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Interleukin-6 and exercise; early evidence of a novel myokine

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INTERLEUKIN-6 AND EXERCISE; EARLY EVIDENCE OF A NOVEL MYOKINE

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ABSTRACT

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Interleukin-6, a novel myokine

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Interleukin-6 (IL-6) was first identified as a pleiotropic cytokine, with a host of body-wide functions. Cytokine functions are characterized by chronically elevated levels within various inflammatory states. In this regard, IL-6 is largely associated with the acute phase response to many stimuli and performs specific actions when produced from certain cell types. Accrued evidence indicates IL-6 release from skeletal muscle often includes myokine functions. Novel myokine functions are adaptive in nature, and as compared to inflammatory/cytokine roles, exhibit a transient time course. Following exercise plasma IL-6 peaks and returns to resting levels within 1-2 hours. In contrast, IL-6 is observed to be consistently elevated in a much longer time course during inflammatory disease states. Production and release of IL-6 is reliant on many stress dependent factors, but from a skeletal muscle perspective, the release appears to be bioenergetic/fatigue dependent. Moreover, long duration in conjunction with high intensity exercise results in the greatest IL-6 production. Association between IL-6 and the metabolic status of skeletal muscle, in regards to glycogen content and glucose uptake, has provided a new avenue of research. The acute increase in circulating IL-6 following exercise indicates divergent roles for IL-6. Recent evidence indicates that IL-6 exerts regulatory cyto-protection in regards to insulin sensitivity and metabolic stress. In addition, recent data suggest that IL-6 is central within mechanisms regarding cardio-protection from ischemic injury. Given these widespread benefits, there is reason to suspect additional myokine roles of IL-6 are on the horizon. Further research efforts with mechanistic approaches should be directed toward investigating skeletal muscle derived IL-6 in regards to exercise-induced protection from metabolic and ischemic injury.

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Interleukin-6 (IL-6): Cytokine Vs Myokine

Over the last decade investigations designed to target interleukin-6 (IL-6) provide new understanding of health and disease progression. Overarching conclusions reveal that interleukin-6 is largely associated with inflammatory and auto-immune processes observed in various diseases such as diabetes, atherosclerosis, systemic lupus erythematosus and rheumatoid arthritis (Kristiansen & Mandrup-Poulsen, 2005; Dubiński, et al., 2007; Tackey et al., 2004; Nishimoto, et al., 2006). Moreover, mechanistic studies report that IL-6 overexpression leads to systemic inflammation, altered metabolic function as well as severe liver inflammation (Franckhauser, et al., 2008). IL-6 is not exclusively observed in detrimental disease states as evidenced by its acute appearance in circulation during and following exercise. Furthermore, ample evidence exists indicating that IL-6 possesses anti-inflammatory and even cyto-protective properties (Pedersen, 2017). IL-6 communicates with various cell types, in turn exerting systemic effects, which highlight a far-reaching influence within human biology (Kishimoto, 1992). The initially perplexing and pleiotropic characteristics of IL-6 have recently been revealed within the context of health and disease.

Cytokine research provides a better understanding of homeostatic function and disease progression. Cytokines are signaling proteins produced from a variety of immune cells and act in paracrine, autocrine and endocrine manners influencing the adaptive and innate immune systems (Kian Fan Chung, 2009). IL-6 is one of the most extensively documented cytokines, with attention being justified due to its ubiquitous nature and chronic elevation within various inflammatory disease states. Interleukin-6 was first identified in 1980 after researchers aimed to clone the interferon (IFN)- β gene within human fibroblasts (Weissenbach, et al., 1980; Van Snick, et al., 1990). However, it was not until 1989 that researchers came to agreement and labeled the

mysterious protein “interleukin-6” (Akira, et al., 1993). Early evidence suggested appearance of IL-6 in circulation was mainly the result of stimulated immune cells such as macrophages, fibroblasts, as well as T-cells, B-cells, and white blood cells (Andus, et al., 1998; Akira, et al., 1993). IL-6 production from immune cells and subsequent action is influenced by the presence of other pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) and interleukin-1 β (IL-1 β). Ample evidence supports a cytokine role of IL-6 in the host defense mechanism/acute phase response to trauma, burns and infection; hematopoiesis and stimulating the synthesis and release of acute phase proteins and antibodies (Andus, et al., 1988; Tanaka, Narazaki and Kishimoto, 2014). Due to the association between IL-6 and inflammatory states, it is understandable that early published findings promoted the notion that plasma IL-6 elevations in response to exercise were a result of tissue damage and inflammatory processes.

IL-6 release following exercise is not dependent on muscle damage. Initial findings of increased circulating IL-6 concentrations post-exercise were first observed by Northoff & Berg in 1991. In the context of the conventional understanding of IL-6 as an important moderator in immune cell function and proliferation, the increase in IL-6 post-exercise was thought to be due to immune cell recruitment following muscle damage. Bruunsgaard et al., (1997) observed larger and prolonged circulating IL-6 concentrations following muscle damaging-eccentric exercise compared to that of concentric exercise (30 min at >100 % VO_{2max} vs. 65 % VO_{2max} , respectively). In their now classic work, this team of researchers suggested that peak IL-6 concentrations following eccentric modalities were associated with elevated creatine kinase (CK) levels following exercise, depicting a possible dependency on muscle damage (Bruunsgaard, et al., 1997). The early assumptions that tissue damage serves as a prime initiator have now been rebutted by multiple groups of investigators that observed a much more prominent peak in IL-6

concentration following concentric exercise compared to previously executed eccentric models (~100-fold vs. ~5-fold) (Ostrowski et al., 1999). Ostrowski, et al., (1999) observed peak CK levels 24 hours post concentric exercise with no relationship between peak IL-6 levels and peak CK levels (Ostrowski et al., 1999). Additionally, it is well established that adhesion molecules following muscle damage attract leukocytes to the inflamed location and IL-6 when acting as a cytokine signaler from immune cells stimulates the binding of adhesion molecules to leukocyte and endothelial cells (Akimoto, et al., 2002; Chen et al., 2006, Kaplanski, et al., 2003). Yet, published findings from multiple research teams report unaltered expression of adhesion molecules following exercise despite an exercise induced IL-6 increase, indicating two things: 1) IL-6 production occurs independent of muscle damage and 2) IL-6 production during/following exercise exhibits an alternate function than that of traditional cytokine processes (Smith et al., 2000, Chaar, et al., 2011). Accordingly, the more novel idea of circulating IL-6 post-exercise, does not negate the well-established fact that muscle damaging eccentric exercise likely initiates more traditional cytokine actions of IL-6 from immune cells. As such, dynamic exercise with a concentric emphasis provides muscle-derived IL-6 release into circulation; a response that appears to be largely independent of inflammation.

