training on human growth hormone response to exercise

Introduction

You are being asked to participate in a research study that is being done by Len Kravitz, PhD, who is the Principal Investigator, and Hung-Sheng Hsu, MS, ABD, from the Department of Health, Exercise and Sports Sciences. This research is being done to evaluate the blood concentration of human growth hormone in response to the combination training of a Wingate sprint (a 30-second cycle test) and circuit training. Human growth hormone (HGH) is one of the most important hormones for muscle growth. HGH release after resistance training has been suggested to be correlated with the degree of muscle growth. You are being asked to participate because you are a resistance-trained individual between the age of 18 and 35 years. However, if you are a weight lifter who currently takes any supplements that claim they increase muscular strength and power or stimulate anabolic hormones (i.e. testosterone, human growth hormone), you cannot be included in this study. You should be otherwise healthy and in very good physical shape.

Approximately 20 people will take part in this study at the University of New Mexico. A small grant from the Graduate Professional Student Association at UNM is providing partial funding for this study.

This form will explain the research study, and will also explain the possible risks as well as the possible benefits to you. We encourage you to talk with your family and friends before you decide to take part in this research study. If you have any questions, please ask one of the study investigators.

What will happen if I decide to participate?

If you agree to be in this study, you will be asked to read and sign this Consent Form. After you sign the Consent Form, the following things will happen:

Exercise testing sessions:

Session #1 (Day 1): Before the first test session, you will be called or emailed to remind you of your appointment. You will be asked to come to the UNM exercise physiology lab in Johnson Center, Room B143 in the morning (before 9 am). You will be instructed not to perform vigorous exercise 24 hours prior to the visit or eat that morning. You will be asked to first complete a health history and physical activity questionnaire and then we will measure your height and weight without shoes. Your %body fat will be estimated via a three-site skinfold measurement which involves squeezing your skin and measuring the skin folds with calipers. Your one-repetition max, also called 1RM (for maximal muscular strength) for each exercise in the circuit weight program used in this study will be measured. However, due to the limitation of our equipment, there is a chance that you may be too strong for us to be able to accommodate you for our particular routine. In this case we may have to exclude you from the study. The following gives you detailed information about the questionnaires and maximal strength tests.

Questionnaire: you will be asked to complete one questionnaire consisting of a part of health history questionnaire and a part of physical activity questionnaire. This should take approximately 20 minutes.

Maximal strength testing: for each of 6 exercises, you will perform 2 warm-up sets at your comfortable weight for 10 repetitions with 3-5 minutes of rest between sets. The research staff will help you to determine the loading progression to reach your 1RM based on your feelings during warm-up. If you know your 1RM for the exercises, the research staff will monitor you to ensure a proper warm up. From the third set, the weight will be adjusted to be close to your 1RM load based on the training experience you provide as well as how you feel during the warm up. If you are able to complete the third set for more than 4 repetitions, an additional 5-10% of the existing weight will be added for the upper body exercises and 10-20% of the weight will be added for the lower body exercises. A 3-5 minute rest period between sets for 1RM will be allowed for a full recovery. A successful lift to determine your 1RM test will be the weight which you cannot complete for more than 3 repetitions using correct form with no extra motions such as jerking and/or momentum during the lift. The 1RM is then calculated using the National Strength and Conditioning Association training load chart (3RM=0.93 x 1RM, 2RM=0.95 x 1RM).

Session #2 (Day 2): no more than a week from Day 1, you will come to Johnson Center (Rm. B143) in the morning (before 8 am) for a familiarization session. You

will receive a reminder call or email with the following instructions for the familiarization session:

-Please fast overnight before coming in (nothing to eat, drink only water)

