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Acute Sprint Interval Exercise Induces a Greater FGF-21 Response in Comparison to Work-Matched Continuous Exercise

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Kinesiology

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ACUTE SPRINT INTERVAL EXERCISE INDUCES A GREATER FGF-21
RESPONSE IN COMPARISON TO WORK-MATCHED CONTINUOUS EXERCISE

(Thesis format: Monograph)

by

Blair Mackay Segsworth

Graduate Program in Kinesiology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

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Abstract

Sprint interval training (SIT) has been associated with substantial reductions in body fat. Recent evidence suggests that myokines (small protein compounds produced in muscle) may promote the fat loss with SIT. The purpose of this project was to compare the plasma accumulation of three myokines (IL-15, Irisin and FGF-21) with sprint interval exercise (SIE) vs work-matched continuous exercise (CE). Nine male subjects completed an acute SIE session consisting of four-30 second sprints and a work-matched CE session. Both exercise sessions were completed on an electromagnetically braked cycle ergometer. Blood samples were collected before and at 5, 30, 90, and 180 min post exercise to determine any changes in plasma myokine concentration. Plasma FGF-21 was increased at five ($P=0.04$) and 30 ($P<0.001$) min with SIE vs baseline and was increased at 30 min ($P=0.03$) when compared to CE. Neither IL-15 nor Irisin were altered significantly although there were some methodology concerns and intersubject variability was substantial so a Type II Error might have occurred. These findings suggest that exercise intensity is a key determinant of plasma FGF-21 accumulation and that FGF-21 may serve as a surrogate measure for both sympathetic activation and exercise-induced lipolysis.

Keywords

Irisin, Fibroblast Growth Factor-21, Interleukin-15, Myokines, fat loss

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List of Abbreviations

FGF-21	Fibroblast Growth Factor-21
PPAR- α	Peroxisome Proliferator-Activated Receptor Alpha
FFA	Free Fatty Acids
mRNA	Messenger Ribonucleic Acid
CI	Continuous Interval
SIE	Sprint Interval Exercise
TFAM	Transcription Factor Alpha, Mitochondrial
AMP	Adenosine Monophosphate
UCP-1	Uncoupling Protein 1
UCP-3	Uncoupling Protein 3
IGF-1	Insulin Growth Factor 1
GLP-1	Glucagon-Like Protein 1
ADP	Adenosine Diphosphate
SIT	Sprint Interval Training
AMPK	AMP Activated Protein Kinase
P38MAPK	P38 Mitogen-Activated Protein Kinase
SIRT-1	Sirtuin 1
PGC-1 α	Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha
FNDC5	Fibronectin Type III Domain-Containing Protein 5

IL-6	Interleukin 6
GLUT-4	Glucose Transporter 4
NRF-1	Nuclear Respiratory Factor 1
BDNF	Brain Derived Neurotrophic Factor
ERK	Extracellular Signal Regulator Kinase
PCr	Phosphocreatine
ATP	Adenosine Triphosphate

1 Introduction

The current state of global health is at a crossroads between improvements in longevity and infant survival and the looming crisis associated with the rising rates of obesity. At this juncture, about 937 million people worldwide are considered to be overweight and ~396 million considered obese. Further, these numbers are projected to grow so that by the year 2030, it is expected that a staggering 1.12 billion people will be obese (Kelly et al., 2008). Many pharmacological interventions have been developed to battle this issue and its comorbidities such as hypertension, dyslipidemia and diabetes; however, it may be that the universal remedy to the obesity epidemic could simply involve consistent physical activity. For example, regular exercise has been shown previously to prevent excessive mass gain and even promote fat losses (up to 386 cubic centimetres of adipose tissue) in obese adults over an 8-wk period (Keating et al., 2015). Unfortunately, regardless of these benefits, many people are unable to maintain physically active lifestyles, citing the lack of time as a major reason for ineffective quantities of exercise (Stutts, 2002). A possible solution to this issue is the use of sprint interval training (SIT) which results in significant fat loss and very brief time commitments (Hazell et al., 2014; MacPherson et al., 2010).

Typically, sprint interval exercise (SIE) consists of four-six bouts of “all-out” intensity sprinting with each bout of sprints separated by a four min rest interval, often performed on a cycle ergometer (Burgomaster et al., 2005) but any exercise mode appears to work. As little as three min of “all-out” sprinting per week has been shown to increase activity of oxidative enzymes such as citrate synthase, cytochrome oxidase IV and β -hydroxy acyl CoA dehydrogenase as well as decreasing average 24-h blood glucose (Gillen et al., 2014). These trends in glucose homeostasis also apply to clinical populations as similar exercise modalities have caused reduced time spent with hyperglycemia both at rest and post-prandial (Gillen et al., 2012). Further, regular SIE can cause significant

improvements in body composition causing a decrease in fat mass and waist circumference by 8% and 3.5%, respectively, while increasing lean mass by 1.5% over a 6 wk training period (Hazell et al., 2014). In terms of oxygen consumption, just two min of SIE induces similar 24-h oxygen consumption as 30 min of continuous aerobic exercise (CE), despite SIE requiring 150% less in VO_2 load during the actual exercise (Hazell et al., 2012). These findings indicate a significant contribution to 24-h oxygen consumption due to an anaerobic exercise component and/or a residual change in post exercise metabolism. This resulting prolonged change in metabolism (increased baseline energy expenditure and/or elevated fat catabolism) may explain some of the fat losses often observed with SIT.

When compared to moderate intensity CE training, meta-analyses demonstrate that SIT produces similar 8% increases in $\text{VO}_{2\text{max}}$ (Gist et al., 2013) due primarily to an increase in mitochondrial density and the upregulation of oxidative enzymes such as cytochrome oxidase IV and β -hydroxy acyl CoA dehydrogenase (Sloth et al., 2013). Interestingly, SIT promotes similar changes in exercise capacity vs CE with exercise volumes of $\sim 10\%$ and total exercise time of $\sim 21\%$ (Gibala et al., 2006). Moreover and importantly, interval training, even intense SIT has also been associated with higher ratings of perceived pleasure when compared to CE (Bartlett et al., 2011).

Many of the peripheral adaptations to SIT are due to increased mitochondrial content that occurs along with improved oxidative capacity (Jacobs et al., 2013, Scalzo et al., 2014). On a molecular level, it is thought that the increased mitochondrial biogenesis is caused by the phosphorylation of p38 MAPK and AMPK which promotes an increase in PGC-1 α mRNA content (Gibala et al., 2008). This connection is critical as PGC-1 α is known to be the master regulator of mitochondrial biogenesis as well as many of the oxidative genes linked to mitochondrial function (Wu et al., 1999). Although there is an established link between SIT and the induction of oxidative adaptations, the precise mechanism regulating these changes has not been elucidated fully. However, the

biochemical signaling process responsible for the adaptations appears to be more powerful because these metabolic changes are induced by substantially less total work volume and time.

A new theoretical model describing the function of muscle has also been described concurrently with the development of SIE as an effective exercise mode. Like adipose tissue, skeletal muscle is now known to act much like an endocrine organ, capable of secreting several hormonal signals to communicate with a number of other body tissues (Ilzuka et al., 2014, Pedersen and Febbraio, 2013). Rather than communicating via fat-soluble hormones, skeletal muscle utilizes contraction-induced cytokines to implement functional changes throughout the body (Pedersen and Febbraio, 2013). These have become known as myokines because the tissue of origin for these cytokines is muscle and, as of 2014, there are at least 69 identified myokines within the secretome of muscle (Catoire et al., 2014). One of the most well characterized myokines is IL-6, traditionally a pro-inflammatory cytokine, it is increased chronically in the obese (Pal et al., 2014). IL-6 is also elevated acutely following exercise and improves insulin and glucose sensitivity through the up-regulation of glucagon-like peptide-1 (Pal et al., 2014). Although much effort has been directed to the study of IL-6, a new subgroup of myokines has been identified as perhaps playing a role in how exercise promotes fat loss.

Irisin, Fibroblast Growth Factor-21 (FGF-21) and Interleukin-15 (IL-15) are three novel myokines that have been implicated with fat metabolism, oxidation and phenotypical changes. Specifically, Irisin promotes a brown/beige adipose phenotype, in which an induction of gene related mitochondrial biogenesis (PGC-1 α , UCP-1, GLUT-4) has been reported (Vaughn et al., 2014, Huh et al., 2014). Further, in conjunction with FGF-21, Irisin increases non-shivering thermogenesis in beige/brown adipose via uncoupling of the electron transport chain (Lee et al., 2014). Interestingly, non-shivering thermogenesis induction by Irisin and FGF-21 are similar between both cold environmental conditions and exercise, with greater plasma concentrations of the induced myokines found

post-exercise (Lee et al., 2014). With respect to phenotype, beige/brown adipose is advantageous because 60 g of brown adipose is capable of metabolizing ~4 kg of white adipose over the course of a year (Virtanen et al., 2009). Moreover, FGF-21 may also provide a substantial body fat reduction benefit in that it is capable of increasing glucose uptake (Kharitonov et al., 2005), the deacetylation and activation of PGC-1 α (Fu et al., 2009), as well as help coordinate the metabolic response to exercise-induced lipolysis (Lee et al., 2013, Cuevas-Ramos et al., 2012).

Traditionally, IL-15 has been viewed as an anabolic myokine, capable of inducing the accretion of myosin heavy chain proteins within human muscle cells (Furmanczyk and Quinn, 2003) as well as assisting in lipid oxidation and storage. The treatment of adipocytes with IL-15 can reduce lipid deposition by 50% (Quinn et al., 2005), enhance the expression of PGC-1 α in muscle cells, and increase total mitochondrial volume (O'Connell and Pistilli, 2015). Interestingly, mice over-expressing IL-15 have been shown to have lower resting RER, up-regulation of PGC-1 α , and increased oxidative muscle fibre content (Quinn et al., 2013). Considering the role of these myokines in producing a more oxidative (β -oxidation) phenotype, it is imperative to discern their role and induction post-exercise.

Recently, Irisin, FGF-21 and IL-15 have been studied with some types of exercise. For example, Irisin with both resistance (strength) and aerobic (endurance) exercise paradigms and appears to respond to intensity of exercise in a dose-dependent manner (Huh et al., 2012, Huh et al., 2014). Apparently acute sprint exercise causes Irisin to accumulate at a concentration 15% greater than an endurance bout (Huh et al., 2014); however, in this study the exercise conditions were not matched for total work output so these data may be confounded. FGF-21 has been reported to increase one h after an acute bout of modest aerobic exercise (50% of VO_{2max}), yet the effect of acute sprinting on the secretion of FGF-21 has not been researched extensively (Kim et al., 2013). In this regard, FGF-21 has only been studied in a SIT regimen consisting of nine

sessions of four-eight 30s sprinting bouts on a cycle ergometer (Scalzo et al., 2014). Similarly, the relationship between exercise and the induction of IL-15 in humans has not been well documented but significant increases in circulating IL-15 after with moderate intensity (50-70% of VO_{2max}) cycling or running have been reported (Christiansen et al., 2013, Tamura et al., 2011). There have been no studies profiling the acute effect of SIE on the secretion of IL-15.

Therefore, the aim of this thesis was to conduct a work-matched comparison (per-joule basis) of Irisin, FGF-21 and IL-15 between acute SIE and CE to determine which exercise modality induces the greatest myokine response. In essence this will determine the role of exercise intensity- defined as a percentage of VO_{2max} - in the accumulation of plasma myokines. It was hypothesized that SIE would promote the greatest increase in plasma Irisin, FGF-21 and IL-15 and thereby perhaps be the mechanistic link between SIE and its noted ability to stimulate both oxidative adjustments to exercise and the resulting positive changes in body composition.

2 Literature Review

2.1 Sprint Interval Exercise in Humans

Typically, sprint interval exercise (SIE) is defined as four-six, 30 sec “all-out” sprints separated by a four min recovery interval that may be either active or passive (Burgomaster et al., 2005). These sprints are often repeat Wingate tests, i.e., 30 sec sprints on a cycle ergometer at a resistance of eight-10% of body mass (Burgomaster et al., 2005, Hazell et al., 2012). Two min of SIE has been reported to induce a similar oxygen consumption (VO_2) over a 24-h period as a 30-min continuous, moderate intensity endurance bout of exercise (CE) (Hazell et al., 2012). In the aforementioned study, it is interesting to note that, although the oxygen consumption was significantly less during the SIE session, the 24-h consumption was not different vs the 30-min endurance exercise, indicating that SIE may cause biochemical or physiological changes that induce an extended increased oxygen consumption post exercise. Further, in both men and women, SIE has been shown to result in substantial body fat loss despite very little training time. In women, SIT on a self-propelled treadmill three times per wk for six wk reduced fat mass by 8%, waist circumference by 3.5% and also increased maximal oxygen consumption by 8.7% (Hazell et al., 2014). During this training, it is noteworthy that the participants did not appear to change their diet significantly. In men, a similar training protocol involving a progressive increase in sprints (from four to six over six wk) induced a 3kg loss of body fat (MacPherson et al., 2010). Further, in a subsequent study, acute SIE had little effect on energy intake despite increased appetite and energy expenditure (Beaulieu et al., 2014). These observations indicate that changes in food intake are not the main factor responsible for the improved body composition.

Another study with untrained, middle aged men who underwent SIT, consisting of four-six bouts of 30 sec all-out sprints for a total of just six training sessions observed an increase in VO_2 peak of 6%, as well as an increase in muscle glucose uptake (Eskelinen et al., 2015). Interestingly, the effect of increased glucose uptake was isolated to the quadriceps femoris muscles. When compared

to other muscle groups the changes in glucose and fatty acid uptake during exercise was only present within the quadriceps, indicating a local effect of the SIT perhaps induced by a form of autocrine/paracrine signaling. All four muscles of the quadriceps responded to SIT, while in comparison, only the vastus lateralis and vastus medialis responded to moderate intensity exercise (Eskelinen et al., 2015).

Metabolically, acute SIE has resulted in increased phosphorylation of AMPK and p38MAPK immediately after four bouts of 30-sec sprints (Gibala et al., 2008). In the same study there was also a two-fold up-regulation of PGC-1 α mRNA at three h into recovery. Since SIE was capable of inducing an increase in PGC-1 α and the aforementioned regulators of oxidative metabolism, the authors concluded that SIE was also capable of increasing mitochondrial content. Therefore these findings contribute further to the idea that SIE is capable of inducing similar increases in mitochondrial content and metabolic remodeling to that caused by traditional endurance training. To support these findings, electron transport chain protein cytochrome c oxidase, a marker for mitochondrial content, has been shown to increase with SIT (Gibala et al, 2006). Another study using 60 sec intervals have found increases in cytochrome c oxidase content, a concurrent 7.9% increase in VO_{2max} , and a 5.1% increase in mean power output (Jacobs et al., 2013). In a similar study, Perry et al. (2008) observed an 18% increase in cytochrome c, increases in B-hydroxyacyl-CoA dehydrogenase, and GLUT4 content within muscle after the completion of a high intensity interval training regime consisting of 10 x four min intervals at 90% VO_{2max} , three times per wk, for six wk. Further, during 60% VO_2 peak exercise there was a 60% increase in fat oxidation after six wk of training when compared to pre-training values at the same intensity. These findings indicate that high-intensity spectrum training such as SIT may induce long-term changes in the efficacy of energy substrate utilization during exercise.

