

MELLICK, PAUL F., Ph.D. The Effect of High-Intensity Intermittent Exercise and Carbohydrate Supplementation on Plasma Visfatin (2013)  
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Plasma visfatin has been proposed to act as an insulin-mimetic hormone and to enhance glucose uptake. However, research regarding its insulin-like properties has been highly variable. Furthermore, its response to exercise, carbohydrate ingestion, and its overall relationship with blood glucose is not fully understood. PURPOSE: To examine the effect of high-intensity intermittent exercise and a carbohydrate supplementation (CHO) on plasma visfatin. METHODS: On 2 days separated by at least 3 days, 10 sprint-trained male subjects (age =  $26.4 \pm 5.3$  yrs; Ht =  $1.77 \pm 0.03$  m; Wt =  $78.78 \pm 9.10$  kg; BF% =  $13.96 \pm 7.28\%$ ) completed 4 bouts of cycling (3 min each), at 50% of mean anaerobic power (based on a Wingate Test), with 6 min of rest between bouts. On the CHO day, subjects ingested 50g of CHO 30 min prior to exercise. On the control day, subjects ingested a sugar free artificially sweetened drink (CON) 30 min prior to exercise. Blood was drawn prior to supplement ingestion, 15 minutes before exercise, before and after each bout of exercise, and 15 and 30 minutes post exercise. Plasma visfatin, blood glucose, and plasma insulin were determined. Body composition and truncal fat were also assessed (DXA; Prodigy Advanced, GE Lunar). RESULTS: Visfatin was not significantly different between treatments (CHO vs CON) at any time point ( $p = 0.163$ ), and was not significantly altered by exercise ( $p = 0.692$ ). Insulin [ $25.65$  vs  $8.35$  mU/l, CHO vs CON, respectively] and glucose [ $138.57$  vs  $98.10$  mg/dl, CHO vs CON, respectively] were both elevated after CHO ingestion and remained elevated throughout the first half of exercise. Baseline visfatin was significantly correlated with truncal fat ( $r^2 = 0.6162$ ,  $p < 0.05$ ), but was not correlated with glucose or insulin. CONCLUSION: Although visfatin was correlated to truncal fat in healthy, sprint-

trained males, it was not altered by high-intensity intermittent exercise or CHO supplementation.

THE EFFECT OF HIGH-INTENSITY INTERMITTENT EXERCISE AND CARBOHYDRATE  
SUPPLEMENTATION ON PLASMA VISFATIN

by

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## CHAPTER I

### INTRODUCTION

One in 10 Americans suffers from type 2 diabetes mellitus (T2D), which has caused annual health care costs to exceed 174 billion dollars (Ong, Cheung et al. 2008). T2D is a result of several compounding problems, one of which is decreased sensitivity of the insulin receptors both in the periphery and in the pancreas. This decreased peripheral sensitivity is partially due to decreased phosphorylation of certain regulatory proteins involved in the cellular signaling pathway (Reaven 1992). A physiological result of this decreased sensitivity of insulin receptors is a decrease in cellular glucose uptake and by extension, elevated blood glucose concentrations. Additionally, there is a decrease in amino acid uptake if insulin is not working as well. Exercise has been shown to counteract this diabetic chain of events by increasing glucose uptake via muscle contraction (independent of insulin) and by increasing insulin action through an increase in phosphorylation of certain regulatory proteins of the insulin signaling pathway (Hawley and Lessard 2008) which can also influence protein synthesis.

In 2005, Visfatin was shown to increase glucose uptake independently of insulin when administered to mice and in cell cultures of adipose tissue (Fukuhara, Matsuda et al. 2005). The possibility that visfatin may play a role in the regulation of glucose, makes it a viable target as a treatment for T2DM. An inability to regulate blood glucose due to decreased insulin action may be attenuated by visfatin. The extent to which visfatin acts on glucose uptake is unknown as are the complexities of its relationship with insulin and blood glucose levels. In order to know more about the effect of visfatin on glucose

uptake and metabolism, it is necessary to examine it during conditions of altered blood glucose.

Visfatin was originally discovered as a cytokine that was highly expressed in bone marrow, liver, and muscle tissue (Samal, Sun et al. 1994). It was originally shown to facilitate the colonization of B cells. It is important to note that while visfatin has been shown to bind to the insulin receptor in one study but not in the same place as insulin (Fukuhara, Matsuda et al. 2005), no other receptor interaction has been shown. Its mode of action is not currently understood.

In addition to its proposed metabolic properties, visfatin is elevated in individuals who are obese or have T2D (Arner 2006; Dedoussis, Kapiri et al. 2009). A recent meta-analysis of the visfatin literature showed that visfatin is elevated in both T2D and type I diabetes (Chang, Chang et al. 2011) and visfatin is elevated in obese, non-diabetic, hyperglycemic individuals (Tilg and Moschen 2008). In summary, visfatin has been shown to have a link to both obesity and diabetes.

Recently, several studies have shown visfatin to play a role in glucose homeostasis. Its specific role is debated, but it has been positively correlated with changes in blood glucose and insulin after high-intensity sprint exercise (Ghanbari-Niaki, Saghebjoor et al. 2010), as well as enhanced insulin signaling through its action on the insulin receptor (Fukuhara, Matsuda et al. 2005; Xie, Tang et al. 2007). Visfatin was first discovered as a mediator of glucose homeostasis by Fukuhara (2005). In this study, visfatin administration was shown to improve glucose uptake by influencing insulin signaling via the phosphoinositide-3-kinase (PI3K) pathway in obese mice. While this study was retracted shortly after publication for reasons unrelated to these findings, other studies have also implicated visfatin as an insulin-mimicking hormone (Xie, Tang

et al, Song, Lee et al. 2008). Improved glucose uptake via visfatin administration has also been shown *in vitro* in human osteoblasts (Xie, Tang et al. 2007) and mesangial cells (Song, Lee et al. 2008).

While the aforementioned studies have focused on the glucose-mediating properties of visfatin, several studies have also identified visfatin as a pro-inflammatory marker (Romacho, Azcutia et al. 2009; Friebe, Neef et al. 2011). As previously mentioned, visfatin is elevated in obese subjects. Since obesity has been identified as a state of chronic low grade inflammation, the elevated plasma visfatin seen with obesity may be partially related to chronic inflammation. In addition, two key inflammatory markers (IL-6 and TNF- $\alpha$ ) have been shown to directly influence plasma visfatin levels and visfatin mRNA within adipose tissue (Frydelund-Larsen, Akerstrom et al. 2007; Sheu, Chang et al. 2008).

There is some debate as to whether visfatin is more strongly correlated to visceral adipose tissue or subcutaneous adipose tissue, but visfatin has been shown to be strongly correlated with overall body fat (Fukuhara, Matsuda et al. 2005; Chang, Chang et al. 2010). Although visfatin does appear to be released primarily from adipose tissue, it has also been shown to be released from liver and muscle tissue (Samal, Sun et al. 1994).

The effects of exercise on visfatin have been varied. Previous studies have shown plasma visfatin levels to be decreased after 45 minutes of moderate intensity exercise and these lower levels were correlated with decreases in blood glucose (Jurimae, Ramson et al. 2009; Koltai, Szabo et al. 2010; Lee, Shin et al. 2010). Conversely, one study has shown an increase in plasma visfatin after high intensity sprint exercise (Ghanbari-Niaki, Saghebjoos et al. 2010). The reason for this disparity in

the exercise-induced visfatin response is likely due to differences in exercise intensity, subject characteristics, and could also reflect the simultaneous alterations in blood glucose and muscle glycogen that coincide with these different exercise intensities.

High intensity intermittent exercise has been shown to elicit acute increases in insulin levels and to increase glucose uptake (MacDougall, Ward et al. 1977). The magnitude of these changes is dependent on the intensity of the exercise. While visfatin was shown to be elevated with high intensity sprint exercise, its relationship with exercise-induced alterations in blood glucose has only been examined in one study (Ghanbari-Niaki, Saghebjojo et al. 2010). They noted elevated glucose and insulin levels after sprint exercise (n =6 males) were accompanied by increased plasma visfatin. However, there is no research regarding the relationship between visfatin and carbohydrate supplementation. In order to fully understand the metabolic role of visfatin, it is necessary to determine its response to carbohydrate supplementation. If visfatin does act in a matter similar to insulin, then carbohydrate supplementation should lead to an increase in plasma visfatin.

In a pilot study (unpublished) of the proposed methods for this proposal, intermittent exercise performed at 50% of absolute mean power (as determined by a Wingate test) for 3-minute intervals after 50g of carbohydrate supplementation using cycle ergometry in highly trained subjects (n=2 males), caused a significant decrease in blood glucose and plasma visfatin during exercise with a concomitant increase in both during a recovery period. When compared to a control (exercise session without carbohydrate supplementation), the change in blood glucose and visfatin was markedly less. Ghanbari-Niaki et al. (2010) reported a similar response using sprint exercise.

These data suggest that high intensity exercise followed by an appropriate recovery will elevate blood glucose above resting levels during recovery; the main source of blood glucose is likely from the liver (MacDougall, Ward et al. 1977). This increase in blood glucose is likely used to replenish muscle glycogen after high intensity exercise (Costill and Hargreaves 1992).

In summary, visfatin has been shown to be elevated in one study after high intensity exercise, decreased after low intensity exercise, and elevated in obese individuals, and diabetics. Additionally, visfatin is strongly correlated to overall body fat. One mechanism for further elucidating the metabolic action(s) of visfatin, is to simultaneously investigate the exercise-induced visfatin response and the corresponding alterations in blood glucose.

## **Purpose**

The primary purpose of this study is to examine the response of plasma visfatin to high intensity intermittent exercise and to assess how that response is affected by carbohydrate supplementation.

## **Specific Aims and Hypotheses**

Specific Aim #1: Determine the response of plasma visfatin to high intensity intermittent exercise.

H1: High intensity intermittent exercise will increase plasma visfatin.

Specific Aim #2: Determine the effect of carbohydrate supplementation on the response of visfatin to high intensity exercise.

H2: Carbohydrate supplementation will augment the effect of exercise on plasma visfatin.

Specific Aim #3: Determine if plasma visfatin is correlated to truncal fat in trained young males.

H3: Plasma visfatin will be positively correlated with truncal fat in trained young males.

### **Limitations**

- 1.) One limitation of the current study is that the proposed glucose load is not guaranteed to offset the exercise-induced decrease in blood glucose in all subjects. The proposed glucose load is based on recent data from Kraemer and colleagues (Kraemer, Francois et al. 2011) which will be discussed in further detail in chapter 3.
- 2.) All blood draws will be done at an antecubital vein, so blood draws may not be completely indicative of what is taking place at the working muscle.
- 3.) Conclusions drawn from this study may not be generalizable due to a limited age range and the use of highly trained subjects.

### **Delimitations**

Subject age (18-30), gender (male), training status (sprint-trained), and health (apparently healthy), will all be controlled to limit between-subject variability.

### **Definition of Terms**

1. Pre-B Cell Colony Enhancing Factor (PBEF) - The original name given to visfatin based on its action in the inflammatory process.

2. Nicotinamide Phosphoribosyltransferase (NAMPT) - Another term for visfatin based on its enzymatic action in the NAD<sup>+</sup> salvage pathway.
3. Visfatin – A possible insulin-mimicking hormone found in body fat, muscle tissue, and liver tissue.
4. Phosphoinositide-3-Kinase (PI3K) – Enzyme with specific actions in the insulin-signaling pathway to facilitate GLUT4 translocation
5. Interleukin-6 (IL-6) – Cytokine involved in acute inflammatory phase; also released from muscle during exercise.
6. Insulin Receptor Substrate 1 (IRS1) – Insulin receptor substrate protein with a phosphotyrosine binding domain. Phosphorylation leads to GLUT4 translocation
7. Glucose Transporter type 4 (GLUT4) – Insulin-regulated protein responsible for translocation of glucose into the cell.
8. Nicotinamide Adenine Dinucleotide (NAD<sup>+</sup>) – Coenzyme responsible for carrying electrons.
9. Sirtuin Proteins (SIRT) – Proteins with deacetylase or ribosyltransferase activity.
10. Absolute Mean Power (AMP) – Average power output over 30 seconds during a Wingate test.
11. Adenosine Monophosphate-Activated Kinase (AMPK) – Energy sensing enzyme which plays a key role in exercise-induced increases in glucose uptake.
12. Mesangial Cells – Specialized smooth muscle cells which control renal capillary blood flow.

