THE EFFECT OF TRAINING BACKGROUND AND ACUTE EXERCISE TYPE ON THE CYTOKINE RESPONSE TO EXERCISE

By

CHRISTOPHER E. ORDWAY

A thesis submitted to the

School of Graduate Studies

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Master of Science

Graduate Program in Kinesiology and Applied Physiology

Written under the direction of

Shawn M. Arent

And approved by

_____

_____

_____

New Brunswick, New Jersey

[October 2020]

ABSTRACT OF THE THESIS

The Effect of Training Background and Acute Exercise Type on the Cytokine Response to Exercise

By CHRISTOPHER E. ORDWAY

Thesis Director

Shawn M. Arent

Acute aerobic and resistance training exercise provide different stimulus to the body. Chronic training in either of these modalities leads to adaptations that alter the body's response to acute exercise bouts. Growth Hormone (GH), Interleukin-6 (IL-6), and Interleukin-10 (IL-10) are all well studied biomarkers in regard to both their acute response to exercise, as well as the adaptations to that response from chronic training. **Purpose:** To compare the effect of chronic endurance (END) or resistance (RES) training on the acute GH, IL-6 and IL-10 responses to trained versus novel exercise bouts. **Methods:** Chronically END (n=10; M_{age}= 24.8±4.92 years; M_{ht}= 177.3±5.8 cm; M_{wt}= 68.9±4 kg; $M_{\%BF}$ = 10.1±4.1%) and RES (n=10; M_{age}= 23.2±2.8 years; M_{ht}= 173.1±8.1 cm; M_{wt}= 76.8±8.8 kg; $M_{\%BF}$ = 15.5±6%) trained men were taken through separate prescribed weight training (WT) and aerobic (AER) exercise bouts based on earlier testing. Subjects arrived 2hr fasted and euhydrated and had blood drawn pre- (T0), 5 minutes (T1) and 60 minutes (T2) post exercise bout. Blood was then processed, and GH, IL-6, and IL-10 values were ascertained by hormone specific ELISA and custom human cytokine panels, respectively. **Results:** GH ad a significant main effect for time (P<.05) and a significant effect for time by condition (P<.05). For IL-6, there was a trend for a main effect of time (P<.10), with no significant differences between groups for either condition. IL-10 had a trend for a main effect of condition as well as time (P<.10), with the RES group having no difference in response between bouts and the END group only differing at T2 with the AE bout eliciting a greater response (P<.05).

Conclusions: GH's role as a differential energy sensor was demonstrated by the difference in responses between conditions, while the lack of response by IL-6 provides insights to other factors that may need to be controlled for in future studies. The unexpected disconnect between the IL-6 and IL-10 response also demonstrates the need for continued exploration into the cytokine signaling mechanisms in response to exercise.

Acknowledgement

I would like to start by thanking those individuals who have helped me significantly through my many years as a graduate student at Rutgers University. First, I would like to thank my advisor, Dr. Shawn Arent. The last few years may not have gone exactly according to plan, the past few years that I was fortunate to work under you, learn from you, both inside and out of the lab and classroom, and the experiences you provided to me have been invaluable. If it were not for my time here with you, I would not have been able to develop into the person I am today and take these next steps forward in my career with such confidence. I'd like to thank my committee members, Dr. Sara Campbell and Dr. Kenneth McKeever, for helping me develop my manuscript and for your expertise. I would like to thank my fellow lab mates whom I have had the opportunity to learn, lift, and experience life with over the past few years. First I would like to thank Joe Pellegrino and Marissa Bello specifically for being so integral to the collection of the data I needed for this thesis. I would also like to thank my fellow labmates Alan Walker, Bridgette McFadden, Dave Sanders, Brittany Bozzini, Harry Cintineo, Traci McCarthy, Ozlem Tok, Meaghan Rabideau, Morgan Hofacker, Sean Conway, Anthony Poyssick, Nick Mackowski, Marissa Bello, Morgan Murray, William Maldonado, Albert Offredo, Kevin Prior, and Alexa Chandler. You are all wonderful human beings and I'm excited to see how far each one of you go in your respective careers. Next, I would like to the thank my second family at Stealth. There are too many of you to name here but I want to specifically acknowledge my owners, John Leonardis and Matt Gozzi, for giving me the opportunity to learn from you, develop my skills as a coach, and build a community who's values I am proud to be a part of. I would also be remiss if I did not thank Dr. Erin Comollo, a fellow coach, a colleague at Rutgers, and above all else, one of my best friends. You pick me up and support me when I'm down, you don't hesitate to give it to me straight when I'm messing up, and are always a source

of wisdom when I am struggling to figure something out. You have truly been one of the most important people in my life the last 5 years and I do not know that I would be who I am today without you. To the rest of the coaching staff: David Colas, Faith Qualshie, Robert Rabouin, Jenny Leonardis, Dennis Chu, Bobby Brown, and Julia Masciandaro, thank you for all being wonderful people to work with and helping to build a truly elite community. Finally, I would like to thank my entire family and all their support over the past 30+ years, especially my parents, Elise and Ed Ordway. I know I have put you through quite the roller coaster over the years. You taught me how to live with integrity and stand up for what I believe in; you showed me how to be empathetic and put others before myself; you have always been a source of support when times got tough, but knew when it was time to stop being upset, dust myself off, and get back to work. Most importantly, you taught me the importance of being true to myself no matter what happened, and that is something I will take with me, wherever I go. Thank you.

Contents

List of Tables

Table **Page**

List of Figures

Figure **Page**

Chapter 1

GH and Cytokine Responses to Acute Exercise and Chronic Training

By

Christopher E Ordway

Introduction

The acute and chronic growth hormone (GH) and inflammatory responses to both aerobic and resistance exercise are widely studied topics. However, little is known regarding how chronic adaptation to one form of exercise can affect the acute response to another. The purpose of this review is to identify and assess the current understanding of acute responses and chronic adaptations to either aerobic or resistance training and examine how an acute bout of a novel exercise type will alter the GH and inflammatory response, if at all.

During an acute aerobic exercise session, submaximal force production in the working muscle is maintained for an extended period of time. To accommodate this stimulus, fuel and oxygen utilization increase while carbon dioxide and other byproducts increase in concentration as a result of the increased metabolic activity. Adaptations occur both within and outside of the muscle as result of these demands. Internally, chronic endurance exercise training increases muscle fatigue resistance and performance efficiency by increasing mitochondrial content and enzyme activity to increase fuel mobilization and utilization efficiency (Hood, 2001). Externally, adaptations occur in the cardiovascular system, including increased stroke volume and increased capillary density, as well as to better fuel mobilization from stores outside of the working muscle (Hood, 2001).

Acute resistance training requires greater overall force production, typically over shorter working periods. While metabolic activity increases as a result, the main stressor of this type of training is the demand to produce larger forces by recruiting a greater number of muscle fibers simultaneously. This incorporates fibers into the training that produce more force but that tend to fatigue quickly. Chronic resistance training, therefore, stimulates increased size and contractility of the musculature, and increased overall neural drive to the fibers. (Bosquet et al., 2013; Schoenfeld, 2010).

Growth Hormone (GH)

In humans, GH is a family of pleiotropic polypeptide hormone variants (at least 22kDa in length), synthesized and released by the anterior pituitary (Giustina & Veldhuis, 1998). GH effects on local tissues include cell division and growth, calcification of cartilage, and a protein anabolic effect (Giustina & Veldhuis, 1998). It also alters both fatty acid and carbohydrate metabolism through increased lipolysis in adipose tissue, increased triglyceride uptake in the muscle and liver, increased hepatic glucose production via glycogenolysis, and reduced skeletal glucose uptake (Vijayakumar et al., 2010). The secretion pattern of GH is pulsatile in nature (McArdle, 2015), indicating a need for careful consideration and control of time of day when comparing the acute GH response to different bouts of exercise. Despite work showing that time of day does not change the acute GH response to exercise (Kanaley et al., 2001), comparison between different exercise sessions requires accounting for baseline values. This makes comparisons of initial GH concentrations between groups and sessions more difficult. As a result, controlling the time of day and the time elapsed since last feeding for all exercise bouts for all subjects may be the simplest and most effective way to enable accurate comparisons of the GH response.

Early studies characterized the acute GH response to aerobic exercise as an initial increase in plasma values occurring 15 minutes after the onset, and peak GH concentrations being seen at or near the end of exercise (Lassarre et al., 1974; Raynaud et al., 1983; Sutton & Lazarus, 1976). Lasserre et al. had 10 subjects exercise for 1 hour on a cycle ergometer with exercise intensity varying between subjects from 37 to 71% of VO_2 max, resulting in maximal GH values from 5 to 41 ng/mL (Lassarre et al., 1974). This was the start of exercise intensity being identified as a key modifier of exercise-induced GH release. Sutton & Lazarus furthered this idea by having subjects exercise on a cycle ergometer for 20 minutes at varying intensities: 300, 600, and 900 kpm/min

(approximately 50, 100, and 150 Watts, respectively) (1976). For the subjects involved, these work rates represented 25-33%, 40-66%, and 75-90% of VO_2max, respectively. They found that the highest work intensity resulted in the greatest peak in GH, with a reduced response to the medium intensity, and no change in GH values in response to the lowest intensity (Sutton & Lazarus, 1976).

Other work demonstrated that intensity was more influenital than volume. When comparing an hour of steady state with an hour alternating 30 seconds on at double the work rate with 30 seconds of rest, the intermittent protocol produced a higher GH response (Raynaud et al., 1983). It was then suggested that GH release required a threshold intensity (Chang, et al., 1986; Felsing et al., 1992; Viru, 1985). More recently, however, the magnitude of GH release has been shown to linearly rise with increasing intensity (Pritzlaff-Roy et al., 2002; Pritzlaff et al., 1999), with increases above baseline GH values occurring with exercise intensities as low as 25% of lactate threshold.

The identification of intensity's key role led to the question of how anaerobic exercise would affect the GH response. Vanhelder et al. (1984) supported the notion of anaerobic exercise producing a higher response by comparing 20 minutes of aerobic cycling with 20 minutes of supramaximal bike intervals. They found a significantly higher elevation in GH following the intervals both immediately post and 30 minutes after exercise termination. There have also been a number of studies addressing the anaerobic stimulus question by using acute resistance exercise protocols. Regardless of the variation in resistance protocol used, as well as sampling intervals chosen by the researchers, the pattern of GH release in resistance exercise is similar to that of aerobic exercise. GH concentration generally peaks at or just after the termination of exercise, with a return to baseline levels by 90 minutes after termination (Hakkinen & Pakarinen, 1995; Kraemer et al., 1995; Kraemer et al., 1992; Kraemer et al., 1991; Kraemer et al., 1990; Nindl et al., 2001; Raastad et al., 2000; Takarada et al., 2000; Vanhelder et al.,

1984). Contrary to the aerobic exercise studies, the acute GH response to resistance exercise is highly impacted by total workload, rather than just intensity, at least when expressed as a function of percentage of 1-RM used (Kraemer et al., 1990; Raastad et al., 2000). As a result of this, the two ends of the exercise spectrum (i.e. aerobic versus resistance exercise bouts) needs to be addressed. This stands to be a difficult task as the two exercise forms do not make it easy to equate intensity and volume, though this may lend itself to greater external validity given the nature of prescription of the modalities.

Cytokines

Cytokines are regulatory proteins secreted by a host of body cells, including white blood cells, in response to a number of inducing stimuli. Cytokines generally function as intercellular messenger molecules that evoke particular biological activities after binding to a receptor on a responsive target cell (Pedersen & Nieman, 1998). When tissue damage occurs in the body, cytokines mediate the acute-phase inflammatory response (Richards, 1995). Traditionally, inflammation has been discussed in a negative context. However, it serves to promote repair of damaged tissue and fight pathogens. As with other forms of stress, exercise induces the release of cytokines to help manage strain placed on the system (Cannon & Kluger, 1983). Similarly, the inflammation following an acute exercise bout functions to hasten regenerative processes through pro-inflammatory cytokine release, while reductions in chronic low-grade inflammation are seen with habitual exercise due to upregulation of anti-inflammatory cytokines (Jankord & Jemiolo, 2004).