IL-6 is expressed, produced and released from skeletal muscle. Ostrowski, et al., (1998b) as well as Steensberg et al. (2000) were frontrunners in identifying IL-6 release directly from skeletal muscle in response to exercise. More recent research indicates vesicles at the sarcolemma and T-tubule that contain IL-6 protein, which upon muscular contraction decrease in content (Lauritzen, et al., 2013). Bente Pedersen and colleagues (2003) were an innovative team of researchers who coined the term “myokine.” A myokine is described as a hormone-like protein produced, expressed and released from skeletal muscle tissue (Pedersen et al., 2003). Similar to

cytokine function, IL-6 is currently the most thoroughly studied myokine. Early attention given to IL-6 is appropriate in that a post-exercise spike in circulating IL-6 precedes the appearance of other myokines. To this end, IL-6 appears in circulation within 10 min of exercise (Macdonald, et al., 2003, Nieman, et al., 2007). Unlike its cytokine functions, the time course of skeletal muscle derived IL-6 release and disappearance in circulation is transient, with levels returning to baseline within two hours post-exercise. Furthermore, production via skeletal muscle fibers during exercise does not activate pro-inflammatory pathways (Pedersen & Febbraio, 2008). For example, monocytes produce and initiate the cytokine role of IL-6 during sepsis, yet monocytes do not express an increase in IL-6 protein during exercise (Moldoveanu, et al., 2000; Starkie, et al., 2001). IL-6 is produced from all fiber types of skeletal muscle, but IL-6 mRNA concentrations following exercise increase predominately in type II muscle fibers, which also contain higher glycogen content. While this is not fully understood, early scientific speculation demonstrates that type II fibers are central to the production of IL-6 during muscular contraction (Hiscock, et al., 2004). Supporting this tentative conclusion, McGinnis et al., (2015) observed in exercised muscle that fiber type specific IL-6 mRNA expression occurs. Tentative findings are supported by findings from real-time PCR (polymerase chain reaction) performed in skeletal muscles of the lower limb. Specifically, cardiomyocytes had the lowest expression while extensor digitorum longus (largely type II) contained the largest expression. With higher expression in type II fibers, it is clear that IL-6 release from skeletal muscle is influenced by exercise intensity due to type II (glycolytic) skeletal muscle fibers being highly recruited with increased exercise intensity (Yamano, et al., 2010).

Cell signaling is heavily influenced by cytokines in a vast range of bodily processes. Over expression of certain cytokines occurs in numerous disease states, highlighting the importance of

investigating the regulatory processes in which these substances are involved. Cytokine secretion is not limited to immune cells, as seen by IL-6 production from skeletal muscle during and following exercise. A body of published data underpins a growing consensus that skeletal muscle acts in endocrine fashion, highlighting the prospect of distinct IL-6 signaling pathways involved with organ cross-talk (Pedersen & Febbraio, 2008). Collectively, a wealth of knowledge surrounding exercise and IL-6 signaling depicts specific responses to other cytokines, exercise duration, exercise intensity, as well as glucose and glycogen availability.

Relationship between circulating TNF- α and IL-6

The action of circulating IL-6 is altered when in the presence of TNF- α . TNF- α is a multifaceted inflammatory cytokine produced predominantly by macrophages, natural killer cells and lymphocytes (Clyde & Glaunsinger, 2010). Antagonists of TNF- α are effectively utilized to treat inflammatory disorders such as psoriasis, rheumatoid arthritis and inflammatory bowel disease (Esposito, & Cuzzocrea, 2009). During severe infections (i.e. sepsis) and cardiometabolic disease, chronically elevated plasma concentrations of TNF- α and IL-6 are consistently observed (Pedersen, 2017). Upon stimulation, in scenarios such as sepsis, macrophages secrete a combination of pro-inflammatory cytokines: TNF- α , IL-1 β and IL-6. Compared to sepsis, exercise results in different cytokine responses: transient increases of IL-6 with no acute increases in TNF- α nor IL-1 β . Rather, skeletal muscle derived IL-6 has downstream anti-inflammatory effects such as inhibition of TNF- α and stimulation of cortisol and anti-inflammatory cytokines IL-10 and IL-1ra (interleukin-1 receptor antagonist) (Steensberg, et al., 2003). TNF- α is a potent inflammatory cytokine, and acts in union with IL-6 during inflammation (Pedersen, 2017). IL-6, unaccompanied by TNF- α , exhibits different and even anti-inflammatory effects.

IL-6 in response to exercise provides an anti-inflammatory environment by suppressing TNF- α . For instance, Starkie, et al., (2003) identified direct inhibition of TNF- α following exercise and recombinant IL-6 infusion. Researchers induced a low grade inflammatory state by administering *E. coli* to healthy subjects following 3h rest, 3h cycling or 3h infusion of recombinant IL-6. The intravenous bolus of *E. coli* resulted in a two- to three-fold increase in TNF- α while subjects were at rest, however following exercise TNF- α was completely blunted. The exercise induced blunting effect of exercise was nearly identical to exercise independent IL-6 infusion (eliciting same concentration as stimulated by exercise). These findings support the notion that IL-6 myokine action attenuates the appearance of TNF- α and may serve as a cytoprotective mechanism. In regards to metabolic function, TNF- α induces peripheral insulin resistance whereas recent findings indicate that IL-6 protects against metabolic damage and improves glucose tolerance (Plomgaard, et al., 2005; Carey, et al., 2006; Ellingsgaard, et al., 2008). IL-6 is no longer regarded as solely a synergist to TNF- α , with evidence indicating counter regulatory effects. In scenarios when TNF- α is not present, such as exercise, IL-6 undergoes phenomenological differences in magnitude, time and action than its customary cytokine roles (Reihmane et al., 2012; Steensberg, et al., 2002; Ostrowski, et al., 1998b).

Tissue specificity and IL-6 signaling

Actions of IL-6 are dependent on the source of production/release. Cytokine and myokine properties of IL-6 are clearly differentiated by their unique upstream and downstream signaling cascades as seen by the altered pathways in skeletal muscle and immune cell (macrophages) IL-6 secretion (Fig. 1). Stimulation of nuclear factor-kappaB (NF-kappaB) due to the activation of the TLR (toll like receptor) signaling cascade results in TNF- α , IL-1 β and IL-6 release from

macrophages in scenarios such as sepsis. When produced in union with one another TNF- α , IL-1 β and IL-6 act in synergy. However, upon muscular contraction IL-6 is not dependent on this stimulatory profile. Rather, the exclusive release of IL-6 from myocytes is suggested to be due to transcriptional control mediated by nitric oxide (NO) (Steensberg, et al., 2007). Steensberg, et al. (2007) observed that inhibiting NO production during exercise attenuated IL-6 protein release. Further downstream pathways involve a distinguishing network of cellular interactions involving nuclear factor of activated T cells (NFAT) and/or p38 mitogen activated protein kinase (p38-MAPK). Moreover, during exercise the sarcoplasmic reticulum releases calcium (Ca²⁺) resulting in muscular contractions. Lower intracellular concentrations of Ca²⁺, as seen during prolonged exercise, activates NFAT through the action of calcineurin (Im & Rao, 2004). NFAT expression is substantially higher in skeletal muscle compared to other cell types and NFAT has been shown to increase IL-6 mRNA expression within human muscle cells (Keller, et al., 2006). In addition to the sustained fatiguing mechanism of lowered Ca²⁺ during long durations, decreased fuel reserves (i.e. glycogen) is important. Prolonged exercise decreases muscle glycogen content, which in turn increases the phosphorylation of p38 MAPK within nuclei (Chan, et al., 2004). To this end, inhibition of p38 MAPK phosphorylation decreases IL-6 mRNA expression in muscle tissue (Chan, et al., 2004). Fatigue processes associated with exercise appears to be a potent stimulus of IL-6 production from skeletal muscle. In addition to source of production and receptor type, the unique myokine production pathways of IL-6 further highlight the difference between traditional cytokine processes.