## CHAPTER II

### REVIEW OF LITERATURE

#### **Visfatin, NAMPT, and PBEF**

Visfatin is a 52 kilodalton cytokine which is transcribed in bone marrow, liver, muscle, and adipose tissue. It was originally coined as pre-B-cell colony enhancing factor (PBEF) because it was found to synergize the pre-B-cell colony formation activity of stem cell factor and interleukin 7 (Samal, Sun et al. 1994). The 3' untranslated region of visfatin mRNA has multiple TATT motifs which makes it similar to many inflammatory cytokine mRNA constructs. This suggests that the final protein structure of visfatin is similar to that of other inflammatory cytokines. Visfatin has a hydrophobic amino terminus with six cysteine residues. Additionally, it has 2 sites for asparagine glycosylation, four potential protein kinase C phosphorylation sites, and five creatine kinase 2 phosphorylation sites. Protein kinase C includes a variety of isoenzymes which serve multiple functions. In relation to this study, novel PKC's (particularly PKC $\theta$ ) have been shown to be stimulated by free fatty acid accumulation, inhibit insulin receptor substrate 1 (IRS1) function and are elevated in insulin-resistant individuals. Conversely, atypical PKC's (e.g. PKC $\lambda$ ) have been shown to increase GLUT4 translocation. The presence of potential protein kinase C phosphorylation sites on visfatin suggests that it may be influenced by these signaling proteins which are part of the insulin signaling pathway. The presence of creatine kinase phosphorylation sites may further indicate a possible metabolic role of visfatin, as creatine kinase is an energy-sensing enzyme which facilitates the production of ATP and creatine from creatine phosphate and ADP.

Plasma visfatin has been shown to be upregulated in states of inflammation (Tilg and Moschen 2008; Li, Yang et al. 2009; Chang, Chang et al. 2010; Friebe, Neef et al. 2011), hyperglycemia, and obesity (Fukuhara, Matsuda et al. 2005; Frydelund-Larsen, Akerstrom et al. 2007; Brema, Hatunic et al. 2008; Haus, Solomon et al. 2009). The preprotein lacks a typical signal sequence for secretion; however, this does not preclude its secretion from tissue. Visfatin has been found in the conditioned medium of multiple cell types as well as the blood, suggesting that it is secreted. Research is lacking, but it has been suggested that visfatin is secreted through a non-classical secretory pathway (Revollo, Korner et al. 2007).

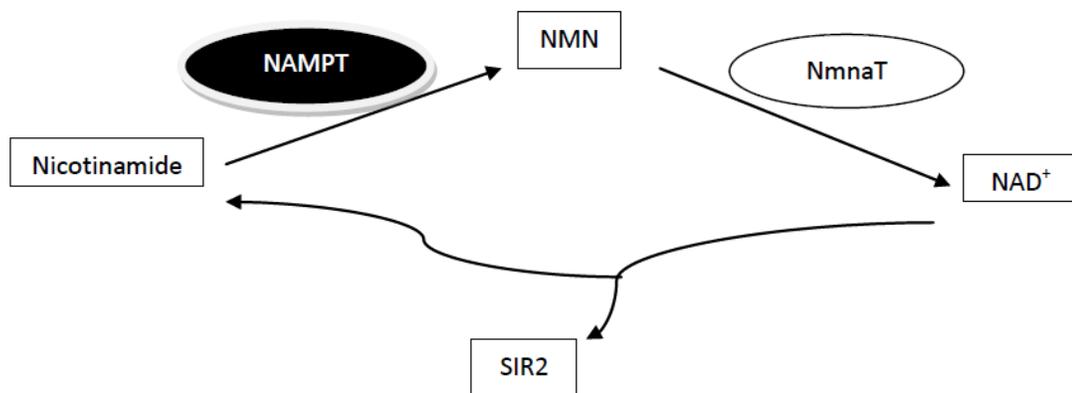
Visfatin also acts as a rate-limiting enzyme in the NAD biosynthetic pathway (Revollo, Grimm et al. 2004). Visfatin has been shown to catalyze the conversion of nicotinamide and phosphoribosylpyrophosphate (PRPP) into nicotinamide mononucleotide (NMN). Following this step, NMN is then converted to NAD<sup>+</sup>. Because of this activity, Revollo and colleagues (2004) coined visfatin as nicotinamide phosphorybiosyltransferase (NAMPT). This pathway is shown in Figure 1.

In 2005, Fukuhara and colleagues found that PBEF is preferentially expressed in the visceral fat of humans when compared to subcutaneous fat of the same subjects and renamed it visfatin. In addition, they found visfatin to act as an insulin-mimetic by binding to the insulin receptor at a site different from insulin. Lastly, independent of insulin binding, visfatin caused autophosphorylation of the beta subunits of the receptor. The specifics of visfatin's proposed insulin-sensitizing abilities will be discussed in the next section.

In summary, this protein has many possible actions and as a result of its association with numerous physiological pathways, it has been given three different

names. However for consistency, in the current review of the relevant literature, the term visfatin will consistently be used regardless of which name was used in the publication cited.

**Figure 1. NAD Biosynthesis Pathway.** NAMPT represents the rate-limiting enzyme of the pathway. NAMPT – Nicotinamide phosphoribosyltransferase, NMN – Nicotinamide mononucleotide, NmnaT – Nicotinamide mononucleotide adenylyltransferase, SIR2 – Sirtuin Protein



### Visfatin as an Insulin Sensitizer

The first study which suggested that visfatin had a possible insulin-sensitizing role was completed by Fukuhara et al (2005). They found that an injection of recombinant visfatin into C57BL/6J obese mice lead to an insulin-independent increase in glucose uptake with a concomitant decrease in blood glucose. Blood glucose was further decreased as the visfatin dose was increased. Furthermore, this effect was unchanged in type II diabetic mice and when insulin production was inhibited by streptozotocin in healthy mice. Through western blotting, they found that this increase in glucose uptake was related to an increase in phosphoinositide-3-kinase (PI3K) activity, and insulin receptor substrate (IRS) activity. This was the first study to examine the metabolic effects of visfatin. Fukuhara and colleagues (2005) concluded that visfatin

has the potential to act as an insulin-mimetic and play a significant role in insulin sensitivity. In 2007, this study was retracted due to disputes regarding the human adipose tissue used to determine a correlation between body fat and visfatin (Fukuhara, Matsuda et al. 2007). Their findings concerning visfatin's correlation to visceral fat were later refuted, but the glucose-mediating properties of visfatin were supported in other studies (Xie, Tang et al. 2007; Song, Lee et al. 2008).

The first study to support the findings from Fukuhara and confirm that visfatin had an insulin sensitizing effect was completed by Xie and colleagues (2007). In this study, cultured human osteocytes were used to further examine the effect of visfatin on insulin signaling proteins. Glucose uptake was increased in a dose-dependent fashion when visfatin was administered to cultured cells without the presence of insulin. An identical response was seen when insulin was administered to cultured cells without visfatin present. This visfatin-induced increase in glucose uptake was shown to be due to an increase in the tyrosine phosphorylation of IRS-1, as well as the insulin receptor. These data support the claims made by Fukuhara and colleagues that visfatin acts as a non-competitive insulin-mimetic hormone and thus, has the potential to partially improve insulin sensitivity.

Similarly, Song and colleagues (2008) used cultured mesangial cells (renal smooth muscle cells) from the renal cortex of healthy Sprague-Dawley rats to examine the relationship between glucose and visfatin. Similar to the aforementioned studies, they found that administration of visfatin increased glucose uptake in cultured cells. This study was novel in that they also investigated the effect of high glucose levels on visfatin synthesis. They found that a higher dose of glucose administered to cultured cells lead to an increase in visfatin mRNA and protein concentration. Furthermore, this was the

first study to look at the effect of altered levels of glucose on visfatin and the first study to show that mesangial cells can produce visfatin.

Revollo et al (2007) further examined visfatin's role in insulin sensitivity by comparing normal mice with visfatin-deficient mice using visfatin-knockout models. In this study, visfatin was not found to act as an insulin-mimetic. This was shown both *in vivo* and *in vitro*. This was determined by examining phosphorylation of the insulin receptor and IRS-1. While these results refute data from Xie et al (2007) PI3K phosphorylation was not determined. PI3K is another proposed site for visfatin action, so data regarding PI3K phosphorylation would have provided further evidence for the claim in this study that visfatin has no insulin-mimetic capabilities. Interestingly, this study showed that visfatin-deficient mice had impaired glucose tolerance and reduced glucose-induced insulin secretion. Furthermore, visfatin was shown to enhance the secretion of insulin from pancreatic  $\beta$ -cells. This is a significant finding as it is the first evidence that visfatin has direct action on insulin secretion at the level of the pancreas, but the specific mechanism of action is not fully understood. While the authors concluded that visfatin does not act as an insulin-mimetic, they did concede that visfatin plays a major role in metabolism, but suggest this role is based on visfatin's action as an enzyme in the NAD salvage pathway. The metabolic significance of this enzymatic role is explained in a later section and is shown in figure 1.

The correlation of visfatin to glucose homeostasis has been summarized in a recent meta-analysis by Chang and colleagues (Chang, Chang et al. 2011). In this study, 46 articles which investigated the level of visfatin in cardiovascular disease, T2D, metabolic syndrome, and insulin resistance were analyzed. They found visfatin to be

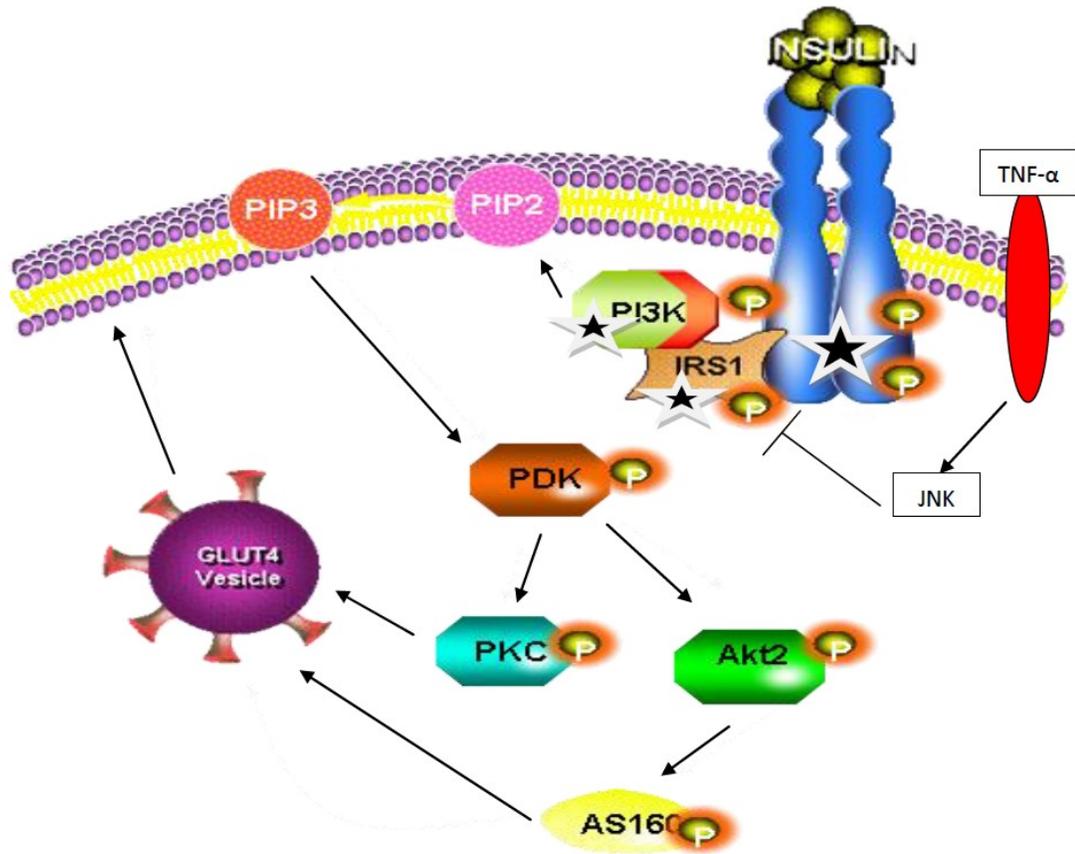
significantly elevated and correlated to other inflammatory markers in each study regardless of the chronic disease investigated.

In summary, multiple studies have shown visfatin plays a role in glucose uptake. Visfatin has also been shown to be elevated in multiple conditions associated with impaired glucose homeostasis. The action that visfatin has on the insulin signaling pathway may be at the insulin receptor as well as IRS1 and PI3K in a manner independent of insulin, but these claims are highly controversial. A summary of the signaling pathway alterations relative to these findings are shown in figure 2.

### **Visfatin and Inflammation**

Chang and colleagues investigated the relationship between visfatin mRNA and TNF- $\alpha$  (Chang, Chang et al. 2010). They found that visfatin mRNA was positively correlated to both TNF- $\alpha$  and the macrophage-specific marker CD68<sup>+</sup>, while the levels of visfatin mRNA were not significantly different in visceral compared to subcutaneous adipose tissue. While the authors concluded that visfatin must be strongly involved in the inflammatory process, there are several limitations to this study. A major limitation of this study is that visfatin mRNA was measured and protein concentration was not. This limits the study in that mRNA levels are not always indicative of protein levels or of protein action as mRNA must be translated in order to form the active protein. Additionally, subjects involved in this study were obese, non-diabetic subjects. Obesity is associated with a state of chronic low grade inflammation and high levels of TNF- $\alpha$  and CD68<sup>+</sup> would be expected. Furthermore, since visfatin has been shown to be correlated to the amount of fat present, a high level of visfatin would be expected in an obese population. The fact that the two are correlated does not necessarily indicate any kind of causal relationship, and the correlation noted in the study may be coincidental.