In a meta-analysis of the cardiovascular adaptations to SIT, Gist et al. (2013) found that SIT and moderate-intensity continuous interval training were similar in

their relative ability to increase VO_{2max} . However, there was a 20% reduction in the time required to accomplish these endpoints with SIT. These findings are supported by another meta-analysis, which found that VO_{2max} increases in a range of four-13% with SIT over two-eight wk (Sloth et al., 2013). It was also shown that there are similar changes in exercise performance, glycemic control and insulin sensitivity with SIT when compared to more traditional high-volume training regimes (Sloth et al., 2013). These findings suggest that there may be separate biochemical pathways or an amplification of factors that are inherent within SIT, making it more efficient at inducing the benefits associated with exercise.

The effect of SIT on the secretion of plasma cytokines/myokines is also important. As outlined above, SIT has been observed to decrease the resting plasma concentrations of FGF-21, after nine sessions of four-eight, 30 sec bouts of all out sprints (Scalzo et al., 2014). Under the same training program, Irisin has a sexually dimorphic response, increasing in women after SIT and decreasing in men (Scalzo et al., 2014), while SIE increases plasma Irisin acutely (Huh et al., 2014). Outside of the aforementioned myokines, SIE has also been reported to have an effect on a wide variety of plasma inflammatory markers. Meckel et al. (2011) showed that SIE increases both pro- and anti-inflammatory factors such as IL-1 and IL-6 immediately after a series of successive sprints of 100-400 metres, while IL-6 remained elevated one h into recovery. These data suggest that along with other cyto/myokines, there may be a global response to sprint exercise, rather than the production of a single subset of secreted myokines. On the other hand, SIT has also been shown to have no effect on resting plasma myokines IL-6, IL-10 and C-reactive protein after 2 wk of thrice weekly bouts of four-six 30s sprints (Hovanloo et al., 2013). It is difficult to determine the long term effects of SIT on plasma protein concentrations because there are such divergent results in the effect of SIT and plasma inflammation markers. Although there are a limited number of studies presented focusing on the effect of SIE on myokine concentrations, it is apparent that there is a gap within the literature and this must be addressed in the context of both SIE and SIT.

In conclusion, SIE is a powerful exercise modality that is capable of inducing similar benefits as traditional endurance exercise with far less total energy and time commitment. It appears that SIE mediates these functions through the up-regulation of mitochondrial transcription factors as well as supporting mitochondrial biogenesis and growth. However, it is unclear by which mechanisms that these processes occur. The effect of SIE on plasma inflammatory markers and myokines has not been studied systematically in the literature and thus requires attention.

2.2 Irisin

In the groundbreaking study first describing Irisin, Boström et al. (2012) found that a product of the proteolytic cleavage of the skeletal muscle integral membrane protein fibronectin type 3 domain containing 5 (FNDC5) was secreted into the bloodstream during exercise. Given the name Irisin for the Greek messenger goddess Iris, this myokine circulates as a 112-amino acid protein (Wrann et al., 2013). The exact process by which FNDC5 is cleaved and released into the bloodstream remains unclear, but it is postulated to be similar in fashion to other transmembrane polypeptide hormones such as Transforming Growth Factor- α (Boström et al., 2012). Previous biochemical studies have shown that while Irisin is a 16 stranded β -sheet dimer of two cleaved FNDC5 proteins for intercellular signaling, there is also a dimerization of FNDC5 endodomains for intracellular cell signaling (Schumacher et al, 2013). This leads to the possibility that activation of the Irisin receptor can induce both endocrine functions within a whole organism and autocrine functions on skeletal muscle. Irisin is thought to mediate its activity through the master regulator of mitochondrial biogenesis, PGC-1 α , and morphogenic changes due to Irisin are believed to be mediated by changes in ERK and p38 MAPK regulation (Zhang et al., 2014).

2.2.1 Cell Studies

Irisin is believed to be sourced directly from skeletal muscle cells, contributing to 72% of all circulating Irisin (Boström et al., 2012; Roca-Rivada et al., 2013). However, this assumption may be subject to change. Using immunohistochemistry to stain for Irisin within different cell cultures taken from human cadaveric tissue, Aydin et al. (2014), found that the main source of Irisin is not from skeletal muscle, but rather from the nerve sheaths that spread within muscle tissue with smaller other contributions derived from the perimysium and epimysium. Among the most responsive human tissues were the testis, pancreas, liver, spleen, brain, stomach and cardiac tissues. By following the staining patterns of Irisin and their tissue relationships, Aydin et al. (2014) concluded that Irisin may play an important role in hypothalamic-pituitary-gonadal axis.

Further, Irisin has been shown to be produced and secreted into cell media by both human and C2C12 myocytes at rest, yet electrical pulse stimulation has been shown to cause no significant increase in FNDC5 gene expression (Raschke et al., 2013). Considering that Irisin has been shown to be related inversely to PCr and ATP concentrations within muscle tissue (Huh et al., 2012) and its downstream effects can be nullified by AMPK inhibitor, Compound C (Huh et al., 2014), it may be that the secretion of Irisin is dependent on the accretion of exercise-induced metabolites, rather than by simple muscular contraction.

Tissues treated with Irisin have shown a wide range of effects. Human muscle cells taken from biopsies of obese individuals treated with Irisin produced increased IGF-1 and decreased myostatin mRNA expression (Huh et al., 2014). In adipose cells derived from the same human donors, both physiological and below-normal concentrations of Irisin inhibited lipid accumulation and significantly inhibited fatty acid synthase. Moreover, after eight days of Irisin treatment, mRNA of genes for thermogenic programs such as cell death-inducing DFFA-like effector A (CIDEA) and uncoupling protein 1 (UCP-1) increased, as well as markers of mitochondrial biogenesis, PGC-1 α and TFAM (Huh et al., 2014). In

the same study, adipose triglyceride lipase was also up regulated, indicating that with the increased expression of UCP1, there is also an increased use of fat substrate for oxidation and heat production. As a final measure, Irisin treatment inhibited adipocyte differentiation and maturation in human and mouse pre-adipocytes.

As suggested by biochemical characteristics, it has been shown through in-vitro studies that Irisin exerts an autocrine effect on skeletal muscle. For example, C2C12 myocytes treated with Irisin have an increase in expression of genes related to mitochondrial biogenesis including PGC-1 α , NRF-1, TFAM, GLUT4, UCP3 and Irisin (Vaughan et al., 2014). The noted increase in Irisin gene expression suggests that circulating Irisin exerts an autocrine, positive feedback loop back on skeletal muscle. The Irisin-treated myocytes also had a greater amount of global mitochondrial organelle content and expressed a greater total carbohydrate oxidation and decreased lactate production (Vaughan et al., 2014). Combined with its positive feedback loop and increased mitochondrial biogenesis, it appears that Irisin exerts a shift to an oxidative phenotype within skeletal muscle and adipose tissue. It is important that the secreted portion of Irisin is also highly conserved within all mammalian species; mouse and human Irisin are 100% identical (Bostrom et al., 2012). Recently, it has been shown that both shivering and exercise cause a similar secretion of Irisin, although marginally greater plasma concentrations are achieved through exercise (Lee et al., 2014). Combined with another myokine, FGF21; Irisin induces the browning of adipose as well as non-shivering thermogenesis in response to lower body temperature in human subjects (Lee et al., 2014). From these experimental data and the conservation of Irisin structure through mammals, it is believed that Irisin is a remnant of evolutionary homology to cold changes within the environment.

2.2.2 Animal Studies

In mice, submaximal treadmill exercise to volitional fatigue induces a two fold increase in plasma Irisin concentration, while free running and control mice showed no increase in plasma Irisin (Brenmoehl et al., 2014). There were also higher concentrations of Irisin within homogenates of femoral muscles, when compared to those of crus (forelimb) muscles in mice. Irisin was also not correlated with the running distance of either the voluntary free-wheel running or the treadmill test, suggesting the determinant of the Irisin response may be exercise intensity not duration (Brenmoehl et al., 2014). Through immunohistochemical staining, it was also shown that Irisin was localized around muscle cell membranes and intercellular spaces. This corresponds with the findings of Aydin et al. (2014) who suggested Irisin might be a product of the nerve sheaths permeating through muscle tissue with smaller contributions also originating from the epimysium and perimysium. Of the various muscle types, slow/oxidative fibres secrete ~40% more Irisin than glycolytic fibres (Roca-Rivada et al., 2013). Further, in a separate study, three wk of endurance training reduced the concentration of Irisin in plasma, suggesting a role of muscle and exercise training in the down-regulation of Irisin signaling (Roca-Rivada et al., 2013).

Animal studies have also shown that adipose tissue may play a role in Irisin homeostasis. Roca-Rivada et al. (2013), suggested that a secreted form of FNDC5 in rats is also produced by adipose tissues and that obese rats over-secrete this protein in adipose. These findings may illustrate a feedback mechanism between muscle and adipose for Irisin and further, that there may be an Irisin resistance during obesity and other metabolic disorders. Comparing adipose types, subcutaneous adipose secretes 40% more FNDC5/Irisin compared to visceral fat. Contrary to these findings, Roberts et al. (2013) found that circulating Irisin tended to be lower in obese/diabetic prone Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Triceps brachii biopsies showed that FNDC5 and PGC-1 α mRNA were found to be 50% and 40% less in OLETF rats, respectively and were correlated with body composition. Total body fat and

plasma Leptin concentrations are associated positively with greater skeletal muscle FNDC5 mRNA expression. It is speculated that Leptin may be a key in the cross talk between muscle and fat because Leptin increases PGC-1 α through AMPK mediated signaling. Therefore, Irisin may serve as a compensatory mechanism to stimulate thermogenesis and increase fat loss in the obese state (Roberts et al., 2013).

A unique and interesting role of Irisin may be located in the central nervous system. Wrann et al. (2013) have proposed the link between exercise and brain BDNF production to be Irisin and the FNDC5 receptor. Via endurance exercise, activation of FNDC5 within the hippocampus of mice was observed to cause an increase in BDNF mRNA. It is believed that the effects of FNDC5 on BDNF transcription is mediated through a complex formed by PGC-1 α and Estrogen Related Receptor- α (ERR α) as both PGC-1 α and ERR α knockout mice had dramatically reduced FNDC5 activity (Wrann et al., 2013). Hippocampal expression of BDNF was also shown to have a negative feedback effect on FNDC5 activity within the brain, indicating a homeostatic loop between FNDC5 and BDNF. The most significant finding from Wrann et al. (2013), is that the peripheral delivery of FNDC5 to the liver via adenoviral vectors caused an increase in the central expression of BDNF. This is a clear indication that a secreted factor influences the central activity of FNDC5 and implies a communication axis between exercising muscle, the liver and the brain; however, it has yet to be determined if the main signaling molecule is Irisin or another cleavage product from muscle FNDC5 (Wrann et al., 2013).

As promising as the animal studies surrounding Irisin may be, it is often difficult to translate research from rodent studies to humans. Raschke et al. (2013) outlined the fact that there is a mutation in a start codon of the FNDC5 gene between rodents and humans. This mutated form of FNDC5 has low translation efficiency in humans and only resulted in 1% of full length FNDC5 protein, when compared to rodents. Raschke et al. (2013) also found that FNDC5 gene expression was not increased during in-vitro electrical pulse stimulation of

primary skeletal muscle cells. As previously stated, these data must be taken with some skepticism because electrical pulse stimulation is far from exercise and often does not result in the accretion of exercise metabolites. Many of these metabolites such as AMP and the subsequent activation of AMPK are implicated in the activation of FNDC5 gene pathways and secretion of Irisin into the bloodstream.

2.2.3 Human Studies

Many studies have profiled the existence of Irisin and its association with exercise. While studying the physiological variation in plasma Irisin, Anastasilakis et al. (2014), found that a diurnal rhythm of Irisin exists, with maximum plasma concentrations being reached at 2100h and minimums at 600h. Interestingly, this day-night rhythm is opposite of FGF-21 (Anastasilakis et al., 2014). It was suggested that the inverse relationship between plasma Irisin and FGF-21 concentrations may be due to temperature, where Irisin plays a role in the formation of brown adipose tissue (Vaughn et al., 2014) and FGF-21 then activates that tissue for heat production during lower overnight temperatures as outlined by Anastasilakis et al. (2014) and Lee et al. (2014).

Huh et al. (2012), using biceps circumference as a gauge, showed that muscle mass was the single greatest indicator of circulating plasma Irisin. Plasma Irisin is correlated positively with BMI and negatively correlated with age, insulin, and cholesterol within the blood. Subjects who had undergone bariatric surgery were observed to have a reduction in both total and lean body mass which coincided with a subsequent decrease in resting plasma Irisin when compared to pre-surgery (Huh et al., 2012). It was also shown that a 7% reduction in body mass due to a hypocaloric diet also caused a reduction in plasma Irisin (De La Iglesia et al., 2014), suggesting a relationship between Irisin production and skeletal muscle. Young, male, athletes were found to have the highest resting plasma concentrations of Irisin (Huh et al., 2012); however, when adjusted for lean body mass, females were found have even greater resting Irisin concentrations (Anastasilakis et al., 2014). In a separate study involving a population of 17 post-

menopausal women, Swick, Orena, & O'Connor (2013) found that the subgroup of women who had daily energy expenditures greater than the predicted values of energy expenditure·kg fat free mass⁻¹ also had the greatest concentrations of resting plasma Irisin. From this information it was hypothesized that the increased energy expenditure may be due at least partially to the browning effect of Irisin on adipose tissues.

With respect to Irisin, the effect of exercise and exercise intensity has been studied but remains controversial. Acutely, Irisin has been shown to increase within the blood in response to endurance exercise by about 20% (Anastasilakis et al., 2014). Sprint interval exercise has also been shown to induce an increase in plasma Irisin (Huh et al., 2012). For example, three sets of two, 80 metre sprints with 20 min of rest between sets induced an 18% increase in plasma Irisin, 30 min after exercise. In the same study, the increase in Irisin was correlated with the decrease in ATP and PCr, while having no relationship with ADP or pyruvate. The same sprint protocol was conducted three times per wk over an eight wk period and at the conclusion of the training the acute induction of Irisin immediately after the sprints Irisin was no longer significant. Interestingly, there was also no acute decrease in ATP, suggesting that Irisin may respond to ATP depletion and that the intensity of the sprint exercise was no longer great enough to induce an Irisin response (Huh et al., 2012). Plasma Irisin has also been observed to be elevated after acute static exercise performed on vibration platforms in untrained women (Huh et al., 2014). However, contrary to their previous findings, Huh et al. (2014) found that the acute Irisin response to exercise was maintained after nine wk of vibration training suggesting that vibration may be different than exercise per se.