-Drink plenty of water

-Refrain from any exercise for 24 hours before visit

-Avoid any alcohol and caffeine consumption for 24 hours before visit

The familiarization trial consists of a Wingate sprint prior to circuit weight training. You will start with a warm-up (5 min cycling at 100 Watts) and will finish with a cool-down (5 min cycling at 30 Watts) on a bike. The Wingate anaerobic sprint is a 30-second all-out cycling sprint at the highest cadence (revolutions per minute) possible against a high resistance. Five seconds before the resistance is dropped on the flywheel, you will start to pedal as fast as possible to reach maximal cadence. At the end of the 5 seconds, the cycle ergometer program applies resistance to the flywheel for 30-seconds during which you are encouraged to maintain as high a cadence as possible. You will then have a 3-min rest period between the Wingate sprint and the next tests which consist of circuit weight training. The circuit training session consists of 6 exercises in the following order: bench press, bend-over barbell row, Smith machine parallel squat, dumbbell shoulder press, Romanian deadlift (RDL), and latissiumus dorsi (lat) pull-down. You will perform 3 circuits with a 30-second rest period between exercises and a 3-minute rest period between circuits. This trial will help us ensure that your fitness level is high enough to participate in this study. During this trial, you must be able to complete a Wingate sprint and the first 2 resistance circuits (the warm-up circuit at 40% of 1RM and the first workout circuit at70% of 1RM) without any help from the spotters. Failure to make it through the first workout circuit will exclude you from continuing the study.

Session #3-#6 (Day 3 – Day 6): you will come to Johnson Center Rm. B143 for 4 exercise trials on non-consecutive days (in the morning before 8 am, no more than 4 days between trials). The following four exercise trials will be assigned to in a non-randomized order (manually set) to avoid trial order effect on human growth hormone response:

-a Wingate sprint prior to circuit weight training.

-a Wingate sprint after circuit weight training.

-a Wingate sprint only.

-a circuit weight training only.

Upon arrival for each of these trials, your hydration status will be checked by asking you to give us a urine sample. If you are dehydrated (urine-specific gravity > 1.2), you will drink some water and we will re-check your urine 20 min later. After 15 minutes of seated rest, 15 ml (~1 Tablespoon) of blood will be drawn from an arm vein by an experienced phlebotomist. Exercise starts after the blood draw site is well clotted (usually around 20 min). Each trial starts with a standard warm-up (5 min cycling at 100 Watts) and finishes with a standard cool-down (5 min cycling at 30 Watts) on a bike. The circuit training session consists of 6 exercises in the following order: bench press, bend-over barbell row, Smith machine parallel squat, dumbbell shoulder press, Romanian deadlift (RDL), and latissiumus dorsi (lat) pull-down. There are 3 circuits with a 30-second rest period between exercises and a 3-minute rest period between circuits. The exercise intensity of the first circuit is 40%-50% of 1 repetition-maximum (1RM) for all exercises as a warm-up set, followed by 2 circuits at an intensity of 70% of 1RM. The Wingate anaerobic sprint is performed on a stationary cycle ergometer and will be performed as described above. You will be given a 3-min rest period between the Wingate sprint and circuit weight training if a trial includes both exercises. Heart rate will be recorded using a wireless transmitter/ receiver unit with a chest strap before and after the warm-up, after the Wingate sprint, each circuit, during cool-down, and at 5 minutes interval within an hour period post-exercise. You will be asked to rate your perceived exertion using a 6-20 scale (6 represents no effort and 20 is maximal effort) immediately after exercise and before cool-down. Your blood lactate will be measured by pricking your ear lobe and collecting a drop of blood 10 min after exercise. Please see the table below for the detailed schedule for each trial.

After a 5-minute cool-down on a bike, you will be instructed to sit on a chair. The post- exercise blood draws will be performed at 5, 10, 20, 30, 45, 60, and 240 min after cool-down. At 5 min post exercise, a 15 ml (~1 teaspoon) blood sample will be collected for the assay of blood hemoglobin mass, hematocrit, HGH concentration, heat shock protein, and autophagy. 6 ml (~1/3 teaspoons)of blood will be collected at 10, 20, 30, 45, and 60 min post exercise for the assay of blood hemoglobin mass, hematocrit, and HGH concentrations. A nutrition bar and a bottle of sport drink will be provided at this point. There is one last blood draw of 8 ml (~½ tablespoon) that will be drawn four hours after completion of the exercise. If you choose to leave then, you will be instructed to return to the

exercise physiology lab at least 15 minutes before your scheduled 4 hours post-exercise blood draw. You also can stay and rest in the lab until the 4 hours post-exercise blood draw is done. Other than the nutrition bar provided by the research team, you will be asked to refrain from eating, drinking anything but water, or exercise until the last blood sample (4 hours post-exercise) is collected.