**Figure 2. Proposed Action of Visfatin on IRS1.** Stars represent sites where visfatin has been shown to enhance phosphorylation. TNF- $\alpha$  and JNK are also shown as they have a direct influence on IRS1 activity.



Finally, this study also demonstrated that visfatin was correlated to blood glucose. While the authors concluded their findings to show that visfatin is more of a cytokine than a metabolic hormone, this conclusion seems premature considering visfatin protein levels were not measured and the characteristics of the subjects used could significantly alter the relationships investigated.

Li and colleagues (2008) examined plasma visfatin as well as visfatin mRNA by examining the effect of tumor necrosis factor alpha (TNF- $\alpha$ ) on visfatin, insulin, and blood glucose in obese mice. They found that a high dose of TNF- $\alpha$  administered via jugular catheter resulted in a decrease in plasma visfatin and visfatin mRNA in adipose

tissue of healthy mice. Additionally, they found that TNF- $\alpha$  administration lead to dose-dependent increases in blood glucose and insulin levels. This study demonstrates a negative correlation between the pro-inflammatory cytokine TNF- $\alpha$  and visfatin. While TNF- $\alpha$  has been previously shown to be a significant pro-inflammatory cytokine, the reason for this correlation may not necessarily be related to inflammation. In addition to its pro-inflammatory function, TNF- $\alpha$  also influences metabolism by regulating lipogenesis, lipolysis, and decreasing insulin action (Uysal, Wiesbrock et al. 1997; Uysal, Wiesbrock et al. 1998). While visfatin has been shown to be correlated to inflammation, the reason for the relationship found in this study may be due to metabolic properties of both cytokines. If visfatin does increase glucose uptake, it is possible that the decrease in glucose uptake elicited by TNF- $\alpha$  may be partially due to inhibition of visfatin release. As this is the only study which has investigated TNF- $\alpha$  administration on visfatin, their relationship needs further investigation.

Romacho and colleagues further investigated the pro-inflammatory function of visfatin by examining its effect on signaling proteins associated with vascular inflammation (Romacho, Azcutia et al. 2009). In this study, cultured human aortic smooth muscle cells were treated with recombinant visfatin. Visfatin elicited an increase in extracellular-signal regulated kinase (ERK) 1 and 2 and nuclear factor kB-inducible and nitric oxide synthase (iNOS). Nitric oxide synthase activation is associated with vascular damage and inflammation. The increase in iNOS found was dose-dependent and lead the authors to conclude that visfatin was a direct contributor to inflammation. This is the only current research which shows visfatin to have a direct effect on the inflammatory signaling process. If these results are substantiated, it makes visfatin a unique hormone in that there is evidence showing it to be pro-inflammatory as well as

insulin-sensitizing. This is unique and somewhat paradoxal, since the majority of pro-inflammatory cytokines (TNF- $\alpha$ , IL-6, etc) have been associated with insulin resistance.

Moschen and colleagues (2009) examined the relationship between visfatin and inflammation by investigating its role in inflammatory bowel disease and bone disease. They found that visfatin levels were positively correlated with disease activity and negatively correlated with bone mineral density. Additionally, visfatin inhibited osteoclast differentiation *in vitro*. While visfatin was concluded to be an inflammatory mediator, this study correlated visfatin to states of inflammation, but showed no causation. Additionally, glucose was not measured, so no conclusions can be drawn regarding the action of visfatin on glucose mediation.

Friebe and colleagues (Friebe, Neef et al. 2011), correlated plasma visfatin levels with a variety of variables. They found plasma visfatin levels to be positively correlated to body mass index (BMI) and blood glucose concentration, as well as strongly correlated to white blood cell counts in adolescent children. These findings are not novel, but they do further support the concept that visfatin is associated with obesity and glucose metabolism. Interestingly, they also found that visfatin was decreased 60 minutes after oral glucose ingestion. This finding is in direct contrast to previous data (Hofso, Ueland et al. 2009), which showed that visfatin transiently increased 60 minutes after oral glucose ingestion. The disparity between these 2 studies may be related to the study population investigated or the time of sampling. Hofso et al. (2009) looked at morbidly obese adults, while Friebe et al. (2011) looked at lean and obese children.

Another novel result of the Friebe et al. study was a strong correlation between neutrophils and visfatin. This is significant since it is the first study to show a relationship between neutrophils and visfatin in non-diseased individuals. Furthermore, the highest

level of visfatin mRNA was found in neutrophils when compared with mRNA levels in adipose and liver tissue. The finding of leukocytes as a major source of visfatin is significant as well, considering visfatin was originally believed to be preferentially expressed by adipose tissue. It is important to note, however, that once again, this was found to be true in obese individuals. This provides evidence that visfatin may contribute to the low-grade chronic inflammation associated with obesity as well as contribute to alterations in glucose metabolism.

In summary, visfatin appears to be linked to inflammation. This is true in instances of obesity as well as inflammation in otherwise healthy individuals. While several studies have shown correlations between visfatin and certain inflammatory markers, research investigating the pathways connecting visfatin to other inflammatory markers is lacking. Additionally, visfatin may be elevated in cases of both altered elevated blood glucose and inflammation. Obesity was first shown to be connected to inflammation in 1993 when it was demonstrated that TNF- $\alpha$  is highly expressed in adipose tissue (Hotamisligil, Shargill et al. 1993) furthermore, inflammatory cytokines have been shown to be highly expressed in both obese and non-obese hyperglycemic individuals (Esposito, Nappo et al. 2002). In a previous study, ingestion of 75g of glucose was found to stimulate reactive oxygen species production by leukocytes in non-obese subjects (Mohanty, Hamouda et al. 2000). It is difficult to separate these findings on inflammation and blood glucose, as subjects who are hyperglycemic are often obese and therefore in a state of low-grade inflammation.

### **Visfatin and Acute Exercise**

To date, limited studies have been conducted investigating the response of visfatin to acute exercise. In 2006, Frydelund-Larsen and colleagues investigated the

response of visfatin mRNA in adipose tissue and skeletal muscle after three hours of cycle ergometer exercise done at 60% VO<sub>2</sub> max in healthy young men. Visfatin mRNA in subcutaneous abdominal adipose tissue was elevated three fold immediately after exercise, 3, 4.5, and 6 hours post exercise. Visfatin mRNA in skeletal muscle was not affected by exercise, nor was plasma visfatin. Visfatin mRNA in subcutaneous adipose tissue was elevated post exercise and accompanied by a significant increase in IL-6 mRNA in subcutaneous adipose tissue as well as a significant decrease in blood glucose. Long-term endurance exercise has been shown to increase glucose uptake independent of insulin. The increase in visfatin mRNA in adipose tissue and not in skeletal muscle may be due to an alteration in the insulin/glucagon ratio seen with such long-term exercise. The reason for the lack of change in plasma visfatin may be due to the fact that the subjects were healthy young men with low visfatin concentrations. Additionally, how visfatin is secreted and where it is secreted from is still not fully known. Visfatin mRNA's role in glucose metabolism post-exercise is difficult to fully understand, but according to these data, there is a correlation.

The first study to measure peripheral (plasma) visfatin concentration after acute exercise was an endurance (aerobic) exercise study using highly-trained rowers as subjects (Jurimae, Ramson et al. 2009). In this study, nine young rowers took part in a two hour rowing session performed at 80% of maximum heart rate. Visfatin decreased by 0.06 ng/ml 30 minutes post-exercise. Insulin decreased from 12.4 ul/ml to 4.1 ul/ml 30 minutes after exercise. These values were also corrected for shifts in plasma volume. The decrease in visfatin seen with this study may be due to the insulin-independent increase in glucose uptake that is accompanied with acute exercise (Hawley and Lessard 2008). An acute bout of endurance exercise decreases insulin

output partially due to an increase in epinephrine and norepinephrine which both inhibit insulin release. Additionally, blood glucose decreases with acute exercise due to insulin-independent increased glucose uptake. If visfatin responds to blood glucose, then visfatin would decrease similarly to insulin as blood glucose is lowered after acute exercise. The results from this study, again, provide evidence for visfatin as an insulin-mimetic as it shows visfatin to mirror the exercise-induced changes in blood glucose in a pattern similar to insulin.

The other study which examined acute exercise and plasma visfatin response was significantly different in that high intensity exercise was used as a treatment (Ghanbari-Niaki, Saghebjo et al. 2010). In this study, physically fit men performed 7 sets of six 35 meter sprints after a 10-minute warm up. Immediately after exercise, plasma visfatin, insulin, and blood glucose were all increased almost two fold. Forty five minutes post-exercise, all variables had returned to pre-exercise levels. Both blood glucose and insulin have previously been shown to be increased after short-term high intensity exercise (MacDougall, Ward et al. 1977), this is however the only study to show visfatin to increase after acute exercise. As the subjects used in this study were healthy (non-obese, non-diabetic), the increase in visfatin was not likely due to impaired glucose uptake. Instead, visfatin may mediate the insulin response to exercise or possibly augment insulin's effect on glucose uptake at the muscle.

Studies have shown glucose and visfatin to be linked, and research suggests that visfatin has an insulin-mimetic role (Fukuhara, Matsuda et al. 2005; Xie, Tang et al. 2007). It is still unclear what exactly drives visfatin alterations in the blood. Some research suggests that glucose alterations drive visfatin concentrations (Ghanbari-Niaki, Saghebjo et al. 2010), but initial studies suggest the opposite may be true (visfatin

drives glucose) (Fukuhara, Matsuda et al. 2005). Current literature does not allow a definitive conclusion about this and studies investigating the glucose and visfatin response in healthy individuals is lacking. Thus, the currently proposed research is paramount for understanding this simple issue; does glucose drive visfatin concentrations, does visfatin drive glucose concentrations or are visfatin and glucose changing in parallel due to another driving force. A summary of these studies are shown in table 1.

**Table 1. Summary of Acute Exercise Studies**

Study	Subjects	Length/Intensity of Treatment	Pre-Test Visfatin (ng/ml)	Post-test Visfatin (ng/ml)	Insulin Response (pre - post)	Blood Glucose Response (pre - post)
Frydelund et al. (2006)	Healthy Young Men	3 hr cycling @60% of VO <sub>2</sub> max	20.6*	24.9	49.3 - 30.6* (pmol/l)	5.1 - 4.6 (pmol/l)
Jurimae et al. (2008)	Elite Rowers	2 hr rowing @80% Heart Rate Reserve	6*	5.4*	12.7 - 3.9* (IU/l)	4.1-4.1 (mmol/l)
Niaki et al. (2010)	Healthy Kickboxers	7 sets of 6 x 35 m sprints (about 20 min)	12*	26*	7 - 13* (IU/l)	6 - 8* (mmol/l)

\* $\alpha < 0.05$

### Visfatin and Chronic Exercise Training

While research regarding exercise training and visfatin is somewhat varied, the consensus is that exercise training elicits a decrease in plasma visfatin. The first study which examined plasma visfatin with exercise training was done in 2006 with type 1 diabetic individuals (Haider, Pleiner et al. 2006). Aerobic exercise sessions were performed on a cycle ergometer working up to 70% maximal oxygen consumption (VO<sub>2</sub>max) twice per week for eight months. Visfatin was found to be decreased 2 months into training, further decreased 4 months into training, and even further

decreased after training when compared to baseline. Neither BMI nor fasting blood glucose was changed with exercise training. Baseline visfatin in type I diabetic subjects was significantly higher than healthy controls. The elevated visfatin level seen in type I diabetics may be an attempt to enhance glucose uptake through visfatin's insulin-like effect on GLUT4 translocation or its action to increase insulin secretion from the pancreas. Exercise training augments glucose uptake independent of insulin (Hawley and Lessard 2008) by increasing GLUT4 translocation and by increasing adenosine monophosphate dependent kinase (AMPK). Because glucose uptake is enhanced by exercise training, plasma insulin levels are decreased in a healthy population. If visfatin is an insulin-mimetic hormone, then a decrease in visfatin with exercise training would be expected (similar to insulin). The training-induced decrease in insulin is often accompanied by a decrease in blood glucose in those with elevated blood glucose. While other training studies have shown decreases in plasma visfatin to be related to decreases in blood glucose, blood glucose did not change in this study. It is possible that the decrease in visfatin was not metabolic, but more due to its enzymatic NAMPT activity or the inflammatory action of visfatin.