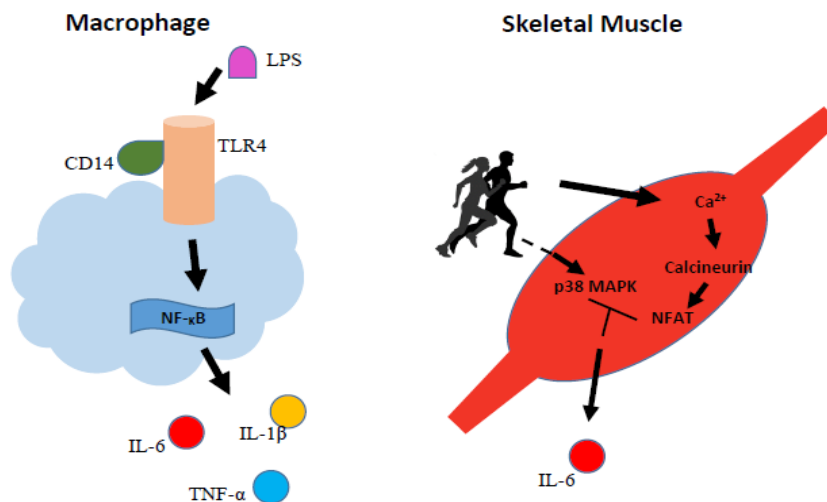


Figure 1. Differences between macrophage and skeletal muscle stimulation of IL-6 release into circulation. Adapted from Pedersen & Febbraio, 2008.

IL-6 production from various cell types initiates subsequent action on target tissues via unique receptor complexes. Receptor complexes, source of production as well as upstream and downstream signaling pathways influence IL-6 action. IL-6 has two receptor-mediated signaling approaches, referred to as *classic signaling* and *trans-signaling* (Fig. 2). In *classic signaling*, activity of IL-6 is mediated via two membrane bound proteins; the ligand-binding receptor (IL-6R) and the signal transducing glycoprotein-130 (gp130) (Kishimoto, 1992). All mammalian cells display the gp130 on their cell membranes, thereby eliciting downstream effects on various cell types (Scheller, et al., 2011). However, not all cells express the membrane bound IL-6R, thus the importance of having another signaling mechanism, *trans-signaling*. Components of this alternate pathway comprise of an extracellular soluble IL-6 receptor (sIL-6R) formed primarily via proteolytic shedding of the membrane bound IL-6R (Scheller, et al., 2011). Whether *classical* or *trans-signaling* occurs, the complex of the receptor and gp130 leads to the signal transduction of IL-6 which then activates the pathways: JAK/STAT (janus kinase/signal transducer and activator

of transcription), ERK (extracellular signal-regulated kinase), and PI-3K (phosphoinositide 3-kinase).

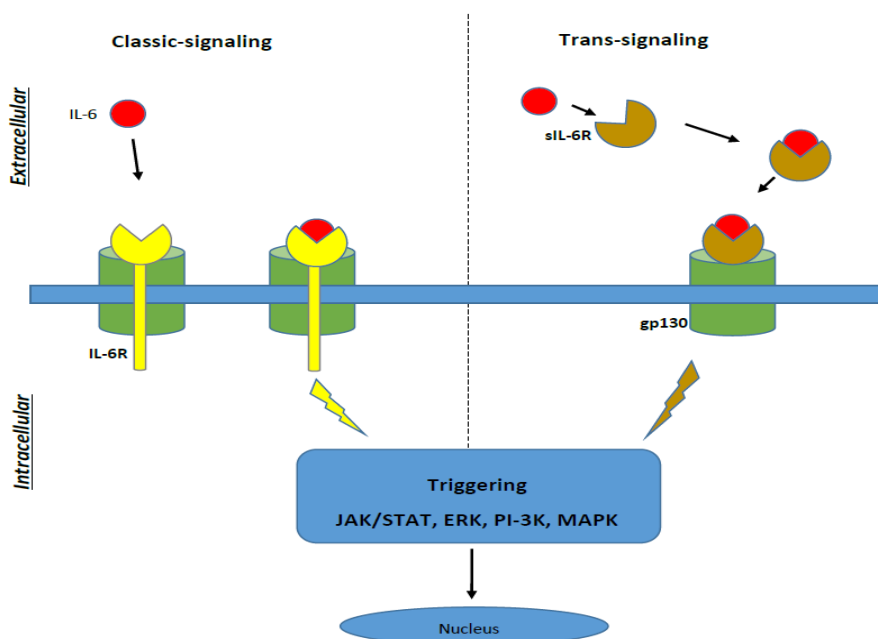


Figure 2. *IL-6 classical and trans-signaling. Proteolytic shedding of the membrane bound receptor results in a soluble IL-6 receptor that then binds to gp130 and produces signal transduction of JAK/STAT, ERK, PI-3K pathways which activate transcription factors. Adapted from Reihmane & Dela, 2014.*

Recently published preliminary evidence suggests that IL-6 activates different upstream and down-stream signaling pathways leading to specific cellular interactions, clarifying the discrepancy between cytokine and myokine functions. Skeletal muscle derived IL-6 signals the myocardium post-exercise through alternate pathways compared to cytokine signaling. Downstream of the IL-6R/gp130 complex, IL-6 signaling pathways are different when IL-6 is secreted as a cytokine versus a myokine (Fig. 3). During ischemic preconditioning (IPC) trials, IL-6 acts through the JAK/STAT and PI-3K pathways initiating protection from ischemic reperfusion injury (I/R) by activating downstream IPC mediators iNOS (inducible nitric oxide synthase) and COX-2 (cyclooxygenase-2). McGinnis, et al. (2015) observed that following exercise

preconditioning and subsequent I/R, IL-6 did provide protection from myocardial necrosis, however not through traditional cytokine signaling. Following exercise, investigators observed that JAK/STAT and PI-3K pathways did not initiate the protective effects (downstream activation of COX-2 and iNOS) of IL-6 signaling within the heart. Based on these findings, one might infer that exercise induced release of IL-6 follows an alternative route by which the myokine IL-6 initiates transcription. Further discussion of these novel findings will be provided in a later section (*Cardioprotection*), due to the ground breaking notion that skeletal muscle derived IL-6 exhibits endocrine properties albeit through unidentified signaling pathways. In addition to the apparent differences in myokine and cytokine signaling within the myocardium, secretory pathways of IL-6 change from immune cells to myocytes.

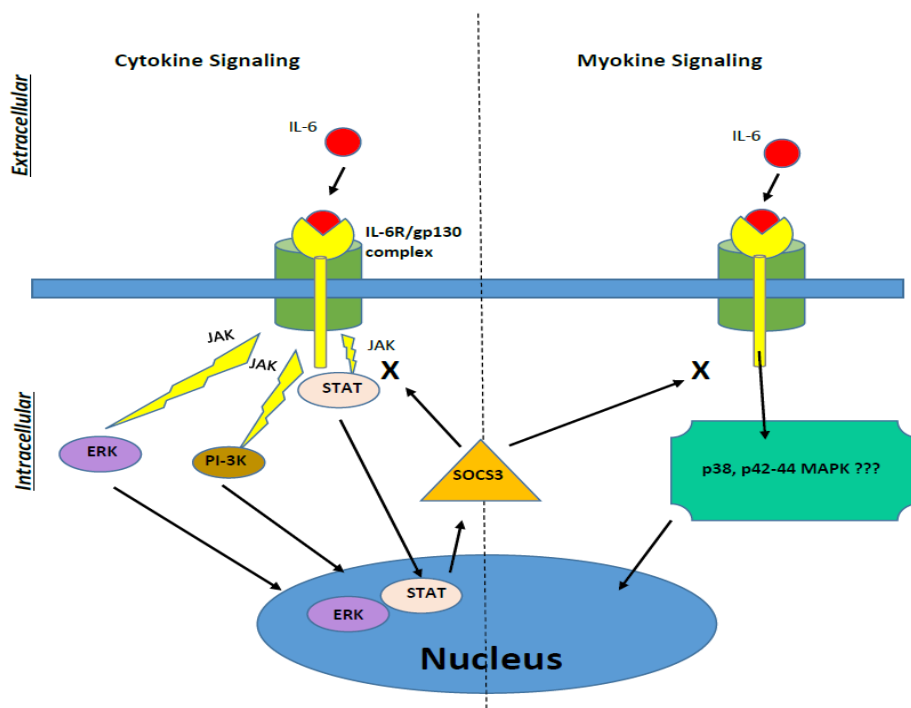


Figure 3. Cytokine signaling compared with myokine signaling as proposed by McGinnis et al., (2015).