Three needle sticks per trial will be required using a vein in the elbow area of your arm. Sterile techniques will be used. The first needle stick is a standard blood draw with a needle. For the second needle stick, we will insert a flexible needle in the same area of your arm. It will be secured with tape and will stay in to collect the 6 post-exercise blood samples. To prevent your blood from clotting in the needle, a small amount (2 ml) of saline (sterile water) will be injected into the catheter after each blood collection. The 3rd needle stick will be done with a standard needle at the 4 hour post-exercise time point.

During the entire study period, you are asked not to engage any type of exercise outside of what is required for the study. Participation in this study will take a total of 29.5 hours over 6 non-consecutive days over a period of 2-3 weeks. The amount of blood collection is 60 ml (4 tablespoons) pre-exercise blood draw, 180 ml (12 tablespoons) within the 1 hour post-exercise (5, 10, 20, 30, 45, & 60 min), and 32 ml (~2 tablespoon) at 4 hours post-exercise. The total amount of blood drawn over the study will be less than 272 ml (~18 tablespoons).

Table 1. The protocols for each trial.

Circuit weight training only	Wingate + circuit weight training	Circuit weight training + Wingate	Wingate only
Data collection (rest)	Data collection (rest)	Data collection (rest)	Data collection (rest)
Bike warm-up (5 min @ 100W)	Bike warm-up (5 min @ 100W)	Bike warm-up (5 min @ 100W)	Bike warm-up (5 min @ 100W)
Rest (1 min)	Rest (1 min)	Rest (1 min)	Rest (1 min)
Circuit warm up @ 50% 1RM (5 min)	Wingate anaerobic test @ 0.7Nm/kg (30 sec)	Circuit warm up @ 50% 1RM (5 min)	Wingate anaerobic test @ 0.7Nm/kg (30 sec)
Recovery: 3 min	Recovery: 3 min	Recovery: 3 min	Cool-down(5 min bike @ 30W)
Circuit 1 @ 70% 1RM (5 min)	Circuit warm up @ 40% 1RM (5 min)	Circuit 1 @ 70% 1RM (5 min)	Data collection after cool-down (Blood draws & lactate)
Recovery: 3 min	Recovery: 3 min	Recovery: 3 min	
Circuit 2 @70% 1RM (5 min)	Circuit 1 @ 70% 1RM (5 min)	Circuit 2 @70% 1RM (5 min)	
Cool-down(5 min bike @ 30W)	Recovery: 3 min	Recovery: 3 min	
Data collection after cool-down (Blood draws & lactate)	Circuit 2 @ 70% 1RM (5 min)	Wingate anaerobic test @ 0.7Nm/kg (30 sec)	
	Cool-down (5 min bike @ 30W)	Cool-down(5 min bike @ 30W)	
	Data collection after cool-down (Blood draws)	Data collection after cool-down (Blood draws & lactate)	

What are the risks or discomforts of being in this study?

Every effort will be made to protect the information you give us. However, there is a small risk of loss of privacy and/or confidentiality. Other risk and discomforts may result in stigmatization or hardship. There are small risks associated with maximal muscular strength test including the following: brief feelings of nausea, lightheadedness, muscle cramps, dizziness during or after completion of exercise, or muscle soreness 1-2 days following exercise. Muscle soreness (also called delayed onset muscle soreness) may or may not occur after 1RM strength tests in the first session.

Drawing blood may cause temporary pain and discomfort from the needle stick, occasional bruising, sweating, feeling faint or lightheaded, and in rare cases, infection. Trained, experienced phlebotomists will draw your blood.

You also may feel uncomfortable because you are required to refrain from having any caffeine, drinking alcohol or eating any food in the morning before muscular strength test and exercise trials. This study requires a lot of your time (6 visits, ~29.5 hours). You may feel bored waiting for the blood sampling throughout the hour after exercise and inconvenience waiting for the final blood draw four hours after the exercise. There are risks of stress, emotional distress, inconvenience and possible loss of privacy and confidentiality associated with participating in a research study.