A separate study by Choi and colleagues found a similar visfatin response in non-obese subjects (Choi, Kim et al. 2007). In this study, both obese and non-obese subjects trained aerobically five times per week for 12 weeks. Exercise was performed using a cycle ergometer at an intensity of 60-70% of maximum heart rate. Training elicited a significant decrease in resting blood glucose, weight, BMI, body fat percentage, and plasma visfatin in obese individuals. In non-obese individuals, training did not lead to a decrease in visfatin but did lead to a decrease in resting blood glucose and BMI. The decrease in visfatin in the obese individuals in response to exercise

training may be due to several reasons. First, as previously stated, visfatin has been correlated to fat mass and a reduction in fat (37.6% to 32.2%) was seen with this study. Additionally, after training, fasting blood glucose was decreased, which could have led to a decrease in visfatin, as visfatin has also been correlated to blood glucose. Similar to the Haider (2006) study, this decrease in blood glucose and visfatin may also be due to an enhancement in glucose metabolism seen with exercise training. As previously stated, exercise training enhances glucose uptake independent of insulin. The fact that visfatin was not altered in non-obese subjects is somewhat difficult to explain. As fasting blood glucose and BMI both were decreased, one would expect plasma visfatin to also decrease. The reason that visfatin did not decrease may be due to the fact that these subjects were not hyperglycemic or obese and did not have elevated visfatin levels at baseline. It is possible that one reason for the associative decreases in plasma visfatin, blood glucose, and BMI seen in obese individuals with exercise training is that visfatin levels are returning to those of healthier individuals. Further research regarding plasma visfatin and healthy individuals is needed to further explain these results.

In T2D patients, 12 weeks of aerobic training led to a decrease in plasma visfatin (Brema, Hatunic et al. 2008). This study was novel in that it was the first study to show a change in plasma visfatin with exercise training in T2D patients. Similar to a type I diabetic population, plasma visfatin is elevated in the presence of T2D when measured at rest. Plasma visfatin appears to be elevated in all instances of impaired insulin function (T2D, T1D, and insulin resistance). This suggests that visfatin may play a significant role in insulin action. In type I diabetes, insulin is not produced by pancreatic beta cells. Visfatin may be elevated in order to increase glucose uptake in the absence of insulin. In T2D, insulin action is impaired. In order to maintain glucose homeostasis

and reduce blood glucose, more insulin is produced. If visfatin is an insulin-mimetic, then elevations in plasma visfatin would be correlated to high levels of insulin. Furthermore, the fact that plasma visfatin is elevated in a T2D population is probably due to both an impairment of insulin signaling and an increase in body fat associated with T2D. This study, however, showed a decrease in visfatin with exercise training without showing any decreases in body fat or BMI. This evidence suggests that the elevated visfatin seen in T2D patients is attenuated by exercise training, independent of changes in body fat or weight. This however needs to be confirmed with other studies.

Haus and colleagues further investigated the impact of exercise training on plasma visfatin by studying non-diabetic obese men (Haus, Solomon et al. 2009). Aerobic exercise was done at 60% of HR max at the start of the study and increased up to 85% of HR max by the end of the 12-week training program. Training elicited a significant decrease in overall body fat, body weight, BMI, resting blood glucose, and a decrease in plasma visfatin. Additionally, a strong correlation ( $r = 0.80$ ) was found between percentage change in visceral adipose tissue and percentage change in plasma visfatin. Furthermore, correlations were found between percentage change in plasma visfatin and percentage change in resting insulin as well as percentage change in plasma visfatin and percentage change in resting blood glucose ( $r = 0.52$  and  $r = 0.53$  respectively). While the decrease in visfatin with training was not a novel finding, this study showed a correlation between visfatin and resting blood glucose, and consequently a link between visfatin and improved glucose tolerance or regulation in an obese population.

A recent study examined plasma visfatin and glucose metabolism in adolescent obese females after a 12 week training program (Lee, Shin et al. 2010). Training in this

study consisted of 40-50 minute aerobic sessions with a caloric expenditure of 400-500 kcal. Exercise was performed 4 times per week for 12 weeks. Once again a decrease in plasma visfatin was shown. The decrease in plasma visfatin reported in this study was greater than previous studies (almost a 35% decrease). Furthermore, insulin and insulin resistance (via HOMA-IR) were reduced after training. This alteration in plasma visfatin and glucose regulation was accompanied by a decrease in overall body fat and BMI which is to be expected with obese subjects.

The most recent study involving plasma visfatin and exercise training was done using older (mean age of 53yrs ) T2D patients as subjects (Jorge, de Oliveira et al. 2011). This study used the novel approach of resistance training as well as aerobic training. Again, a 12-week protocol was used. Aerobic training was performed 3 days per week for 60 minutes on a cycle ergometer at an intensity equal to the lactate threshold (moderate intensity). Resistance training consisted of a 7-exercise circuits involving major muscle groups done 3 times per week. In this study, visfatin was found to be increased after training. The increase was not different between resistance exercise, aerobic training, or a combined protocol in this study. Additionally no changes were observed in BMI, insulin resistance (assessed via HOMA-IR), or TNF- $\alpha$ , but a decrease was seen in C-reactive protein (CRP). These data are in contrast to other research which has shown visfatin to correlate to inflammatory markers as well as glucose metabolism. The increase in visfatin was not only in opposition to the other inflammatory markers measured, but also did not correspond to changes in fat or other markers of insulin resistance. One possible reason for this increase could be due to the increase in ATP demand in this study. This mechanism will be discussed further in a later section.

In summary, aerobic exercise training tends to lower plasma visfatin levels. This has been shown to be true in multiple studies. Often, this decrease is accompanied by a decrease in body fat or a decrease in BMI (Choi, Kim et al. 2007; Haus, Solomon et al. 2009; Lee, Shin et al. 2010) and is often accompanied by decreases in resting blood glucose or insulin (Brema, Hatunic et al. 2008; Haus, Solomon et al. 2009; Lee, Shin et al. 2010). The majority of training studies have also examined the visfatin response during states of altered glucose metabolism such as diabetes (Type I and II) or obesity (Haider, Pleiner et al. 2006; Brema, Hatunic et al. 2008; Haus, Solomon et al. 2009; Lee, Shin et al. 2010; Jorge, de Oliveira et al. 2011). The decrease in plasma visfatin seen with exercise training may be due to an enhancement of glucose uptake as blood glucose has been shown to be decreased in the majority of training studies. Additionally, the decrease in plasma visfatin may be due to a decrease in visfatin secretion after blood glucose is decreased. The link between blood glucose and plasma visfatin is still not fully understood, but based on the exercise training literature, it does appear that blood glucose and plasma visfatin are related. However, two studies have shown visfatin to be increased independent of blood glucose concentration or insulin (Haider, Pleiner et al. 2006; Jorge, de Oliveira et al. 2011). The reason for a change in plasma visfatin without a change in blood glucose or insulin is difficult to discern. This may be related to visfatin release or inflammation independent of glucose regulation. It is possible that the reason plasma visfatin increased may be due to its enzymatic action or possible pro-inflammatory effects. A summary of these studies is presented in table 2.

**Table 2. Summary of Chronic Exercise Studies**

Study	Subjects	Length of Study	Pre-Training Resting Visfatin (ng/ml)	Post-Training Resting Visfatin (ng/ml)	Insulin (iu/l pre - post)	Blood Glucose (mg/dl pre - post)
Haider et al. (2006)	Type I Diabetic	4 Months	64.1	27.8*	N/A	151 – 125
Choi et al. (2007)	Obese	12 weeks	16.4	7.7*	13.8-10.9*	82-77*
	Non-Obese	12 Weeks	7.7	7.7	13-10	90- 81*
Brema et al. (2008)	T2DM	12 weeks	64.7	29.5*	N/A	N/A
	Obese	12 Weeks	55.8	11.6*	N/A	N/A
Haus et al. (2009)	Obese	12 weeks	17	13*	45.8 - 35.3* (mmol)	450 – 392* (mmol)
Jorge de Oliveira et al. (2011)	Obese	12 weeks	112	131*	156 – 148	N/A

\*  $\alpha = 0.05$

In summary, the response of visfatin to exercise appears to be dependent on the duration, intensity, and the characteristics of subjects involved. Visfatin has been shown to be decreased after exercise training, and this decrease is often associated with a decrease in body fat and resting blood glucose. In contrast, visfatin has been shown to be both increased and decreased after acute exercise. The differences in these results are likely due to exercise intensity and subject characteristics. Generally speaking, visfatin will decrease in subjects who have higher resting visfatin levels and higher blood glucose levels after training. This is more evident in studies which have used obese individuals or T2D patients as subjects. Furthermore, although studies are limited, higher intensity exercise has been shown to initiate an increase in plasma visfatin in a manner similar to that of insulin.

## **Visfatin and Energy Demand**

In addition to the role that visfatin may play in glucose homeostasis, visfatin may be involved during exercise through its enzymatic action as NAMPT. This was shown in rats by Koltai and colleagues in 2010 (Koltai, Szabo et al. 2010). In this study, rats were trained for 6 weeks, at 60%  $VO_2$  max. After exercise, relative enzymatic activity of NAMPT was increased as was sirtuin1 (SIRT1) in the gastrocnemius. SIRT1 is an  $NAD^+$ -dependent protein deacetylase which helps to regulate muscle differentiation and repair, and also plays a role in metabolism (Bhakat, Mokkalapati et al. 2006). Exercise training led to an increase in the availability of NAMPT protein and a concomitant increase in  $NAD^+$ . The reason for increases in both visfatin and SIRT1 may be due to SIRT1's muscle cell differentiation ability but are more likely metabolically related. With exercise training, an increase in  $NAD^+$  availability is expected as is an increase in glycolytic flux. As blood glucose was not measured, it is impossible to establish a connection between glucose, visfatin, SIRT1, and exercise in this study. That being said, it does appear that visfatin's connection to blood glucose may also be affected by its significant role in  $NAD^+$  availability.

A similar study was conducted by Hokari and colleagues in male rats which were trained 7 days per week (Hokari, Kawasaki et al. 2010). In this study, a significant increase was seen in SIRT3, and in  $NAD^+$ , but not in NAMPT in the soleus, plantaris, and triceps muscles of exercised rats. These data suggest that NAMPT may be more active in SIRT1 activation or that a greater increase in  $NAD^+$  is required to see significant changes in NAMPT.  $NAD^+$  increased by almost 100% in the previous study (Koltai, Szabo et al. 2010) compared to 49% in this study. Furthermore, sirtuins have been shown to improve insulin sensitivity (Sun, Zhang et al. 2007), again providing a link

between visfatin and enhanced insulin signaling. The exact relationship between visfatin, sirtuin proteins, and insulin sensitivity is not fully known and further investigation is required.

The relationship between visfatin and energy demand has also been investigated in humans (Costford, Bajpeyi et al. 2010). In this study, visfatin was significantly correlated to mitochondrial mass, AMPK activity, and glucose disposal rate in the gastrocnemius. This was determined by a cross-sectional analysis of athletes and obese individuals as well as through a moderate training intervention. After the cross-sectional analysis, obese individuals underwent a 3-week aerobic training program (intensity and duration not stated). After 3 weeks of training, NAMPT protein concentration was increased 127%. Furthermore, when AMPK was activated via AICAR *in vitro*, visfatin mRNA was increased 3.4 fold. These data suggest that visfatin plays a vital role in energy demand within muscle. Exactly what this role is remains unclear.

### **Visfatin and Feeding**

To our knowledge, no research has been done to investigate the acute response of visfatin to feeding of any kind in healthy individuals. Considering visfatin has been shown to be higher in those with higher fat diets and higher blood glucose (de Luis, Gonzalez Sagrado et al. 2008; de Luis, Aller et al. 2010), one could speculate that visfatin is elevated by a higher caloric intake. Furthermore, this study did not control for body fat. Considering visfatin is correlated to total body fat, the elevated visfatin seen with chronic overeating of fat and glucose may be due to excess body fat. The acute response of visfatin to general caloric intake or specific macronutrients is unknown.

## **High-Intensity Exercise and Metabolism**

In 1977, MacDougall and colleagues demonstrated that muscle glycogen was drastically decreased immediately after high intensity intermittent exercise (MacDougall, Ward et al. 1977). Twenty-four hours after exercise, muscle glycogen levels were increased back to pre-exercise levels, and were increased beyond pre-exercise levels in carbohydrate-loaded subjects. These changes in muscle glycogen were accompanied by elevations in blood glucose. Decreases in muscle glycogen were followed by an increase in blood glucose delivery to the muscles and vice versa. The reason for this is that high intensity exercise requires muscle glycogen as a fuel source. As exercise intensity (or duration during long-term exercise) increases and muscle glycogen is further decreased, glucose output from the liver is increased to replenish muscle glycogen stores, and thus, blood glucose delivery to the muscle is increased.