Huh et al. (2014) further described Irisin as being present in lower concentrations in both physically active and older individuals at rest, when compared to sedentary or young individuals. However, acute increases in plasma Irisin immediately after exercise were unrelated to fitness or age. In a comparison between five, 50 metre swimming sprints and a single 2,000 metre endurance

swim, Huh et al. (2014), found that Irisin was elevated in plasma by 30% immediately after and by 15% one h after the sprint condition, while the endurance group showed no significant change. As stated earlier, there was no interaction between fitness, age and the increase in plasma Irisin, thus the authors concluded that, regardless of baseline plasma Irisin, the response to acute exercise is similar (Huh et al., 2014). Moreover, similar plasma Irisin concentrations have been found with VO_{2max} treadmill tests (34% increase in plasma Irisin) as with 10 min at 70% VO_{2max} or 10 min at an absolute (75W) workloads (Daskaloupoulou et al., 2014). Using 90 min of continuous treadmill running, Kraemer et al. (2014) reported that plasma Irisin was only significantly different from rest at 54 min of exercise rather than at the completion of the 90 min exercise bout. These findings indicate that the Irisin response of muscle may only be limited to a small window of activity after the initial onset of exercise. Lastly, Tsuchiya et al. (2014) showed that 20 min of high intensity exercise at 80% VO_{2max} produced plasma Irisin concentrations greater than pre-exercise values at six and 19h after exercise by 18% and 23%, respectively. In contrast, 40 min of low intensity exercise (40% VO_2 max) caused no increase from pre-exercise values. Further, low intensity exercise produced a decrease in plasma Irisin. The findings of Tsuchiya et al., (2014) suggest that the Irisin response from muscle may, although unlikely, be delayed in nature because there was no Irisin response until six hours post-exercise.

The Irisin response to both endurance and sprint training is not nearly as clear as the acute effect of exercise. In the original paper outlining the existence of Irisin, Boström et al. (2012) found that endurance training (10 wk of four-five sessions of 20-35 min cycling at 65% VO_{2max} per wk) promoted a twofold increase in plasma Irisin concentrations in humans at rest. SIT has been shown to decrease resting plasma Irisin in males, while increasing resting plasma Irisin in females Scalzo et al. (2014). Scalzo et al. (2014) also determined that the secretion of Irisin into the plasma is unaffected by the sympathetic stimulation associated with exercise as there were no changes in plasma Irisin due to either inhibition by

clonidine (a central acting adrenergic agonist), or by hypoxia- induced sympathetic activation.

In terms of resistance exercise, 12 wk of progressive resistance training in untrained women had no effect on FNDC5 expression or serum Irisin (Ellefsen et al., 2014). Further, it was found that FNDC5 expression was closely correlated to the proportion of aerobic muscle fibres pre-training, but this correlation disappeared post-training. In the untrained state, Irisin appeared to be correlated to both lean body mass and fat mass, yet in the trained state, was only related to fat mass (Ellefsen et al., 2014). These findings suggest that there may be a change in Irisin regulation within muscle due to strength training.

There is a great deal of controversy surrounding the existence of Irisin and its production as a result of exercise. Pekkala et al. (2013), have shown that there is an inconsistent Irisin response to resistance and low intensity endurance training. No differences in FNDC5 expression in muscle were found after 21 wk of aerobic or aerobic/resistance combined training; however, an acute high-intensity resistance bout increased PGC-1 α mRNA expression four-fold in older men, while increasing FNDC5 mRNA expression 1.4 times in young men, reinforcing the notion that the function of FNDC5 and Irisin, if any, may only be acute in nature. Hecksteden et al. (2013) showed similar results indicating that there were no changes in resting Irisin after a 26 wk combined resistance and aerobic training program. Further, they also noted that there was an effect of storage time for Irisin, where longer time in freeze storage resulted in reduced Irisin concentrations as determined by ELISA. Hecksteden et al. (2013) found that at -20°C, there was a degradation slope that was calculated to be 0.184 ng·ml⁻¹·day⁻¹. Using only gene chip analysis, (Timmons, Baar, Davidsen, & Atherton, 2012) showed that out of 200 subjects, an increase in FNDC5 expression was only evident in a small subgroup of highly active elderly subjects. After undergoing aerobic or resistance training protocols, there was no increase in FNDC5 expression, while an increase in PGC-1 α was consistent throughout all groups. These data allowed Timmons et al. (2012) to conclude that FNDC5 and Irisin are

perhaps non-existent in humans or, as claimed by (Raschke et al., 2013), a pseudo gene. In a rebuttal published in *Nature*, (Boström et al., 2012) responded to these issues, stating that gene chip analyses are not a robust quantitative measure and are more qualitative in design, limiting any conclusions based on the change in FNDC5 transcription. It must also be noted that the subjects for Timmons et al. (2012), had undergone endurance exercise training and the acute secretion of Irisin was not evaluated. From many of the studies reviewed, it appears that FNDC5 activation and the subsequent release of Irisin is acute in nature and that it may be the chronic exposure to the pulsatile release of Irisin during exercise that is the true mediator of any beneficial effects.

Regardless of what controversy that may exist, it is safe to conclude that Irisin is indeed a myokine that is induced during exercise. It is likely that any secretion of Irisin is dependent on total muscle mass, but presents a dichotomy between men and women, where females appear to have greater resting concentrations and increase plasma Irisin as a response to exercise training. Irisin appears to be responsible for morphogenic changes within adipose tissue, being capable of inducing a brown/beige adipose phenotype. It is likely that the secretion of Irisin is dependent on the intensity of exercise as displayed by the studies featuring SIE; however, this concept requires further attention.

2.3 Fibroblast Growth Factor-21

Discovered by Nishimura et al. (2000), Fibroblast Growth Factor-21 (FGF-21) was identified as a 210 amino acid protein and found to be expressed within the liver. The amino acid sequence of FGF-21 is almost entirely identical between mice and humans and relative to the other Fibroblast Growth Factors, is most similar to FGF-19 in structure. FGF-21 responds to starvation and stress within the liver. Unlike most FGF family members that have autocrine or paracrine functions requiring heparin sulfate binding, FGF-21 is capable of exerting endocrine effects and is dependent on β -Klotho for receptor binding (Bae, Kim, & Park, 2014). Interestingly, β -Klotho is almost exclusively expressed within the liver, adipose tissue and pancreas, indicating that the endocrine target tissues of FGF-21 are most likely to be the pancreas and adipose tissue.

2.3.1 Cell Studies

Many cell studies have focused on the capabilities of FGF-21 as a signaling molecule as well as the nature of its regulation. Using Murine C2C12 myocytes, Ribas et al. (2012), showed that FGF-21 was induced upon the differentiation of myoblasts into myotubes. It was found that the transcription co-factor, MyoD, controls FGF21 mRNA expression as the overexpression of MyoD caused an induction of both FGF-21 mRNA and an increase in FGF-21 protein within the cell media (Ribas et al., 2012). Mitochondrial dysfunction induced by oligomycin- an inhibitor of complex II within the electron transport chain- also showed an increased amount of FGF-21 protein production. This response was blunted by the presence of reactive oxygen species scavenger Trolox, a soluble form of Vitamin E. These data implicate FGF-21 as a marker for mitochondrial dysfunction or stress. FGF-21 has been implicated as a possible avenue for the treatment of Metabolic Syndrome, as the treatment of FGF-21 in both mouse 3T3-L1 adipocytes and human primary adipocytes causes an increase in glucose uptake (Kharitonov et al., 2005).

2.3.2 Animal Studies

The relationship between energy homeostasis and FGF-21 has been thoroughly studied in both rats and mice. In conjunction with their evaluation of the effects of FGF-21 on adipocytes, Kharitonov et al. (2005), also showed that the therapeutic administration of FGF-21 was capable of reducing plasma triglycerides and blood glucose to normal concentrations in both leptin-deficient and diabetic-obese rats. Obesity is also implicated to be an FGF-21 resistant state as Fisher et al. (2010), showed that diet-induced obese rats had elevated resting plasma FGF-21 and that rats treated with FGF-21 have a reduced response to treatment as measured by the phosphorylation of ERK 1/2. It may be that this is caused by the up regulation of micro RNA-43a (miR-43a) (Fu et al., 2014). MiR-34a was shown to be a key in the inhibitor of FGF-21 receptor components and may explain the FGF-21 resistance that is observed within the obese state. Downregulation of this micro RNA by the use of the anti-sense strand for miR-43a also showed improved lipid and glucose profiles and promoted the formation of brown and beige adipose tissue (Fu et al., 2014). In addition, Fu et al. (2014) also showed that FGF-21 is a key mechanism for the deacetylation and activation of the PGC-1 α gene and its subsequent effects in promoting an oxidative phenotype through mitochondrial biogenesis. The authors concluded that FGF-21 may exert this effect through increasing the activity of the enzyme AMPK.

FGF-21 has been reported to also be involved in the response to starvation and malnutrition. Kubicky et al. (2012), showed that with four wk of food restriction, wild type mice had decreased body mass and tibial growth when compared to FGF-21 knockout mice. The knockout mice showed normal growth patterns, but these differences from wild type were abrogated when they were treated with daily recombinant FGF-21. In the food restricted group, FGF-21 mRNA was up-regulated in comparison to a group feeding ad libitum as well as a reduction in circulating growth hormone and growth hormone receptor protein at the tibial growth plate, indicating that FGF-21 reduces the functionality of growth hormone

during starvation (Kubicky et al., 2012). The authors concluded from these data that FGF-21 plays a causative role in reducing growth in malnutrition as an adaptive stress response. In addition to these findings, Laeger et al. (2014) found that circulating FGF-21 in rats and mice increased 10 fold while on a low-protein, isoenergetic diet when compared to controls. Protein restriction in humans also caused an increase in circulating FGF-21, inducing a 121% increase in circulating FGF-21 after 28 days on a low-protein diet. FGF-21 was found to be responsible for the behavioural adaptations to low-protein diet, where wild-type rats increased food intake and energy expenditure while FGF-21 knockout mice showed no changes in eating behaviour or energy expenditure. Over the course of the diet, the FGF-21 knockout mice incurred a significant increase in body and fat mass, further implicating that FGF-21 restricts body mass and assists in regulating metabolism (Kubicky et al., 2012). It is thought that this regulation is part of the liver-brain axis, where fasting has been shown to cause FGF-21 to be released from the liver and activates downstream hypothalamic ERK1/2. Subsequently, this increases the expression of corticotrophin releasing hormone and corticosterone, increasing the rate of gluconeogenesis within the liver (Liang et al., 2014).

Transgenic mice over expressing the thermogenic protein UCP-1, showed an increase in the induction of the FGF-21 gene and also had a fivefold increase in circulating FGF-21 protein (Keipert et al., 2013). Interestingly, the treatment of white adipocytes with the serum of the UCP-1 transgenic mice also caused the expression of UCP-1 within the wildtype adipocytes, indicating that FGF-21 is likely a cause for morphogenic changes that occur within adipose tissue (Keipert et al., 2013). In support of the findings by Kubicky et al. (2012) and Keipert et al. (2013) also described that the UCP-1 transgenic mice also had reduced bone growth and smaller overall size, a key indication of the growth restriction that can be induced by FGF-21.

Very little has been studied in terms of the relationship between exercise and FGF-21 in animal models. A progressive ramp exercise test for 60 min or until

reaching exhaustion caused a significant increase in plasma FGF-21 in mice (Kim et al., 2013). There was no significant increase in FGF-21 mRNA in muscle, but there was a significant increase in hepatic FGF-21 mRNA. PPAR- α and ATF4, known positive regulators of FGF-21, were also elevated in the exercised mice. These data indicate that the FFA released during exercise may be the cause of the observed increase in plasma FGF-21 because PPAR- α is heavily regulated by the presence of free fatty acids (FFA) (Kim et al., 2013). The authors also concluded that it is also likely that FGF-21 is not a true myokine, but is secreted as a result of lipolysis induced by exercise.

2.3.3 Human Studies

FGF-21 in humans appears to behave similarly to the findings in both animal and cellular studies. In human subjects with specific mitochondrial myopathies such as lacking Iron-Sulfur cross bridges, FGF-21 protein has been reported to be circulating at greater concentrations (Crooks et al., 2013). Many of these myopathies act at Complex II of the electron transport chain, which is in accordance with the findings of Ribas et al. (2012), who used oligomycin to inhibit Complex II and induce an FGF-21 response. These studies indicate that FGF-21 may be a sensor for the energy availability or productive capabilities of the cell.

A diurnal rhythm of FGF-21 exists, where plasma concentrations peak at 0800h and are lowest at 1700h, with a half life of ~two h in humans (Lee et al., 2013; Scalzo et al., 2014). A study investigating the effect of ambient temperature on the secretion of FGF-21 found cooler temperatures induced a far greater FGF-21 response and the change in FGF-21 correlated positively with changes in glycerol, an indicator of FFA (Lee et al., 2013). Once again these findings indicate FGF-21 may be a consequence of FFA circulating in the body, helping orchestrate a response to energy substrate availability. Moreover, when examining patients with metabolic syndrome, FGF-21 was elevated at rest (Bobbert et al., 2013). When adjusted for age, sex, BMI, fasting glucose and cholesterol, FGF-21 remained an independent predictor of metabolic syndrome.

This knowledge allowed the authors to conclude that FGF-21 may act to counterbalance very early pathophysiological conditions such as elevated plasma lipids and is thus elevated earlier on in the development of metabolic syndrome (Bobbert et al., 2013). In men, it was found that plasma FGF-21 is negatively related to cardiorespiratory fitness as defined as peak VO_2 (Taniguchi et al., 2014). These data support the findings of Scalzo et al. (2014), who found that aerobic training caused a reduction in resting FGF-21 concentrations, indicating that there may be an inverse relationship between aerobic fitness and resting FGF-21 plasma concentrations. Alternatively, FGF-21 and visceral adipose were positively related. These results suggest that as visceral fat increases within the body and its associated consequential increase of FFA within the blood, so will FGF-21 (Taniguchi et al., 2014). This relationship may serve as the mechanism for the elevated and resistant state of FGF-21 in obesity.

In relation to exercise, FGF-21 is influenced by both the sympathetic activation of exercise and muscular contraction. Basal concentrations of FGF-21 are not influenced by sympathetic inhibition via administration of clonidine, yet increases during hypoxia-induced sympathetic activation and its concurrent increase in circulating epinephrine (Scalzo et al., 2014). Any increase in FGF-21 due to hypoxia was abrogated with the co-administration of clonidine, solidifying the causal nature of sympathetic input for the release of FGF-21. It is likely that exercise-induced FGF-21 is highly related to epinephrine and lipolysis because sympathetic inhibition does not affect basal FGF-21, yet acute activation of the sympathetic system does (Scalzo et al., 2014). Humans undergoing either a 50 or 80% VO_{2max} treadmill run for 30 min have been observed to induce an increase in plasma FGF-21 one hour after the cessation of exercise (Kim et al., 2013). At one h there was also a significant difference between the 50% and 80% VO_{2max} tests, indicating a potential dose-response relationship between exercise intensity and FGF-21 secretion. Kim et al. (2013) also measured peak FFA concentrations, which occurred immediately after exercise. Considering that immediate response of FFA and the delayed increases in FGF-21, it is likely that

the FFA response of exercise may be the cause of the subsequent release of FGF-21. Contrary to these findings, Cuevas-Ramos et al. (2012) found no acute changes in plasma FGF-21 concentrations at one and four h after a treadmill ramp test producing an energy output of 22 metabolic equivalents (energy required as measured by oxygen consumption for resting/basal metabolism) at its peak. In the same study conducted in overweight women, aerobic training in the form of nine treadmill ramp tests caused resting FGF-21 to increase as well as epinephrine and FFA. Considering that the FGF-21 gene is responsive to the effect of FFA on the PPAR- α transcription factor, it may be due to increased lipolysis and its resulting increase in circulating FFA. It was postulated that FGF-21 becomes elevated to increase lipid metabolism to prevent any ectopic deposition of lipid that may be caused by the increase in FFA post exercise (Cuevas-Ramos et al., 2012). Additional training studies have found that a combined aerobic and resistance training program consisting of 45 min of aerobic training at 70% of age predicted maximum heart rate and 20 min of resistance training caused a 44% reduction in resting plasma FGF-21 after three months (Yang et al., 2011). The training was conducted five d per week for 12 wk which also resulted in a significant decrease in body mass index, indicating the decline in resting FGF-21 may be induced by a loss of body fat (Yang et al., 2011). A sprint training modality has also been studied where an interval exercise program consisting of four-eight, 30 sec, all-out sprints caused a reduction in the resting concentration of FGF-21 after nine sessions of training (Scalzo et al., 2012).