To our knowledge, the incidence of resistance-training induced cardiovascular (heart) injuries has not been studied. Therefore, the risk of cardiovascular injury during participation in this study is unknown. We estimate the incidence of weight training induced muscle and joint injuries is approximately 2.8 injuries per 1,000 hours of training. However, since you are an experienced weightlifter, this risk may be lower,

How will my information be kept confidential?

Your name and other identifying information will be maintained in locked files, available only to authorized members of the research team, for the duration of the study. For any information entered into a computer, the only identifier will be a unique study identification (ID) number. Any personal identifying information and any record linking that information to study ID numbers will be destroyed when the study is completed. Information resulting from this study will be used

for research purposes and may be published; however, you will not be identified by name in any publications.

Information from your participation in this study may be reviewed by federal and state regulatory agencies, and by the UNM Human Research Review Committee (HRRC) which provides regulatory and ethical oversight of human research. There may be times when we are required by law to share your information. However, your name will not be used in any published reports about this study.

What are the benefits to being in this study?

There are no direct benefits to your participation in this study. You may better understand how your body responds to exercise trials by knowing your post-training HGH concentrations. However, please note we are not able to process your HGH results unless you complete all 4 exercise trials.

What other choices do I have if I do not want to be in this study?

You have the option to not participate if you don't want to. You have the option not to take part in this study at any time, even if you are part way through or have almost completed the study. There will be no penalties involved if you choose not to take part in this study.

What are the costs of taking part in this study?

There is no cost for you to participate in this study.

Will I be paid for taking part in this study?

Upon the time you complete the last trial in this study, you will receive giftcard(s) worth $50.

What will happen if I am injured or become sick because I took part in this study?

If you are injured or become sick as a result of this study, UNM will provide you with emergency treatment, at your cost.

No commitment is made by University of New Mexico (UNM) to provide free medical care or money for injuries to participants in this study.

In the event that you have an injury or illness that is caused by your participation in this study, reimbursement for all related costs of care will be sought from your insurer, managed care plan, or other benefits program. If you do not have insurance, you may be responsible for these costs. You will also be responsible for any associated co-payments or deductibles required by your insurance.

It is important for you to tell the investigator immediately if you have been injured or become sick because of taking part in this study. If you have any questions about these issues, or believe that you have been treated carelessly in the study, please contact the Human Research Review Committee (HRRC) at the University of New Mexico, Albuquerque, New Mexico 87131, (505) 272-1129 for more information.

How will I know if you learn something new that may change my mind about participating?

You will be informed of any significant new findings that become available during the course of the study, such as changes in the risks or benefits resulting from participating in the research or new alternatives to participation that might change your mind about participating.

If we find any abnormal values from the blood testing, our physician will inform you of these findings and recommend follow-up if necessary. Any abnormal results from the strength tests will be discussed with you by a National Strength and Conditioning Association certified strength and conditioning specialist.

Can I stop being in the study once I begin?

Your participation in this study is completely voluntary. You have the right to choose not to participate or to withdraw your participation at any point in this study without affecting your future health care or other services to which you are entitled.

The investigators have the right to end your participation in this study if they determine that you no longer qualify to take part, if you do not follow study procedures, or if it is in your best interest or the study's best interest to stop your participation.

HIPAA Authorization for Use and Disclosure of Your Protected Health Information (HIPAA)

As part of this study, we will be collecting health information about you. This information is "protected" because it is identifiable or "linked" to you.

Protected Health Information (PHI)

By signing this Consent Document, you are allowing the investigators and other authorized personnel to use your protected health information for the purposes of this study. This information may include: levels of human growth hormone, height, weight, age, % body fat, health history, and results of 1RM muscular strength tests. You will be asked to let us know if you are taking certain

supplements which may take you ineligible to participate (supplements that claim to improve human performance, i.e. muscular strength and power, and/or to stimulate anabolic hormone secretion or concentration, i.e. testosterone, growth hormone, etc.).