As a generalization, the sIL-6R is associated with undesirable processes while the membrane bound IL-6R is more often associated with beneficial effects on homeostasis. For instance, pro-inflammatory effects of IL-6 are mostly mediated by *trans-signaling* (Kraakmen et

al., 2013; Rose-John, et al., 2007, Kraakmen et al, 2015). Early conclusions are supported by the observation that blockage of IL-6 *trans-signaling* in mice resulted in decreased inflammation in atherosclerosis (Scheutt, et al., 2012). Furthermore, use of tissue-specific deletion of membrane bound IL-6R (*classical signaling*) in murine hepatocytes, mice demonstrated detrimental metabolic phenotypes in regards to insulin resistance and glucose intolerance and liver inflammation (Wunderlich et al., 2010; Mauer et al., 2014). However, caution is warranted from broad based conclusions since the respective roles of membrane bound and soluble IL-6 receptor activation are still being defined in the context of myokine stimuli. Case in point, in addition to increasing circulating IL-6 protein, exercise also increases both membrane bound IL-6R and the sIL-6R in plasma and skeletal muscle (Leggate, et al., 2010; McGinnis, et al., 2015). The production of this myokine is heavily influenced by fatigue mechanisms, which are stimulated by higher intensity and longer duration exercise. The phenomenological differences between IL-6 cytokine and myokine signaling have yet to be completely resolved. However, the upstream and downstream signaling pathways accompanied by the alternate receptor complexes provide a solid foundation in identifying fundamental differences between cytokine and myokine actions.

Role of exercise intensity and duration on IL-6 production

The magnitude in the spike of circulating IL-6 during and post-exercise appears to be influenced by the amount of muscle mass recruited. Running, which recruits a large amount of muscle mass by virtue of lower and upper body movement, typically results in the largest circulating spike in post-exercise IL-6 (Nieman et al., 1998, Starkie, et al., 2000, 2001, Ostrowski, 1998b). In agreement, exercise limited to the upper extremities does not produce a dramatic IL-6 elevation post-exercise (Bergfors, et al., 2005; Hirose, et al., 2004). Although the amount of

contracting skeletal muscle influences IL-6 magnitude within circulation, the intensity and duration of exercise are the most potent stimulators of IL-6 production, independent of modality. With higher intensity and longer duration, human skeletal muscle performance is hindered by inevitable fatigue. As noted earlier, muscular fatigue is a powerful promoter of IL-6 release from skeletal muscle.

Intensity

Elevated levels of IL-6 are consistently observed following strenuous exercise indicating the influence of exercise intensity on IL-6 production. For instance, early published findings indicate a two-fold increase in plasma IL-6 following 6 min of “all out” rowing at a workload of 400 watts (Nielsen, et al., 1996). Similar findings of a mild increase were observed following 2 hours of whole body resistance training consisting of four sets of ten repetitions at 40-60% 1RM (1 set at 40% and 3 at 60% 1RM). A six-fold increase in plasma IL-6 from pre to post-exercise was observed (Nieman, et al., 2003). Later investigations revealed that IL-6 release is directly influenced by exercise intensity (Helge et al., 2003; Ostrowski et al., 2000). Helge et al. (2003) reported that plasma IL-6 concentrations increased proportionally to exercise intensity with a main effect of $p < 0.05$. Subjects performed double knee extension for 45 min at 25% W_{max} followed by an additional 35 min where one leg exercised at 65% W_{max} and the other at 85% W_{max} . Using the Fick principle (femoral arterial-venous difference X blood flow) researchers identified differences in IL-6 release between both legs. The highest IL-6 release was observed at 85% W_{max} , eliciting nearly a six-fold increase from resting values ($p < 0.05$), whereas release at 65% and 25% W_{max} did not exceed a three-fold increase. Notably, at both intensities (65% and 85% W_{max}) IL-6 release increased over time, suggesting a positive associative relationship with exercise duration.

Duration

The majority of plasma IL-6 elevations following exercise is attributed to exercise duration (Fischer, 2006). Rest intervals do not significantly affect IL-6 production as seen by Nieman et al. (2007). Nieman, et al., reported a 40-fold increase in plasma IL-6 concentrations in both continuous and intermittent exercise groups where individuals cycled for 2 hours at an intensity of 75% VO₂max. Comparatively, a mere five-fold increase in arterial IL-6 concentrations was observed after cycling for 1 hour at a similar intensity; 70% VO₂max ($p < 0.05$) (Macdonald, et al., 2003). Ostrowski et al. (1998a) observed during treadmill running at 75% VO₂max that circulating IL-6 levels were elevated 30 min into the bout (four-fold increase from rest) but peaked at 2.5 hr of exercise (25-fold from rest), supplying further evidence that increases in plasma IL-6 during exercise are largely affected by duration. Ostrowski and colleagues continued their investigation, by observing IL-6 response following a marathon, where IL-6 levels increased markedly following the race (completion time: 3hr 17min \pm 7.39 min) (Ostrowski, et al., 1998b). IL-6 plasma concentrations collected immediately post-race, revealed a near 100-fold increase from 1.5 ± 0.7 at rest to 94.4 ± 12.6 pg/ml. In the same study a significant decline in IL-6 plasma concentration was observed 2 hour post-exercise, resulting in 22.1 ± 3.8 pg/ml, elucidating the transient response to exercise. Another group of researchers observed similar findings of a 100-fold increase in plasma IL-6 post marathon, (mean finish time = 2hr 37min) (Suzuki, et al., 2003). Contrarily, other teams of investigators failed to report the same magnitude of IL-6 appearance following marathon running (Bernecker, et al., 2013; Nieman, et al., 2001). The large disparity among finish times between the two sets of studies (1-1.5 hr) may be the mitigating factor behind the different amplitudes of circulating IL-6, although sample collection and handling are also likely

explanations. Nonetheless, IL-6 secretion from skeletal muscle is heavily influenced by the length of exercise and when combined with a higher intensity the largest release is observed.

Influence of glucose and glycogen availability

With longer duration and higher intensity of exercise active human skeletal muscle will inevitably achieve some degree of glycogen depletion. Ostrowski et al., (1998a) revealed that while exercising at 75% VO_2max , the highest concentrations of plasma IL-6 occurred at 2.5 hr, a time point at which total glycogen depletion is imminent. Thus, appearance of IL-6 likely reflects the energy status/fatigue level of skeletal muscle, i.e. glycogen availability. Data from Steensberg et al. (2001) as well as Macdonald, et al. (2003) support this hypothesis, where larger release of IL-6 from skeletal muscle occurred in a glycogen depleted state.