The most frequently and thoroughly studied of the cytokines, particularly in relation to exercise, is interleukin 6 (IL-6). IL-6 expression and action is well-established, with both pro- and anti-inflammatory effects being identified (Biffl et al., 1996; Pedersen et al., 2001). IL-6 has been labeled as the “keystone cytokine in health and disease”

(Hunter & Jones, 2015).

IL-6 was first sequenced and characterized as B-lymphocyte differentiator over 2 decades ago (Hirano et al., 1986). Since then, other roles and similar cytokines have been identified and grouped together (Kishimoto et al., 1995). Typical IL-6 plasma concentration is around 1 pg/ml or even lower, but has been shown to reach upwards of 10000 pg/ml in cases of severe systemic infection (Friedland et al., 1992; Ostrowski et al., 1998). Typical elevation of IL-6 in response to acute infection follows that of TNFα and IL1β (Petersen & Pedersen, 2006) and has been justified as the primary mediator of the physiologic acute phase response to injury (Biffl et al., 1996). During acute injury or infection, IL-6 is produced and binds to its receptor, inducing a JAK/STAT transduction pathway that then induces expression of acute phase proteins (APP) (Wegenka et al., 1993). These APPs are then associated with differentiation and proliferation of immune cells and processes known collectively as the acute phase response, part of the innate immune response geared towards healing and reestablishing homeostasis (Cray et al., 2009). Conversely, IL-6 has been demonstrated to inhibit expression of TNF and IL1 (Schindler et al., 1990), enhance synthesis of glucocorticoids which play anti-inflammatory and immunosuppressive roles (Dougall & Nick, 1991), and induce macrophage expression of IL1-receptor agonist (IL1ra) and soluble TNFα receptor, both of which truncate the effects of their respective pro-inflammatory cytokines (Tilg et al., 1994). As a result, if IL-6 levels remain elevated for a prolonged period of time, there is an increased risk of immunosuppression and sepsis (Biffl et al., 1996). Chronic elevations in IL-6 (~10 fold above resting) have also been associated with various health conditions including, but not limited to, obesity (Bastard et al., 2000), type 2 diabetes (Kado et al., 1999), and cardiovascular disease (Fisman et al., 2006).

While the typical response to acute infection involves other pro-inflammatory cytokines (i.e. TNF-alpha and IL1β), the response to exercise is led by a massive (up to

100 fold) increase in IL-6 with limited rises in the other pro-inflammatory cytokines and independent of markers of muscle damage (Petersen & Pedersen, 2006). An important factor distinguishing IL-6 release triggered by infection versus exercise stress is whether there is a concomitant rise in TNFα concentration prior to the IL-6 appearance. This differentiates the nature of the stress of infection and the acute phase response from that of exercise. This difference may stem from variation in the source and kinetics of IL-6 as a result of exercise. During exercise, IL-6 concentrations increase in a near exponential manner and peak near the end of the exercise bout, followed by a fairly rapid return to baseline levels (Ostrowski et al., 1998). Rather than macrophage production, the contracting skeletal muscle during exercise appears to be the major source of IL-6 based on notable increases in IL-6 mRNA content at the end of exercise (Steensberg et al., 2002). This theory is also supported by micro dialysis measurements showing a increases in IL-6 concentrations within the interstitium of contracting muscle above that found in circulation (Rosendal et al., 2005).

In addition to its role in the stress response, IL-6 serves as a mobilizer of energy substrates during exercise. Several studies have demonstrated the ability of IL-6 to increase insulin-stimulated glucose disposal in humans at rest and glucose uptake in vitro (Carey et al., 2006) and whole-body glucose production and uptake during exercise (Febbraio et al., 2004), as well as downregulate pyruvate dehydrogenase activity in the skeletal muscle of fed mice (Bienso et al., 2014). Interestingly, studies have found that carbohydrate ingestion during endurance training attenuates the exercise-induced increase in plasma IL-6 without affecting IL-6 mRNA content of the working muscle (Nehlsen-Cannarella et al., 1997; Starkie et al., 2001), suggesting glucose availability plays a role in the regulation of IL-6 mRNA processing and/or IL-6 release. Contributing to this idea, it has been shown that beginning exercise in a glycogen-depleted state results in an elevated plasma IL-6 response (Gleeson & Bishop, 2000). Furthermore, IL-

6 infusion in humans increases whole-body lipolysis and fat oxidation (van Hall et al., 2003; Wolsk et al., 2010). As such, IL-6 appears to influence fuel utilization through sensing of muscle glycogen stores and manipulation of circulating macronutrient levels.

Exercise mode, intensity, and duration appear to be the main determinants of the IL-6 response magnitude. Previously, it was suggested that the IL-6 response was related to muscle damage (Bruunsgaard et al., 1997). Ostrowski et al. instead showed that the IL-6 response was sensitive to intensity (2000), an appropriate finding as intensity can be viewed as an indirect representation of the contracting muscle mass involved. While this could imply that increased muscle damage would lead to just a greater magnitude in IL-6 release, it has been clarified that eccentric exercise results in a delayed peak and slower decrease of plasma IL-6 concentrations Keeping with the idea that muscle mass affects the magnitude of the response, exercise involving solely the musculature of the upper extremities may not elicit a significant IL-6 response (Bergfors et al., 2005), while exercise that incorporates larger muscle groups, such as running, elicits a much more dramatic plasma IL-6 increase (Nieman et al., 2005; Nieman et al., 2001; Niess et al., 2003). Also, the use of elbow flexor muscles limits the amount of contracting muscle mass involved in the bout, a factor that has already been discussed as a contributor to the overall cytokine response. These results do align with the conclusions made in an earlier review (Hirose et al., 2004), however there was very little research on resistance training at that time. (MacIntyre et al., 2001; Willoughby et al., 2003). More recent studies continue to question the assumption that higher muscle use and damage is central to the exercise induced cytokine response Hirose et al (2004) determined eccentric resistance exercise produced a smaller cytokine response compared to the typical response to endurance training and Mendham et al. found no significant differences in the IL-6 response to either a cycle ergometer or full-body resistance training session (2011). A recent review by Calle and Fernandez (2010),

failed to come to any clear conclusions regarding the effect of resistance exercise training on specific cytokine responses and suggested further studies were needed to elucidate their relationship.

Despite the effect of mode and intensity, duration appears to have the greatest influence on the IL-6 response (Ostrowski et al., 1998). A previous review reported that more than 50% of the variation in plasma IL-6 after exercise was explained by duration and that while running generally elicited the highest increases, the log-log linear relationship between fold increase of plasma IL-6 and time was “remarkably insensitive to the mode of exercise” (Fischer, 2006).

Interleukin 10 (IL-10) is another key cytokine known for its powerful anti-inflammatory actions (Moore et al., 2001). When challenged with a low dose of endotoxin, it was shown that IL-10 reduces serum levels of TNFα, IL-6, IL8, IL1ß, neutrophil accumulation, elastase, production and cortisone (Pajkrt et al., 1997). Originally identified as cytokine synthesis inhibitory factor (Vieira et al., 1991), IL-10 has been shown to severely blunt cytokine production by both T cells and NK cells. This inhibition comes through an indirect method, namely inhibition of macrophage and monocyte ability to present antigens (de Waal Malefyt, 1991; Ding & Shevach, 1992; Fiorentino et al., 1991). In humans, it has been shown that IL-10 concentrations are augmented by increases in circulating IL-6 (Steensberg et al. 2003). This helps to explain why at the Copenhagen Marathon, Ostrowski et al. (1999) found that both IL-6 and IL-10 peaked immediately after the race.

Conclusion

Chronic training appears to have anti-inflammatory effects overall. Jankord and Jemiolo (2004) found that among healthy older men, those with greater weekly volume of aerobic activity had decreased serum IL-6 and increased serum IL-10 concentrations. Smith et al. (1999) produced a significant increase in IL-10 utilizing a 6 month hospital

based exercise intervention. Conversely, Oberback et al. (2006) did not find significant changes in IL-6 or IL-10 after a 4 week training intervention in adults with varying degrees of glucose tolerance. Unfortunately, the study protocol was not fully disclosed so it is difficult to determine if mode or intensity were sufficient to elicit an adaptive effect. This is especially true given the relatively short duration of the intervention, 4 weeks, is not a long enough period of time to be considered “chronically’ trained. Continuing focus needs to be placed on elucidating specific cytokine responses to both acute and chronic resistance exercise.

Works Cited

Bastard, J. P., Jardel, C., Bruckert, E., Blondy, P., Capeau, J., Laville, M., . . . Hainque, B. (2000). Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab, 85*(9), 3338-3342. doi:10.1210/jcem.85.9.6839

Bergfors, M., Barnekow-Bergkvist, M., Kalezic, N., Lyskov, E., & Eriksson, J. W. (2005). Short-term effects of repetitive arm work and dynamic exercise on glucose metabolism and insulin sensitivity. *Acta Physiol Scand, 183*(4), 345-356. doi:10.1111/j.1365-201X.2005.01407.x

Bienso, R. S., Knudsen, J. G., Brandt, N., Pedersen, P. A., & Pilegaard, H. (2014). Effects of IL-6 on pyruvate dehydrogenase regulation in mouse skeletal muscle. *Pflugers Arch, 466*(8), 1647-1657. doi:10.1007/s00424-013-1399-5

Biffl, W. L., Moore, E. E., Moore, F. A., & Peterson, V. M. (1996). Interleukin-6 in the injured patient. Marker of injury or mediator of inflammation? *Ann Surg, 224*(5), 647-664. doi:10.1097/00000658-199611000-00009

Bosquet, L., Berryman, N., Dupuy, O., Mekary, S., Arvisais, D., Bherer, L., & Mujika, I. (2013). Effect of training cessation on muscular performance: a meta-analysis. *Scand J Med Sci Sports, 23*(3), e140-149. doi:10.1111/sms.12047

Bruunsgaard, H., Galbo, H., Halkjaer-Kristensen, J., Johansen, T. L., MacLean, D. A., & Pedersen, B. K. (1997). Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *The Journal of Physiology, 499*(3), 833-841. doi:10.1113/jphysiol.1997.sp021972

C. Richards, J. G. (1995). Role of cytokine in acute-phase response. In R. K. P. Bharat B. Aggarwal (Ed.), *Human Cytokines: Their Role in Disease and Therapy* (pp. 253-269). Cambridge: Blackwell Science Inc.

Calle, M. C., & Fernandez, M. L. (2010). Effects of resistance training on the inflammatory response. *Nutr Res Pract, 4*(4), 259-269. doi:10.4162/nrp.2010.4.4.259

Cannon, J., & Kluger, M. (1983). Endogenous pyrogen activity in human plasma after exercise. *Science, 220*(4597), 617-619. doi:10.1126/science.6836306

Carey, A. L., Steinberg, G. R., Macaulay, S. L., Thomas, W. G., Holmes, A. G., Ramm, G., . . . Febbraio, M. A. (2006). Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes, 55*(10), 2688-2697. doi:10.2337/db05-1404

Chang, F. E., Dodds, W. G., Sullivan, M., Kim, M. H., & Malarkey, W. B. (1986). The acute effects of exercise on prolactin and growth hormone secretion: comparison between sedentary women and women runners with normal and abnormal menstrual cycles. *J Clin Endocrinol Metab, 62*(3), 551-556. doi:10.1210/jcem-62-3-551

Cray, C., Zaias, J., & Altman, N. H. (2009). Acute phase response in animals: a review. *Comp Med, 59*(6), 517-526.

de Waal Malefyt, R. (1991). Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *Journal of Experimental Medicine, 174*(4), 915-924. doi:10.1084/jem.174.4.915

Ding, L., & Shevach, E. M. (1992). IL-10 inhibits mitogen-induced T cell proliferation by selectively inhibiting macrophage costimulatory function. *J Immunol, 148*(10), 3133-3139.