From the studies outlined above, FGF-21 appears to be a myokine that is an early indicator of obesity which can be up regulated by mitochondrial abnormalities and is responsive to exercise and exercise training. FGF-21 is capable of inducing an oxidative phenotype and is responsible for shifts in adipose phenotypes. It is possible that FGF-21 is a response to the adrenergic and lipolytic effects of exercise, but these conclusions are currently unclear. The relationship between FGF-21 and exercise has not been thoroughly researched

and thus focus on this myokine is warranted, specifically in the area of both SIE and SIT.

2.4 Interleukin-15

Discovered in 1994 by Grabstein et al. and Giri et al., Interleukin-15 (IL-15) is a 59 amino acid member of the four α -helix family of cytokines. IL-15 was originally identified as a T-cell growth factor of the immune system which interacts with subunits of the Interleukin-2 receptor (Grabstein et al., 1994). IL-15 was first implicated in the function of muscle when it was found to be highly expressed in muscle (Grabstein et al., 1994) and when the treatment of IL-15 induced the accretion of myosin heavy chain proteins in murine and bovine myocytes (Quinn et al., 1995). Based on the findings of these original studies, IL-15 was classified as an anabolic growth factor.

2.4.1 Cell Studies

Cell studies focusing on the effects of IL-15 have been able to show both an anabolic role and the ability to induce an oxidative phenotype in muscle. In one of the original papers recounting the anabolic effects of IL-15, Quinn et al. (2002) described the induction of hypertrophy independent of IGF-1 in differentiated myotubes from mice. Over-expression of IL-15 in same-cell lineage via retroviral injection of the IL-15 gene into the cells resulted in 400-fold greater concentration of myosin heavy chain and alpha-actinin accretion. The hypertrophic effect occurred without proliferation of myoblasts, indicating a sarcoplasmic hypertrophic response. It is important to note that the treatment of IL-15 also prevented the degradation of protein within the cultured myotubes, making a conclusion related to any increase in protein synthesis difficult. Furmanczyk and Quinn (2003) were able to replicate the previous findings regarding the ability of IL-15 to induce the accretion of myosin heavy chain protein within differentiated human myotubes and muscle fibres. These data are consistent with both studies from myogenic precursor and rodent studies, demonstrating an anabolic effect of IL-15.

The effect of IL-15 on lipid deposition and oxidation is also pronounced within cell studies. By treating murine 3T3-L1 adipocytes with recombinant IL-15, Quinn et al. (2005) were able to reduce lipid deposition by more than 50% and stimulate the release of adiponectin. These findings prompted the authors to conclude that there is a direct modulating effect of IL-15 on the function of adipose tissue and there is a communication axis between muscle and fat. Furthermore, O'Connell and Pistilli (2015) found that the treatment of muscle cells derived from mice with recombinant IL-15 had a distinct and significant increase in PGC-1 α and PPAR- γ expression and greater mitochondrial density when compared to control cell lines. These findings indicate that IL-15 may be able to not only reduce the accretion of adipose tissue, but also enhance β -oxidation of circulating lipids in skeletal muscle.

2.4.2 Animal Studies

The role of IL-15 within animal models has been studied extensively in both rats and mice. When compared to wild-type mice, transgenic mice over-expressing IL-15 have been described as having both elevated muscle IL-15 protein and circulating IL-15 in plasma (Quinn et al., 2013). In a treadmill run to exhaustion, the IL-15 transgenic mice ran twice the length of time to fatigue when compared to wild type mice and also had a lower amount of visceral adipose tissue. In the same study, indirect calorimetry of the IL-15 transgenic mice indicated that the over-expression of IL-15 induced a lower RER suggesting the mice utilized a greater proportion of fat as primary energy substrate. Alongside the over-expression of IL-15, there was also increased expression of oxidative regulators such as PGC-1 α , an increase in the oxidative Myosin Heavy Chain Type I and markers of mitochondrial lipid oxidation, indicating an induction of oxidative adaptations associated to exercise. In a continuation of the previous study, IL-15 knockout mice did not show any up-regulation of PGC-1 α , SIRT-1 or any other pro-oxidative mediators after undergoing a bout of exhaustive exercise running (Quinn et al., 2014). Wild type mice exhibiting IL-15 expression also had a much longer run time to exhaustion. Injection of recombinant IL-15 into the knockout

mice was able to up-regulate the aforementioned oxidative mediators, suggesting that many of the oxidative adaptations due to exercise are mediated directly through IL-15.

IL-15 is present in mammalian systems in two forms, one long and one short signaling domain peptides. In a study examining the effect of either peptide form Quinn et al. (2008) identified the short signal peptide as the most efficient endocrine signal and that any functional changes in body composition are due to the appropriate signal peptide being secreted into the bloodstream. Elevated IL-15 resulted in significantly reduced body fat (50% for males, 25% for females) and increased bone mineral density. In a group of mice over-expressing the efficient short signal IL-15 peptide, there were reduced concentrations of common pro-inflammatory cytokines such as IL-6 in groups that were fed a high fat diet. In the same study, Quinn et al. (2008) found there was no increase in muscle protein synthesis under a milieu of IL-15, but a reduction in protein degradation. This suggests that IL-15 may not be an anabolic factor, but an anti-catabolic factor in-vivo.

In 2010, Quinn et al. identified that physiological concentrations of IL-15 decrease with age, but expression of IL-15 mRNA did not. However, the expression of the soluble IL-15R α subunit decreased 5-fold with age and correlated significantly with circulating IL-15. Based on these data, Quinn et al. (2010) suggested that the α -subunit of IL-15 both influences the secretion of IL-15 into circulation and the responsiveness of tissues to IL-15. Lastly, in clinical populations 12 wk of endurance treadmill running led to an increase in IL-15 expression in the muscle of diabetic rats and also improved intraperitoneal glucose tolerance (Kim et al., 2013).

2.4.3 Human Studies

In comparison to the myokines outlined above, IL-15 has not been studied to the same extent in humans. However, IL-15 has been evaluated in the context of both strength and aerobic training. For strength training, Reichman et al. (2004),

trained men three days per week for 10 wk. The subjects performed 13 exercises each, containing four sets of six-10 repetitions at their 80% one-rep maximum. By studying the effect of different haplotypes for the IL-15 receptor α -subunit, it was found that 7.1% of the variability of muscle mass gained from the training was due to a single receptor haplotype, the IL-15 short signal peptide. These findings are consistent with the findings of Quinn et al. (2008), who found that the short signal peptide of IL-15 was most efficient as an endocrine signal in animal models. The strength exercise increased plasma IL-15 acutely both before and after the 10 wk of training and this effect was still significant when accounting for any changes in plasma volume due to exercise. In an acute bout of aerobic exercise, lean and obese subjects exercised on a cycle ergometer for 120 min at a moderate intensity (55-60% of age predicted maximum heart rate). IL-15 was increased two-three fold in both lean and obese subjects, with no difference between the groups as defined by body mass (Christiansen et al., 2013). In a similar study, 30 min of treadmill running at 70% of age predicted maximal heart rate in untrained men induced a significant response with a peak plasma IL-15 concentration reached 10 min after the cessation of exercise (Tamura et al., 2011). The authors concluded that the release of IL-15 was due to contraction induced stimuli rather than from muscular damage caused by exercise because the maximum observed creatine kinase response from the exercise was greatest at three h post-exercise, while the IL-15 concentration was reduced to normal concentrations at three h. These findings are critical in defining IL-15 as a true myokine being secreted from muscle as a response to contraction, rather than a global inflammatory response to exercise stress.

The change in plasma IL-15 due to aerobic training has also been evaluated. Christiansen et al. (2010), found that an exercise intervention consisting of 12 weeks of tri-weekly aerobic running for ~60 min and an energy expenditure of 500-600 kJ had no effect on circulating IL-15 (with a concurrent loss of three kg of body fat) in obese subjects. However, the combination of energy restriction and exercise (with a loss of 12.1kg of body fat) caused a reduction of 25% of plasma IL-15. This suggests that plasma IL-15 may be related to the absolute

amount of body fat and in obese individuals and that IL-15 may be chronically elevated as a marker of inflammation associated with the obese state.

However, there are also instances where exercise has been shown to have no effect on the concentration of plasma IL-15. In endurance trained athletes, Ostrowski et al. (1998) found no change in serum IL-15 after 2.5 h of treadmill running at 75% of maximum oxygen consumption. It is likely that in this situation the athletes may have had a body fat content that was below a threshold value to produce IL-15 as proposed by Christiansen et al. (2010) or due to their training status, the stimuli of the running was not great enough to illicit a myokine response from the muscle tissue. Neilsen et al. (2007) also demonstrated that acute, heavy resistance leg exercise was unable to produce a significant increase in plasma IL-15. However, there was an increase in muscle IL-15 mRNA. These findings allowed the authors to speculate that IL-15 may be present as a translationally inactive pool. Interestingly, IL-15 mRNA was enhanced in muscle groups dominated by Type II fibres, suggesting that activation of these fibres may result in the specific secretion of IL-15. If so, it is likely that the intensity of exercise is key to the secretion of IL-15 and very high intensity weightlifting or sprinting is most likely to induce the strongest myokine response from muscle.

In summary, IL-15 appears to be a muscle-derived signaling protein that is capable of inducing both hypertrophy and oxidative adaptations to exercise. It is possible that IL-15 is dependent on the absolute fat content of the body in mammalian models and that the beneficial effects of exercise such as increased fat oxidation and mitochondrial proliferation are directly mediated through IL-15. Although there is a significant amount of data supporting the function of IL-15 in animal models, much less research exists in human models. This is particularly evident in evaluations of sprint exercise, as no studies currently exist profiling the effect of SIE on the secretion of IL-15.

3 Methods

3.1 Participants

Twelve exercise-trained men started and nine finished this study ($n=9$, age 23.3 ± 2.4 years, height 177.7 ± 4.9 cm, mass 82.1 ± 11.1 kg, body fat $17.2 \pm 8.4\%$; mean \pm SD). All subjects were recreationally active and participated in regular strength and/or aerobic exercise ~ 3 d/wk, but did not follow specific training programs and were not sprint-trained. Potential participants were excluded if they had any known metabolic, musculoskeletal or neurological diseases. In addition, participants completed a PAR-Q (Canadian Society for Exercise Physiology, 2002, Appendix D) and a health information form (Appendix C) to screen out any potential contraindications to the exercise. Participants could not have engaged in any sprint interval training (SIT) within two months of the beginning of the experimental procedures.

All risks and discomforts were explained fully prior to any testing and all participants provided written, informed consent. This study was conducted in the Exercise Nutrition Research Laboratory (ENRL) and was approved by the Office of Research Ethics at The University of Western Ontario.

3.2 Preliminary Visits

Prior to experimental testing, participants were required to visit the ENRL on two separate occasions for familiarization to laboratory testing procedures and screening/baseline measures.

On the first visit, participants had their body composition measured and were familiarized with the computerized, electromagnetically braked Velotron™ cycle ergometer (RacerMate, Inc., Seattle, Washington USA), which was used for all cycling tasks throughout the study (maximal oxygen consumption [VO_{2max}] and sprint exercise). Previous research confirmed the power accuracy of the Velotron™ to be within 3% of the power recorded via a dynamic calibration rig during high intensity interval exercise (Abbiss et al., 2009). All individual

adjustments made to seat height, seat distance, handle bar height and handle bar reach were recorded and used for subsequent tests. Once familiarized, participants completed a VO_{2max} test.

On a subsequent day and not within 48 hours of the completion of the VO_{2max} test, participants completed a familiarization of the experimental sprint protocol. The protocol was described originally by Burgomaster et al. (2005). Briefly, participants completed a series of four Wingate tests (30 sec max efforts; resistance of 9% body mass), with a rest interval of four min separating each test. During the resting periods, the load against the Velotron™ flywheel was reduced to zero and participants were given the option of either active or passive recovery. Each received a three min warm-up on a Monark 874E Ergometer (Monark, Inc, Vansbro, Sweden) at an absolute resistance of one kg. After the three min warm-up, the participants were then allotted a two-min stretching period. During the familiarization session total work output or the area under the curve of instantaneous power output was recorded as well as peak power output and anaerobic power. The total work output was recorded for each Wingate within the familiarization session and added together to represent the total work output for the sprint session.

3.3 Experimental Overview

Participants underwent two experimental conditions: Sprint Interval Exercise (SIE) and Continuous Exercise (CE). Each condition consisted of a five h test day in the laboratory. Conditions were conducted via a systemically rotated, crossover design and were separated by at least one week. Briefly, the first participant was assigned randomly to an experimental condition and thereafter treatments were systematically rotated to avoid order or training effects. Each subject completed both conditions (SIE and CE).

On the eve of testing, participants were provided a standardized high carbohydrate pasta meal (two g of carbohydrate·kg body mass⁻¹) in order to

minimize the intra and inter-variability of nutritional status (Jeacocke & Burke, 2010). Participants consumed the pasta meal between 1700-2000 h with no additional carbohydrates besides 250 mL of their preferred pasta sauce.

On test days, participants reported to the ENRL at 0800 h after a 12 h overnight fast having been instructed to not exercise or consume alcohol for 24 h prior to testing and no caffeine on the morning of the test day. Participants were given a standardized breakfast 0830h (~25 kJ·kg⁻¹ body mass) which consisted of 10 kJ·kg⁻¹ of Dempster's® Original 100% Whole Wheat Bread (Maple Leaf Foods Inc., Toronto, Ontario), 11 kJ·kg⁻¹ of Kraft® Smooth peanut butter (Kraft Canada Inc., Don Mills, Ontario) and 200mL of Tropical Grove Apple Cocktail (Lassonde Beverages Canada, Toronto, Canada).

At 0805 h, a resting venous blood sample was taken directly into a four ml EDTA vacutainer. A second one ml sample was taken by a polyurethane syringe directly from the catheter into which it was aliquoted into triplicate 70-microlitre hematocrit samples, used to determine any changes in blood plasma concentration. This sampling procedure was repeated for all time points at which a blood sample was taken. At 0925 h, subjects completed an exercise warm-up that was identical to that of the SIE familiarization day. At 0930h subjects completed either the SIE session or a work-matched (Joules) CE experimental trial. Four more blood and hematocrit samples were taken as follows: five, 30, 90, and 180 min after the completion of exercise. At 1115h subjects were fed a standardized lunch (~17kJ·kg⁻¹ body mass), consisting of the same ingredients as breakfast.