Muscle glycogen stores as well as exogenous glucose affect the magnitude of IL-6 release from skeletal muscle during exercise. Many published findings report a blunting effect of IL-6 following ingestion of carbohydrates (CHO). In this regard, when fed CHO during 3 hours of running, participants exhibit lower plasma IL-6 concentrations compared to their non-CHO fed counterparts (Nieman, et al., 2003). A number of additional studies where endurance athletes where fed CHO (6% in solution), also resulted in lowered circulating IL-6 (Nehlsen-Cannarella, et al., 1997; Nieman, et al., 2001; Nieman, et al., 1998). However, Nieman et al. (2003) reported no difference in plasma IL-6 concentrations between a CHO fed group and a control group following 2 hr of heavy resistance training. However, the extent of circulating IL-6 did not reach that of the previously mentioned studies, therefore endogenous CHO may only attenuate IL-6 release at a certain magnitude.

Muscle glycogen content is a powerful stimulus for IL-6 release from muscle. While performing one legged exercise (40% W_{max}), Steensberg et al. (2001) observed that a significant net release of IL-6 from a glycogen depleted leg (40% less, $p < 0.05$) occurred 1 hr before an elevation in IL-6 was observed in a non-depleted exercised leg ($4.38 \pm 2.80 \text{ ng} \cdot \text{min}^{-1}$ vs. $0.36 \pm 0.14 \text{ ng} \cdot \text{min}^{-1}$, respectively). Moreover, following a randomized cross-over design (two week washout), subjects cycled for one hour at 70% VO_{2peak} where muscle biopsies were taken from the vastus lateralis (opposite legs for respective trials). Higher IL-6 release was observed at each 10 min increment in glycogen depleted trials compared with high glycogen trails ($p < 0.05$) (Macdonald et al., 2003).

Although IL-6 mRNA is influenced by glycogen availability, circulating IL-6 exhibits the strongest associations. For example, muscle glycogen depletion (vastus lateralis, $35.3 \pm 4.2\%$) following treadmill running for 2.25 hr (70% VO_{2max}) was inversely correlated with plasma IL-6 concentrations ($r = -0.72$, $p < 0.001$), yet it was not significantly associated with skeletal muscle IL-6 mRNA post-exercise ($r = -0.211$, $p = 0.321$) (Nieman, et al., 2015). In a subsequent study where subjects performed a 75 km cycling time trial ($169 \pm 26 \text{ min}$), depleted muscle glycogen stores (vastus lateralis, $77.2\% \pm 17.4\%$) were once again significantly correlated with the changes in plasma IL-6 ($r = 0.462$, $p = 0.04$) (Nieman, et al., 2016). Interesting to note, plasma IL-6 concentrations in both of these studies increased roughly 40-fold during similar durations and intensity, yet muscle glycogen concentrations were substantially different within the biopsied muscle. These puzzling results may be attributed to two mechanisms: 1) running recruits more muscle mass resulting in a larger systemic release of IL-6 and 2) cycling isolates the vastus lateralis more so than running, resulting in a larger glycogen depletion and subsequent IL-6 release. IL-6 levels in circulation are related to muscle glycogen content, however there are other elusive fatigue

mechanisms/pathways that may influence IL-6 production. The effect of glucose and glycogen availability on IL-6 production led to the understanding of IL-6 having a mediatory role in glucose metabolism. In this regard, prolonged exercise results in IL-6 inhibition of glycogen synthase while glycogen phosphorylase activity is promoted (Kanemaki, et al., 1998). Collectively, these findings suggest that IL-6 in circulation during/following exercise is highly influenced by glycogen depletion and glucose availability.

IL-6 as a metabolic regulator

Early evidence suggests that IL-6 may serve as a bioenergetic regulator, and exhibits roles within substrate metabolism. In support, mice lacking IL-6 expression develop mature onset obesity as well as elevated basal glucose levels in accordance with impaired glucose tolerance (Wallenius, 2002). Furthermore, in humans, infusion of IL-6 induces intramuscular as well as whole body lipolysis and fat oxidation (van Hall, et al., 2003; Wolsk, et al., 2010; Bruce et al., 2004; Petersen, et al., 2005). When released in a fasted state, IL-6 mediates free fatty acid mobilization (Wueest, et al., 2014). Although, IL-6 alters fat metabolism, other evidence points towards a more central role within CHO metabolism during exercise. IL-6 clearly influences glucose kinetics at rest and during exercise. In a dose dependent manner, infusion of recombinant human IL-6 increased whole body glucose oxidation within human subjects (Stouthard et al., 1995). Furthermore, Steensberg et al. (2001) concluded that a relationship exists between increased glucose uptake and net IL-6 release during exercise at low intensities (40% W_{max}). If correct, this notion is consistent with subsequent observations which indicate a positive correlation exists between IL-6 release and glucose uptake at 25% W_{max} was observed ($R = 0.82$, $p = 0.03$) (Helge et al. 2003). Accrued evidence demonstrates that IL-6 increases the delivery of glucose, glucose

uptake, lipolysis and fatty acid oxidation via 5'-adenosine monophosphate-activated protein kinase (AMPK).

AMPK is central to metabolic responses to exercise via stimulation of fatty acid oxidation, glucose uptake and GLUT4 translocation (Ruderman, et al., 2006; Kahn, et al., 2005; Fisher, et al., 2002). IL-6 directly affects AMPK activity within skeletal muscle and adipose tissue, subsequently influencing substrate utilization (Kelley, et al., 2004; Carey, et al., 2006). Infusion of IL-6 results in insulin stimulated whole body glucose uptake, increased glucose oxidation as well as exercise induced whole body glucose production (Carey, et al., 2006; Febbraio, et al., 2004). As little as 1 ng/ml of IL-6 elicits 2-fold increases GLUT4 translocation within skeletal muscle (Carey et al., 2006). Additionally, GLUT4 content in inguinal white adipose tissue of IL-6 KO mice reduced post-exercise, indicating that IL-6 contributes to the regulation of CHO metabolism in adipose tissue in addition to skeletal muscle (Knudsen, et al., 2015; Knudsen, et al., 2017). IL-6 appears to influence metabolic regulation and it is likely that IL-6 has an integral role within glucose metabolism at rest, during and post-exercise. In addition to enhancing AMPK, IL-6 also influences pyruvate dehydrogenase (PDH) activity (Bienso, et al., 2014; Gudiksen, et al., 2016).

PDH is central in the regulation of substrate utilization, with higher activity leading towards more CHO usage (Mourtzakis, et al., 2006). In mouse models (C57BL/6), injection of IL-6 reduced pyruvate dehydrogenase (PDH) activity within skeletal muscle in a fed state but increased PDH in a fasted state (Bienso, et al., 2014). These findings suggest that IL-6 exerts physiologic influence on glucose metabolism differently when fed or fasted. As such, IL-6 release appears to be part of complex concert of processes responsible for sensing whole body fuel availability. Such as, IL-6 may assist in signaling the hypothalamus, like leptin, influencing whole

body fuel balance (Wueest, et al., 2014). Concomitantly, IL-6 function is associated with altered PDH activity during prolonged exercise. PDH activity is reported to be higher within IL-6 KO mice, suggesting more CHO utilization. Gudiksen, et al., (2016) observed higher skeletal muscle PDH levels within IL-6 skeletal muscle-specific KO mice at rest and at 60 min of exercise compared to littermate floxed controls ($p < 0.05$). Additionally, in a sub study conducted by Gudiksen and colleagues, mice lacking IL-6 ran for a shorter duration (> 10 min, $p < 0.05$) during a graded exercise test to exhaustion. These findings suggest that IL-6 is in some way incorporated in the overall regulation of CHO fuel usage at rest and during exercise, specifically sparing CHO during prolonged exercise resulting in a longer durations. A role for IL-6 in CHO metabolism is not exclusive to skeletal muscle, with evidence suggesting that IL-6 communicates with the liver in an effort to regulate glucose availability. Bertholdt, et al. (2017) utilized skeletal muscle tissue-specific deletion of IL-6 to identify an influence on hepatic CHO metabolism. At rest, IL-6 KO compared to wild type mice had higher PDH activity within the liver. Following 120 min of exercise, hepatic glucose was significantly lower in IL-6 KO mice ($p < 0.05$). Taken together, these data indicate that skeletal muscle IL-6 influences hepatic CHO oxidation at rest and hepatic glucose release during exercise (Bertholdt, et al., 2017). All in all, research involving IL-6 and metabolic regulation provide new insights into the orchestration of metabolic processes between skeletal muscle and other tissues.