Furthermore, insulin levels and glucose concentrations have been shown to be decreased during, and elevated shortly after a Wingate test (Moussa, Zouhal et al. 2003; Vincent, Berthon et al. 2004), exhaustive high-intensity exercise (MacDougall, Ward et al. 1977; Felsing, Brasel et al. 1992; Higaki, Kagawa et al. 1996; Zouhal, Vincent et al. 2009), and circuit resistance training (Kraemer, Durand et al. 2004; Ghanbari-Niaki 2006). This increase in plasma glucose after high-intensity exercise is likely due to the inhibitory effect of cortisol and GH on glucose uptake which has been shown to lead to transient insulin resistance after high intensity exercise (Ghanbari-Niaki, Saghebjo et al. 2010). Furthermore, elevated epinephrine decreases glucose uptake in non-working muscle, and increased norepinephrine stimulates liver glycogenolysis to enhance blood glucose. This leads to an increase in glucose uptake, and muscle glycogen stores are replenished. Whether or not this glucose uptake is partially mediated by visfatin is

unknown. Finally, glucose ingestion prior to exercise has been shown to augment these alterations in blood glucose levels (Bacurau, Bassit et al. 2002).

Additionally, high intensity exercise has been shown to elicit significant increases in glucagon. This has been shown with an exercise volume as low as 3 x 300m sprints (Naveri, Kuoppasalmi et al. 1985). In this study, a twofold increase seen in blood glucagon immediately after exercise was accompanied by a twofold increase in blood glucose as well. Furthermore, this increase was similar to an increase seen with 15 minutes of continuous running. The results seen in this study further show that short-term high intensity exercise causes an immediate increase in blood glucose with a concomitant increase in glucagon.

In summary, exercise done at a high enough intensity and for long enough duration will lead to a decrease in muscle glycogen. In order to replenish muscle glycogen stores, glucose uptake at the muscle is increased and blood glucose decreases. In order to raise blood glucose, glucose output from the liver is increased, and blood glucose levels return to pre-exercise values.

### **Visfatin and Insulin**

Several studies have reported visfatin to act in the same matter as insulin. This has been shown both after acute exercise (Ghanbari-Niaki, Saghebjoos et al. 2010; Jurimae, Gruodyte et al. 2011) and chronic exercise (Choi, Kim et al. 2007; Haus, Solomon et al. 2009). Furthermore, resting visfatin levels have been shown to be elevated in individuals who have elevated insulin levels due to insulin impairment (T1D or T2D) (Haider, Pleiner et al. 2006; Choi, Kim et al. 2007; Haus, Solomon et al. 2009; Jorge, de Oliveira et al. 2011). The possible link between insulin and visfatin was originally proposed to be due to visfatin's potential action on the insulin receptor

(Fukuhara, Matsuda et al. 2005), but a more recent study has suggested that visfatin may act on the release of visfatin from the pancreas (Revollo, Korner et al. 2007). In this study, nicotinamide mononucleotide (NMN) was intravenously administered to mice. This increase in NMN lead to an increase in visfatin and concomitant increase in insulin release from pancreatic beta cells. The authors further suggested that the action visfatin may have on insulin release or action is due to its enzymatic action as NAMPT (figure 1). This is the only study to have examined the action of visfatin on the pancreas. More research is needed to fully understand the enzymatic role of visfatin at the level of the pancreas.

### **Exercise and Carbohydrate Supplementation**

Multiple studies have investigated the use of carbohydrate supplementation prior to exercise to maintain blood glucose levels. In 1986, Coyle and colleagues found ingestion of 2 grams of carbohydrates per kilogram of body weight taken in a solution of 50% water improved performance and maintained blood glucose levels (Coyle, Coggan et al. 1986). After 105 minutes of cycling at 70% of  $VO_2$ max, carbohydrate-fed subjects did not have a significant decrease in blood glucose while the blood glucose of subjects not fed a carbohydrate solution decreased by 50%. Additionally, fatigue was delayed by 33% in carbohydrate-fed subjects. Carbohydrate ingestion prior to prolonged exercise increases muscle glycogen synthesis by increasing available glucose as a substrate for glycogen and by increasing insulin to increase glucose uptake at the muscle. Furthermore, carbohydrate feeding during 4 hours of prolonged cycling increased blood glucose levels above pre-exercise levels (Hargreaves, Costill et al. 1984).

Since the publication of these initial, ground breaking studies, multiple studies have shown glucose supplementation to sustain blood glucose and spare muscle

glycogen during prolonged exercise, short-term exercise, and resistance exercise (Jeukendrup, Brouns et al. 1997; Lancaster, Jentjens et al. 2003; Fernandez, Da Silva-Grigoletto et al. 2010; Ghanbari-Niaki, Saghebjo et al. 2010). To summarize the effects of glycogen supplementation, The International Society of Sports Nutrition released a position statement. In it, the following is stated;

During exercise, CHO should be consumed at a rate of 30 - 60 grams of CHO/hour in a 6 - 8% CHO solution (8 - 16 fluid ounces) every 10 - 15 minutes. Adding PRO to create a CHO: PRO ratio of 3 - 4:1 may increase endurance performance and maximally promotes glycogen re-synthesis during acute and subsequent bouts of endurance exercise. 3.) Ingesting CHO alone or in combination with PRO during resistance exercise increases muscle glycogen, offsets muscle damage, and facilitates greater training adaptations after either acute or prolonged periods of supplementation with resistance training. 4.) Post-exercise (within 30 minutes) consumption of CHO at high dosages (8 - 10 g CHO/kg/day) have been shown to stimulate muscle glycogen re-synthesis, while adding PRO (0.2 g - 0.5 g PRO/kg/day) to CHO at a ratio of 3 - 4:1 (CHO: PRO) may further enhance glycogen re-synthesis. (Kerksick, Harvey et al. 2008)

A more recent study done by Kraemer and colleagues (2011) showed that ingestion of a 350 kcal shake composed of 57% carbohydrates, 28% fat, and 15% protein maintained blood glucose levels for 90 minutes of prolonged exercise at 60%  $VO_2$  max in healthy individuals. Subjects were supplemented 60 minutes prior to exercise and after an overnight fast. While this study shows similar results to the aforementioned studies regarding glucose supplementation, it is relevant to this study as the methodology is similar. Although in the current study, protein and fat are not being supplemented, the timing will be identical to the Kraemer study.

Carbohydrate supplementation of 50g of carbohydrate has also been shown to maintain blood glucose levels in anaerobic exercise more so than aerobic exercise (Fernandez, Da Silva-Grigoletto et al. 2010). In this study, trained males performed two exercise sessions. One session involved 30 minutes of cycling performed just below

anaerobic threshold while the other involved a series of squats at 80% of 1RM. Glucose supplementation administered 15 minutes prior to exercise led to elevated blood glucose in both situations, but the elevation was augmented in the anaerobic group (7.5mmol/l in anaerobic and 6.0mmol/l in aerobic). Again, both groups had higher blood glucose levels compared to no supplement.

In summary, ingestion of a carbohydrate mixture prior to exercise will attenuate the decrease in blood glucose usually seen with exercise. While a variety of methods, solutions, and exercise intensities have been used, 50g of carbohydrates administered prior to exercise has been shown to augment elevations in blood glucose during and after anaerobic exercise (Fernandez, Da Silva-Grigoletto et al. 2010; Kraemer, Francois et al. 2011). Furthermore, high-intensity intermittent exercise has been shown to increase plasma visfatin more so than moderate intensity exercise (Jurimae, Ramson et al. 2009; Ghanbari-Niaki, Saghebjo et al. 2010).

### **Summary**

In summary, the response of visfatin to exercise is varied and dependent on many factors. The majority of data regarding visfatin and exercise has been done using exercise training and obese individuals whose glucose homeostasis is altered. Additionally, only one study to date has examined visfatin after high intensity exercise. In order to further understand visfatin's role in metabolism and exercise, it is necessary to investigate visfatin in a healthy population so that dysfunctional insulin signaling is not an issue. Another possible link between visfatin and metabolic function is visfatin's action as a rate-limiting enzyme in the NAD<sup>+</sup> biosynthesis pathway. Alterations in energy balance and ATP production (e.g. exercise) have been shown to lead to alterations in visfatin. Since NAD<sup>+</sup> availability plays a significant role in the rate of

glycolysis, increased visfatin may be linked to its ability to increase NAD<sup>+</sup> availability as would be needed during exercise or when glucose uptake is increased.

The current study is an attempt to examine the response of visfatin to alterations in blood glucose without the extraneous influence of altered glucose metabolism, obesity, or chronic inflammation. While much research is still needed regarding all areas of visfatin function, the current study will attempt to delineate how visfatin changes in response to acute exercise coupled with elevations or decrements in blood glucose.

## CHAPTER III

### METHODS

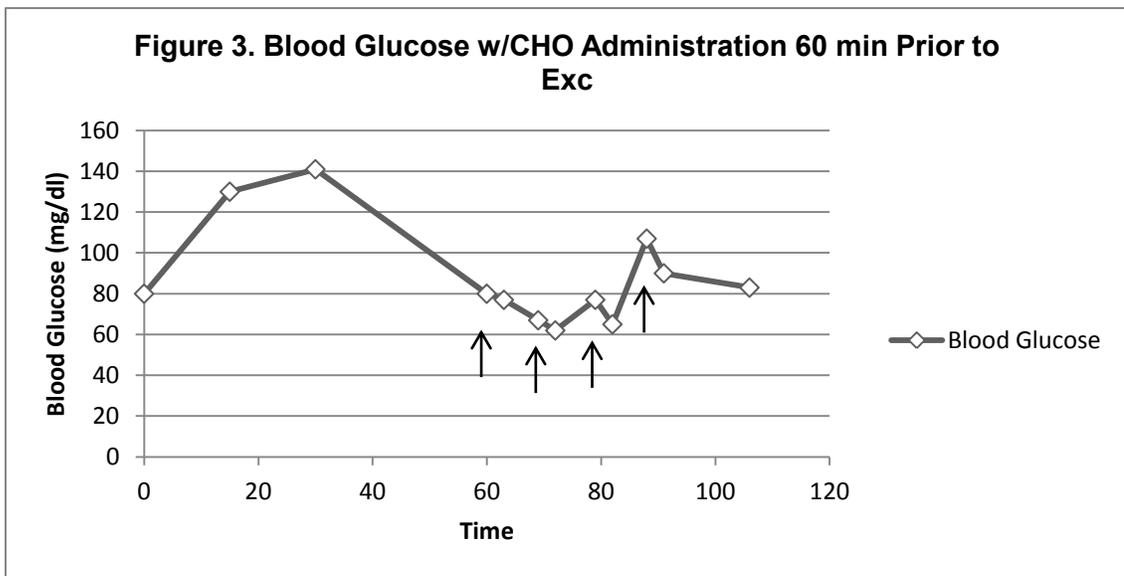
#### **Pilot Study**

To determine the timing of glucose feeding and exercise intervals required to elicit the desired alterations in blood glucose and plasma visfatin, a pilot study was completed. Two sprint-trained males (ages 24 & 28) were recruited and completed a series of exercise bouts. Absolute mean power (AMP) was previously determined for both subjects using a Wingate test. The intensity for the exercise bouts was 50% of the subject's AMP. In all sessions, after a six-minute warm up at an intensity of 50 watts, 4 3-minute bouts of exercise were performed at 50% of AMP. Each bout was separated by a six-minute active recovery period performed at an intensity of 50 watts. Blood was drawn immediately prior to and immediately after each exercise bout. In all testing sessions, blood glucose was determined using a commercially available glucometer (Bayer Contour). Visfatin levels were determined in only one subject due to cost.