3.4 Exercise Protocol

The aim of the experiment was to conduct a work-matched (per-joule basis) comparison of exercise intensity on the effect of plasma myokine accumulation between SIE and CE. For this project, exercise intensity is referred to as a percentage of measured VO_{2max} . To do this the exercise protocols were designed

to have the same total work output. The length of the CE session was thus determined by the total work output from the SIE familiarization session or the SIE experimental trial in the situation where the systematic rotation of the experimental trials determined that the SIE trial was performed first.

3.4.1 Continuous Exercise Trial

The intensity of the CE trial was set at a load that would elicit a VO_2 response of ~65% of VO_{2max} because this is a moderate intensity and is common for many aerobic exercise training regimes (American College of Sports Medicine. ACSM Guidelines for Exercise Testing and Prescription. Whaley MH, ed. 7th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2006). The load of the CE trial in $kgm \cdot min^{-1}$ was determined using the following equation:

$$0.65VO_{2max} = 1.8(kgm \cdot min^{-1})/Body\ mass\ (kg) + 2(MET)$$

Note that for this experiment that MET (Metabolic Equivalents) was set to a value of 2.6 ml of $O_2 \cdot kg^{-1} \cdot min^{-1}$ as it has been shown that the traditional value of 3.5 ml of $O_2 \cdot kg^{-1} \cdot min^{-1}$ is a significant overestimation and that 2.6 is a more accurate value (Byrne et al., 2005, McMurray et al., 2013). Metabolic Equivalents are defined as the as energy required for basal/resting metabolism as measured by oxygen consumption. The VO_{2max} value was taken from the VO_{2max} test that all participants completed during their preliminary visit to the laboratory.

Once the load for the CE trial was determined in $kgm \cdot min^{-1}$, it was converted to watts using the equation:

$$1\ Watt = 6.12kgm \cdot min^{-1}$$

The length of the CE trial was then calculated using the total work output in Joules from either the familiarization trial or the SIE experimental trial, depending on the participant's order of experimental trial rotation. The length of the CE trial in sec was determined using the following equation:

$$\text{Watts} = \frac{\text{Joules}}{\text{Time(s)}}$$

OR

$$\begin{aligned} & \text{Time of CE Trial(s)} \\ & = \text{Total Work output of Sprints(J)} \div \text{Load of } 65\%VO_{2max}(\text{Watts}) \end{aligned}$$

The CE was then conducted on the Velotron™ cycle ergometer interfaced with computer based, interactive Coaching Software (RacerMate Inc, Version 1.15). The Coaching Software's ergometer mode ensured participants maintained the prescribed workload irrespective of cadence. Participants were provided with a schematic profile of power output throughout the trial as well as real-time feedback of time elapsed and time remaining.

3.4.2 Sprint Interval Exercise Trial

The SIE trial was conducted as described by Burgomaster et al. (2005), consisting of a series of four Wingate tests each separated by four min of recovery. The Wingate cycle test is a computerized measure of anaerobic power, described originally by Barr-Or and colleagues (1977). Briefly, participants sat on the Velotron ergometer and cycled as fast as possible for 30 sec against a flywheel resistance set to 9% body mass in kg. Total work output, peak power (highest power output over any five sec) and anaerobic power were determined using an online data acquisition system (Computrainer, RacerMate Inc, Seattle, WA). As explained earlier, the load against the flywheel during the recovery periods was set to zero so that the subject's choice of active recovery would not affect the total work output.

3.5 Measurements

3.5.1 Body Composition

Air displacement plethysmography body volume (BodPod®) and body mass were used to determine body density. Participants were required to fast three h prior to entering the BodPod®, and to wear approved clothing (compression shorts and lycra swim cap) to minimize errors due to air in hair or under clothing. Thoracic volume was estimated via a calculation integral to the BodPod® software. In order to estimate body composition, the attained body density was imputed into the Siri (Siri, 1961) equation

3.5.2 Aerobic Capacity

VO_{2max} was determined via a $25\text{ W}\cdot\text{min}^{-1}$ incremental ramp protocol on a Velotron™ cycle ergometer. Briefly, the ramp protocol consisted of a two min warm-up at a self-selected wattage followed by an increase to an initial resistance of 90-125 W (depending on body mass) with subsequent five W increases every 12 sec. Expired gases were collected via a breath-by-breath collection system (SensorMedics Vmax 29, Yorba Linda, CA). The greatest value achieved over a 30 sec collection period was considered max whenever a plateau in VO_2 occurred (<50% of the expected increase in oxygen uptake for the increased workload) or when two of the following three criterion measures were attained (95% of age predicted maximum HR, $RER > 1.15$ [$RER = \text{volume of } CO_2 \text{ produced} / \text{volume of } O_2 \text{ consumed}$] or volitional exhaustion).

3.5.3 Blood Sampling and Hematocrit

To determine any changes of plasma myokines concentration, blood samples were taken pre-exercise, five, 30, 90 and 180 min post-exercise. All venous blood samples were drawn by a certified nurse, who employed standard sterile blood handling techniques to prevent infection or contamination. Prior to any specimen collection, the participants were debriefed regarding the blood draw

process as well as informed of the procedure in place in case of an adverse reaction.

Prior to exercise a 20 gauge indwelling catheter (BD Angiocath™, Becton, Dickinson and Company®, New Jersey, USA) was inserted into a vein in the antecubital area of either one of the subject's arms and an IV set three-way stopcock (MED-RX®, Benian Inc., Oakville, Canada) was attached. For any of the blood samplings, blood was drawn into a four ml BD Vacutainer® EDTA collection tube (Lavender top, Becton, Dickinson and Company®, New Jersey, USA), inverted eight times and immediately centrifuged. The vacutainers were centrifuged for 10 min at 3000x g at four°C (Allegra™ 21R, Beckman Coulter™, California, USA). Plasma obtained was aliquoted into two, two ml Eppendorf tubes (Eppendorf Inc., Mississauga, Ontario) and frozen at -20°C for ~three weeks and then transferred to -70°C until later analysis. After the blood samples were taken, two ml of 0.9% Injectable Saline USP (Hospira, Montreal, QC, Canada) was injected back through the catheter to act as a saline lock to keep the catheter patent in between samples. Any remaining saline was drawn off from the catheter using a separate BD Luer Lok™ 3ml syringe (Becton, Dickinson and Company®, New Jersey, USA) and discarded before any Vacutainer samples were taken.

For hematocrit, an additional one ml of blood was drawn into a BD Luer Lok™ one ml syringe (Becton, Dickinson and Company®, New Jersey, USA) and then drawn via capillary action into Drummond® Heparinized microcapillary tubes (Drummond Scientific Co. Broomall, PA, USA). The microcapillary tubes were then sealed at one end by Critoseal® (Oxford Labware®, St. Louis, MO, USA). The microcapillary tubes were centrifuged for three min in a Triac™ Hematocrit Centrifuge (Clay Adams, Division of Becton, Dickinson and Company®, New Jersey, USA). The centrifuged microcapillary tubes were then measured against a ruler to determine the Packed Cell Volume (PCV) as a proportion of the total volume within the microcapillary tube. The PCV was used to determine any changes in plasma volume due to the pressure gradient effects of exercise (Van

Beaumont, 1972). Plasma volume may also have been affected by osmotic pressure due to the effect of exercise metabolites increasing in concentration within the cellular compartment. These two factors were considered when analyzing any changes in plasma volume.

3.5.4 Blood Analysis

Blood was analyzed and myokines were quantified using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) Kits. FGF-21 and IL-15 were measured using the Human FGF-21 and IL-15 Quantikine ELISA kits (R&D Systems, Minneapolis, Minnesota, USA). Irisin was measured using Phoenix Pharmaceuticals Inc. Irisin ELISA assay kit (EK-052-67).

3.6 Statistical Analysis

Statistical analyses were conducted using SigmaPlot for Windows (Version 12.0). All data were analyzed using a two-way repeated measures ANOVA (condition x time). Tukey's HSD was used for post-hoc analysis to determine any significant effects. In order to determine effect sizes, a partial eta-squared analysis was conducted. Linear regressions were also performed to determine any association between, fat mass, lean mass and the absolute peak concentration of plasma myokines. Significance was set at $p \leq 0.05$. Data are presented as means \pm SD.

4 Results

4.1 Descriptive Statistics

Of the original 12 participants, three could not finish the entire study. One subject withdrew due to an illness unrelated to the study and two others withdrew because they relocated away from the London area. Subject 6 was eliminated from all analyses because all samples consistently had negative absorbances and thus below zero concentrations of myokines. Subject 9 was removed from the Irisin analysis due to the fact that its ELISA readings were also all negative absorbances. The catheters clotted at the following timepoints and thus no samples were obtained: Subject 2 and 11 at 180 min in sprint condition and subject 2 and 3 at 180 min in the endurance condition. Subject 8 also temporarily clotted 5 min post endurance condition. The clot did not remain and thus it was the only data point that was lost.

The training history of the participants was varied. Of the participants three were competitive power lifters, one was a trained hockey player, one was a competitive ultimate frisbee athlete and four others exercised approximately three days per week, but did not follow specific aerobic or resistance training programs. It must be noted that only one of the subjects elected to choose active recovery while resting between sets of Wingates while performing the sprint condition.

Table 4.1. Participant characteristics and performance test results

Participant #	Age (years)	Mass (kg)	Height (cm)	Lean Body Mass (kg)	Fat Mass (kg)	Body Fat (%)	VO _{2max} (mL O ₂ •kg ⁻¹ •min ⁻¹)	SIE Trial Work output (J)	65% VO _{2max} (W)	Length of CE Trial (min)
1	25	90.1	182.0	76.0	14.1	15.6	58.3	81902.2	255	5.41
2	25	98.2	183.0	69.0	29.2	29.7	42.7	85149.7	184	7.71
3	22	79.0	178.0	67.2	15.8	20.2	49.7	65882.0	182	6.03
4	22	89.5	180.5	81.1	8.8	9.8	61.1	91924.8	267	5.74
6	23	67.6	167.0	62.5	3.6	5.4	56.8	60573.0	185	5.45
7	22	86.9	175.0	65.1	21.9	25.1	41.8	65640.0	160	7.89
8	24	83.4	177.0	67.3	16.3	19.5	38.9	64410.2	143	7.52
9	19	60.0	182.0	56.3	3.1	5.2	52.0	43106.2	147	5.19
11	28	84.5	174.5	63.6	20.9	24.7	35.1	60409.7	135	7.18
Mean	23.3	82.1	177.7	67.1	14.9	17.2	48.5	68777.5	184.2	6.55
SD	2.4	11.1	4.9	7.1	8.1	8.4	8.7	13452.7	44.7	1.00

Values are mean±SD; y = years; kg = kilograms; cm = centimetres; % = percent; min = minutes; VO_{2max} = maximal oxygen uptake; J = joules; W = watts

4.2 Hematocrit and Hemoconcentration

There was no significant main effect of time or exercise condition. There was a significant interaction effect (exercise condition x time; $p= 0.04$). Pairwise comparisons (Tukey) indicated that PCV was significantly greater at 5 min post-exercise with SIE condition when compared to CE condition ($p<0.001$). At 5 min post SIE, PCV increased from 46.4 percent to 51.9 percent. The CE condition had no significant effect on PCV. Neither exercise condition was significantly different at any other time point (Fig 4.1).

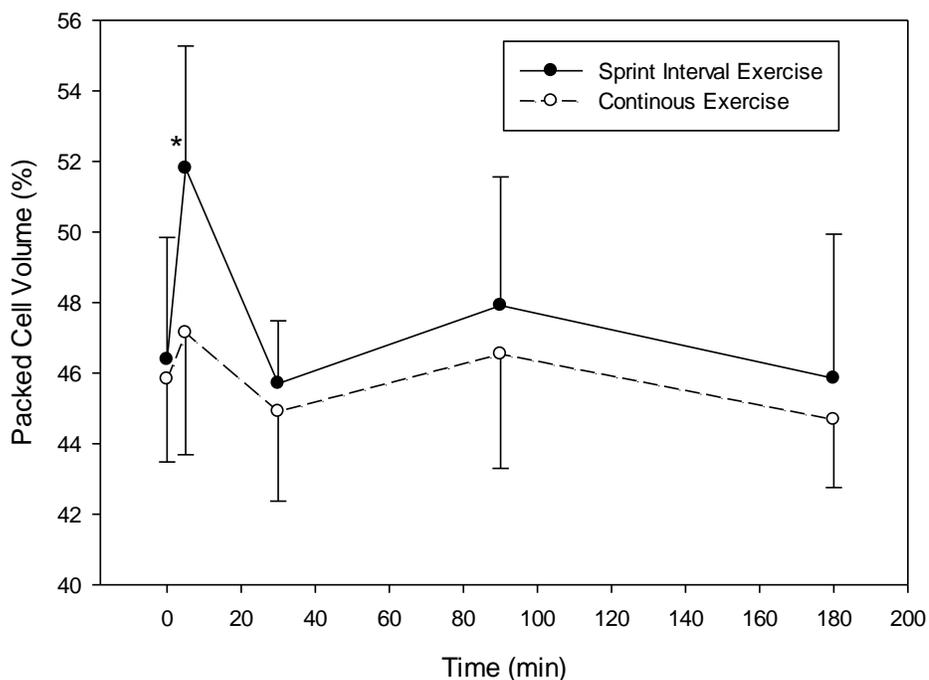


Figure 4.1. Hematocrit changes after SIE or work-matched CE as denoted by Packed Red Blood Cell Volume as percent of total microcapillary tube volume. (* = $p<0.05$) Data are means mean \pm SD (n=8).

Table 4.2. Percent changes in plasma volume after SIE or work-matched CE.

	5 min post exercise	30 min post exercise	90 min post exercise	180 min post exercise
SIE	-19.5	2.8	-6.0	2.1
CE	-5.2	3.8	-2.8	4.7

The change in plasma volume (% Δ PV) was calculated with the following equation:

$$\% \Delta PV = \frac{100}{100 - Hct_{pre}} * \frac{100(Hct_{pre} - Hct_{post})}{Hct_{post}}$$

Effect size estimates (η^2) indicated that 6% of the variability in hematocrit was due to exercise condition and 11% of the variability was due to time. These values translate to medium-large effect sizes.

4.3 IL-15

There was no significant main effect of time ($p=0.55$) or exercise condition ($p=0.71$) on the plasma accumulation of IL-15. There was also no significant interaction effect (condition \times time, $p=0.34$) (Fig 4.2). Further, due to a high degree of variability within duplicates from the IL-15 ELISA assay, IL-15 was excluded from any remaining analysis.