Emerging data suggests myokine functions of IL-6 may assist in regulating CHO metabolism at rest as well as immediately following exercise. Influence on AMPK and PDH highlights a role for IL-6 within substrate utilization. Evidence does not indicate that CHO metabolism is dependent on IL-6, rather, IL-6 likely serves as a potentiator of the foundational roles of CHO regulation. IL-6 has been observed to influence insulin activity and improve GLUT4

transport post-exercise, hinting at a protective role in regards to glucose tolerance. Furthermore, numerous studies applying knock-out models have reported adverse metabolic profiles within rodents lacking skeletal muscle IL-6.

Protection against metabolic stress

Skeletal muscle derived IL-6 likely contributes to cytoprotection against metabolic stress by improving glucose tolerance, enhancing insulin sensitivity and preventing pancreatic cell damage. Although chronically elevated plasma IL-6 exist in low grade inflammatory states such as obesity and type 2 diabetes IL-6 however is accompanied by TNF- α as well as IL-1 β in these states. Both TNF- α and IL-1 β are detrimental to one's metabolic profile. IL-1 β is responsible for pancreatic β -cell damage within type 2 diabetes and inhibiting IL-1 β , pancreatic β -cell dysfunction and glucose homeostasis is attenuated (Eguchi, et al., 2013; Ehses, et al., 2007). TNF- α is a prime actor within peripheral insulin resistance (Hotamisligil, et al., 1996; Uysal, et al., 1997). Specifically, TNF- α inhibits the phosphorylation of a vital substrate within the insulin signaling cascade of GLUT4 translocation (Plomgaard, et al., 2005). Exercise induced IL-6 release inhibits both of these detrimental cytokines upon infusion and exercise (Starkie, et al., 2003, Steensberg, et al., 2003). In addition to blocking these damaging cytokines, IL-6 enhances insulin's action post-exercise and at rest.

Insulin sensitivity increases with repeated exercise bouts and is enhance immediately post-exercise (King, et al., 1988; Wojtaszewski, et al., 2000). Carey et al (2006) specifically observed that IL-6 infusion results in insulin stimulated glucose uptake. Furthermore, increased IL-6 levels following exercise also stimulates glucagon-like peptide-1 (GLP-1) secretion from pancreatic β -cells consequently improving insulin secretion (Ellingsgaard, et al., 2011). The influence of IL-6

on insulin not only provides a mechanism to replenish energy stores post-exercise but it may serve as protective mechanism against metabolic damage.

IL-6 prevents α -cell apoptosis as a result of metabolic stress, indicating protection against successive metabolic stressors (Ellingsgaard, et al., 2008). Additionally, IL-6 is reported to stimulate pancreatic α -cell proliferation and regulation (Ellingsgaard, et al., 2008). Impaired IL-6 signaling within hepatocytes leads to liver inflammation as well as insulin insensitivity (Wunderlich, et al., 2010). Upon deletion of the membrane bound IL-6 receptor from hepatocytes, glucose tolerance is reduced due to mitigated insulin-stimulated glucose transport within skeletal muscle and fat (Wunderlich, et al., 2010). Although IL-6 appears to have beneficial outcomes on metabolic homeostasis, over-expression of IL-6 exerts detrimental effects on liver and muscle metabolic function due to hyperinsulinemia and decreased glucose uptake (Franckhauser, et al., 2008). Using an electro-transfer in C57 mice, chronic overexpression of IL-6 resulted in a marked inflammatory state within the liver with reported increases in serum amyloid A, TNF- α and SOCS-3 (suppressor of cytokine signaling) gene expression ($p < 0.05$) (Franckhauser, et al., 2008). IL-6 overexpression also resulted in elevated levels of insulin within beta cells accompanied by reduced glucose production from the liver. Furthermore, skeletal muscle metabolic function was significantly altered as observed by reduced glucose uptake and GLUT-4 content as well as decreases in mitochondrial genes: COX-2, ATP synthase and PGC1- α ($p < 0.05$) (Franckhauser, et al., 2008). In addition, Kubaszek et al., (2003) identified a specific polymorphism within the promoter region of the IL-6 gene in which individuals who possessed it had lower rates of whole-body glucose uptake and exhibited more resistance to insulin administration.

Together, these findings suggest further evidence that IL-6 is influential within CHO metabolism. While overexpression of IL-6 appears to be detrimental, skeletal muscle derived IL-

6 appears to have the ability to provide cytoprotection by battling TNF- α and IL-1 β , enhancing insulin and GLUT-4 action as well as promoting beneficial pancreatic function. In addition to metabolic protection, IL-6 provides protection against ischemic bouts within the myocardium, as seen by lessened necrotic tissue, and decreased markers of autophagy and apoptosis (McGinnis, et al., 2015). Interestingly, the specific biological processes due to IL-6 are likely to be stimulus-dependent in that exercise and ischemic preconditioning evoke different outcomes in terms of indirect markers of autophagic and apoptotic involvement (McGinnis, et al., 2015; Smart, et al., 2006; Dawn, et al., 2004; Matsushita, et al., 2005).

Future horizons

The ubiquitous nature of IL-6 is now well documented in a wealth of diverse research findings from basic science to integrative physiology. Most recently, efforts have led to the discovery of potential cytoprotective effects of skeletal muscle derived myokines. For instance, IL-6 improves insulin action following exercise and likely protects against metabolic damage due to its inhibitory effects on TNF- α and IL-1 β . Aside from findings of IL-6 facilitating protection from metabolic stress, recent evidence reveals potential cardioprotective properties. Different genotypes of IL-6 exist, facilitating a new area of genetic and epigenetic research. With over expression leading to disease states and the transient increases post-exercise exerting metabolic benefits comes the question of whether or not certain individuals are at risk for disease due to more or less expression of IL-6.

IL-6 Genotype Alterations

Polymorphisms within cytokines are hypothesized to influence disease prevalence and progression. Also, among the many exercise protocols reviewed, a majority reported large standard deviations of plasma IL-6 concentrations within subjects, suggesting a responder versus non-responder effect, a polymorphism may contribute to these observations (Nieman, et al., 2016). In an effort to identify genetic predisposition to elevated IL-6 expression, Fishman et al., (1998) identified a polymorphism in the 5' flanking region of the IL-6 gene. The 5' flanking region is vital to the regulation of IL-6 gene expression. Researchers identified a single nucleotide alteration from the G allele to C allele at position -174 (rs1800795). In vivo observations of Fishman and colleagues indicated that the CC genotype resulted in lower IL-6 levels compared with the GC or GG genotype. However, conflicting evidence exists in regards to the effects of these polymorphisms. For instance, stimulation of monocytes with lipopolysaccharide resulted in higher IL-6 production within GC and CC compared to GG genotypes (Patel, et al., 2010). The C allele is associated with skeletal muscle damage following exercise and rhabdomyolysis (Funghetto, et al., 2013; Yamin, et al., 2008). Specifically, Funghetto, et al., (2013) observed higher IL-6 activity from zero to 48 hr post-eccentric exercise within CC and GC genotypes compared to GG. Together, findings suggest that the C allele may promote more cytokine and subsequent inflammatory actions. As mentioned earlier, Kubaszek et al., (2003) identified that subjects expressing the CC genotype display insulin resistance and higher serum glucose concentration. Individuals who expressed this CC genotype also exhibited lower energy expenditure along with higher BMI compared with the G allele. In agreement, a lower walking distance during the six minute walk test in elderly individuals was reported with individuals carry the C allele ($p < 0.05$) (Nicklas, et al., 2005). Although these results indicate polymorphisms in IL-6 promoter sites play

a role in IL-6 levels and ensuing effects, more research is needed to identify if these alterations in genotypes factor into the myokine and cytoprotective properties of IL-6.