In addition to researchers and staff at UNM and other groups listed in this form, there is a chance that your health information may be shared (re-disclosed) outside of the research study and no longer be protected by federal privacy laws. Examples of this include disclosures for law enforcement, judicial proceeding, health oversight activities and public health measures.

Right to Withdraw Your Authorization

Your authorization for the use and disclosure of your health information for this study shall not expire unless you cancel this authorization. Your health information will be used or disclosed as long as it is needed for this study. However, you may withdraw your authorization at any time provided you notify the UNM investigators in writing. To do this, please send letter notifying them of your withdrawal to:

Len Kravitz

MSC 04 2610

1 University of New Mexico

Albuquerque New Mexico 87131

Please be aware that the research team will not be required to destroy or retrieve any of your health information that has already been used or shared before your withdrawal is received.

Refusal to Sign

If you choose not to sign this consent form and authorization for the use of your PHI, you will not be allowed to take part in the research study.

Whom can I call with questions or complaints about this study?

If you have any questions, concerns or complaints at any time about the research study, Len Kravitz, PhD., or his associates will be glad to answer them at 505-277-4136, Monday-Friday 8-5, or after hours call Hung-Sheng Hsu at 318-607-8353. If

you would like to speak with someone other than the research team, you may call the Human Research Review Committee (HRR) at (505) 272-1129. The HRRC is a group of people from UMHSC and the community who provide independent oversight of safety and ethical issues related to research involving human participants.

Whom can I call with questions about my rights as a research participant?

If you have questions regarding your rights as a research participant, you may call the UNM Human Research Review Committee (HRRC) at (505) 272-1129. The HRRC is a group of people from UNM and the community who provide independent oversight of safety and ethical issues related to research involving human participants. For more information, you may also access the HRRCwebsite at http:// http://hsc.unm.edu/som/research/hrrc/committees.shtml

Consent and Authorization

You are making a decision whether to participate in this study. Your signature below indicates that you read the information provided (or the information was read to you). By signing this consent form, you are not waiving any of your legal rights as a research participant.

_____

I have had an opportunity to ask questions and all questions have been answered to my satisfaction. By signing this consent form, I agree to participate in this study and give permission for my health information to be used or disclosed as described in this Consent Form. A copy of this consent form will be provided to me.

_____ _____/_____

Name of the Participant (print) Signature of the Participant Date

I have explained the research to the participant and answered all of his questions. I believe that he understands the information described in this consent form and freely consents to participate.

_____ _____/_____

Name of Research Team Member (print) Signature of Research Team Member Date

APPENDIX 4: Flyer

Male subjects needed for a research project at the UNM Exercise Physiology Lab!

HRPO 14-279

What is the study about? This study is going to investigate the effect of a combination training of a Wingate sprinting and circuit training on human growth hormone (HGH) response. HGH is one of the most important anabolic hormones for muscle building. The elevated change of HGH levels after resistance training is correlated with an increase in skeletal muscle size.

Who can volunteer? We are seeking male subjects, 18-35 yrs of age, who are healthy and have engaged in resistance training at least 2 days/week for more than 6 months.

What will you be asked to do if you participate in this study? Your health history questionnaire, your weightlifting technique for muscular strength tests, and a familiarization sprint trial will determine if you can enter the study. After joining the study, your blood samples will be collected at and after you complete 4 different circuit exercise trials. The exercise trials will be performed over a period of 2-3 weeks.

Is there any compensation for completing this study?
Yes. Once you complete this study, you will receive gift cards worth $50 in person. If you withdraw or leave the study early, you will not receive any compensation.

Who can I contact for more information?
Hung-Sheng Hsu, 318-607-8353 or 505-277-2658, hhs007@unm.edu;
Dr. Len Kravitz, lkravitz@unm.edu

APPENDIX 5: Health History Questionnaire

Name _____ D.O.B __/__/__ Date __/__/__

Age __ yrs Height ___ cm Weight ___ kg Gender __ Ethnicity

Sitting blood pressure _____/_____ mmHg

MEDICIAL HISTORY QUESTIONNAIRE

Section A

1. When was the last time you had a physical examination?

2. If you are allergic to any medications, foods, or other substances, please name them.

3. If you have been told that you have any chronic or serious illnesses, please name them.

4. Give the following information pertaining to the last 3 times you have been hospitalized. Note: Women, do not list normal pregnancies.