Dougall, W. C., & Nick, H. S. (1991). Manganese Superoxide Dismutase: A Hepatic Acute Phase Protein Regulated by Interleukin-6 and Glucocorticoids*. *Endocrinology, 129*(5), 2376-2384. doi:10.1210/endo-129-5-2376

Febbraio, M. A., Hiscock, N., Sacchetti, M., Fischer, C. P., & Pedersen, B. K. (2004). Interleukin-6 Is a Novel Factor Mediating Glucose Homeostasis During Skeletal Muscle Contraction. *Diabetes, 53*(7), 1643-1648. doi:10.2337/diabetes.53.7.1643

Felsing, N. E., Brasel, J. A., & Cooper, D. M. (1992). Effect of low and high intensity exercise on circulating growth hormone in men. *J Clin Endocrinol Metab, 75*(1), 157-162. doi:10.1210/jcem.75.1.1619005

Fiorentino, D. F., Zlotnik, A., Vieira, P., Mosmann, T. R., Howard, M., Moore, K. W., & O'Garra, A. (1991). IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *146*(10), 3444-3451.

Fischer, C. (2006). Interleukin-6 in acute exercise and training: what is the biological relevance? *Exercise Immunology Review, 12*, 6-33.

Fisman, E. Z., Benderly, M., Esper, R. J., Behar, S., Boyko, V., Adler, Y., . . . Tenenbaum, A. (2006). Interleukin-6 and the risk of future cardiovascular events in patients with angina pectoris and/or healed myocardial infarction. *Am J Cardiol, 98*(1), 14-18. doi:10.1016/j.amjcard.2006.01.045

Friedland, J. S., Suputtamongkol, Y., Remick, D. G., Chaowagul, W., Strieter, R. M., Kunkel, S. L., . . . Griffin, G. E. (1992). Prolonged elevation of interleukin-8 and interleukin-6 concentrations in plasma and of leukocyte interleukin-8 mRNA levels during septicemic and localized Pseudomonas pseudomallei infection. *60*(6), 2402-2408.

Giustina, A., & Veldhuis, J. D. (1998). Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. *Endocr Rev, 19*(6), 717-797. doi:10.1210/edrv.19.6.0353

Gleeson, M., & Bishop, N. C. (2000). Special feature for the Olympics: effects of exercise on the immune system: modification of immune responses to exercise by carbohydrate, glutamine and anti-oxidant supplements. *Immunol Cell Biol, 78*(5), 554-561. doi:10.1111/j.1440-1711.2000.t01-6-.x

Hakkinen, K., & Pakarinen, A. (1995). Acute hormonal responses to heavy resistance exercise in men and women at different ages. *Int J Sports Med, 16*(8), 507-513. doi:10.1055/s-2007-973045

Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., . . . et al. (1986). Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature, 324*(6092), 73-76. doi:10.1038/324073a0

Hirose, L., Nosaka, K., Newton, M., Laveder, A., Kano, M., Peake, J., & Suzuki, K. (2004). Changes in inflammatory mediators following eccentric exercise of the elbow flexors. *Exerc Immunol Rev, 10*, 75-90.

Hood, D. A. (2001). Invited Review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. *J Appl Physiol (1985), 90*(3), 1137-1157. doi:10.1152/jappl.2001.90.3.1137

Hunter, C. A., & Jones, S. A. (2015). IL-6 as a keystone cytokine in health and disease. *Nat Immunol, 16*(5), 448-457. doi:10.1038/ni.3153

Jankord, R., & Jemiolo, B. (2004). Influence of Physical Activity on Serum IL-6 and IL-10 Levels in Healthy Older Men. *Medicine & Science in Sports & Exercise, 36*(6), 960-964. doi:10.1249/01.Mss.0000128186.09416.18

Kado, S., Nagase, T., & Nagata, N. (1999). Circulating levels of interleukin-6, its soluble receptor and interleukin-6/interleukin-6 receptor complexes in patients with type 2 diabetes mellitus. *Acta Diabetol, 36*(1-2), 67-72. doi:10.1007/s005920050147

Kanaley, J. A., Weltman, J. Y., Pieper, K. S., Weltman, A., & Hartman, M. L. (2001). Cortisol and growth hormone responses to exercise at different times of day. *J Clin Endocrinol Metab, 86*(6), 2881-2889. doi:10.1210/jcem.86.6.7566

Kishimoto, T., Akira, S., Narazaki, M., & Taga, T. (1995). Interleukin-6 family of cytokines and gp130. *86*(4), 1243-1254.

Kraemer, R. R., Heleniak, R. J., Tryniecki, J. L., Kraemer, G. R., Okazaki, N. J., & Castracane, V. D. (1995). Follicular and luteal phase hormonal responses to low-volume resistive exercise. *Medicine & Science in Sports & Exercise, 27*(6). doi:10.1249/00005768-199506000-00004

Kraemer, R. R., Kilgore, J. L., Kraemer, G. R., & Castracane, V. D. (1992). Growth hormone, IGF-I, and testosterone responses to resistive exercise. *Med Sci Sports Exerc, 24*(12), 1346-1352.

Kraemer, W. J., Gordon, S. E., Fleck, S. J., Marchitelli, L. J., Mello, R., Dziados, J. E., . . . Fry, A. C. (1991). Endogenous anabolic hormonal and growth factor responses to heavy resistance exercise in males and females. *Int J Sports Med, 12*(2), 228-235. doi:10.1055/s-2007-1024673

Kraemer, W. J., Marchitelli, L., Gordon, S. E., Harman, E., Dziados, J. E., Mello, R., . . . Fleck, S. J. (1990). Hormonal and growth factor responses to heavy resistance exercise protocols. *J Appl Physiol (1985), 69*(4), 1442-1450. doi:10.1152/jappl.1990.69.4.1442

Lassarre, C., Girard, F., Durand, J., & Raynaud, J. (1974). Kinetics of human growth hormone during submaximal exercise. *J Appl Physiol, 37*(6), 826-830. doi:10.1152/jappl.1974.37.6.826

MacIntyre, D. L., Sorichter, S., Mair, J., Berg, A., & McKenzie, D. C. (2001). Markers of inflammation and myofibrillar proteins following eccentric exercise in humans. *Eur J Appl Physiol, 84*(3), 180-186. doi:10.1007/s004210170002

Mendham, A. E., Donges, C. E., Liberts, E. A., & Duffield, R. (2011). Effects of mode and intensity on the acute exercise-induced IL-6 and CRP responses in a sedentary, overweight population. *Eur J Appl Physiol, 111*(6), 1035-1045. doi:10.1007/s00421-010-1724-z

Moore, K. W., de Waal Malefyt, R., Coffman, R. L., & O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol, 19*, 683-765. doi:10.1146/annurev.immunol.19.1.683

Nehlsen-Cannarella, S. L., Fagoaga, O. R., Nieman, D. C., Henson, D. A., Butterworth, D. E., Schmitt, R. L., . . . Davis, J. M. (1997). Carbohydrate and the cytokine response to 2.5 h of running. *J Appl Physiol (1985), 82*(5), 1662-1667. doi:10.1152/jappl.1997.82.5.1662

Nieman, D. C., Dumke, C. L., Henson, D. A., McAnulty, S. R., Gross, S. J., & Lind, R. H. (2005). Muscle damage is linked to cytokine changes following a 160-km race. *Brain Behav Immun, 19*(5), 398-403. doi:10.1016/j.bbi.2005.03.008

Nieman, D. C., Henson, D. A., Smith, L. L., Utter, A. C., Vinci, D. M., Davis, J. M., . . . Shute, M. (2001). Cytokine changes after a marathon race. *J Appl Physiol (1985), 91*(1), 109-114. doi:10.1152/jappl.2001.91.1.109

Niess, A. M., Fehrenbach, E., Lehmann, R., Opavsky, L., Jesse, M., Northoff, H., & Dickhuth, H. H. (2003). Impact of elevated ambient temperatures on the acute immune response to intensive endurance exercise. *Eur J Appl Physiol, 89*(3-4), 344-351. doi:10.1007/s00421-003-0809-3

Nindl, B. C., Hymer, W. C., Deaver, D. R., & Kraemer, W. J. (2001). Growth hormone pulsatility profile characteristics following acute heavy resistance exercise. *J Appl Physiol (1985), 91*(1), 163-172. doi:10.1152/jappl.2001.91.1.163

Oberbach, A., Tonjes, A., Kloting, N., Fasshauer, M., Kratzsch, J., Busse, M. W., . . . Bluher, M. (2006). Effect of a 4 week physical training program on plasma

concentrations of inflammatory markers in patients with abnormal glucose tolerance. *Eur J Endocrinol, 154*(4), 577-585. doi:10.1530/eje.1.02127

Ostrowski, K., Hermann, C., Bangash, A., Schjerling, P., Nielsen, J. N., & Pedersen, B. K. (1998). A trauma-like elevation of plasma cytokines in humans in response to treadmill running. *The Journal of Physiology, 513*(3), 889-894. doi:10.1111/j.1469-7793.1998.889ba.x

Ostrowski, K., Rohde, T., Asp, S., Schjerling, P., & Pedersen, B. K. (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *The Journal of Physiology, 515*(1), 287-291. doi:10.1111/j.1469-7793.1999.287ad.x

Ostrowski, K., Rohde, T., Zacho, M., Asp, S., & Pedersen, B. K. (1998). Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *The Journal of Physiology, 508*(3), 949-953. doi:10.1111/j.1469-7793.1998.949bp.x

Ostrowski, K., Schjerling, P., & Pedersen, B. K. (2000). Physical activity and plasma interleukin-6 in humans--effect of intensity of exercise. *Eur J Appl Physiol, 83*(6), 512-515. doi:10.1007/s004210000312

Pajkrt, D., VanderPool, T., Levi, M., Cutler, D. L., Affrime, M. B., vandenEnder, A., . . . vanDeventer, S. J. H. (1997). IL-10 inhibits activation of coagulation and fibrinolysis during human endotoxemia. *Thrombosis and Haemostasis, 89*(8), O2452-O2452.

Pedersen, B. K., & Nieman, D. C. (1998). Exercise immunology: integration and regulation. *Immunology Today, 19*(5), 204-206. doi:10.1016/s0167-5699(98)01255-9

Pedersen, B. K., Steensberg, A., & Schjerling, P. (2001). Exercise and interleukin-6. *8*(3), 137-141.

Petersen, A. M., & Pedersen, B. K. (2006). The role of IL-6 in mediating the anti-inflammatory effects of exercise. *J Physiol Pharmacol, 57 Suppl 10*, 43-51.

Pritzlaff-Roy, C. J., Widemen, L., Weltman, J. Y., Abbott, R., Gutgesell, M., Hartman, M. L., . . . Weltman, A. (2002). Gender governs the relationship between exercise intensity and growth hormone release in young adults. *J Appl Physiol (1985), 92*(5), 2053-2060. doi:10.1152/japplphysiol.01018.2001

Pritzlaff, C. J., Wideman, L., Weltman, J. Y., Abbott, R. D., Gutgesell, M. E., Hartman, M. L., . . . Weltman, A. (1999). Impact of acute exercise intensity on pulsatile growth hormone release in men. *J Appl Physiol (1985), 87*(2), 498-504. doi:10.1152/jappl.1999.87.2.498

Raastad, T., Bjoro, T., & Hallen, J. (2000). Hormonal responses to high- and moderate-intensity strength exercise. *Eur J Appl Physiol, 82*(1-2), 121-128. doi:10.1007/s004210050661

Raynaud, J., Capderou, A., Martineaud, J. P., Bordachar, J., & Durand, J. (1983). Intersubject viability in growth hormone time course during different types of work. *J Appl Physiol Respir Environ Exerc Physiol, 55*(6), 1682-1687. doi:10.1152/jappl.1983.55.6.1682

Rosendal, L., Sogaard, K., Kjaer, M., Sjogaard, G., Langberg, H., & Kristiansen, J. (2005). Increase in interstitial interleukin-6 of human skeletal muscle with repetitive low-force exercise. *J Appl Physiol (1985), 98*(2), 477-481. doi:10.1152/japplphysiol.00130.2004

Schindler, R., Mancilla, J., Endres, S., Ghorbani, R., Clark, S. C., & Dinarello, C. A. (1990). Correlations and interactions in the production of interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. *Blood, 75*(1), 40-47.