Cardioprotection

IL-6 provides cardioprotection due to a regulatory role in ischemic pre-conditioning (McGinnis, et al., 2015; Smart, et al., 2006, Dawn, et al., 2004). An IL-6R gene single nucleotide polymorphism has been linked to the predisposition of atrial fibrillation and elderly individuals with GC genotype have been associated with higher levels of CVD and all-cause mortality (Lin et al., 2014; Bruunsgaard, et al., 2004). IL-6 can be deleterious, however, it plays a distinct role in the cardioprotective benefits of ischemic preconditioning (IPC). IPC is characterized as the process of administering brief ischemic episodes which then increases the heart's ability to withstand ensuing ischemic reperfusion injury (I/R) (Dawn, et al., 2004). Dawn et al. (2004) identified that IL-6 is necessary for beneficial IPC cardioprotection. Investigators identified IPC increases IL-6 expression within cardiomyocytes. Increased expression of IL-6 was reported to be responsible for the activation of the JAK/STAT pathway and the succeeding increase in iNOS (inducible nitric oxide synthase) and COX-2 (cyclooxygenase-2), both of which are mediators of IPC. In IL-6 KO mice, researchers reported JAK/STAT suppression as well as abrogated expression of IPC downstream mediators, iNOS and COX-2. Furthermore, Smart et al. (2006) observed that pre-treatment of IL-6 (10ng/ml) 24 hr prior to ischemic reperfusion injury (I/R) increased iNOS expression and induced a NO-dependent protective mechanism against reperfusion injury. More interesting, upon blocking the PI-3K pathway, both iNOS and the apparent IL-6 dependent protection were negated (Smart, et al., 2006). Data suggests that IL-6 acts through the canonical pathways JAK/STAT and PI-3K in order to provide the cardioprotective benefits of IPC.

Exercise prevents myocardial necrosis following I/R injury, although it is thought to provide protection through different mechanisms than that of IPC. The aforementioned work by McGinnis et al., (2015) served as groundwork in identifying inter-organ cross talk involving myokines and the myocardium. Specifically, an exercise protocol was implemented in an effort to identify if IL-6 produced from skeletal muscle signals the heart during the acute post-exercise period. Knowing the influence of IL-6 on IPC, investigators hypothesized that skeletal muscle derived/exercise induced IL-6 provides protection against I/R injury by signaling the heart. Upon analysis of exercised and non-exercised myocardium, there were no differences in COX-2 and iNOS between C57 wild type and IL-6 KO mice. Reports indicated that exercised wild type mice were protected against I/R injury 24 hr post-exercise as observed by the larger infarct areas and necrosis within exercised IL-6 KO mice ($p = 0.049$). Additionally, cleaved PARP (poly ADP-ribose polymerase), a marker of cellular apoptosis, was lower within ischemic and perfused tissues of exercised wild-type mice compared to exercised IL-6 KO. Therefore, mice lacking IL-6 appeared to suffer increased apoptosis following I/R, which is in agreement with findings from Matusushita, et al., (2005). Autophagy marker, cardiac Atg3, was also altered depending on the presence of IL-6. For instance, ischemic as well as perfused tissue of exercised IL-6 KO mice had higher cardiac Atg3 compared to the sedentary IL-6 KO counterparts, while exercise C57 mice exhibited lower Atg3 levels when exercised. The difference in this autophagy marker further suggests that without IL-6 present, exercise does not provide the same levels of protection from I/R. Furthermore, despite the same COX-2 and iNOS expression within exercised and non-exercised mice, exercise protected against cardiac injury only in the mice that expressed IL-6. IL-6 and its role in protection from I/R was believed to be dependent on COX-2 and iNOS action due to the IPC models of Smart, et al. (2006) and Dawn et al. (2004). However, results from the

McGinnis group shed light on the fact that IL-6 exerts protective effects during exercise precondition through an alternate pathway than that of IPC. IL-6 was suggested to possibly act through MAPK pathways due to the “protected” wild type exercised mice exhibiting larger p44/42 MAPK and p38 MAPK expression compared with exercised IL-6 KO mice. More specifically, the increased MAPK levels were observed in both ischemic and perfused tissues, likely indicating that I/R did not cause the increased signaling, instead exercise preconditioning was the suggested source. Following exercise the Quindry group (McGinnis, et al., 2015) observed transient increases of the membrane bound IL-6 receptor within the myocardium of wild type mice only, indicating that this myokine has the ability to signal the heart in an endocrine fashion. Taking the data from McGinnis et al. (2015), research should investigate the effects of IL-6 using tissue specific KO models targeting myocardial IL-6 receptor in addition to skeletal muscle IL-6. These exciting findings will propel future research into identifying additional cytoprotective capabilities of this myokine. Skeletal muscle derived IL-6 serves as an archetype for understanding skeletal muscle’s endocrine capabilities, which will only continue to progress.

Conclusions

IL-6 exhibits alternate cellular actions when produced as a cytokine versus a myokine. Cytokine processes are mostly involved in inflammatory responses, such as infection and tissue damage following eccentric exercise. When produced directly from skeletal muscle, IL-6 acts in a different manner than when produced from immune cells. Differences in IL-6 action are attributed to IL-6 receptor signaling, location of production and subsequent upstream and downstream secretory pathways, as well as the presence of pro-inflammatory cytokines (TNF- α and IL-1 β).

IL-6 is a myokine with the ability to deter pro-inflammatory cytokines, such as TNF- α and IL-1 β , providing a protective mechanism against disease progression. When in the presence of TNF- α , IL-6 performs inflammatory processes. However, exercise induced increases in IL-6 are not accompanied by TNF- α , rather IL-6 has the capability to inhibit pro-inflammatory cytokines and stimulate anti-inflammatory molecules (Steensberg, et al., 2003). The anti-inflammatory effects of exercise, as seen via IL-6, suggest an acute yet potent mechanism to fight progression of low-grade inflammatory disease states, such as obesity and type 2 diabetes.

IL-6 release from skeletal muscle during exercise is strongly influenced by the energy status of skeletal muscle. IL-6 elevations during endurance exercise are reduced when fed CHO and increased when glycogen stores are diminished (Nehlsen-Cannarella et al., 1997; Nieman et al. 1998; Nieman et al., 2001). In 2001, Starkie et al. published results indicating carbohydrate ingestion mitigated plasma IL-6. As such these findings suggest that when the body is supplied ample CHO, IL-6 is not produced significantly. Furthermore, exercising in a glycogen-depleted state elevates plasma IL-6 (Steensberg et al., 2001, Helge, et al., 2003, Nieman, et al., 2015, Nieman et al., 2016). From this evidence, there appears to be an interrelationship between IL-6 and the energy state of active skeletal muscle although, the precise mechanisms are not fully understood. Collectively, a wealth of research has generated a developing hypothesis that IL-6 production following exercise is dependent on fatigue of skeletal muscle by exercise duration, exercise intensity and ensuing glycogen depletion. Glycogen depletion is at the forefront due to data indicating IL-6 having vital roles within CHO metabolism at rest and during exercise.