	Hospitalization	Hospitalization
Reason for hospitalization	_____	_____
Month and year of hospitalization	_____	_____
Hospital	_____	_____
City and state	_____	_____

Section B

During the past 12 months

1. Has a physician prescribed any form of medication for you? Yes No
2. Has your weight fluctuated more than a few pounds? Yes No
3. Did you attempt to bring about this weight change through diet or exercise? Yes No
4. Have you experienced any faintness, light-headedness, or blackouts? Yes No
5. Have you occasionally had trouble sleeping? Yes No
6. Have you experienced any blurred vision? Yes No
7. Have you had any severe headaches? Yes No
8. Have you experienced chronic morning cough? Yes No
9. Have you experienced any temporary change in your speech pattern,

such as slurring or loss of speech? Yes No

10. Have you felt unusually nervous or anxious for no apparent reason? Yes No

11. Have you experienced unusual heartbeats such as skipped bests or palpations? Yes No

12. Have you experienced periods in which your heart felt as though it were racing for no apparent reason? Yes No

At present

1. Do you experience shortness or loss of breath while walking with others your own age? Yes No

2. Do you experience sudden tingling, numbness, or loss of feeling in your arms, hands, leg, feet, or face? Yes No

3. Have you ever noticed that your hands or feet sometimes feel cooler than other parts of your body? Yes No

4. Do you experience swelling of your feet and ankles? Yes No

5. Do you get pains or cramps in your legs? Yes No

6. Do you experience any pain or discomfort in your chest? Yes No

7. Do you experience any pressure or heaviness in your chest? Yes No

8. Have you ever been told that your blood pressure was abnormal? Yes No

9. Have you ever been told that your serum cholesterol or triglyceride level was high? Yes No

10. Do you have diabetes? Yes No

If yes, how is it controlled?

□ Dietary means □ Insulin injection

□ Oral medication □ Uncontrolled

11. How often would you characterize your stress level as being high? Yes No

□ Occasionally □ Frequently □ Constantly

12. Have you ever been told that you have any of the following illness? Yes No

□ Myocardial infarction □ Arteriosclerosis □ Heart disease □ Thyroid disease

□ Coronary thrombosis □ Rheumatic heart □ Heart attack □ Heart valve disease

□ Coronary occlusion □ Heat failure □ Heart murmer

□ Heart block □ Aneurysm □ Angina

13. Have you ever had any of the following medical procedures? Yes No

□ Heart surgery □ Pacemaker implant

□ Cardiac catheterization □ Defibrillator

□ Coronary angioplasty □ Heart transplantation

Section C

Has any member of your immediate family been treated for or suspected to have any of these conditions? Please identify their relationship to you (father, mother, sister, brother, etc.).

A. Diabetes
B. Heart disease
C. Stroke
D. High blood pressure

Section D

Smoking habits

1. Have you ever smoked cigarettes, cigars, or a pipe? Yes No
2. Do you smoke presently? Yes No

Cigarettes _____ a day

Cigars _____ a day

Pipefuls _____ a day

3. At what age did you start smoking? _____ years
4. If you have quit smoking, when did you quit?

PHYSICAL ACTIVITY/EXERCISE QUESTIONARE

1. Do you exercise vigorously on a regular basis? Yes No
2. What activities do you engage in on a regular basis?

3. If you walk, run, or jog, what is the average number of miles you cover each workout?
_____ Miles
4. How many minutes on the average is each of your exercise workouts?