Schoenfeld, B. J. (2010). The mechanisms of muscle hypertrophy and their application to resistance training. *J Strength Cond Res, 24*(10), 2857-2872. doi:10.1519/JSC.0b013e3181e840f3

Smith, J. K. (1999). Long-term Exercise and Atherogenic Activity of Blood Mononuclear Cells in Persons at Risk of Developing Ischemic Heart Disease. *Jama, 281*(18). doi:10.1001/jama.281.18.1722

Starkie, R. L., Arkinstall, M. J., Koukoulas, I., Hawley, J. A., & Febbraio, M. A. (2001). Carbohydrate ingestion attenuates the increase in plasma interleukin-6, but not skeletal muscle interleukin-6 mRNA, during exercise in humans. *The Journal of Physiology, 533*(2), 585-591. doi:10.1111/j.1469-7793.2001.0585a.x

Steensberg, A., Fischer, C. P., Keller, C., Moller, K., & Pedersen, B. K. (2003). IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *Am J Physiol Endocrinol Metab, 285*(2), E433-437. doi:10.1152/ajpendo.00074.2003

Steensberg, A., Keller, C., Starkie, R. L., Osada, T., Febbraio, M. A., & Pedersen, B. K. (2002). IL-6 and TNF-alpha expression in, and release from, contracting human skeletal muscle. *Am J Physiol Endocrinol Metab, 283*(6), E1272-1278. doi:10.1152/ajpendo.00255.2002

Sutton, J., & Lazarus, L. (1976). Growth hormone in exercise: comparison of physiological and pharmacological stimuli. *J Appl Physiol, 41*(4), 523-527. doi:10.1152/jappl.1976.41.4.523

Takarada, Y., Nakamura, Y., Aruga, S., Onda, T., Miyazaki, S., & Ishii, N. (2000). Rapid increase in plasma growth hormone after low-intensity resistance exercise with vascular occlusion. *J Appl Physiol (1985), 88*(1), 61-65. doi:10.1152/jappl.2000.88.1.61

Tilg, H., Trehu, E., Atkins, M., Dinarello, C., & Mier, J. (1994). Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. *83*(1), 113-118.

van Hall, G., Steensberg, A., Sacchetti, M., Fischer, C., Keller, C., Schjerling, P., . . . Pedersen, B. K. (2003). Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J Clin Endocrinol Metab, 88*(7), 3005-3010. doi:10.1210/jc.2002-021687

Vanhelder, W. P., Radomski, M. W., & Goode, R. C. (1984). Growth hormone responses during intermittent weight lifting exercise in men. *European Journal of Applied Physiology and Occupational Physiology, 53*(1), 31-34. doi:10.1007/bf00964686

Vieira, P., de Waal-Malefyt, R., Dang, M. N., Johnson, K. E., Kastelein, R., Fiorentino, D. F., . . . Moore, K. W. (1991). Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: homology to Epstein-Barr virus open reading frame BCRFI. *Proc Natl Acad Sci U S A, 88*(4), 1172-1176. doi:10.1073/pnas.88.4.1172

Vijayakumar, A., Novosyadlyy, R., Wu, Y., Yakar, S., & LeRoith, D. (2010). Biological effects of growth hormone on carbohydrate and lipid metabolism. *Growth Horm IGF Res, 20*(1), 1-7. doi:10.1016/j.ghir.2009.09.002

Viru, A. (1985). *Hormones in Muscular Activity* (1st ed. Vol. 1). Boca Raton: CRC Press.

Wegenka, U. M., Buschmann, J., Lütticken, C., Heinrich, P. C., & Horn, F. (1993). Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6 at the posttranslational level. *Molecular and Cellular Biology, 13*(1), 276-288. doi:10.1128/mcb.13.1.276

William D. McArdle, F. I. K., Victor L. Katch. (2015). *Exercise Physiology: Nutrition, Energy, and Human Performance* (8th ed.). Philadelphia: Lippincott Williams & Wilkins.

Willoughby, D. S., McFarlin, B., & Bois, C. (2003). Interleukin-6 expression after repeated bouts of eccentric exercise. *Int J Sports Med, 24*(1), 15-21. doi:10.1055/s-2003-37197

Wolsk, E., Mygind, H., Grondahl, T. S., Pedersen, B. K., & van Hall, G. (2010). IL-6 selectively stimulates fat metabolism in human skeletal muscle. *Am J Physiol Endocrinol Metab, 299*(5), E832-840. doi:10.1152/ajpendo.00328.2010

Chapter 2

The Effect of Training Background and Acute Exercise Type on the Cytokine Response to Exercise

By

CHRISTOPHER E. ORDWAY

Introduction

Two of the most prevalent forms of exercise today are aerobic and resistance training. Acute exercise bouts cause disruptions to normal physiological conditions that, when repeated regularly and appropriately, result in chronic adaptations (Lambert, 2016). Aerobic exercise requires the body to increase oxygen and fuel delivery to the working muscle, while subsequently removing carbon dioxide and metabolic by products. To achieve this, not only must cardiorespiratory system work harder, but the blood vessels and capillaries must respond to various signals to increase blood flow to specific parts of the body and the working muscle must work harder to use the resources available. Chronic training with aerobic exercise leads to endurance adaptations in muscle fatigue resistance and performance efficiency through improvements in capillary density, mitochondrial content, and fuel mobilization and utilization efficiency (Hood, 2001). Resistance exercise, on the other hand, challenges the muscle's ability to generate force. This is achieved through recruitment of more muscle fibers, specifically higher force producing ones, as well as the synchronization of motor unit contraction to produce sufficient force to overcome the resistance. Chronic resistance training leads to increases in muscle fiber hypertrophy, contractile strength, and neural drive (Bosquet et al., 2013; Schoenfeld, 2010).

While these chronic adaptations vary based on training type, the end results are the same: increased ability to handle the acute stressors that caused a disruption to the system. Missing from this literature, however, is understanding if and how the chronic adaptations to one exercise type affects the system's ability to respond to an acute bout of a novel stimulus.

One well studied physiological marker in exercise is growth hormone (GH). GH is a significant modifier of substrate utilization both during and after exercise through regulation of gluconeogenesis and glucose uptake (Vijayakumar et al., 2010). This

regulatory control over glucose metabolism aids in optimizing muscle performance and fatigue resistance through improved energy production, especially as plasma free fatty acid levels decline (Giustina & Veldhuis, 1998). In addition, GH is known for its role in cell division and protein synthesis with receptors for GH found throughout the body (Giustina & Veldhuis, 1998). These factors suggest controlling for food intake prior to exercise a necessity, GH's pulsatile secretion pattern (William D. McArdle, 2015) suggests it necessary to carefully consider and control when subjects complete study sessions. Conversely, Kanaley et al. (2001) demonstrated that time of day does not change the acute GH response to 30 minutes of treadmill running once resting values are accounted for. Early GH studies demonstrated that aerobic exercise caused an initial increase in plasma values 15 minutes after onset, and peak GH concentrations occurred at or near the end of exercise (Lassarre et al., 1974; Raynaud et al., 1983; Sutton & Lazarus, 1976). Recent studies utilizing frequent sampling intervals have shown that the intensity of aerobic exercise has a linear correlation with the magnitude of the GH response (Cappon et al., 1994; Pritzlaff et al., 1999; Wideman et al., 2000; Wideman et al., 1999).

Despite relatively fewer studies on resistance training, current works suggest that the pattern of GH release in response to this form of exercise is similar to that of aerobic exercise. Typically, GH concentration peaks at or just after the termination of resistance exercise, with a return to baseline levels by 90 minutes after termination (Hakkinen, 1995; Kraemer, 1995; Kraemer, 1992; Nindl, 2001 ; Raastad, 2000; Takarada, 2000; Kraemer & Ratamess, 2005; Kraemer et al., 2017). Studies have also indicated that the acute GH response to resistance exercise is highly impacted by total workload, rather than just intensity in terms of percentage of 1-RM used (Kraemer et al., 1990; Raastad et al., 2000).

While both types of exercise appear to stimulate a similar GH response, subtle differences in magnitude and time course are present. Exercise intensity and total work volume have both been identified as the major modifiers of the exercise-induced GH response (Pritzlaff-Roy et al., 2002; Pritzlaff et al., 1999)and, as a result, both of these key variables need to be considered in any study attempting to compare aerobic and anaerobic exercise on the sole basis of modality.

Cytokines generally function as intercellular messenger proteins that respond to a variety of stimuli and evoke particular biological activities after binding to a receptor on a responsive target cell (Bente Klarlund Pedersen & Nieman, 1998). Typically, cytokines play pro- or anti-inflammatory roles to mediate homeostatic disruptions induced by tissue damage. Exercise has been shown to elicit a release of cytokines to help manage strain placed on the system through regulation of inflammatory cascades (Cannon & Kluger, 1983). Inflammation following acute exercise bouts hastens regeneration and repair through pro-inflammatory cytokine release, while habitual exercise appears to reduce chronic low-grade inflammation through upregulation of anti-inflammatory cytokines (Jankord & Jemiolo, 2004).

IL-6 expression, release and action are well-established, with both pro- and anti-inflammatory effects being identified (Biffl et al., 1996; Pedersen et al., 2001). During acute injury or infection, damaged tissue (i.e endothelial cells and fibroblasts) and circulating immune cells (i.e. monocytes) produce and release IL-6. This then binds to its receptor, inducing a JAK/STAT transduction pathway that then promotes expression of acute phase proteins (APP) (Wegenka et al., 1993). These APPs are then associated with the innate immune response geared towards healing and reestablishing homeostasis (Cray et al., 2009).

In its anti-inflammatory role, IL-6 has been demonstrated to inhibit expression of TNF-α and IL-1 (Schindler et al., 1990), enhance synthesis of glucocorticoids, which play

anti-inflammatory and immunosuppressive roles (Dougall & Nick, 1991), and induce macrophage expression of IL-1-receptor agonist (IL-1ra) and soluble TNF-α receptor, both of which truncate the effects of their respective pro-inflammatory cytokines (Tilg et al., 1994). As a result of these effects, chronic elevation of IL-6 can result in an increased risk of immunosuppression and sepsis (Biffl et al., 1996) and has been associated with various health conditions such as obesity (Bastard et al., 2000), type 2 diabetes (Kado et al., 1999), and cardiovascular disease (Fisman et al., 2006).

During exercise, IL-6 is produced and released from contracting muscle (Rosendal et al., 2005; Steensberg et al., 2002), causing plasma concentrations to increase in a near exponential manner and peak near the end of the exercise bout, followed by a quick return to baseline levels (Kenneth Ostrowski et al., 1998). Outside of its inflammatory roles, IL-6 appears to influence fuel utilization through sensing of muscle glycogen stores (Gleeson & Bishop, 2000; Nehlsen-Cannarella et al., 1997; Starkie et al., 2001) and manipulation of circulating macronutrient levels. Specifically, it moderates glucose metabolism (Bienso et al., 2014; Carey et al., 2006; Febbraio et al., 2004) and increases whole-body lipolysis and fat oxidation (van Hall et al., 2003; Wolsk et al., 2010)..