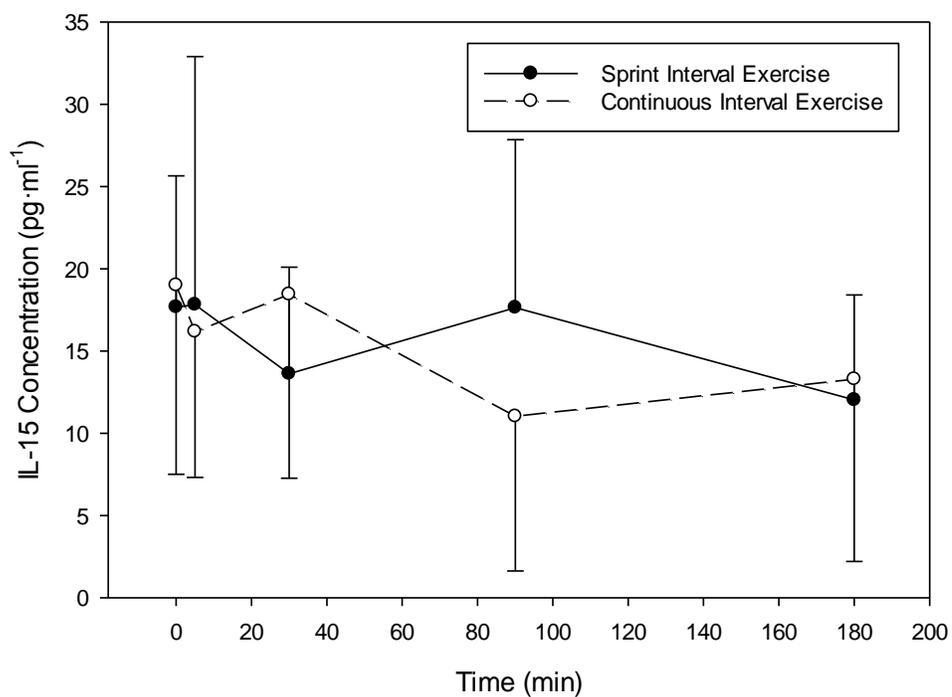


Figure 4.2. Change in plasma IL-15 concentration (pg/ml) with SIE or work-matched CE. Data are presented as a mean \pm SD, $n=8$

4.4 Irisin

There was no significant main effect of exercise condition ($p=0.18$), time ($p=0.28$) or interaction effect ($p=0.32$) (Fig 4.3).

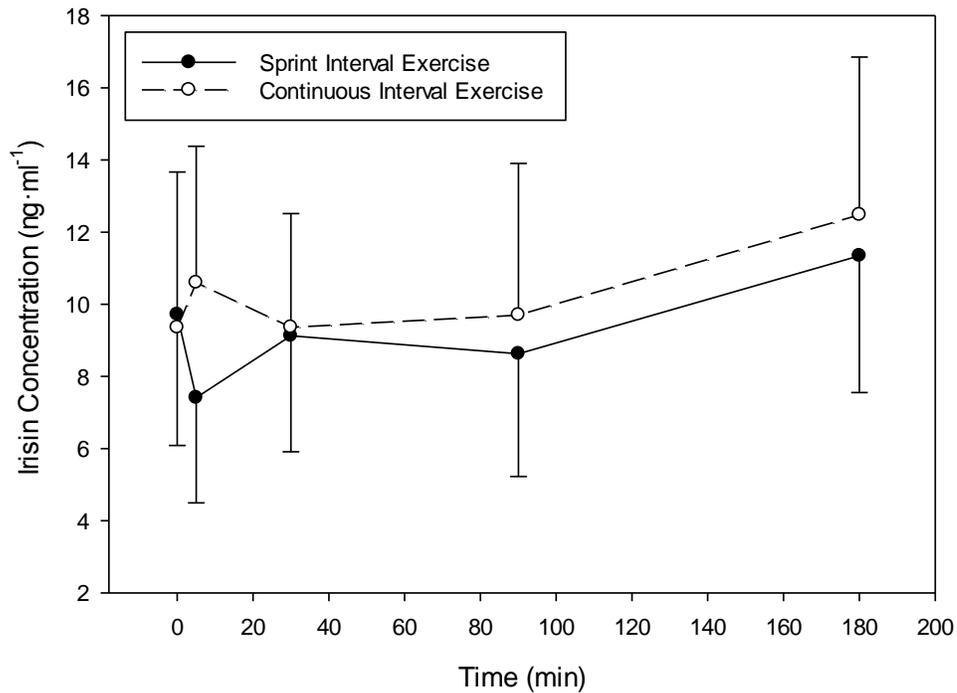


Figure 4.3. Change in plasma Irisin concentration with SIE or work-matched CE. Data are presented as means \pm SD, $n=7$.

4.4.1 Irisin and Body Composition

Plasma Irisin concentration correlated significantly with lean body mass at rest (Correlation coefficient = 0.78, $p=0.001$, $r^2=0.6$; Fig 4.4). After exercise, plasma Irisin was not correlated to muscle mass at either 5 min post exercise or 180 min post exercise.

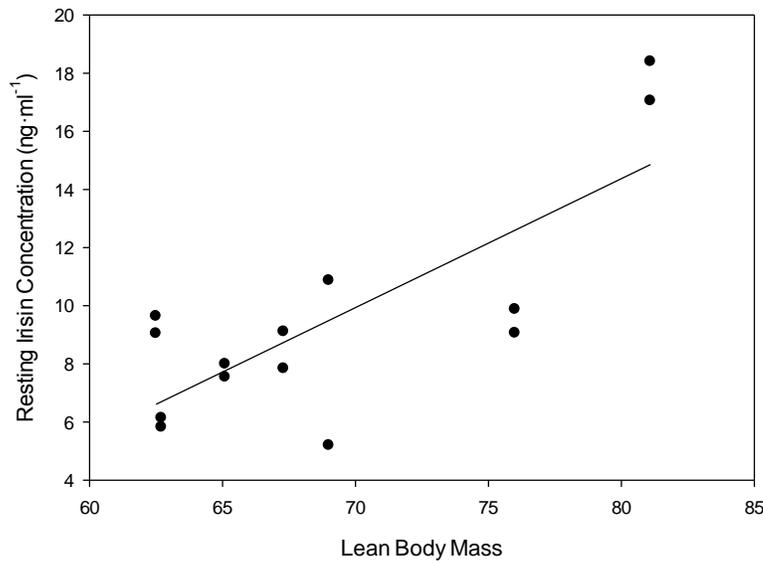


Figure 4.4 Correlation between lean body mass and resting plasma Irisin concentration. Correlation coefficient = 0.78, $p=0.001$, $r^2=0.6$, $n=7$.

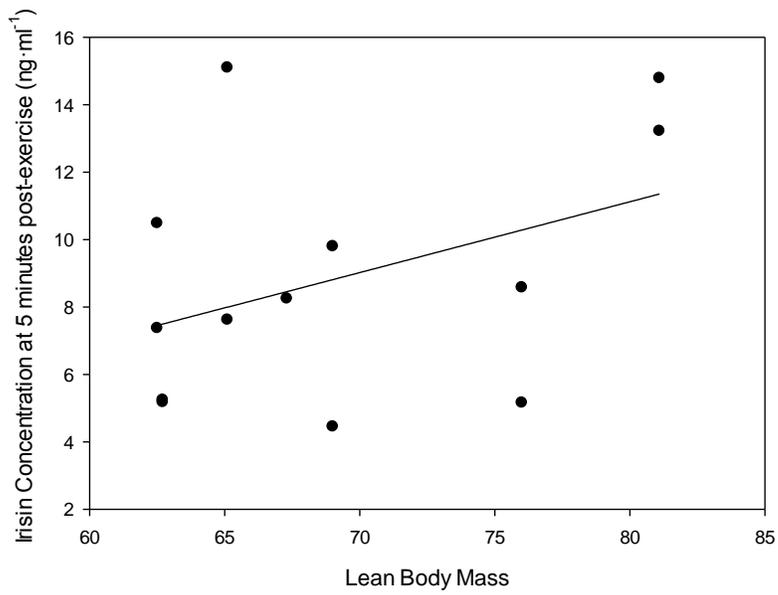


Figure 4.5. Correlation between lean body mass and plasma Irisin 5 minutes post-exercise under either SIE or CE paradigm. Correlation coefficient=0.40, $p=0.17$, $r^2=0.32$, $n=7$. Subject 8 in the sprint condition is missing.

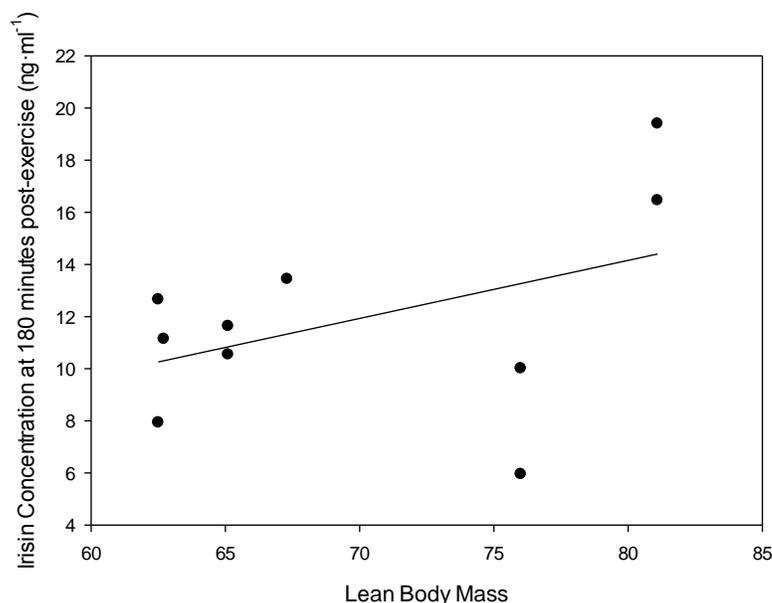


Figure 4.6. Correlation between lean body mass and plasma Irisin 180 minutes post-exercise under either SIE or CE paradigm. Correlation coefficient=0.44, $p=0.20$, $r^2=0.19$, $n=5$.

4.5 FGF-21

There was no significant main effect of time or exercise condition. There was a significant interaction effect (condition X time), $p=0.04$). Pairwise comparisons (Tukey) indicated mean plasma FGF-21 concentration was elevated significantly at 30 min ($p<0.001$) post-exercise within the sprint interval condition (Fig 4.7). At this time point, mean plasma FGF-21 rose from 49.4 ng/ml at rest to 107.64 ng/ml. Further, at 30 min after exercise, SIE induced a significantly greater plasma FGF-21 concentration ($p=0.03$) vs CE, with a mean difference of 57.2 ng/ml between SIE and CE. Continuous exercise had no significant effect on the accumulation of FGF-21.

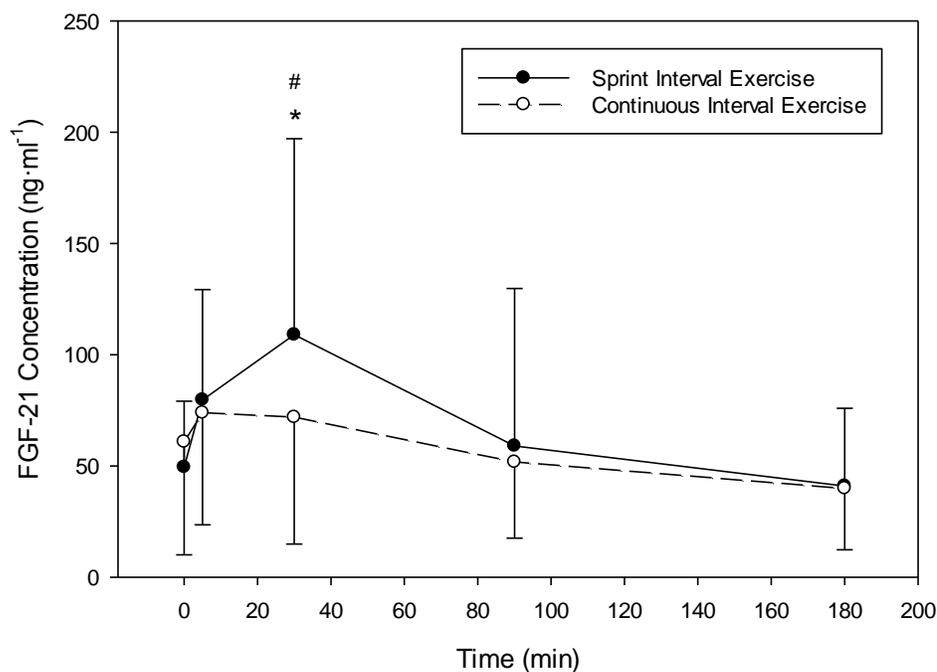


Figure 7. Change in plasma FGF-21 concentration with SIE or work-matched CE. All data are presented as mean \pm SD. * indicates that the concentration of FGF-21 is significantly greater than rest within the sprint interval group ($p=0.04$, $p<0.001$). # indicates significantly greater concentration of plasma FGF-21 for SIE vs CE at 30 min post-exercise ($p=0.03$), $n=8$.

Effect size estimates (η^2) indicated that 3% of the variability in plasma FGF-21 is due to exercise condition, 11% is due to time and 5% due to the interaction effect (exercise condition \times time). These translate to small, medium and small effect sizes, respectively.

All correlations between plasma FGF-21 and body fat %, lean body mass or fat mass at rest or post-exercise were nonsignificant.

5 Discussion

The primary finding from the present study is that an acute bout of SIE increased plasma FGF-21 significantly at 30 min after the cessation of exercise. Further, the SIE bout increased plasma FGF-21 significantly at 30 min post-exercise vs CE, suggesting a physiological difference in the capacity to induce a systemic FGF-21 response between the two exercise modes. In contrast, there was no significant effect of either exercise mode on Irisin or IL-15 plasma concentration. However, Irisin was correlated positively with lean body mass at both rest, suggesting that total muscle mass may influence systemic Irisin concentrations. Interestingly, this relationship was no longer present post-exercise. IL-15 and FGF-21 were not significantly correlated to any body composition parameter.

5.1 IL-15

This is the first study to examine the effects of SIE on the plasma concentration of IL-15 but neither SIE nor CE altered plasma IL-15 concentration significantly. However, the results need to be evaluated in context because there was a high degree of intra-assay variability in duplicate samples as well as variability between subjects. For this reason, it was difficult to make a definitive conclusion regarding these data. Some of this variability is likely attributed to sample preparation because it appeared that some samples were not uniform in their consistency. Learning from this, all FGF-21 and Irisin samples were vortexed, re-centrifuged and aliquoted after thawing to ensure uniformity. Regardless of these sample preparation issues, some comments about the IL-15 data are appropriate. Previously published values of plasma IL-15 with endurance treadmill running and strength exercise in lean, healthy subjects range from ~1.6-12.6 pg/ml (Christiansen et al., 2013; Riechman et al., 2004; Tamura et al., 2011) and the current data are similar. Further, compared to the present investigation (n=9), the previous studies had larger samples sizes; n=13 (Tamura et al., 2011), n=15 (Christiansen et al., 2013), especially the Reichman et al. (2004) study (n=76). Consequently, the present observed IL-15 response may have been nonsignificant due to variability, i.e., a Type II error. Moreover, although the

current study was designed to isolate the effect of exercise intensity on the plasma concentration of IL-15, the absence of an increase may suggest that a minimal exercise duration is necessary for an IL-15 response. Including rest periods, the present SIE protocol took a total of 14 min (exercise was only 2 min) and the work-matched CE took only 6.5 min. This is shorter than previously published studies that reported IL-15 increases. For example, Tamura et al., 2011 reported significant mean increases from 1.71 pg/ml to 1.95 pg/ml after 30 min of running at 70% of age-predicted heart rate maximum. Although the intensity of the CE trial in the current study was comparable (65% $\text{VO}_{2\text{max}}$) to Tamura et al. (2001), the total time of exercise was ~5 times less. This same situation applies to the study by Christiansen et al. (2013), where subjects ran continuously at a moderate intensity for 60-75 min. Also, using high-intensity muscular contractions (strength exercise for more than one h duration), Riechman et al. (2004) observed a significant increase in plasma IL-15. Based on these findings, it is possible that the secretion of IL-15 is dependent on some interaction between total exercise time (or volume) and intensity. Clearly, more study is needed to sort this out.