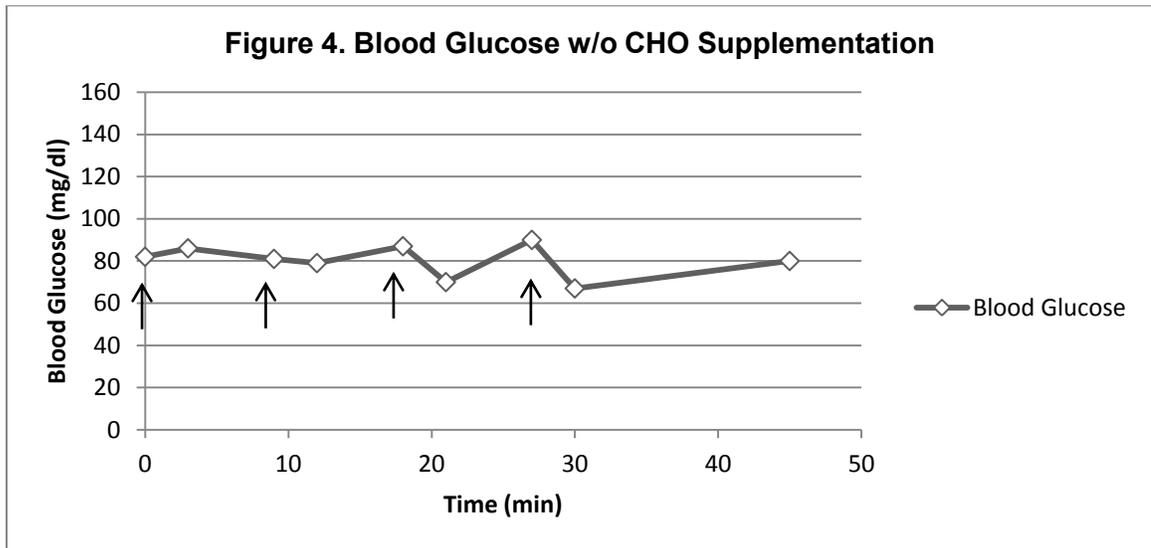
During the glucose feeding trials, resting blood glucose was determined after an overnight fast. Immediately following the resting fasted blood glucose determination, 236 ml of Gatorade G01 prime was then ingested. The macronutrient composition of this supplement included 50 g of carbohydrates, and 0g of fat or protein (200 Kcals). Additionally, there were 35 mg of potassium and 110 mg of sodium. As there is no research regarding carbohydrate supplementation, exercise, and visfatin, timing and dosage of supplementation was based on a recent study done by Kraemer and

colleagues (Kraemer, Francois et al. 2011). In summary, the following pilot testing sessions were completed;

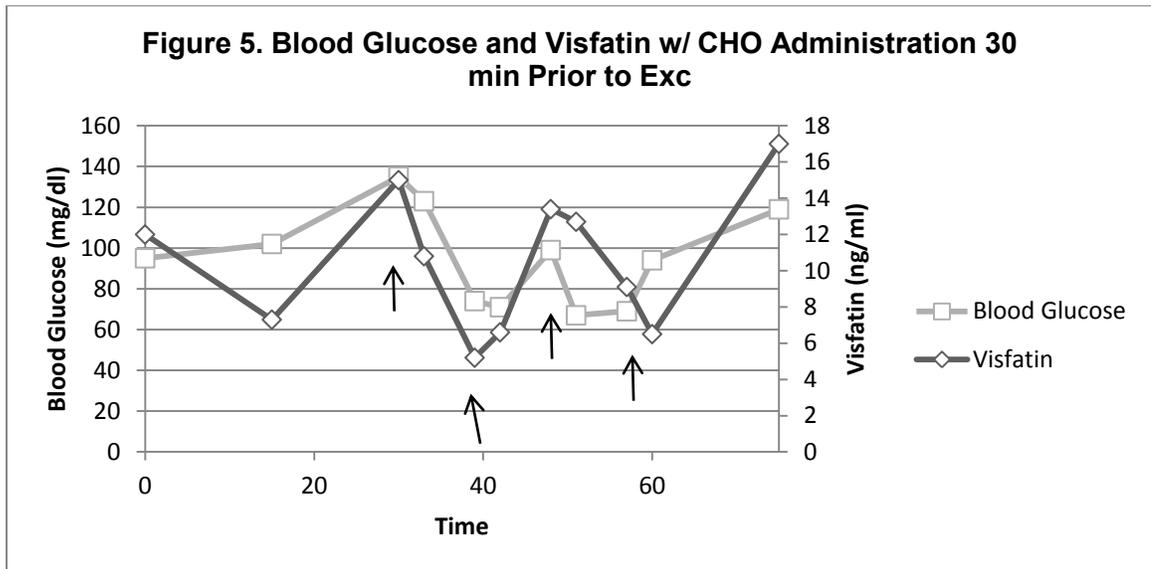
1. Subject 1, Pilot Trial 1; Glucose supplementation was given 60 minutes prior to exercise. Only blood glucose was assessed due to cost. Blood glucose peaked 30 minutes after supplementation but returned to baseline by the time exercise began. These data are presented in Figure 3 with the black arrows representing the onset of each exercise bout.



2. Subject 1, Pilot Trial 2; The timing for trial 2 was identical to trial 1. Glucose supplementation, however, was not given and blood glucose was tested immediately prior to the onset of exercise. Blood glucose remained relatively constant throughout exercise. These data are presented in Figure 4 with the black arrows representing the onset of each exercise bout.



3. Subject 2, Pilot Trial 3; After analysis of the first two trials, glucose supplementation was administered 30 minutes prior to exercise in order to begin exercise at the peak blood glucose value. Both blood glucose and plasma visfatin were assessed in trial 3. Plasma visfatin was assessed via commercially available ELISA kit (Raybiotech, Norcross, GA). Both blood glucose and plasma visfatin appeared to respond similarly to the sprint exercise protocol. These data are presented in Figure 5 with the black arrows representing the onset of each bout.



### Study Design

Ten sprint-trained males, between the ages of 18 and 30, with less than 20% body fat (determined via 3-site skinfold assessment) were used as subjects. A group of ten subjects was determined to be appropriate to yield a power of 0.80 with an alpha value of 0.05. Sprint-trained males were chosen as subjects since this was a high-intensity protocol, and it may have been too intense for someone who was not sprint-trained. Furthermore, as demonstrated by the pilot study, the desired alterations in blood glucose were reached when sprint-trained males acted as subjects. As research is lacking regarding visfatin, females were not included in order to eliminate hormonal influences and increase homogeneity of the subject pool. Sprint-trained was defined as participating in some form of high intensity interval exercise at least 2 times per week for at least the last 4 weeks using leg muscles that would be utilized for the Wingate and cycling. The majority of subjects (N=6) cycled regularly. Four subjects cycled at least 100 miles per week. Three subjects were crossfit competitors, 1 of which had competed on the national level.

Subjects returned to the lab on 2 separate occasions after a screening session. Each exercise session included a high-intensity interval exercise session, and either a carbohydrate supplement or a placebo (236 ml of sugar free kool-aid). Each supplement was given 30 minutes prior to exercise and was randomly determined based on a coin flip.

### **Screening Session**

A screening session was completed prior to the exercise sessions. During the screening session, informed consent was obtained, body composition and body fat distribution were determined through skinfold assessment, and abdominal circumference and BMI were assessed. Resting heart rate, blood pressure, medical history, exercise history were assessed, and a 3-day dietary record was given to all subjects, which was completed for the 3 days prior to each exercise session. Dietary records were analyzed for total caloric intake and macronutrient percentage using the software, my fitness pal. After subjects were considered eligible, total body was also determined through dual-energy x-ray absorptiometry (DXA). Truncal fat was also determined by DXA analysis. Truncal fat was determined as any fat located below the neck, and not on either limb. Truncal fat was assessed since it has been shown to be a direct indicator of the amount of visceral fat (Dwimartutie, Setiati et al. 2010). Additionally, a Wingate test was completed to determine AMP. Resistance during the Wingate test was 7.5% of body weight. Absolute mean power was calculated as;

$$\text{AMP (W)} = \text{load (Kg on flywheel)} \times \text{average revolutions} \times 11.765$$

In addition to AMP, absolute peak power (APP) and fatigue index (FI) were determined.

APP was calculated as;

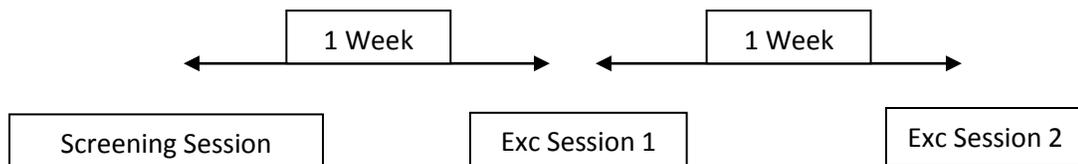
$$\text{APP (W)} = \text{load (Kg on flywheel)} \times \text{peak revolutions} \times 11.765$$

FI was calculated as;

$$\text{FI} = \frac{\text{Highest APP} - \text{Lowest APP}}{\text{Highest APP}} \times 100$$

Exclusion Criteria; Subjects were excluded if body fat percentage was higher than 20% as determined by skinfold assessment, any positive ('yes') response on the physical activity readiness questionnaire (PAR-Q; Appendix A), responses on the health history form (Appendix B), indicative of increased cardiovascular risk for exercise (as outlined by ACSM) or lack of adequate sprint training history on the exercise history form (Appendix C). A timeline of the study is presented in Figure 6.

**Figure 6. Study Timeline**



### **Exercise Sessions**

Subjects reported to the lab after an overnight fast. An indwelling IV catheter was placed in the arm of the subject so that blood draws could be taken throughout the session. The first blood draw was done 30 minutes prior to exercise. Exercise began with a 6 minute warm-up done at a standardized intensity of 50 watts (W). After the warm-up, each subject completed 4 3-minute exercise bouts at 50% of AMP, separated by 6 minutes of active recovery at an intensity of 50 W watts. This timing and intensity was chosen from the pilot study, as it elicited the alterations in blood glucose necessary

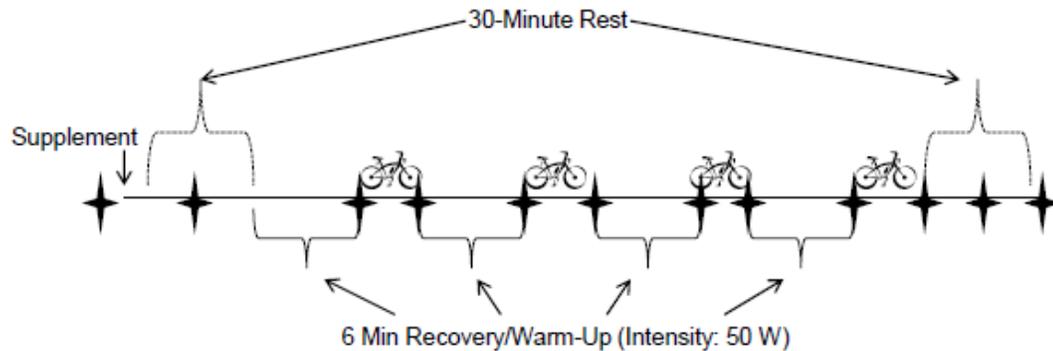
to test the hypotheses. Subjects were given water ad libitum, and water intake was not recorded. Blood draws consisted of 5 ml purple top (EDTA-treated) tubes and 5 ml red top (blank) tubes taken immediately prior to and immediately after each high intensity interval. The final draws took place 15 minutes and 30 minutes post exercise. These time points were chosen since exercise-induced changes in visfatin have been shown to be reduced to baseline levels by 30 minutes post exercise (Jurimae, Ramson et al. 2009). Finally, in order for some subjects to be able to complete each bout, resistance was adjusted. Total work (in watts) was calculated for each bout to control for any difference between exercise sessions. To further assess work, heart rate was recorded at each blood draw.

One exercise session included a carbohydrate supplementation. Supplementation was given 30 minutes prior to exercise as determined through pilot testing. Supplementation consisted of 236 ml of Gatorade G01 Prime carbohydrate solution, as described in the pilot testing session (50g of carbohydrates, 0g of protein, 0g of fat; 35mg of potassium, and 110mg of sodium). A timeline of the blood draw protocol is diagrammed in Figure 7.

### **Blood Processing and Analysis**

EDTA treated tubes were inverted several times after being collected to ensure proper mixing. Blood in the EDTA tubes as well as the blank tubes was centrifuged at 3000 rpm at 4°C for 15 minutes. Plasma was pipetted into centrifuge tubes and stored at -80°C until analysis. Blood was also collected into capillary tubes prior to exercise, after the second session, and after the fourth session. Hematocrit was assessed by centrifuging the capillary tubes for 5 minutes at 10000 RPMs and using a ruler to determine the ratio of compacted red blood cells to plasma.

**Figure 7. Session Timeline.** 3-minute exercise bouts are represented by cycles while blood draws are represented by asterisks.



Insulin resistance was assessed using HOMA-IR, which was calculated as;

$$\text{HOMA-IR} = \text{Resting BG} \times \text{Resting insulin} / 22.5$$

In this equation, resting BG and resting insulin were calculated as the mean values of the 0 time point from both sessions, and the 15 minute time point from the placebo session.

Visfatin; Plasma visfatin concentrations were determined via commercially available sandwich assay ELISA kit (Adipobiosciences, Santa Clara, CA, USA). The analysis was done at 450nm wavelength using a microplate reader (Biotek Instruments, Winesski, VT). All samples were measured in duplicate and compared to standards. The mean interassay coefficient of variance (CV) was 7.32% and the mean intrassay CV was 4.05%

Blood Glucose; Plasma glucose was determined via commercially available assay kit (Cayman Chemical, Ann Arbor, MI, USA). Analysis was done using a microplate reader (Biotek Instruments, Winesski, VT) at 515nm wavelength and all samples were

measured in duplicate and compared to standards. The mean interassay CV was 8.64%, and the mean intrassay CV was 5.49%

Insulin; Plasma insulin was determined via commercially available assay kit (Mercodia, Winston-Salem, NC, USA). Analysis was done using a microplate reader (Biotek Instruments, Winesski, VT) at 450 nm wavelength. All samples were measured in duplicate and compared to standards. The mean interassay CV was 6.84%, and the mean intrassay CV was 3.84%.