Exercise mode, intensity, and duration appear to be the main determinants of the IL-6 response magnitude. Ostrowski et al. (2000) showed that the IL-6 response was sensitive to intensity by demonstrating marathon runners who ran at a higher intensity had a greater peak in plasma IL-6 concentrations. This is further exemplified by comparing studies that incorporated larger muscle groups (i.e. running) vs those looking at smaller muscle groups. Utilizing larger muscle groups, which would correlate with a higher VO_2, appears to stimulate a large IL-6 increase (Nieman et al., 2005; Nieman et al., 2001; Niess et al., 2003) while utilizing only the musculature of the upper extremities, similar to the repetitive, low intensity, and long duration of continuous production line

work does not induce such a response (Bergfors et al., 2005). Despite the effect of mode and intensity, duration appears to have the greatest influence on the IL-6 response (K. Ostrowski et al., 1998). A previous review reported that more than 50% of the variation in plasma IL-6 after exercise was explained by duration and that while running generally elicited the highest increases, the log-log linear relationship between fold increase of plasma IL-6 and time was “remarkably insensitive to the mode of exercise” when comparing exercise modes that utilize similar muscle groups (i.e. dynamic knee-extensor vs bicycling)(Fischer, 2006). While the specific exercise was different in this study, the movement patterns used in the two were similar enough to each other that a difference would not logically be expected to begin with.

Interleukin 10 (IL-10) also plays a powerful role in anti-inflammatory processes (Moore et al., 2001) by blunting inflammatory cytokine production by both T cells and NK cells (de Waal, 1991; Ding & Shevach, 1992; Fiorentino et al., 1991). IL-10 concentrations are stimulated by IL-6 (Steensberg et al., 2003), and a similar time course occurs for IL-6 and IL-10 in response to marathon running (Ostrowski et al., 1999).

Exercise training has been associated with beneficial changes to the baseline anti-inflammatory status of individuals. A survey of healthy older men by Jankord and Jemiolo (2004) found that greater weekly aerobic activity was associated with decreased serum IL-6 and increased serum IL-10 concentrations. A 6-month hospital-based exercise intervention by Smith et al. (Smith, 1999) resulted in a significant increase in IL-10. Conversely, Oberback et al. (2006) did not find significant changes in IL-6 or IL-10 after a 4-week training intervention in adults with varying degrees of glucose tolerance, suggesting a greater duration of regular training is necessary to elicit a positive anti-inflammatory effect.

Adaptations to chronic exercise inherently help individuals to better manage the

stress of acute exercise bouts. However, that is typically due to the acute bout of exercise being similar in nature to the chronic training modality. It has not been determined how chronic adaptations to one type of training influences the response to an acute bout of a novel exercise type. Therefore, the purpose of this study was to compare the responses to practiced and novel acute exercise bouts and how these may differ depending on the type of chronic training.

Materials and Methods

Subjects. Twenty healthy, college-aged male subjects volunteered to take part in this study. Subjects were recruited and categorized by training background: Endurance trained (END, n=10; M_{age}= 24.8±4.92 years; M_{ht}= 177.3±5.8 cm; M_{wt}= 68.9±4 kg; $M_{\%BF}$ = 10.1±4.1%) and Resistance trained (RES, n=10; M_{age}= 23.2±2.8 years; M_{ht}= 173.1±8.1 cm; M_{wt}= 76.8±8.8 kg; $M_{\%BF}$ = 15.5±6%). Inclusion criteria were a minimum of two years of regular training predominantly categorized as endurance or resistance training, at least six months free from injury or training disruption, between the ages of 18 and 39, and absence of any known health issues. Additionally, participants were selected based on fitness testing results - having mismatched results during END and RES testing (i.e. the average END subject fell into the superior category for VO_2 and a 10RM back squat below bodyweight; See Table 1). Individuals with metabolic disorders were excluded from the study. Subjects were permitted to use supplements as long as they had not started or stopped use within six -months of enrollment through completion. We implemented a between-subjects design to compare training background and added within-subjects comparisons between exercise sessions, including resting measures on each day. The research was approved by the Rutgers University Institutional Review Board for the Protection of Human Subjects.

Testing. Subjects arrived at the Rutgers Center for Health and Human Performance (CHHP) on three separate visits to complete baseline testing for

assessment of training status, collection of fitness data, and determination of load and power output to be used during the exercise sessions. In the first visit, subjects completed a health screening to determine eligibility and written informed consent was obtained. All subsequent sessions required the subjects to report at least 2 hours fasted, in a euhydrated state and having refrained from exercise for at least 24 hours.

In the second session, subjects underwent body composition analysis to determine body mass and body fat percentage via air displacement plethysmography using the Siri equation (Siri, 1956) and a Bod Pod (COSMED, Concord USA). Subjects then performed a 5-minute warm-up at a self-selected pace on the bike or treadmill before completing a graded exercise test on a cycle ergometer (Velotron, Racermate, Seattle, WA) to determine VO_{2max} via indirect calorimetry using a gas analyzer (CPET Quark Metabolic cart, COSMED, Concord USA). The starting wattage was determined based on the subject's estimated fitness (Figure 2), with 30 Watt increases every 3 minutes. Subjects were asked to maintain 90 RPM until volitional fatigue, which was assessed as the inability to maintain >80 RPM. The test was considered valid if 3 or more of the following criteria were met: observed HR ≥ age-predicted max minus 10 BPM, rating of perceived exertion score ≥ 18, VO_2 increase ≤ 150 mL/min between stages, or respiratory exchange ratio ≥ 1.15. Heart rate (HR) was monitored using a Polar H7 HR transmitter linked to a Polar M400 monitor (Polar Electro Inc., Bethpage, NY). Maximal aerobic power (PO_{max}) was calculated according to following formula (Naperalsky et al., 2010):

$$\boldsymbol{PO_{max}} = \textit{((W completed) + (Wattage increase)) x (\% time on stage attempted)}$$

After determining PO_{max}, the value at 75% PO_{max} was used as the prescribed intensity for the aerobic exercise (AE) condition. Additionally, ventilatory threshold (VT) was calculated using the graphical ventilatory equivalent method as described in Davis et al. (1980).

The final baseline session tested maximal strength using a 10-repetition maximum (10RM) to evaluate seven lower body exercises: barbell back squat, leg press, barbell back-rack lunge, Romanian deadlift, leg extension, prone leg curl, and seated calf raise. The National Strength and Conditioning Association guidelines for exercise technique and testing were followed and all testing was administered by a Certified Strength and Conditioning Specialist (Haff & Triplett, 2016). 10RM values were obtained over the course of 3-5 working sets. The 10RM value was used for the weight training (WT) condition, with subjects completing their lifts in the same order at 90% of 10RM.

Experimental Sessions. Subjects returned to the CHHP for their assigned experimental visits and were asked to refrain from exercise for the previous 24 hours and avoid intense or prolonged exercise for the previous 48 hours. Prior to their first condition, subjects completed a 24-hour food log to record their diet. They were encouraged to eat normally, and consume a meal 2-3 hours prior to the visit, with only water after. The food log was utilized to help the subjects repeat their diets as closely as possible for all subsequent conditions. Subjects were asked to refrain from consuming caffeine within six hours of their visit. Time of day was matched between conditions, with start time occurring between 8:00AM and 12:00PM for all subjects. Conditions were completed 3-7 days removed from previous sessions (testing or experimental condition). Session order was randomized, and conditions were matched for total duration of about 45 minutes. Water was consumed ad libitum for both sessions.

The AE condition required subjects to arrive and sit quietly for 10-15 minutes before the resting blood draw was performed. The Velotron bike seat and handle position were adjusted for the individual and then the subject was permitted to complete a short self-prescribed warm-up lasting no more than 10 minutes. Subjects then completed 45 minutes on the Velotron at approximately 75% of PO_{max}. Subjects were instructed to maintain a cadence of approximately 90 RPM and were monitored by a

study member throughout the session. If HR did not achieve steady state, reached a value above 90% HR_{max}, or the cadence could not be maintained, the wattage was reduced in 5 W increments until the subject was able to meet the required criteria. The subjects remained in the lab for 60 minutes for additional sample collection.

For the WT condition, subjects repeated the same procedure for their resting blood draw and warm-up. The exercise order from baseline testing was used, and subjects completed three sets of ten repetitions of each exercise at 90% of 10RM with approximately 90 seconds of rest between sets, monitored using a stopwatch. If subjects were unable to complete either of the first two sets of ten reps at the prescribed weight, the weight was reduced for the remainder of the sets of that exercise based on NSCA guidelines (Figure 3). The subjects remained in the lab for 60 minutes for additional sample collection. Water was consumed ad libitum for all experimental sessions.

Biochemical measures were performed on blood drawn during the experimental sessions. For each session, a resting draw (T1) was performed via venipuncture from an antecubital vein by standard procedures. A vacutainer system (Becton Dickinson, Rutherford, NJ, USA) was used to collect approximately 12 mL of blood into a serum separator tube. Upon completion of the prescribed exercise bout, a cannula was placed in an antecubital vein using standard procedures. A vacutainer system was then used to collect 12 mL of blood from the cannula within 5 minutes post-exercise (T2) and 60 minutes post exercise (T3).

***Blood Sample Preparation and Assays*.** After collection, tubes sat for 30 minutes before being centrifuged for 10 minutes at 4,750rpm (Allegra x-15R Centrifuge, Beckman Coulter, Brea, CA). Serum was then transferred into microcentrifuge tubes and stored at -80ºC until analysis. Cytokine values were determined using a custom ordered MILLIPLEX MAP Human Cytokine/Chemokine 9-Plex Magnetic Bead Panel (EMD Millipore, Mahopac, NY). Growth Hormone levels were determined by hormone specific

ELISA (Aviva Systems Biology, San Diego, CA).

Statistics. 2 (group) x 2 (condition) x 3 (time) multivariate analyses of variance with repeated measures on time were conducted for growth hormone, IL-6, and IL-10. Univariate follow ups were performed when significant multivariate effects were found. Significance was set a $p \leq 0.05$. All statistical analyses were completed using SPSS statistical software (IBM®, SPSS® version 23, New York, NY, USA).

Results

GH (figure 1) had a significant main effect for time (P<.05), a trend for condition (P<.10), and a significant effect for time by condition (P<.05). In the END group, the AE bout elicited a significantly higher GH value immediately post exercise than WT (T_1: AE= 19.1±12.1ng/mL vs WT: 11.9±8.9ng/ml, P<.05). In the RES group, a similar difference between conditions occurred, however it only trended towards significance (T_1: AE= 32.03±29.6ng/ml vs WT: 19.31±17.1ng/ml, P=0.07). For IL-6 (figure 2), there was a trend for a main effect of time (P<.10). There were no significant differences between groups for either condition. There was a significant increase for the RES group during the AE session (T_0= 12.43±20.0ng/mL vs T_1= 16.28±23.8ng/mL, P<.05) with no other differences seen between groups or condition. IL-10 (figure 3) had a trend for a main effect of condition as well as time (P<.10). The RES group did not differ in its IL-10 response between the exercise conditions, but had a trend for an increase from T_0 to T_1 for both AE and WT (P<.10) and reached significance above baseline at T_2 (WT: T_0= 11.23±7.8ng/ml vs T_2= 31.72±17.7ng/mL; AE: T_0= 19.66±33.2ng/ml vs T_2= 30.53±27.3ng/mL, P<.05). The END group differed in their response to exercise type. IL-10 values at rest were not different between conditions and displayed no change from pre- to immediately post-exercise for both, however the AE bout elicited significantly higher concentrations at 1 hr post exercise than the WT (T_2: AE= 54.24±50.6ng/mL vs WT= 27.33±29.4ng/mL, p<.05).