5.2 Irisin

No significant effect of exercise on the plasma concentration of Irisin was observed in the current study. From the literature, baseline Irisin concentrations range from ~90-140 ng/ml (Huh et al., 2012, Huh et al., 2014, Tsuchiya et al., 2014, Daskalopoulou et al., 2014). With the present study the mean baseline and post-exercise values of Irisin concentration were approximately 10-fold less than published values. Due to the uniformity of this phenomenon and the fact that the standards from the ELISA kit produced values as expected, it may be that the low observed Irisin concentrations are the result of an error within the sampling process. Huh et al. (2012) point out Irisin sample concentrations are unaffected by freeze-thaw cycles, when stored at -80°C . The current Irisin samples were stored at -20°C initially for ~three weeks before being transferred to -70°C storage so, it is possible that some protein degradation occurred, thus reducing

the concentration of Irisin and perhaps affecting the sensitivity of the experiment to any changes that may have occurred, regardless of the exercise condition. Hecksteden et al. (2013) found that Irisin has a degradation rate of $0.184 \text{ ng}\cdot\text{ml}^{-1}\cdot\text{day}^{-1}$ when stored at -20°C . Therefore, although this may have contributed to some of degradation within our samples, it is unlikely that it is the entire cause for the low Irisin concentrations because if the degradation rate found by Hecksteden et al.(2013) occurred, it would have taken 62 wk to produce the values found within our samples. Obviously, something else was involved. Both SIE and high intensity exercise in general have been reported to produce 16-35% increases in plasma Irisin within 5 min of the completion of exercise (Huh et al., 2012, Huh et al., 2014, Daskalopoulou et al., 2014). In the current study, SIE caused no change in plasma Irisin concentration 5 min post exercise, but did show a 28% increase 3 h post-exercise. Although nonsignificant, this percent increase in plasma Irisin is similar to previous studies.

Tsuchiya et al. (2014), have suggested that there may be a decrease in plasma Irisin immediately after high intensity exercise (20 min treadmill running at $80\% \text{ VO}_{2\text{max}}$) followed by an increase in plasma Irisin 3 h post-exercise. The present increase observed with CE at three h post exercise was smaller (12%) when compared to SIE. As with IL-15 the present results were quite variable and with the small sample size it is possible that the lack of significant change was the result of a Type II error.

A significant increase in plasma Irisin 5 min post exercise was anticipated. However, no such increase in plasma Irisin was observed in either exercise condition. These findings are surprising in that the work load studied is similar or greater to those used in the literature. For example Huh et al. (2012), implemented three sets of two, 80 metre sprints with each set separated by 20 min of rest and reported a 16% increase in plasma Irisin. A second study investigating six, 50 metre maximal swim sprints with a mean completion time of ~ 32 sec observed a 30% increase in plasma Irisin, 5 min post exercise (Huh et al., 2014). The current SIE involved a similar exercise time (four, 30 sec exercise

bouts) as Huh et al. (2014) but two less exercise bouts. Consequently, it is possible that our sprinting protocol was below the required exercise duration to induce an Irisin response, i.e., two additional sets of sprints may have caused a significant change. The rest/recovery intervals within the Huh et al. (2014) were similar (5 min) to those of the current study, thus diminishing the likelihood that the current results were due to rest/recovery interval differences. It is also possible that the mode of exercise played an important role in the lack of an Irisin response. Both Huh et al. studies (Huh et al., 2012, 2014), used full-body exercises in the form of track sprinting and freestyle swimming sprints. These exercises likely engaged a larger muscle mass in comparison to our study which was mainly quadriceps dependent (cycling). Further, the convective properties of water may have influenced these results. Huh et al. (2014) used swimming sprints and the convective ability of water to draw heat away from the bodies of the subjects may have artificially lowered body temperature. In conjunction with FGF-21, Irisin has been found to induce non-shivering thermogenesis through the uncoupling of the electron transport chain (Lee et al., 2014). So with cooler body temperatures induced by water, Irisin may have been released into the bloodstream to contribute to non-shivering thermogenesis. Regardless, the results of the current study appear to show a reduced effect of exercise on plasma Irisin vs the literature.

The observation that resting Irisin is correlated to lean body mass is of particular interest. These findings are consistent with a previous study which found biceps circumference as a measure of lean body mass to be correlated positively with resting plasma Irisin (Huh et al., 2012). Together these data suggest that a major source of plasma Irisin is skeletal muscle. However, it cannot be determined that the source of Irisin is skeletal muscle alone because it could also originate from the motor nerve tissue permeating through skeletal muscle as suggested by Aydin et al. (2014). If correct, this could have contributed to the observed reduced Irisin response in the current study because less motor units would have been active during the cycling exercise used and thus less nerve tissue was active to contribute to an Irisin response.

Paradoxically, the correlation between circulating Irisin and lean body mass was no longer present post-exercise. It is possible that the production of Irisin may be a result of the basal metabolism of skeletal muscle. Therefore at rest, Irisin would be produced equally among all muscle tissue and be correlated to lean body mass. When exercising, it is likely that ATP is diverted away from homeostatic functions such as myokine production and shunted for the purpose of muscle fibre contraction. Thus, the linear relationship would be abrogated, producing the results that were observed within our current study.

The training history of the subject may have played a role in the results. Many of the subjects regularly participated in strength and resistance training and it could be thus assumed that they would have had a larger proportion of Type IIx muscle fibres. It has been shown that in untrained individuals, plasma Irisin is related to the proportion of Type I fibres (Ellefsen et al., 2014). However, the same study showed that the relationship between Irisin and Type I fibres was no longer present after 12 weeks of whole body resistance training. It is likely that the participants within the current study had approximately the same amount of resistance training profiled by Ellefsen et al., and thus plasma Irisin would not be related to their fibre type composition. Physical fitness appears to have no effect on the acute accumulation of Irisin or other myokines. Huh et al, 2014, found that there was no difference in the acute accumulation of Irisin after a treadmill run to exhaustion in relation to participant VO_{2max} . This suggests that the results of the current study was not dependent on the cardiorespiratory fitness of the subjects. Although the literature suggests this conclusion, it is imperative that for further studies a precise training history would be taken and that subjects would be matched on training history and VO_{2max} .

5.3 FGF-21

The present study has shown that there is a significant effect of SIE on the plasma concentration of FGF-21. At both 5 and 30 min post exercise, there was a significant increase in plasma FGF-21 concentrations. Further, at 30 min post exercise, the SIE response was significantly greater than CE. These findings

suggest that there is a physiological difference between SIE and CE relative to plasma FGF-21 accumulation. Finally, current baseline measures of FGF-21 compare favourably to published values at approximately the same daily time (10:00h, 50-60 ng/ml) (Lee et al., 2013). Peak FGF-21 concentrations occur at 0800h and reach a minimum at 1700h (Lee et al., 2013). Following this trend, the natural variation of plasma FGF-21 would be decreasing during the sampling period of the present study. Therefore, it is likely that the increase in plasma FGF-21 concentration was due to exercise and not the natural diurnal rhythm of FGF-21.

Moreover, it is likely that there is a dose-response relationship between exercise intensity and the subsequent plasma concentration of FGF-21 because SIE caused a significantly greater FGF-21 response in comparison to CE. These findings are in congruence with those of Kim et al. (2013), who found that 30 min of treadmill running at 80% VO_{2max} induced a 60% greater FGF-21 response than 30 min at 50% VO_{2max} . It is therefore plausible that as length and intensity of exercise increases, so too will the plasma concentration of FGF-21.

It is important to rule out any possible effect of reduced plasma volume that may be the underlying cause for the significant increase in plasma FGF-21 concentration within the sprint interval condition. There was a significant increase in hematocrit and thus a significant decrease in plasma volume within the sprint condition 5 min post-exercise. This is caused by the increase in intramuscular pressure due to exercise, causing an increase in mean arterial pressure and thus an efflux of fluid from the plasma compartment. Another important factor is the increase of osmotic pressure from the muscular compartment due to increased exercise metabolites within the muscle that had not reached equilibrium with the plasma. The change in plasma volume at 5 min post-sprint therefore influenced the concentration of FGF-21 and after correction for these plasma volume changes, no significant difference was found. 30 min-post exercise, there was no significant change in hematocrit or plasma volume, thus the increase in FGF-21

concentration at this time point is likely not attributable to any change in plasma volume.

Some data suggest that cardiorespiratory fitness can play a role in the determination of FGF-21 concentration, i.e., resting FGF-21 has been inversely related to VO_{2max} (Taniguchi et al., 2014). Therefore it is possible that the wide range of cardiorespiratory fitness of the present subjects ($35-61.1 \text{ ml}\cdot\text{O}_2^{-1}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) may have limited the absolute responses to exercise within each subject. This range of responses may have therefore confounded any other mean observable changes in FGF-21 concentration.

Since there was an observed increase in FGF-21 concentration with SIE, it is of interest to speculate about possible mechanisms behind this response to exercise. First, it is possible that FGF-21 is produced by contracting muscle, but the observed increase in plasma FGF-21 concentration could also be an indirect response to exercise. For example, Scalzo et al. (2014) showed that baseline FGF-21 concentration is not determined by basal sympathetic input, but will increase in response to hypoxia-induced sympathetic activation. When the hypoxia-induced sympathetic activation was inhibited by the administration of clonidine (a central acting adrenergic agonist), the subsequent increase in FGF-21 concentration was also eliminated (Scalzo et al., 2014). Therefore it appears that sympathetic activation is necessary for the accumulation of FGF-21 and that sympathetic activation during exercise may be a primary cause for the accumulation of FGF-21. Second, Kim et al. (2013) have shown that peak FGF-21 concentration is observed ~one h post-exercise and ~one h after peak blood FFA is reached. Cuevas-Ramos et al. (2012) have proposed that because the PPAR- α transcription factor (which plays a significant role in the regulation of the FGF-21 gene) is stimulated by FFA, it is lipolysis that causes the induction of FGF-21. If so, this could explain the latency (30 min -one h) in which peak FGF-21 concentrations are reached. Therefore, it may be that the increase in FGF-21 was due to the sympathetic activation and associated lipolysis of SIE. FGF-21 is highly expressed within the liver (Bae et al., 2014; Nishimura et al., 2000), as well

as its co-receptor, β -Klotho. As theorized by Cuevas-Ramos et al. (2012), FGF-21 is produced by the liver in response to increasing FFA to increase lipid metabolism in order to prevent the ectopic deposition of lipids that had been liberated as a result of exercise. Therefore from these findings, it is possible to suggest that FGF-21 can be used as a surrogate measure for sympathetic activation and lipolysis associated to exercise. This theory may also help explain the dramatic fat loss associated with regular SIT, in that a greater amount of lipolysis may occur within each acute bout of training, as exemplified by relative increases in circulating FFA.

5.4 Limitations

There are a few important limitations to this study that need to be addressed. As mentioned, the study itself was relatively underpowered. Consequently, it is possible with a sample size of 9 that the statistical analyses were subjected to potential Type II errors. The present ELISA results also proved to be highly variable. This needs to be eliminated. Attempts were made to minimize the errors made in the IL-15 sample preparation with the Irisin and FGF-21 analyses, but additional steps for sample preparation should be taken including: 1) pre-chilling EDTA tubes with ice baths before taking blood samples and 2) consistent freezer storage at -80°C . In the present study, some samples were stored at -20°C and then transferred to the colder freezers. It is possible that there was a degree of protein degradation due to storage temperature that may have affected the absolute values of myokine concentrations within our samples. These limitations make definitive conclusions regarding the acute effect of exercise mode on plasma myokine accumulation difficult.

Further, into the future, it is important to test the effect of SIT to determine whether any training adaptations alter the acute response of FGF-21 to SIE. It is possible that the observed changes within the current study may be altered with training and this needs to be determined.

5.5 Future Directions

Based on the current findings, further studies on the effect of SIE on the accumulation of FGF-21, Irisin, and IL-15 in plasma are warranted due to the errors made with sampling. Second, it is necessary to determine if the acute SIE is abrogated by the administration of clonidine to determine if the secretion of FGF-21 is dependent entirely on the sympathetic activation of exercise. Third, FFA could also be measured with SIE to document any relationship between circulating FFA and plasma FGF-21. Fourth, it would be of interest to determine whether PPAR- α is up-regulated by acute SIE to determine the molecular regulators and mechanisms behind the production of FGF-21 after exercise. Fifth, SIT studies should be completed to determine if the acute effect of SIE on plasma FGF-21 is altered with training. Sixth, assessing different SIE modalities, including running, swimming, cycling and strength exercise would be of interest to determine how plasma myokine concentration is affected by exercise modality. Seventh, increasing work volume studies should be investigated because if IL-15 and Irisin are dependent on a threshold volume of exercise, it might not have been met with the current study. This could be accomplished through additional sprint sets or alternatively, using a pre-determined CE protocol i.e. (15 min at 60% VO_{2max}) to then determine the ~number of sprints to match work outputs. It is also important to match the subjects based on training history to determine if there is any effect on the acute accumulation of myokines. This way any variability based on fibre type composition may be eliminated. Eighth, assessing the capture of myokines against a known concentration. This would require spiking a blood sample with a known concentration and processing it with the exact same aliquoting and storage procedure. This will help determine to what extent the yield from the blood sampling was accurate for our results. Finally, muscle biopsies might be utilized to determine if the change in IL-15 is localized more directly within the muscle tissue for paracrine/autocrine signaling, rather than serving as an endocrine molecule. This may apply to myokine protein production or perhaps the induction of its respective mRNA. It is not possible to conclude that the total

change in plasma myokine concentration is attributable to production alone because plasma concentration is a net result of production and degradation. Microdialysis may be one technique that could be used to determine the production of myokines. Acting similarly to a capillary bed, microdialysis probes are capable of measuring solutes flowing directly out of a tissue. The implementation of this technique would allow for the determination of myokine production directly from the tissue without interaction with the plasma.

5.6 Summary and Conclusion

Nine young, physically active men completed four, 30 sec sprints and a work-matched continuous interval bout at 65% VO_{2max} on an electromagnetically braked cycle ergometer. Changes in plasma concentration of three myokines were measured via ELISA assays: IL-15, Irisin and FGF-21. In contrast to the experimental hypothesis, IL-15 and Irisin showed little effect of either exercise treatment; however, plasma FGF-21 was elevated significantly with SIE. Specifically, at 5 ($p=0.039$) and 30 min ($p<0.001$) post-SIE, plasma FGF-21 was increased. At 30 min post exercise, this observed FGF-21 increase was greater vs the work-matched CE condition ($p=0.03$).