## **Statistical Analysis**

### Exercise Variables

AMP, HR, and plasma volume were all analyzed using a paired samples t-test.

### Specific Aims #1-2

Blood glucose, visfatin, and insulin were all analyzed using a 2 (condition: supplementation and non-supplementation) x 12 (time point) multivariate analysis of variance (MANOVA). Where a main effect was found, post hoc testing was done using least squared differences. To further analyze the response of the blood variables to exercise, a time series analysis was done using autoregressive integrated moving average (ARIMA). Blood variables were compared between groups by determining relative area under the curve (AUC), and analyzing AUC with a paired samples t-test.

Finally, to further assess the effect of exercise on visfatin, exercise data was teased apart from non-exercise data and were analyzed using a 2 (pre and post) x 2 (CHO and placebo) x 4 (exercise bout) MANOVA. Area under the curve was also assessed based on exercise using a 2 (CHO and placebo) x 4 (exercise bout) ANOVA. To determine the response of visfatin to exercise, the change in visfatin between time

points and from resting values was also assessed using a 2 (condition: placebo and carbohydrate by 12 (time point) MANOVA was used.

### Specific Aim #3

The relationship between plasma visfatin and body fat were correlated using a pearson product-moment correlation coefficient.

All data was analyzed using an SPSS statistical package (version 21) with statistical significance set at an alpha level of  $\leq 0.05$ .

## CHAPTER IV

### RESULTS

#### Subjects

Each subject completed a DXA scan as part of the study. Body composition and other subject characteristics are shown in Table 3. All subjects reported to the lab at the same time on both treatment days after an overnight fast. Prior to exercise, resting blood pressure and heart rate were determined.

**Table 3. Subject Characteristics (N=10)**

	Mean $\pm$ SD
Age (years)	26.4 $\pm$ 5.3
Height (m)	1.77 $\pm$ 0.03
Weight (kg)	78.78 $\pm$ 9.10
BMI (kg/m <sup>2</sup> )	24.19 $\pm$ 2.82
Body Fat (%)	13.96 $\pm$ 7.28
Resting SBP (mmHg)	113.5 $\pm$ 9.3
Resting DBP (mmHg)	71.6 $\pm$ 8.2
RHR (bpm)	61.5 $\pm$ 7.6

#### Diet

Each subject filled out a 3-day dietary recall (Appendix F). Subjects were asked to try to replicate this diet for the three days prior to the next trial based on the previous recall. Only half the subjects returned both dietary recalls (N = 5). The macronutrient intake of each subject was analyzed from dietary recalls available. These data are

presented in Table 4. Based on a paired samples t-test, there were no macronutrient differences between trials. Furthermore, all subjects refrained from exercise for 48 hours prior to both trials, and no subject was taking any prescription or over the counter medication including multivitamins.

**Table 4. Dietary Record Results**

	CHO Trial (mean $\pm$ SD)	Placebo Trial (mean $\pm$ SD)	P Value
Total Kcal	2398 $\pm$ 241	2417 $\pm$ 303	0.897
Kcal from CHO	1255.1 $\pm$ 172.5	1305.4 $\pm$ 198.2	0.749
Kcal from Protein	667.1 $\pm$ 86.3	708.9 $\pm$ 70.1	0.871
Kcal from Fat	544.6 $\pm$ 103.1	486.5 $\pm$ 189.0	0.782

### **Wingate Test**

Subject workload was determined by a Wingate test on the screening day. Absolute mean power for all subjects was 633.8 W, and mean predicted workload for the trials was 317.4 W. Actual workload results from the 2 trials are shown in Table 5. Workload was adjusted so that every subject could complete each three-minute bout of sprint exercise and mean workload decreased slightly with each sprint bout. While the actual workload was lower than predicted, there was no difference between the two treatments in total workload (CHO mean: 293.5 W, placebo mean: 292.6 W,  $p = 0.726$ ) as determined by paired t-tests. Paired t-tests were also used to compare each bout between treatment groups. Mean workload per trial is shown in Table 5, and mean HR per blood draw is shown in Table 6.

**Table 5. Total Work Done (Watts)**

	CHO Trial (mean ± SD)	Placebo Trial (mean ± SD)	P Value
Predicted	317.4 ± 47.26	317.4 ± 47.26	N/A
Bout 1	303.3 ± 43.98	302.6 ± 41.57	0.787
Bout 2	294.5 ± 46.28	300.3 ± 49.96	0.339
Bout 3	290.7 ± 45.90	286.2 ± 50.22	0.613
Bout 4	285.4 ± 51.39	281.1 ± 54.45	0.416

**Table 6. Exercise Heart Rate (bpm)**

Time Point	CHO Trial (mean ± SD)	Placebo Trial (mean ± SD)
Pre-0	61 ± 13	62 ± 12
Pre-15	58 ± 7	59 ± 9
Pre-Bout 1	101 ± 25	91 ± 20
Post-Bout 1	154 ± 23	159 ± 22
Pre-Bout 2	117 ± 17	122 ± 11
Post-Bout 2	160 ± 26	163 ± 29
Pre-Bout 3	122 ± 21	119 ± 16
Post-Bout 3	170 ± 17	162 ± 33
Pre-Bout 4	125 ± 21	128 ± 23
Post-Bout 4	172 ± 30	169 ± 29
Post-15	96 ± 15	101 ± 12
Post-30	84 ± 18	78 ± 11

**Plasma Volume**

To account for changes in plasma volume, hematocrit was determined at the first blood draw, halfway through exercise, and after exercise. These data are shown in Table 7. As shown, a 2 (treatment) x 3 (time point) ANOVA revealed no significant

interaction effect or main effect of a carbohydrate supplement on plasma volume ( $p=0.697$  and  $0.712$ , respectively). Within a specific treatment, plasma volume was significantly different halfway through exercise compared to pre and post exercise.

**Table 7. Hematocrit Changes**

	Supplement (Mean $\pm$ SD)	Placebo (Mean $\pm$ SD)
Pre (%)	45.98 $\pm$ 3.04	44.47 $\pm$ 6.02
Mid (%)	49.36 $\pm$ 3.74*	50.17 $\pm$ 3.91*
Post (%)	44.93 $\pm$ 4.23	44.11 $\pm$ 3.58

\*  $p < 0.05$  compared to pre and post

## Blood Markers

### Visfatin

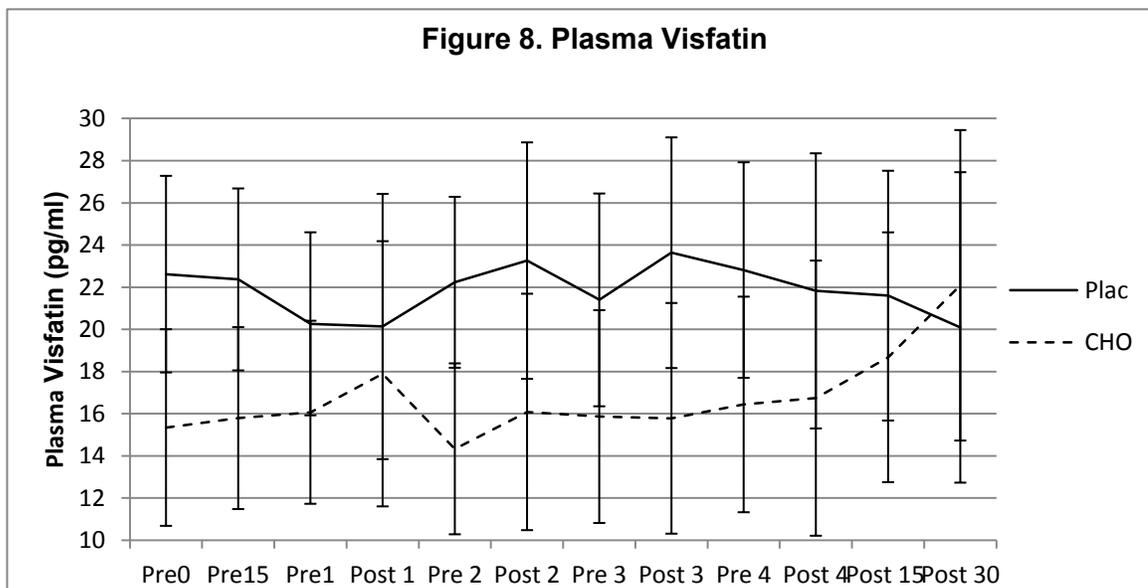
A 2 (treatment) x 12 (time point) RMANOVA showed no statistically significant interaction effect or main effect of exercise or supplement on plasma visfatin (Table 8). The pattern of visfatin during the trial is pictured in Figure 8 and mean visfatin values for each time point are presented in Table 9.

**Table 8. Visfatin RMANOVA Results**

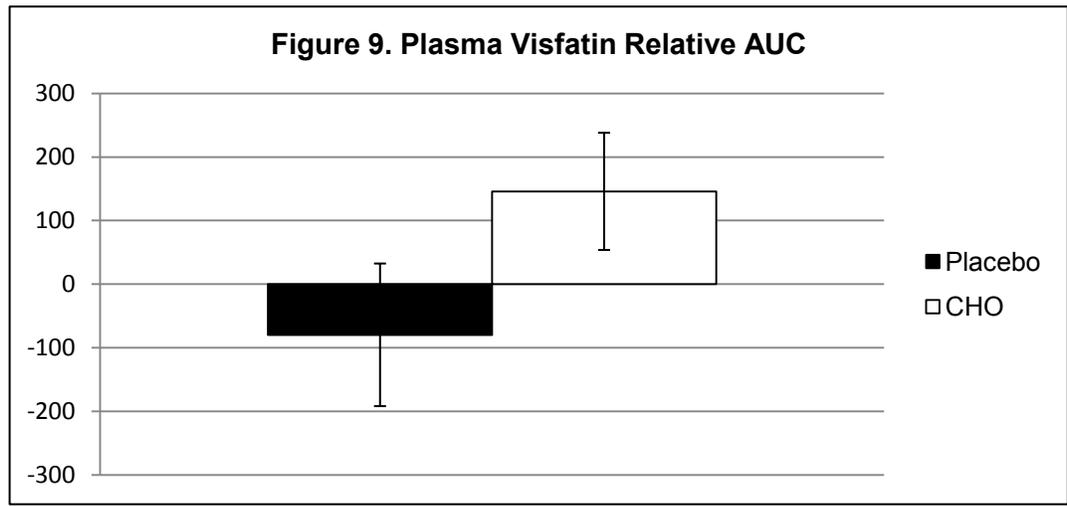
Effect	F-value	p-value
Supplement x Time Point	1.860	0.182
Supplement	2.312	0.163
Time Point	0.413	0.692

**Table 9. Plasma Visfatin (ng/ml)**

Time Point	CHO Supplement (mean $\pm$ SD)	Placebo (mean $\pm$ SD)
Pre-0	15.35 $\pm$ 14.74	22.66 $\pm$ 25.46
Pre-15 min	15.79 $\pm$ 13.64	22.37 $\pm$ 23.74
Pre-Bout 1	16.07 $\pm$ 13.73	20.26 $\pm$ 20.65
Post-Bout 1	17.90 $\pm$ 19.89	20.14 $\pm$ 19.93
Pre-Bout 2	14.34 $\pm$ 12.82	22.23 $\pm$ 28.00
Post-Bout 2	16.09 $\pm$ 17.73	23.26 $\pm$ 28.85
Pre-Bout 3	15.87 $\pm$ 15.95	21.40 $\pm$ 24.78
Post-Bout 3	15.78 $\pm$ 17.30	23.64 $\pm$ 30.50
Pre-Bout 4	16.44 $\pm$ 16.17	22.82 $\pm$ 27.09
Post-Bout 4	16.74 $\pm$ 20.63	21.83 $\pm$ 27.92
Post-15 min	18.68 $\pm$ 18.73	21.60 $\pm$ 25.92
Post-30 min	22.09 $\pm$ 23.28	20.09 $\pm$ 21.65



Treatments were further compared by analyzing the area under the curve (Figure 9). Area under the curve was adjusted to resting value. Statistical analysis was done using a paired t-test and again, no significant difference ( $p = 0.150$ ) was observed between the two treatments.



Autoregressive integrated moving average also showed no statistically significant differences between trials. There was also no significant difference when visfatin was assessed based on change between time points. When exercise was teased apart from rest and recovery using a 2 x 2 x 4 MANOVA, no statistically significant difference was found between trials. Finally, when AUC was assessed per exercise bout, again, no statistically significant difference was observed.

### Blood Glucose

A 2 (treatment) x 12 (time point) RMANOVA was used to analyze differences in blood glucose (Table 10). A significant time by treatment interaction effect ( $p=0.033$ ) and a significant main effect for the treatment ( $p = 0.047$ ) was observed. The blood glucose profile during the trials is shown in Figure 10 and the mean values for blood glucose are presented in Table 11. Since significant interaction and main effects were present, post hoc analysis was completed using least squared differences to compare time points across treatments and these results are included in Figure 10 and Table 11.