Discussion

The effect of training background and acute exercise type were investigated to assess biomarkers related to energy status and inflammation (GH, IL-6, IL-10). While none of the factors had a clear or dominant influence over the response of each marker, a general pattern is still present. The AE bout elicited a greater response in general, from pre to post for GH and IL-6 or during recovery for IL-10. This general pattern makes sense as GH and IL-6 both tend to respond during and peak at the cessation of exercise, while IL-10 is further down the cascade, with the IL-10 response being stimulated by rising IL-6 plasma concentrations. GH's higher response during the AE bout than the WT one points towards it's sensitivity to plasma FFA, which are expected to be taken up by working muscle as a fuel source to a greater extent during an aerobic session than a resistance one. The lack of significant difference in IL-6 response is interesting as several of the resistance exercises required more full body muscle activation (i.e. back squats and lunges), while the entire AE session was performed in a seated position.

Total exercise bout duration was matched. However, inherent to the differences in cycling vs resistance training, the AE bout resulted in a greater duration of muscle contraction than the WT. Additionally, the AE bout continuously used the same muscle groups in the exact same way throughout the bout, and even though there is inherently a recovery portion to each cycling stroke, while one leg relaxes, the other is contracting keeping the body in a constant level of exertion Meanwhile the WT led to increases and decreases in specific muscle group utilization depending on the specific movement being performed and the rest intervals between working sets may have allowed for significant enough levels of recovery despite systemic activation. This difference in the nature of the exercise sessions would explain why the differences in GH response occurred in both the END and RES groups. Previous work has suggested the impact of

intensity as a modifier of the overall biomarker response (Ostrowski et al., 2000; Pedersen et al., 1998; Pritzlaff et al., 1999), however, in line with other studies, the current investigation provides evidence that duration of muscle contractile activity is a decidedly more potent factor.

The condition dependent difference in GH response, especially in the END group contributes to previous work in GH's role in sparing glucose. A higher GH concentration during the AE bout indicates greater reliance upon fatty acid oxidation for energy than in the WT bout. For the END group, the AE bout was likely of low enough intensity to allow for them to remain efficient and productive while utilizing fatty acids, while the WT bout was both a novel exercise type, and a much higher intensity level, necessitating greater utilization of CHO as represented by the reduced GH response. Previous resistance training studies on GH used subjects who were likely already better adapted to the stimulus and, thus, were better able to continue using FFA during their session than our END group. For the RES group, the lack of difference between conditions points towards a reduced aerobic capacity in these individuals which caused the AE bout to be of a sufficiently high intensity to necessitate continued CHO reliance for energy. While not significant, the RES group tended to have higher GH values at T1 than the END group when comparing the same conditions (AE: END T_1= 19.1±12.1ng/mL vs RES T_1=32.03±29.6ng/ml; WT: END T_1= 11.9±8.9ng/ml vs RES T_1= 19.31±17.1ng/ml). This may be indicative of a resistance training adaptation, perhaps stimulating greater FFA use for recovery immediately after the exercise bout.

Interestingly, there was no difference in the IL-6 or IL-10 responses between conditions for either group. The limited number of participants is a likely contributor to the minimal significant results found. Individual variability in even resting values can easily mask the overall response. Also contributing, the END group tended to have a higher IL-6 value at all time points than the RES group for both conditions. Although this

was not significant, this could point to an adaptation to aerobic exercise that maintains slightly elevated circulating IL-6 values. The exercise type-independent cytokine response implies other factors may have a greater influence over the body's handling of exertion than the nature of the acute bout itself. Given that the IL-6 response to exercise is dependent upon the demands placed on the contracting muscle, the inherent differences between the bouts in both intensity and duration of muscle contraction would be expected to elicit differential response. While plasma IL-6 concentrations were not different between bouts, it is unclear if this is due to similar changes in muscle IL-6 mRNA content, or if another, post-transcriptional regulatory process is at play. Glucose availability may not have been reduced significantly enough issue to elicit an increased release of IL-6. Perhaps indicating a fast greater than 2 hours was needed. The significant rise from T_0 to T_1 for only the RES group in the AE bout points towards IL-6's role as an energy sensor. The duration of the session, in a novel exercise type, at a sufficiently high intensity, may have caused a greater stressor to the subjects than the WT bout, leading to greater reliance on glucose as a fuel substrate, and thereby depleting muscle glycogen stores..

The significant IL-10 responses without concomitant IL-6 responses serves as a reminder of the complexity of the cytokine response cascade. While IL-10 is known to respond to rises in IL-6, this study demonstrates that a significant IL-6 rise is not necessary for IL-10 to respond. This could indicate that either IL-10 is sensitive enough to IL-6 levels so as to respond significantly to only minor fluctuations, or that the response is being stimulated by another signaling molecule and IL-6's role as a regulator in the cytokine response cascade is overstated.

In this study, exercise type and training background was investigated to determine their roles in the GH, IL-6 and IL-10 responses to exercise. While significant results were limited potentially due to sample size, a clear enough pattern was seen that

confirms GH role in energy sensing and substrate utilization. The lack of IL-6 response overall may be an unfortunate result of insufficient fasting before the exercise bouts. Future studies may need to alter several factors, such as specific exercise modalities to better control for muscle utilization (i.e. running in the aerobic sessions to incorporate more than just the legs or remove weight training exercises that incorporate other stabilizing muscles) and pre-exercise fasting period, or consider controlling for other, potentially more influential, markers of intensity such as ventilatory threshold for aerobic sessions.

Works Cited

Bastard, J. P., Jardel, C., Bruckert, E., Blondy, P., Capeau, J., Laville, M., . . . Hainque, B. (2000). Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab, 85*(9), 3338-3342. doi:10.1210/jcem.85.9.6839

Bergfors, M., Barnekow-Bergkvist, M., Kalezic, N., Lyskov, E., & Eriksson, J. W. (2005). Short-term effects of repetitive arm work and dynamic exercise on glucose metabolism and insulin sensitivity. *Acta Physiol Scand, 183*(4), 345-356. doi:10.1111/j.1365-201X.2005.01407.x

Bienso, R. S., Knudsen, J. G., Brandt, N., Pedersen, P. A., & Pilegaard, H. (2014). Effects of IL-6 on pyruvate dehydrogenase regulation in mouse skeletal muscle. *Pflugers Arch, 466*(8), 1647-1657. doi:10.1007/s00424-013-1399-5

Biffl, W. L., Moore, E. E., Moore, F. A., & Peterson, V. M. (1996). Interleukin-6 in the injured patient. Marker of injury or mediator of inflammation? *Ann Surg, 224*(5), 647-664. doi:10.1097/00000658-199611000-00009

Bosquet, L., Berryman, N., Dupuy, O., Mekary, S., Arvisais, D., Bherer, L., & Mujika, I. (2013). Effect of training cessation on muscular performance: a meta-analysis. *Scand J Med Sci Sports, 23*(3), e140-149. doi:10.1111/sms.12047

Cannon, J., & Kluger, M. (1983). Endogenous pyrogen activity in human plasma after exercise. *Science, 220*(4597), 617-619. doi:10.1126/science.6836306

Cappon, J., Brasel, J. A., Mohan, S., & Cooper, D. M. (1994). Effect of brief exercise on circulating insulin-like growth factor I. *J Appl Physiol (1985), 76*(6), 2490-2496. doi:10.1152/jappl.1994.76.6.2490

Carey, A. L., Steinberg, G. R., Macaulay, S. L., Thomas, W. G., Holmes, A. G., Ramm, G., . . . Febbraio, M. A. (2006). Interleukin-6 increases insulin-stimulated glucose

disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes, 55*(10), 2688-2697. doi:10.2337/db05-1404

Cray, C., Zaias, J., & Altman, N. H. (2009). Acute phase response in animals: a review. *Comp Med, 59*(6), 517-526.

de Waal Malefyt, R. (1991). Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *Journal of Experimental Medicine, 174*(4), 915-924. doi:10.1084/jem.174.4.915

Ding, L., & Shevach, E. M. (1992). IL-10 inhibits mitogen-induced T cell proliferation by selectively inhibiting macrophage costimulatory function. *J Immunol, 148*(10), 3133-3139.

Dougall, W. C., & Nick, H. S. (1991). Manganese Superoxide Dismutase: A Hepatic Acute Phase Protein Regulated by Interleukin-6 and Glucocorticoids*. *Endocrinology, 129*(5), 2376-2384. doi:10.1210/endo-129-5-2376

Febbraio, M. A., Hiscock, N., Sacchetti, M., Fischer, C. P., & Pedersen, B. K. (2004). Interleukin-6 Is a Novel Factor Mediating Glucose Homeostasis During Skeletal Muscle Contraction. *Diabetes, 53*(7), 1643-1648. doi:10.2337/diabetes.53.7.1643

Fiorentino, D. F., Zlotnik, A., Vieira, P., Mosmann, T. R., Howard, M., Moore, K. W., & O'Garra, A. (1991). IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *146*(10), 3444-3451.

Fischer, C. (2006). Interleukin-6 in acute exercise and training: what is the biological relevance? *Exercise Immunology Review, 12*, 6-33.

Fisman, E. Z., Benderly, M., Esper, R. J., Behar, S., Boyko, V., Adler, Y., . . . Tenenbaum, A. (2006). Interleukin-6 and the risk of future cardiovascular events

in patients with angina pectoris and/or healed myocardial infarction. *Am J Cardiol, 98*(1), 14-18. doi:10.1016/j.amjcard.2006.01.045

Giustina, A., & Veldhuis, J. D. (1998). Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. *Endocr Rev, 19*(6), 717-797. doi:10.1210/edrv.19.6.0353

Gleeson, M., & Bishop, N. C. (2000). Special feature for the Olympics: effects of exercise on the immune system: modification of immune responses to exercise by carbohydrate, glutamine and anti-oxidant supplements. *Immunol Cell Biol, 78*(5), 554-561. doi:10.1111/j.1440-1711.2000.t01-6-.x

Haff, G., & Triplett, N. T. (2016). *Essentials of Strength Training and Conditioning* (Fourth Edition ed.). Champain. IL: Human Kinetics.

Hakkinen, K., & Pakarinen, A. (1995). Acute hormonal responses to heavy resistance exercise in men and women at different ages. *Int J Sports Med, 16*(8), 507-513. doi:10.1055/s-2007-973045

Hood, D. A. (2001). Invited Review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. *J Appl Physiol (1985), 90*(3), 1137-1157. doi:10.1152/jappl.2001.90.3.1137

Jankord, R., & Jemiolo, B. (2004). Influence of Physical Activity on Serum IL-6 and IL-10 Levels in Healthy Older Men. *Medicine & Science in Sports & Exercise, 36*(6), 960-964. doi:10.1249/01.Mss.0000128186.09416.18

Kado, S., Nagase, T., & Nagata, N. (1999). Circulating levels of interleukin-6, its soluble receptor and interleukin-6/interleukin-6 receptor complexes in patients with type 2 diabetes mellitus. *Acta Diabetol, 36*(1-2), 67-72. doi:10.1007/s005920050147

Kraemer, R. R., Heleniak, R. J., Tryniecki, J. L., Kraemer, G. R., Okazaki, N. J., & Castracane, V. D. (1995). Follicular and luteal phase hormonal responses to low-

volume resistive exercise. *Medicine & Science in Sports & Exercise, 27*(6). doi:10.1249/00005768-199506000-00004

Kraemer, R. R., Kilgore, J. L., Kraemer, G. R., & Castracane, V. D. (1992). Growth hormone, IGF-I, and testosterone responses to resistive exercise. *Med Sci Sports Exerc, 24*(12), 1346-1352.