A mediatory role within substrate metabolism has been attributed to IL-6. IL-6 exerts its effects on metabolic processes via its influence on AMPK and in turn PDH. Infusion of IL-6 initiates lipolysis and fat oxidation as well as glucose uptake. Action of IL-6 within metabolism

seems to depend on being in a fed or fasted state. Fasting induced free fatty acid mobilization is influenced by IL-6 activity (Wueest, et al., 2014). Meanwhile, IL-6 appears to play a more vital role in CHO metabolism as seen by IL-6-dependent increases of insulin stimulated GLUT4 translocation and glucose uptake at rest and following exercise (Febbraio, et al., 2004; Carey et al., 2006). The influence on insulin activity leads to speculation of IL-6 and energy restoration processes post-exercise. In addition, IL-6 likely provides beneficial mechanisms at rest as well due to its influence on insulin stimulated glucose uptake. Evidence also suggests a protective role for IL-6 in metabolism due to proliferation and protective effects of pancreatic α -cells (Ellingsgaard, et al., 2008).

In addition to inhibiting pancreatic α -cell apoptosis, IL-6 appears to be essential to the cardioprotective effects of a short term exercise regimen. McGinnis, et al., (2015) observed that the protection from ischemic reperfusion injury due to exercise preconditioning was dependent on IL-6. More specifically, this team suggested that skeletal muscle derived IL-6 influenced signaling within the heart in endocrine fashion protecting the myocardium from reperfusion injury. Subsequent research will use findings from McGinnis et al., (2015) in order to identify specific actions within the myocardium and other tissues in which IL-6 may induce protective mechanisms. The dogma surrounding the relationship between inflammation and IL-6 has long vanished with an abundance of research indicating anti-inflammatory effects, regulatory actions in metabolism and cytoprotective properties.

The roles of IL-6 in health and disease are partially understood through compelling recent evidence suggesting a valuable role of IL-6 (and exercise) in combating morbidity. Stated differently, exercise is medicine and the IL-6 response to exercise is one of the many factors that supports this notion. Exercise is a simple, yet effective treatment in mitigating inflammatory,

metabolic and cardiac damage (Pedersen, et al., 2017; Quindry, 2017). Due to the far reaching benefits of exercise participation, many individuals would benefit from protocols tailored toward stimulating IL-6 release from skeletal muscle. Diabetes, heart disease and obesity are three of the most prevalent disease states within the United States (PAGACR, 2008). Interleukin-6 has the capabilities to combat all three of these diseases. IL-6 regulates CHO metabolism and assist in glucose transport into skeletal muscle (Carey et al., 2006). Individuals suffering from diabetes could greatly improve their metabolic profile through exercise and the IL-6 response that come with it, due to the observed increase of insulin sensitivity and GLUT-4 translocation. Also, exercise induced IL-6 suppresses TNF- α and IL-1 β , which not are “inflammatory cornerstones” in many disease states, including conditions provoked by obesity. By combating TNF- α and IL-1 β , interleukin-6 (and exercise) reduces inflammation within numerous diseases. Furthermore, McGinnis, et al., (2015) reported exercised induced cardio protection 24 hr post-exercise, which was dependent on IL-6. Other investigations report that IL-6 also protects against ischemic injuries during IPC trials (Dawn et al., 2004; Smart et al., 2006). Skeletal muscle acts as an endocrine organ with IL-6 being the most investigated effector myokine to date. Future investigations into exercise myokines are needed to better understand the benefits of regular exercise. Next generation experiments will likely result in the eventual application of myokine-related exercise therapy prescriptions.

Personalization of an exercise plan is crucial to establish an effective stimulus for IL-6 release as well as ensure the safety of an individual. Due to a polymorphism within the promoter region of the IL-6 gene which may predispose an individual to a more pronounced response than others, the following guidelines are a general recommendation for any individual aiming to acquire the benefits of exercise and subsequent IL-6 release. Training methods should target the known

stimulus of IL-6 release; fatigue. High exercise intensity, long duration and low CHO availability are all methods of achieving fatigue, with a combination of these factors resulting in the most potent stimulus (Steensberg, et al., 2001; Ostrowski, et al., 1998; Leggate, et al., 2010; Nieman, et al., 2015, 2016). Concentric modalities result in the largest plasma IL-6 concentrations, with running eliciting the most prominent peaks during/post-exercise (Ostrowski, et al., 1998; Fischer, et al., 2006). With a greater number working muscles the larger an IL-6 response will be, which explains why running elicits some of the largest IL-6 concentrations in circulation (Nieman et al., 1998, Starkie, et al., 2000, 2001, Ostrowski, 1998b). However, if running is not appropriate for an individual, one could easily adapt and isolate a specific muscle group. Isolation exercise(s) are an effective strategy due to an increased amount of glycogen depletion, as seen during cycling bouts (Nieman et al., 2016). A more advanced training tool to elicit glycogen depletion and subsequent IL-6 release would be two-a-days (Gollnick, Piehl & Saltin, 1974). For example, during the initial bout an individual could target their glycogen stores (fast glycolytic energy system) by training at a high intensity followed by an additional bout at a relatively high intensity once again in order to achieve a quicker and more pronounced IL-6 release from muscle which were likely depleted of glycogen in the initial bout. Thus, from the perspective of eliciting a rapid IL-6 spike post exercise, one might limit CHO-replenishing practices. To this end, strategic CHO feeding should be evaluation in ongoing research to better understand the influence on IL-6 myokine release (Nehlsen-Cannarella, et al., 1997; Nieman, et al., 1998; Nieman, et al., 2001; Nieman, et al., 2003). In regard to exercise intensity, evidence suggests that IL-6 release is more pronounced to type II fibers. Thus, to the end of promoting IL-6 release, it now appears that exercise protocols that consists of higher intensities due to type II fibers recruitment are most effective (Yamano, et al., 2010; Hiscock, et al., 2004; McGinnis, et al., 2015). Therefore, models utilizing interval training

serve as an effective strategy to evoke an exercise induced IL-6 response. Specifically, high intensity interval training (HIIT) may be a beneficial option due to the increased demand of glycolytic sources and ensuing glycogen depletion and fatigue (Knuiman, et al., 2015). New investigations reported that HIIT elicits an IL-6 release, however responses are heavily influenced by the number of intervals or total duration (Leggate, et al., 2010). The specific concentration of IL-6 protein and therefore the amount of exercise needed for protective systemic effects remain unknown and almost certainly varies among individuals. However, it is important to recall that the cytoprotective benefits of an acute exercise bout are biochemical in nature and, as demonstrated by McGinnis, et al., (2015), extend at least 9 days after their last exercise bout. More studies are needed to identify how long these protective effects last in addition to clarifying the optimal concentrations of circulating IL-6 and amount of exercise to induce metabolic and cardioprotection. Aside from future investigations, engaged readers should advance this rationale being cognizant of the fact that exercise is an accessible, cost effective and widely beneficial strategy to combat various diseases; and that myokines such as IL-6 are just the “tip of the iceberg” in promoting pleiotropic health benefits.

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