These results suggest that SIE elicits a physiologically different response to exercise when compared to CE at least with FGF-21. They also indicate that per joule of work, sprint interval exercise is capable of generating a significantly greater systemic FGF-21 response. Moreover, elevated FGF-21 may indicate that there is a greater fatty acid release during SIE because FGF-21 may be a surrogate measure of sympathetic activation during exercise. Therefore, it is possible that the fat loss associated with SIT may be related directly to the amount of FFA generated during and following each acute sprint interval training session.

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Appendices

Appendix A: Human Ethics Approval



Research Ethics

Western University Health Science Research Ethics Board HSREB Full Board Initial Approval Notice

Principal Investigator: Dr. Peter Lemon
Department & Institution: Health Sciences/Kinesiology, Western University

HSREB File Number: 105614
Study Title: Effect of Exercise Intensity on the Secretion of Muscle-Derived Signalling Proteins
Sponsor:

HSREB Initial Approval Date: December 15, 2014
HSREB Expiry Date: December 15, 2015

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Instruments	PAR-Q Questionnaire	
Data Collection Form/Case Report Form		
Letter of Information & Consent		2014/12/25
Western University Protocol	including the study schedule	2014/12/26
Recruitment Items		2014/12/26

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review. If an Updated Approval Notice is required prior to the HSREB Expiry Date, the Principal Investigator is responsible for completing and submitting an HSREB Updated Approval Form in a timely fashion.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

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Appendix B: Letter of Information and Informed Consent



Title of Study: The Effect of Exercise Intensity on the Secretion of Muscle-Derived Signaling Proteins

Principal Investigator: Dr. Peter W.R. Lemon (PhD)
Graduate Student: Blair Segsworth (B.Sch.)

Exercise Nutrition Research Laboratory (Room 2235 – 3M Centre)
School of Kinesiology, Western University.

LETTER OF INFORMATION AND CONSENT

INVITATION TO PARTICIPATE

You are being invited to participate in research study at the Exercise Nutrition Research Laboratory (Room 2235, 3M Centre) investigating the effects of Sprint Interval exercise (SIE) on the secretion of muscle-derived signalling proteins.

PURPOSE OF THE LETTER

The purpose of this letter is to provide you with information required for you to make an informed decision regarding participation in this research.

PURPOSE OF THIS STUDY

In recent years, skeletal muscle (a type of muscle that causes movement) has been shown to be capable of secreting signaling proteins to various tissues throughout the body. Signalling proteins are substances that are released from muscle during exercise that are used to communicate with other parts of the body. It is believed that these secreted proteins, called myokines, are important mediators of several beneficial effects of exercise, including fat metabolism, muscle growth and the cardioprotective (protect the heart) effects of exercise.

Sprint interval exercise or SIE has been popularized as an efficient type of exercise in terms of rapidly changing body composition and cardiovascular fitness. SIE is an

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exercise strategy alternating periods of short high intensity physical activity and recovery. Research on SIE has shown that it has a reduced total time commitment (up to 30 minutes) and is capable of reducing body fat percentage and increasing maximum oxygen consumption relative to traditional endurance exercise (e.g., 1-2 hours). SIE varies from traditional endurance exercise in that it involves greater exertion to produce the same energy output as endurance exercise, but within a smaller timeframe. Traditional endurance exercise is commonly characterized as a jog or moderate run up to about an hour. Therefore, SIE vs. Traditional endurance exercise is like compressing the energy spent on a long run into a few sprints with rest in between. As myokines have been previously shown to mediate some of the beneficial effects of exercise, we would like to see if the benefits of SIE are also mediated by a difference in myokine secretion, relative to traditional endurance exercise.

INCLUSION CRITERIA

In order to be eligible to participate in this study you must be a healthy, 18 to 35 year old man.

EXCLUSION CRITERIA

You will be excluded from this study if you:

- You cannot have undergone a SIE regimen within the previous 2 months.
- Smoke
- Have symptoms or take medication for respiratory, cardiovascular, metabolic, neuromuscular disease
- Been diagnosed with a cognitive impairment and/or learning disability
- Use any medications with side effects of dizziness, lack of motor control, or slowed reaction time
- Use any other dietary supplements (excluding protein powder)
- Have a history of concussion/head injuries.
- Have an excessive alcohol intake (>2 drinks/day)

STUDY OUTLINE:

All study activities will be completed in the Exercise Nutrition Research Laboratory (Room 2235, 3M Centre) ***For the test days, you will be asked to arrive at the laboratory at 8 am following an overnight fast (no food or drink except water after 10pm). You will also be asked to refrain from exercise and from consuming caffeine or alcohol for 24 hours prior to your study visits. You will be given a standardized pre-fast meal of 2g/kg of carbohydrate to ensure you will have sufficient energy for the next day of testing ***

STUDY PROCEDURES

If you volunteer to participate in this study, we will ask you to do the following things:

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1. You will visit the lab for two familiarization sessions prior to the study days. The familiarization sessions will be held within one week of each other and the final session will be held no less than one week before the initial trial day. The first session will involve filling out a physical activity readiness questionnaire to ensure your safe participation in the study as well a body composition measurement and a maximum oxygen consumption test. The second familiarization session will be used to acclimate you to the SIE trial day. The total time required for the two familiarization sessions will be ~2h.
2. For the study sessions you visit the laboratory on two additional occasions. Both will involve an exercise bout (either endurance or SIE). The order of these two exercise protocols needs to be equated so some will do the endurance bout first while others will complete the SIE bout first and all will do both. For the SIE visit you will complete 4 Wingates [Wingates are an all-out sprint exercise (maximum intensity) for 30 seconds]. You will be required to do 4 Wingate bouts. In between each bout you will have 4 minutes of rest. For the endurance exercise visit you will complete an exercise session of approximately 10 minutes (the duration will be determined by the amount of time is needed to match the work output of the SIE session).
3. We will collect 4 ml (3/4 teaspoons) of blood pre-exercise, immediately post-exercise, 0.5 hours, 1.5 hours and 3.0 hours after each exercise session. Therefore, we will collect a total of 20 ml (4 teaspoons) of blood during the first study visit and another 20 ml for the second study visit for a total of 10 samples (40 mls 8 teaspoons) over a 2-week period. Your blood will be used to determine the quantity of myokines that are dissolved within the liquid fraction of your blood. This is known as the blood myokine concentration. This will help us determine the amount of myokines that are being released from your muscle tissue.

Familiarization sessions: Before your inclusion in the study sessions, you will be asked to fill out a physical activity readiness questionnaire and a participant information form for personal and familial health history. These evaluation sessions will be held within the same week of one another and the final session will be no less than one week before the exercise trial days. Additionally, during the first familiarization session you will have your body composition determined via BodPod® and maximal oxygen consumption test on a stationary bicycle. The BodPod® is a chamber which determines body volume by measuring the space your body takes up and together with your body weight allows us to calculate muscle, bone, and body fat content. The maximal oxygen consumption test involves a continuous, incremental test on a stationary bike until you reach volitional fatigue. During the second familiarization session you will conduct four, 30 second Wingate sprints in succession, each sprint being followed by a four minute rest period.

Test day 1 (Total duration is approximately 5 hours): You will arrive at the lab at 8:00 am with limited activity (drive/use of the elevator to get to the lab). This strategy is used to ensure that you are in a rested physiological state and thus will not affect any baseline measurements of blood myokine concentration.

At 8:05 am, we will serve you a standardized breakfast and you will be allowed time to read/study. This meal will provide 4kcal/kg of bodyweight for energy and will consist of a peanut butter/hazel nut spread sandwich (allergy status considered) and orange juice.

- At 9:30 am, 4 ml (3/4 teaspoon) of blood will be collected.
- At 10:00 am, you will be given 5 minutes to warm up on the stationary bike.
- At 10:05 am, you will perform sprint interval exercises (four, 30s Wingates each separated by 4 minutes of passive rest).
- At 10:40 am, 4ml (3/4 teaspoon) of blood will be collected.
- At 11:10 am, 4ml (3/4 teaspoon) of blood will be collected.
- At 11:15 am, you will eat a standardized lunch. Lunch will provide 2kcal/kg of energy in the form of a small sandwich and orange juice.
- At 12:15 pm, 4ml (3/4 teaspoon) of blood will be collected from you..
- At 1:00 pm, you will eat a standardized snack, consisting of a fruit cup and juice at 1kcal of energy per kg of bodyweight.
- At 1:45 pm, 4ml (3/4 teaspoon) of blood will be collected from you.
- At 1:55 pm, end of the first test session.

Test day 2 (Total duration is approximately 5 hours): This visit will take place one week after the first study visit. You will perform the same study procedures you did during the first study visit except the sprint interval exercise will be replaced with a continuous endurance session.

1. POSSIBLE RISK AND HARMS

This study involves strenuous exercise that may pose a risk of muscle injury or soreness. This is not unusual with unaccustomed exercise and generally is minor. All exercise involves some health risk (primarily cardiovascular or hydration-related). Importantly, similar exercise to that used in this study is completed by kinesiology students and Mustang athletes daily. Further, the risks of cardiovascular complications are much reduced in young, healthy individuals. Finally, you will be encouraged to drink enough water to stay hydrated.

Maximal oxygen consumption test: you may experience muscle fatigue, discomfort, dizziness and/or nausea.

Blood Collection: the needle stick may cause discomfort and possibly some residual soreness and minor bruising of the skin. There is also a low risk of infection. Infrequently, the procedure causes someone to faint or infections may occur when proper blood handling techniques are not used.

POTENTIAL BENEFITS

Participating in this study may give you some insight in to your exercise capacity by completing the fitness assessment.

COMPENSATION

You will not be compensated for your participation in this study. You will not be reimbursed for additional costs such as parking or transportation.

VOLUNTARY PARTICIPATION

Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time with no effect on your future academic or employment status. The investigator may withdraw you from this research if circumstances arise which warrant doing so.

RIGHTS OF A PARTICIPANT (in the event of a study related injury)

If you suffer any study related injury during your participation in this study care will be provided to you at no cost.

CONFIDENTIALITY

If you agree to join this study, only members of the study team will look at your personal information (e.g., name) and collect only the information they need for the study.

The information that is collected for the study will be kept in a locked and secure area by the study doctor for 5 years. Only the study team or the people or groups listed below will be allowed to look at your records.

Representatives of the University of Western Ontario Health Sciences Research Ethics Board may look at the study records and at your personal health information to check that the information collected for the study is correct and to make sure the study followed proper laws and guidelines.

All information collected during this study, including your personal health information, will be kept confidential and will not be shared with anyone outside the study unless required by law. You will not be named in any reports, publications, or presentations that may come from this study.

If you decide to leave the study, the information about you that was collected before you leave the study will still be used in order to answer the research question. No new information will be collected without your permission.

CONTACT FOR FURTHER INFORMATION

If you have any questions about this research project, feel free to call us (Dr. Peter Lemon _____ or Blair Segsworth _____) for clarification. Further, if you have any questions about the conduct of this study or your

rights as a research subject you may contact the Office of Research Ethics at Western University at 519-661-3036 or at ethics@uwo.ca.

Consent Form

The Effect of Exercise Intensity on the Secretion of Muscle-Derived Signaling Proteins

Investigators: Dr. Peter W.R. Lemon and Blair Segsworth, B.ScH.

I have read the accompanying "Letter of Information", have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

If you wish to participate in future studies in the Exercise Nutrition Research Lab, the research team will collect your contact information.

I wish to be contacted for future studies in the Exercise Nutrition Research Laboratory.

Yes _____ (check mark), No _____ (check mark) Date: _____

By signing below, I agree to participate in this study.

Name of Participant (please print): _____

Signature of Participant: _____

Date: _____

Name of Person Obtaining Informed Consent: _____

Signature of Person Obtaining Informed Consent: _____

Date: _____

You will receive a copy of the consent form after it has been signed. You do not waive any legal rights by signing the consent form.

This letter is for you to keep for future references.

Sincerely,

Dr. Peter Lemon

Blair Segsworth, B.ScH.

25/11/14

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Appendix C: Participant Health Information Form

Participant Health Information Form

Participant I.D.: _____ Date: _____
 Age: _____ Height: _____ Weight: _____ Smoker: **Yes / No**
 Ethnic Background: _____

Medical History (please check any and all that apply)

Family history of heart disease	<input type="checkbox"/>	Diabetes	<input type="checkbox"/>
Heart murmur	<input type="checkbox"/>	Endocrine disorder	<input type="checkbox"/>
Phlebitis	<input type="checkbox"/>	Raynaud's syndrome	<input type="checkbox"/>
Other heart disorder (please specify)	<input type="checkbox"/>	Seizures	<input type="checkbox"/>
_____		Digestive/gastrointestinal problems	<input type="checkbox"/>
Family history of stroke	<input type="checkbox"/>	Asthma	<input type="checkbox"/>
Migraines	<input type="checkbox"/>	Bronchitis	<input type="checkbox"/>
Sinus problems	<input type="checkbox"/>	Other respiratory disorder (please specify)	<input type="checkbox"/>
Hypertension	<input type="checkbox"/>	_____	

Have you ever fainted? **Yes / No**
 If yes, under what circumstances:

Are you taking any medications? **Yes / No**

If yes, please specify: _____

Have you had any major surgeries, illnesses or injuries? **Yes / No**

If yes, please specify (include dates): _____

Have you had a concussion or serious head injury? **Yes / No**

If yes, please specify (include dates): _____

Do you consume alcohol or any caffeinated beverages on a regular basis? **Yes / No**

If yes, please specify the quantity: _____

Do you consume any dietary supplements (including protein powder)? **Yes / No**

If yes, please specify the type, frequency, and typical daily dose: _____

Appendix D: Physical Activity Readiness Questionnaire

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

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

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

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

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

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

**If
you
answered**

YES to one or more questions

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

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

NO to all questions

If you answered NO to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.

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

DELAY BECOMING MUCH MORE ACTIVE:

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

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

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

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

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

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

NAME _____

SIGNATURE _____

DATE _____

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

WITNESS _____

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



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Curriculum Vitae

- Name:** Blair Segsworth
- Post-secondary Education and Degrees:** University of Guelph
Guelph, Ontario, Canada
2008-2012 B.Sch.
- The University of Western Ontario
London, Ontario, Canada
2014-2015 M.Sc
- Honours and Awards:** Ontario Graduate Scholarship
2014-2015
- Western Graduate Research Scholarship
2014-2015
- Related Work Experience** Graduate Teaching Assistant
The University of Western Ontario
2014
- Publications:** O'Brien, L. D., Wills, K. L., Segsworth, B., Dashney, B., Rock, E. M., Limebeer, C. L., & Parker, L. A. (2013). Effect of chronic exposure to rimonabant and phytocannabinoids on anxiety-like behavior and saccharin palatability. *Pharmacology, Biochemistry, and Behavior*, 103(3), 597–602. doi:10.1016/j.pbb.2012.10.008