Kraemer, W. J., Gordon, S. E., Fleck, S. J., Marchitelli, L. J., Mello, R., Dziados, J. E., . . . Fry, A. C. (1991). Endogenous anabolic hormonal and growth factor responses to heavy resistance exercise in males and females. *Int J Sports Med, 12*(2), 228-235. doi:10.1055/s-2007-1024673

Kraemer, W. J., Marchitelli, L., Gordon, S. E., Harman, E., Dziados, J. E., Mello, R., . . . Fleck, S. J. (1990). Hormonal and growth factor responses to heavy resistance exercise protocols. *J Appl Physiol (1985), 69*(4), 1442-1450. doi:10.1152/jappl.1990.69.4.1442

Kraemer, W. J., & Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Med, 35*(4), 339-361. doi:10.2165/00007256-200535040-00004

Lambert, M. I. (2016). General Adaptations to Exercise: Acute Versus Chronic and Strength Versus Endurance Training. In D. Vaamonde, S. S. du Plessis, & A. Agarwal (Eds.), *Exercise and Human Reproduction: Induced Fertility Disorders and Possible Therapies* (pp. 93-100). New York, NY: Springer New York.

Lassarre, C., Girard, F., Durand, J., & Raynaud, J. (1974). Kinetics of human growth hormone during submaximal exercise. *J Appl Physiol, 37*(6), 826-830. doi:10.1152/jappl.1974.37.6.826

Moore, K. W., de Waal Malefyt, R., Coffman, R. L., & O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol, 19*, 683-765. doi:10.1146/annurev.immunol.19.1.683

Naperalsky, M., Ruby, B., & Slivka, D. (2010). Environmental temperature and glycogen resynthesis. *Int J Sports Med, 31*(8), 561-566. doi:10.1055/s-0030-1254083

Nehlsen-Cannarella, S. L., Fagoaga, O. R., Nieman, D. C., Henson, D. A., Butterworth, D. E., Schmitt, R. L., . . . Davis, J. M. (1997). Carbohydrate and the cytokine response to 2.5 h of running. *J Appl Physiol (1985), 82*(5), 1662-1667. doi:10.1152/jappl.1997.82.5.1662

Nieman, D. C., Dumke, C. L., Henson, D. A., McAnulty, S. R., Gross, S. J., & Lind, R. H. (2005). Muscle damage is linked to cytokine changes following a 160-km race. *Brain Behav Immun, 19*(5), 398-403. doi:10.1016/j.bbi.2005.03.008

Nieman, D. C., Henson, D. A., Smith, L. L., Utter, A. C., Vinci, D. M., Davis, J. M., . . . Shute, M. (2001). Cytokine changes after a marathon race. *J Appl Physiol (1985), 91*(1), 109-114. doi:10.1152/jappl.2001.91.1.109

Niess, A. M., Fehrenbach, E., Lehmann, R., Opavsky, L., Jesse, M., Northoff, H., & Dickhuth, H. H. (2003). Impact of elevated ambient temperatures on the acute immune response to intensive endurance exercise. *Eur J Appl Physiol, 89*(3-4), 344-351. doi:10.1007/s00421-003-0809-3

Nindl, B. C., Hymer, W. C., Deaver, D. R., & Kraemer, W. J. (2001). Growth hormone pulsatility profile characteristics following acute heavy resistance exercise. *J Appl Physiol (1985), 91*(1), 163-172. doi:10.1152/jappl.2001.91.1.163

Oberbach, A., Tonjes, A., Kloting, N., Fasshauer, M., Kratzsch, J., Busse, M. W., . . . Bluher, M. (2006). Effect of a 4 week physical training program on plasma concentrations of inflammatory markers in patients with abnormal glucose tolerance. *Eur J Endocrinol, 154*(4), 577-585. doi:10.1530/eje.1.02127

Ostrowski, K., Hermann, C., Bangash, A., Schjerling, P., Nielsen, J. N., & Pedersen, B. K. (1998). A trauma-like elevation of plasma cytokines in humans in response to

treadmill running. *The Journal of Physiology, 513*(3), 889-894. doi:10.1111/j.1469-7793.1998.889ba.x

Ostrowski, K., Rohde, T., Asp, S., Schjerling, P., & Pedersen, B. K. (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *The Journal of Physiology, 515*(1), 287-291. doi:10.1111/j.1469-7793.1999.287ad.x

Ostrowski, K., Rohde, T., Zacho, M., Asp, S., & Pedersen, B. K. (1998). Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *The Journal of Physiology, 508*(3), 949-953. doi:10.1111/j.1469-7793.1998.949bp.x

Ostrowski, K., Schjerling, P., & Pedersen, B. K. (2000). Physical activity and plasma interleukin-6 in humans--effect of intensity of exercise. *Eur J Appl Physiol, 83*(6), 512-515. doi:10.1007/s004210000312

Pedersen, B. K., & Nieman, D. C. (1998). Exercise immunology: integration and regulation. *Immunology Today, 19*(5), 204-206. doi:10.1016/s0167-5699(98)01255-9

Pedersen, B. K., Ostrowski, K., Rohde, T., & Bruunsgaard, H. (1998). The cytokine response to strenuous exercise. *Can J Physiol Pharmacol, 76*(5), 505-511. doi:10.1139/y98-055

Pedersen, B. K., Steensberg, A., & Schjerling, P. (2001). Exercise and interleukin-6. *8*(3), 137-141.

Pritzlaff-Roy, C. J., Widemen, L., Weltman, J. Y., Abbott, R., Gutgesell, M., Hartman, M. L., . . . Weltman, A. (2002). Gender governs the relationship between exercise intensity and growth hormone release in young adults. *J Appl Physiol (1985), 92*(5), 2053-2060. doi:10.1152/japplphysiol.01018.2001

Pritzlaff, C. J., Wideman, L., Weltman, J. Y., Abbott, R. D., Gutgesell, M. E., Hartman, M. L., . . . Weltman, A. (1999). Impact of acute exercise intensity on pulsatile growth

hormone release in men. *J Appl Physiol (1985), 87*(2), 498-504. doi:10.1152/jappl.1999.87.2.498

Raastad, T., Bjoro, T., & Hallen, J. (2000). Hormonal responses to high- and moderate-intensity strength exercise. *Eur J Appl Physiol, 82*(1-2), 121-128. doi:10.1007/s004210050661

Raynaud, J., Capderou, A., Martineaud, J. P., Bordachar, J., & Durand, J. (1983). Intersubject viability in growth hormone time course during different types of work. *J Appl Physiol Respir Environ Exerc Physiol, 55*(6), 1682-1687. doi:10.1152/jappl.1983.55.6.1682

Rosendal, L., Sogaard, K., Kjaer, M., Sjogaard, G., Langberg, H., & Kristiansen, J. (2005). Increase in interstitial interleukin-6 of human skeletal muscle with repetitive low-force exercise. *J Appl Physiol (1985), 98*(2), 477-481. doi:10.1152/japplphysiol.00130.2004

Schindler, R., Mancilla, J., Endres, S., Ghorbani, R., Clark, S. C., & Dinarello, C. A. (1990). Correlations and interactions in the production of interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. *Blood, 75*(1), 40-47.

Schoenfeld, B. J. (2010). The mechanisms of muscle hypertrophy and their application to resistance training. *J Strength Cond Res, 24*(10), 2857-2872. doi:10.1519/JSC.0b013e3181e840f3

Siri, W. E. (1956). Body Composition from fluid spaces and density: analysis of methods. *Lawrence Berkeley National Laboratory*.

Smith, J. K. (1999). Long-term Exercise and Atherogenic Activity of Blood Mononuclear Cells in Persons at Risk of Developing Ischemic Heart Disease. *Jama, 281*(18). doi:10.1001/jama.281.18.1722

Starkie, R. L., Arkinstall, M. J., Koukoulas, I., Hawley, J. A., & Febbraio, M. A. (2001). Carbohydrate ingestion attenuates the increase in plasma interleukin-6, but not skeletal muscle interleukin-6 mRNA, during exercise in humans. *The Journal of Physiology, 533*(2), 585-591. doi:10.1111/j.1469-7793.2001.0585a.x

Steensberg, A., Fischer, C. P., Keller, C., Moller, K., & Pedersen, B. K. (2003). IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *Am J Physiol Endocrinol Metab, 285*(2), E433-437. doi:10.1152/ajpendo.00074.2003

Steensberg, A., Keller, C., Starkie, R. L., Osada, T., Febbraio, M. A., & Pedersen, B. K. (2002). IL-6 and TNF-alpha expression in, and release from, contracting human skeletal muscle. *Am J Physiol Endocrinol Metab, 283*(6), E1272-1278. doi:10.1152/ajpendo.00255.2002

Sutton, J., & Lazarus, L. (1976). Growth hormone in exercise: comparison of physiological and pharmacological stimuli. *J Appl Physiol, 41*(4), 523-527. doi:10.1152/jappl.1976.41.4.523

Takarada, Y., Nakamura, Y., Aruga, S., Onda, T., Miyazaki, S., & Ishii, N. (2000). Rapid increase in plasma growth hormone after low-intensity resistance exercise with vascular occlusion. *J Appl Physiol (1985), 88*(1), 61-65. doi:10.1152/jappl.2000.88.1.61

Tilg, H., Trehu, E., Atkins, M., Dinarello, C., & Mier, J. (1994). Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. *83*(1), 113-118.

van Hall, G., Steensberg, A., Sacchetti, M., Fischer, C., Keller, C., Schjerling, P., . . . Pedersen, B. K. (2003). Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J Clin Endocrinol Metab, 88*(7), 3005-3010. doi:10.1210/jc.2002-021687

Vanhelder, W. P., Radomski, M. W., & Goode, R. C. (1984). Growth hormone responses during intermittent weight lifting exercise in men. *European Journal of Applied Physiology and Occupational Physiology, 53*(1), 31-34. doi:10.1007/bf00964686

Vijayakumar, A., Novosyadlyy, R., Wu, Y., Yakar, S., & LeRoith, D. (2010). Biological effects of growth hormone on carbohydrate and lipid metabolism. *Growth Horm IGF Res, 20*(1), 1-7. doi:10.1016/j.ghir.2009.09.002

Wegenka, U. M., Buschmann, J., Lütticken, C., Heinrich, P. C., & Horn, F. (1993). Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6 at the posttranslational level. *Molecular and Cellular Biology, 13*(1), 276-288. doi:10.1128/mcb.13.1.276

Wideman, L., Weltman, J. Y., Patrie, J. T., Bowers, C. Y., Shah, N., Story, S., . . . Weltman, A. (2000). Synergy of L-arginine and GHRP-2 stimulation of growth hormone in men and women: modulation by exercise. *Am J Physiol Regul Integr Comp Physiol, 279*(4), R1467-1477. doi:10.1152/ajpregu.2000.279.4.R1467

Wideman, L., Weltman, J. Y., Shah, N., Story, S., Veldhuis, J. D., & Weltman, A. (1999). Effects of gender on exercise-induced growth hormone release. *J Appl Physiol (1985), 87*(3), 1154-1162. doi:10.1152/jappl.1999.87.3.1154

William D. McArdle, F. I. K., Victor L. Katch. (2015). *Exercise Physiology: Nutrition, Energy, and Human Performance* (8th ed.). Philadelphia: Lippincott Williams & Wilkins.