5. How many workouts a week you participate in on average?

6. Do you train weight-lifting? Yes No

7. How well trained are you?

8. How many workouts a week do you participate weight-lifting in on average?

9. How many years of weight-lifting experience do you have?

10. Do you know your bench press exercise 1 repetitions maximal (RM) weight?

APPENDIX 6: Data Collection Sheet

Data Collection Sheet (eligibility screen)

Subject ID #: _____ Height:_____
Weight:_____

Pre-screen

- Health and Physical Activity History Questionnaire completion: Yes □ No □
- The subject is able to pass the exclusive criteria in the familiarization trial: Yes □ No □
  - **Exercise HR:**
  - Warm-up: pre _____ Post:_____
  - Wingate HR: Pre _____ Post: _____
  - Circuit training HR:
  - Circuit 1 (warm up), Pre_____ Post_____
  - Circuit 2 (warm up), Pre_____ Post_____
  - Circuit 3 (warm up), Pre_____ Post_____
  - RPE right after end of exercise and before cool down: _____
  - Cool down HR:
  - 1 min_____ 2 min_____ 3 min_____ 4 min_____ 5 min_____
  - Follow up call:_____
- If the subject is able to continue to participate this study: Yes □ No □
- If the subject is excluded, what is the reason?

  _____

  _____

Skinfold measurement

	1	2	3	Average
Chest				
Abdominals				
Mid-thigh				

One Repetition-Maximum test

Smith Machine Squat: _____

1RM:_____ 70%1RM:_____

Bench Press: _____

1RM:_____ 70%1RM:_____

Bend-over Barbell Row: _____

1RM:_____ 70%1RM:_____

Romanian Deadlift: _____

1RM:_____ 70%1RM:_____

Dumbbell Shoulder Press: _____

1RM:_____ 70%1RM:_____

Latissimus Dorsi Pull-down: _____

1RM:_____ 70%1RM:_____

Data Collection Sheet (exercise trial)

Subject #:_____ Weight:_____ Trial:_____

Pre-exercise:

Urine gravity: _____ Resting HR: _____ Hematocrits: _____

Hb concentration: _____

At exercise:

Warm up HR: Pre _____ Post:_____

Wingate HR: Pre _____ Post: _____

Circuit training HR:

Circuit 1 (warm up), Pre_____ Post_____

Circuit 2 (warm up), Pre_____ Post_____

Circuit 3 (warm up), Pre_____ Post_____

RPE right after end of exercise and before cool down: _____

Cool down HR:

1 min_____ 2 min_____ 3 min_____ 4 min_____ 5 min_____

Post-exercise

5 min

HR _____, Hematocrits _____, Hb concentration _____
blood lactate concentration _____, RPE _____

10 min

HR _____, Hematocrits _____, Hb concentration _____,

HR@15 min _____, RPE _____

20 min

HR _____, Hematocrits _____, Hb concentration _____,
RPE _____

HR@25 min _____, RPE _____

HR _____

30 min

HR _____, Hematocrits _____, Hb concentration _____

HR@35 min _____, HR@40min _____,

45 min

HR _____, Hematocrits _____, Hb concentration _____

HR@50 min _____, HR@55min _____,

60 min

HR _____, Hematocrits _____, Hb concentration _____

Follow up call:

_____
_____

40% of 1RM

	Barbell	45 lbs	35 lbs	25 lbs	10 lbs	5 lbs	2.5 lbs	Weight
Bench Press	1							
Bend-over Bar. Row	1							
Smith Machine Squat	1 (5lbs)							
Dumbbell Shoulder Press								
Romanian Deadlift (RDL)	1							

Latissimus Dorsi Pull-down								
Total weights needed	3							

50% of 1RM

	Barbell	45 lbs	35 lbs	25 lbs	10 lbs	5 lbs	2.5 lbs	Weight
Bench Press	1							
Bend-over Bar. Row	1							
Smith Machine Squat	1 (5lbs)							
Dumbbell Shoulder Press								
Romanian Deadlift (RDL)	1							
Latissimus Dorsi Pull-down								
Total weights needed	3							

70% of 1RM

	Barbell	45 lbs	35 lbs	25 lbs	10 lbs	5 lbs	2.5 lbs	Weight
Bench Press	1							
Bend-over Bar. Row	1							
Smith Machine Squat	1 (5lbs)							
Dumbbell Shoulder Press								
Romanian Deadlift (RDL)	1							
Latissimus Dorsi Pull-down								
Total weights needed	3							