Wolsk, E., Mygind, H., Grondahl, T. S., Pedersen, B. K., & van Hall, G. (2010). IL-6 selectively stimulates fat metabolism in human skeletal muscle. *Am J Physiol Endocrinol Metab, 299*(5), E832-840. doi:10.1152/ajpendo.00328.2010

Table 1

	END	RES
Anthropometrics		
Age (yr)	24.75±4.92	23.2±2.8
Height (cm)	177.3±5.8	173.1±8.1
Mass (Kg)	68.9±4	76.8±8.8
%Body Fat	10.1±4.1%	15.5±6%
Strength		
Squat 10RM (Kg)	60.2±19.2	114.9±41.7
Leg Press 10RM (Kg)	167.5±77.2	255.9±58.18
Lunge 10 RM (Kg)	41.4±14.27	52.5±9.37
RDL 10RM (Kg)	59.7±36.4	107.6±30.4
Aerobic Fitness		
VO_{2MAX} (ml/Kg/min)	57.7±7.5	41.5±3.7
PO @VO_{2MAX} (Watts)	293.5±48.8	221±25.3
VT (% of VO_{2MAX})	84.3±8.7%	61.7±6.2%

Table 1: Demographics of each subject group

Table 2:

Cycling VO_{2MAX} Protocol

	Wattage	Elapsed time	
1	**70**	**3:00**	
2	**100**	**+3:00**	**RES**
3	130	+3:00	
4	**160**	**+3:00**	**END**
5	190	+3:00	
6	220	+3:00	
7	250	+3:00	
8	280	+3:00	
9	310	+3:00	
10	340	+3:00	
11	370	+3:00	
12	400	+3:00	

Table 2. VO_{2max} Protocol. Rightmost column demarcates the average start for the indicated group.

Table 3:

Reps completed in set	Weight reduced for remaining sets
10	0%
9	≥2.6%
8	≥6.2%
7	≥10.6%
6	≥11.8%

Table 3: Weight reduction procedures during WT session

Figure 1

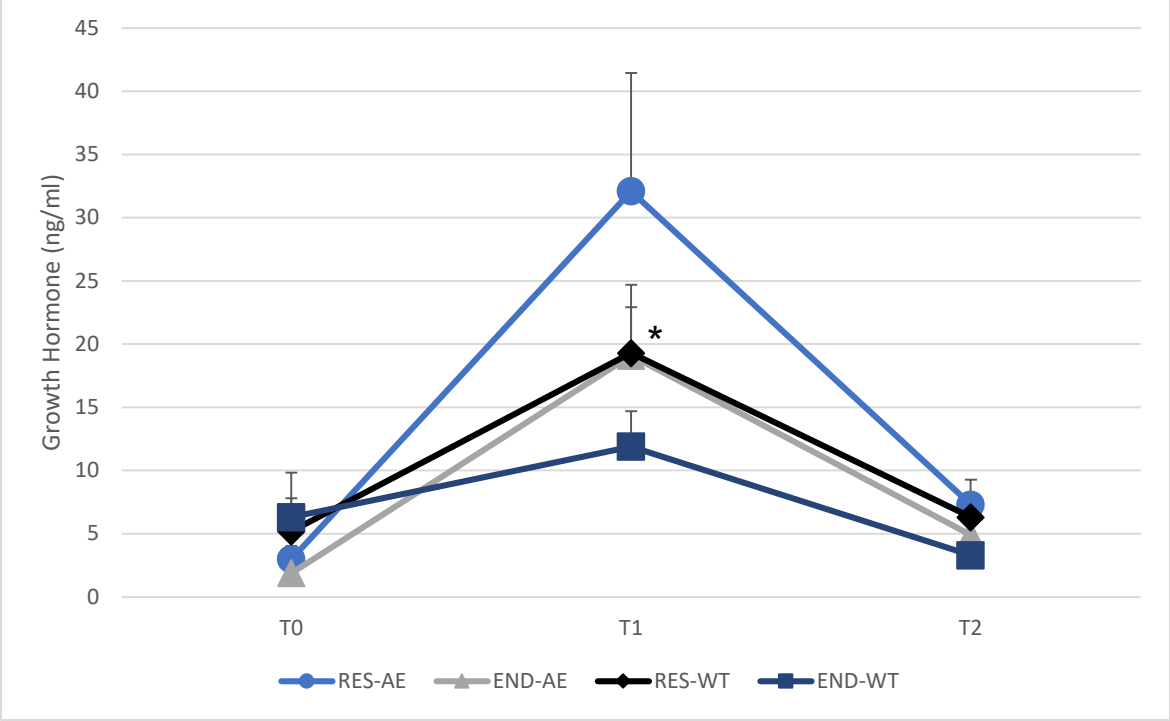

Figure 1: Growth Hormone. There was a significant main effect for time (P<.05), a trend for condition (P<.10), and a significant effect for time by condition (P<.05). For the END group, the AE bout elicited a significantly higher GH value immediately post exercise than WT (T_1: AE= 19.1±12.1ng/mL vs WT: 11.9±8.9ng/ml, P<.05) Denoted by *. The RES group, a similar difference between conditions occurred, however it only trended towards significance (T_1: AE= 32.03±29.6ng/ml vs WT: 19.31±17.1ng/ml, P=0.07).

Figure 2

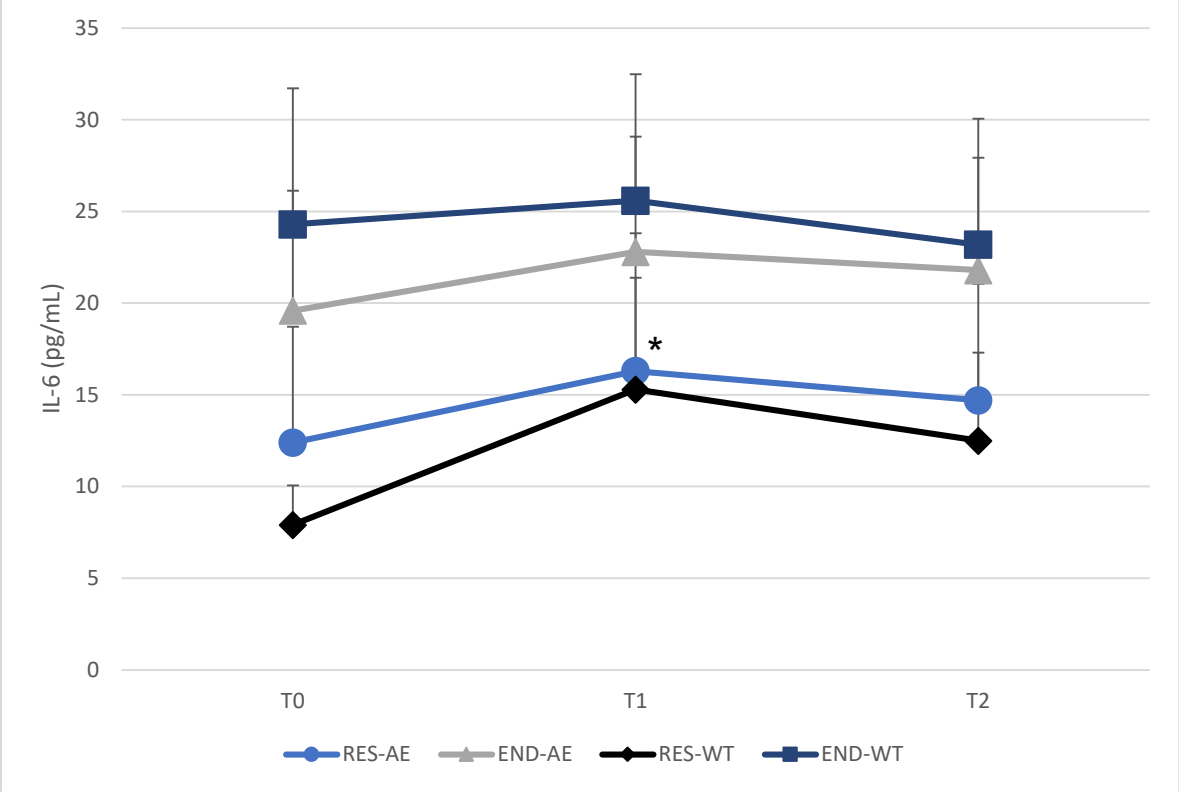

Figure 2: IL-6. There was a trend for a main effect of time (P<.10). There were no significant differences between groups for either condition.
There was a significant increase for the RES group during the AE session (T_0= 12.43±20.0ng/mL vs T_1= 16.28±23.8ng/mL, P<.05) denoted with *. No other differences were seen between groups or condition.

Figure 3

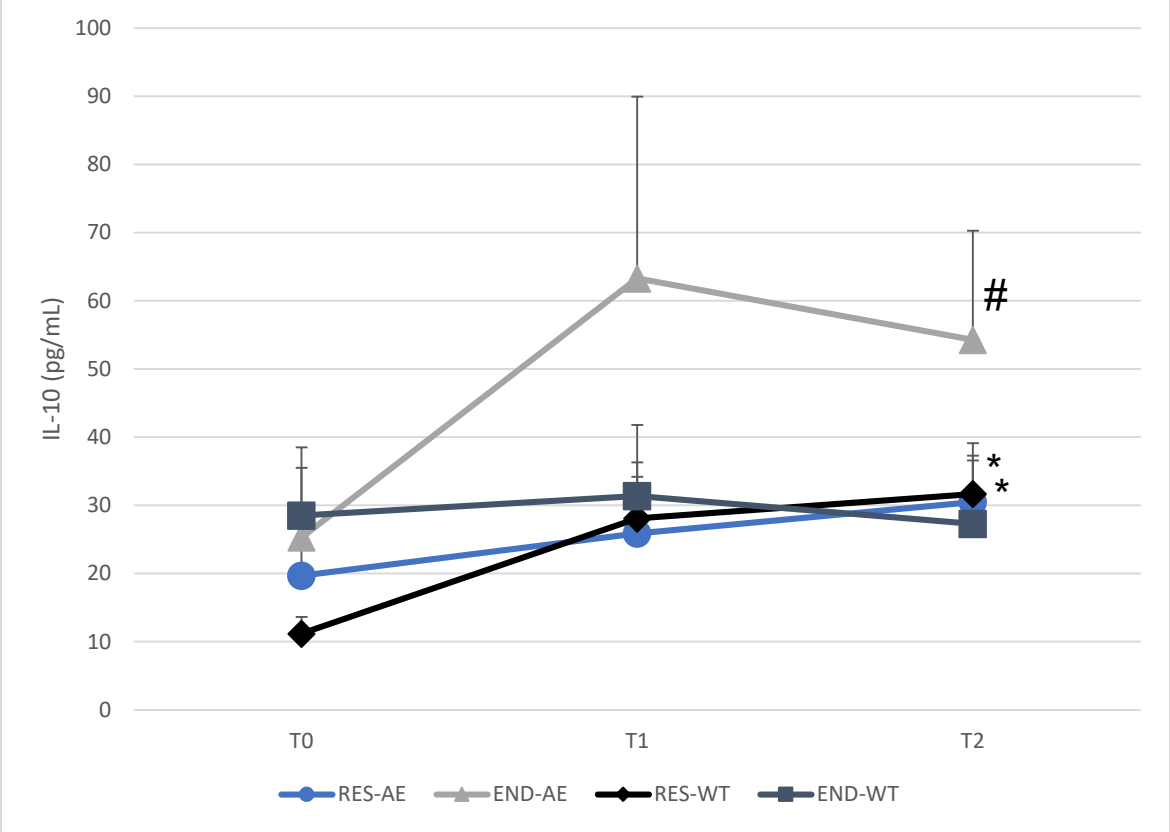

Figure 3: IL-10. There was a trend for a main effect of condition as well as time (P<.10). The RES group did not differ in its IL-10 response between the exercise conditions, but had a trend for an increase from T_0 to T_1 for both AE and WT (P<.10) and reached significance above baseline at T_2 (WT: T_0= 11.23±7.8ng/ml vs T_2= 31.72±17.7ng/mL; AE: T_0= 19.66±33.2ng/ml vs T_2= 30.53±27.3ng/mL, P<.05).
* denotes significant increase above baseline
The END group differed in their response to exercise type. IL-10 values at rest were not different between conditions and displayed no change from pre- to immediately post-exercise for both, however the AE bout elicited significantly higher concentrations at 1 hr post exercise than the WT (T_2: AE= 54.24±50.6ng/mL vs WT= 27.33±29.4ng/mL, p<.05).
# denotes significant difference between conditions at the same time point