**Table 10. Glucose RMANOVA Results**

Effect	F-value	p-value
Supplement x Time Point	2.921	0.033*
Supplement	5.623	0.047*
Time Point	1.534	0.226

\*Significant at level of  $p \leq 0.05$

**Table 11. Blood Glucose (mg/dl)**

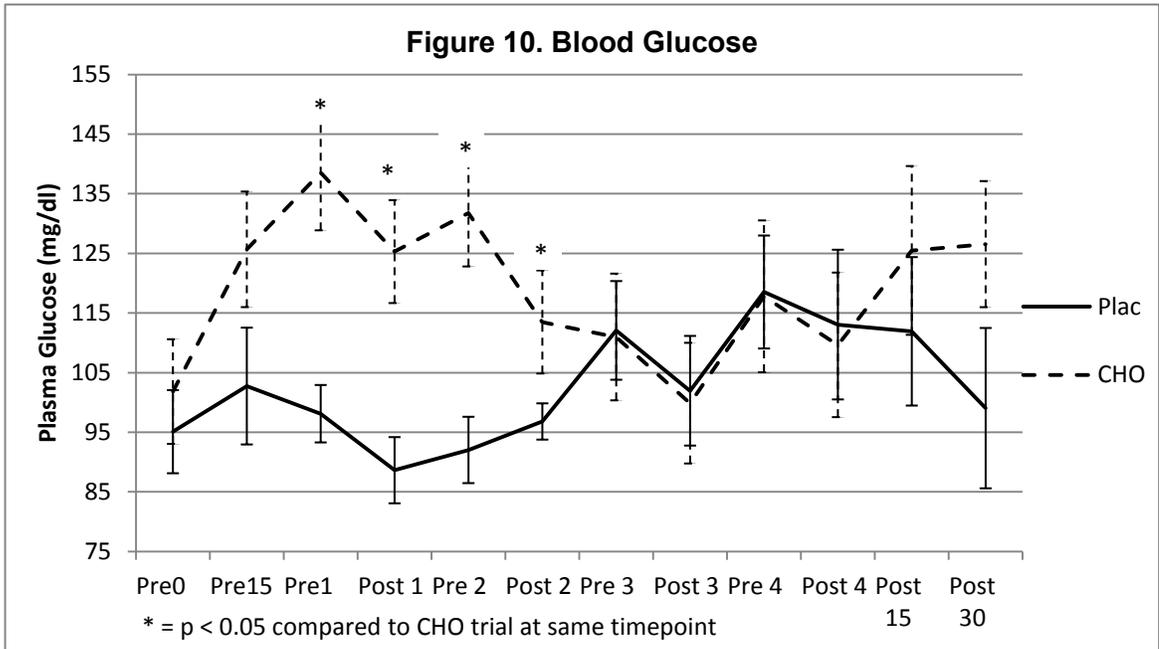
Time Point	CHO Supplement (mean $\pm$ SD)	Placebo (mean $\pm$ SD)
Pre-0	101.81 $\pm$ 27.75	95.09 $\pm$ 22.06
Pre-15 min	125.67 $\pm$ 30.65	102.73 $\pm$ 30.97
Pre-Bout 1	138.57 $\pm$ 30.71+*	98.10 $\pm$ 15.23
Post-Bout 1	125.29 $\pm$ 27.30+*	88.62 $\pm$ 17.55‡
Pre-Bout 2	131.78 $\pm$ 28.43+*	92.02 $\pm$ 17.60‡
Post-Bout 2	113.48 $\pm$ 27.33*	96.79 $\pm$ 9.62
Pre-Bout 3	110.96 $\pm$ 33.60	112.07 $\pm$ 26.16
Post-Bout 3	99.87 $\pm$ 31.99†	101.95 $\pm$ 29.07
Pre-Bout 4	117.78 $\pm$ 40.26	118.51 $\pm$ 29.95
Post-Bout 4	109.64 $\pm$ 38.32	113.05 $\pm$ 39.69
Post-15 min	125.48 $\pm$ 44.75	111.91 $\pm$ 39.36
Post-30 min	126.54 $\pm$ 33.42	99.03 $\pm$ 42.51

\* =  $p < 0.05$  compared to same time point under placebo

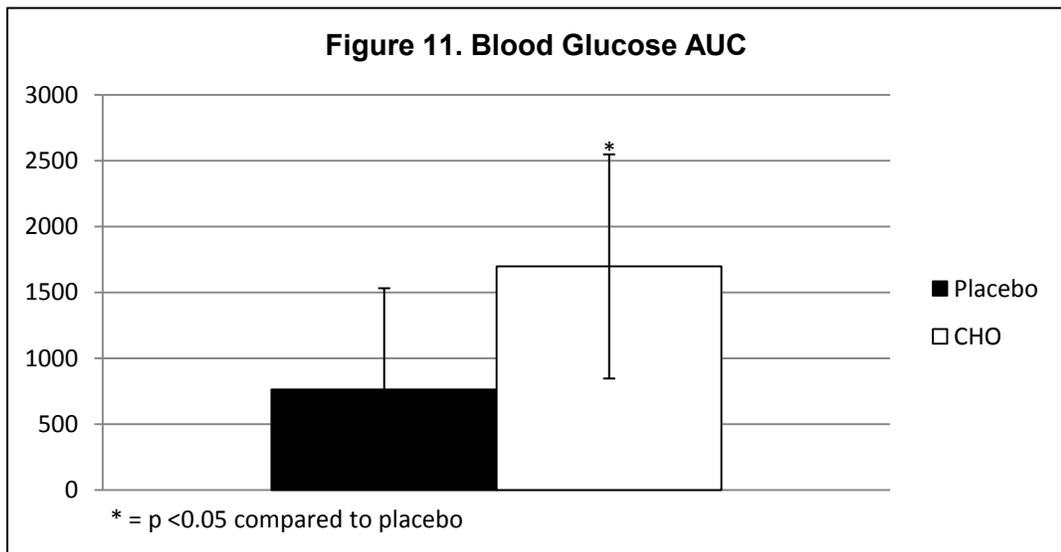
+ =  $p < 0.05$  compared to pre 0

† =  $p < 0.05$  compared to pre 1

‡ =  $p < 0.05$  compared to pre 4



Differences in plasma glucose between treatments were also analyzed by determining total area under the curve. A paired t-test revealed a significantly lower area during the placebo treatment when compared to the carbohydrate supplement ( $p = 0.050$ ; Figure 11).



## Insulin

A 2 (treatment) x 12 (time point) RMANOVA was used to analyze differences in plasma insulin between treatments and time points. A significant interaction effect ( $p = 0.001$ ) as well as significant main effect for treatment ( $p = 0.045$ ), was found for insulin (Table 12). The plasma insulin response to carbohydrate supplement and a placebo is shown in Figure 12 and mean values for insulin are presented in Table 13. Since significant interaction and main effects were present, post hoc analysis was completed using least squared differences to compare time points across treatments and these results are included in Figure 12 and Table 13.

**Table 12. Insulin RMANOVA Results**

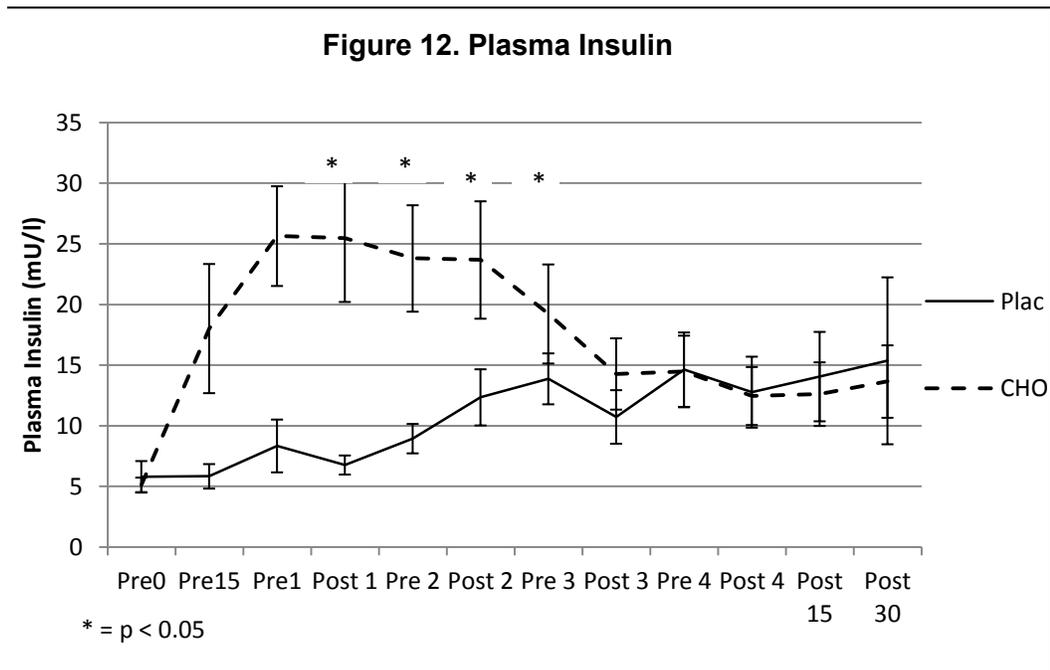
Effect	F-value	p-value
Supplement x Time Point	7.218	0.000*
Supplement	5.434	0.045*
Time Point	2.780	0.50*

\*Significant at level of  $p \leq 0.05$

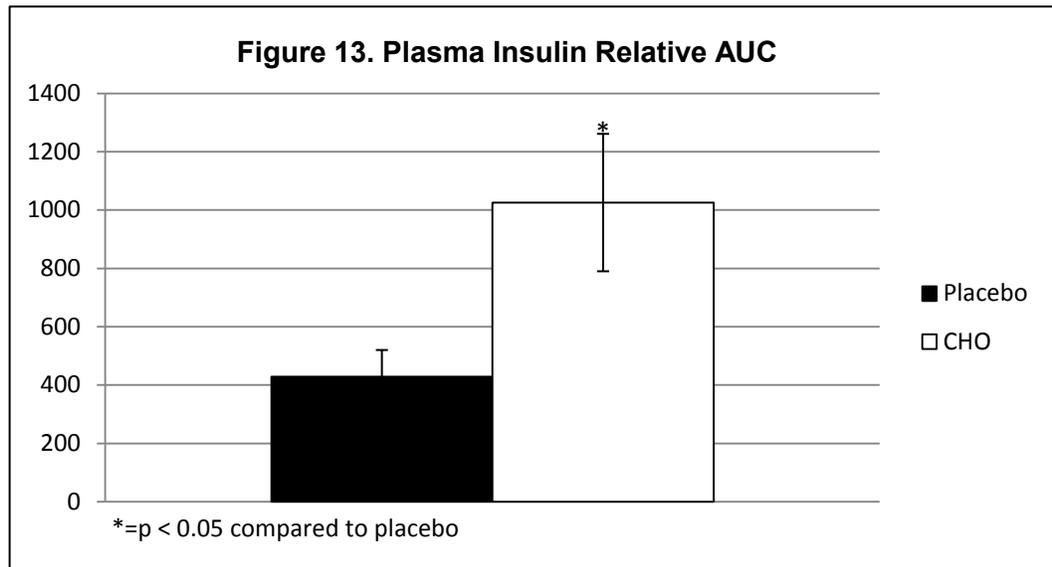
**Table 13. Plasma Insulin (mU/L)**

Time Point	CHO Supplement (mean $\pm$ SD)	Placebo (mean $\pm$ SD)
Pre-0	5.14 $\pm$ 1.89	5.80 $\pm$ 4.11
Pre-15 min	18.02 $\pm$ 16.84+	5.84 $\pm$ 3.19
Pre-Bout 1	25.65 $\pm$ 13.00*	8.34 $\pm$ 6.88
Post-Bout 1	25.48 $\pm$ 16.65*	6.78 $\pm$ 2.49
Pre-Bout 2	23.81 $\pm$ 13.87*	8.95 $\pm$ 3.84
Post-Bout 2	23.68 $\pm$ 15.31*	12.35 $\pm$ 7.32
Pre-Bout 3	19.23 $\pm$ 12.92+	13.88 $\pm$ 6.64
Post-Bout 3	14.28 $\pm$ 9.30†	10.74 $\pm$ 6.98
Pre-Bout 4	14.49 $\pm$ 9.34†	14.64 $\pm$ 9.70‡
Post-Bout 4	12.47 $\pm$ 7.56†	12.78 $\pm$ 9.26
Post-15 min	12.62 $\pm$ 8.30†	14.07 $\pm$ 11.66‡
Post-30 min	13.66 $\pm$ 9.45†	15.37 $\pm$ 21.76‡

- \* =  $p < 0.05$  compared to same time point during placebo trial
- + =  $p < 0.05$  compared to pre 0
- † =  $p < 0.05$  compared to pre 1
- ‡ =  $p < 0.05$  compared to pre 0 and pre 15

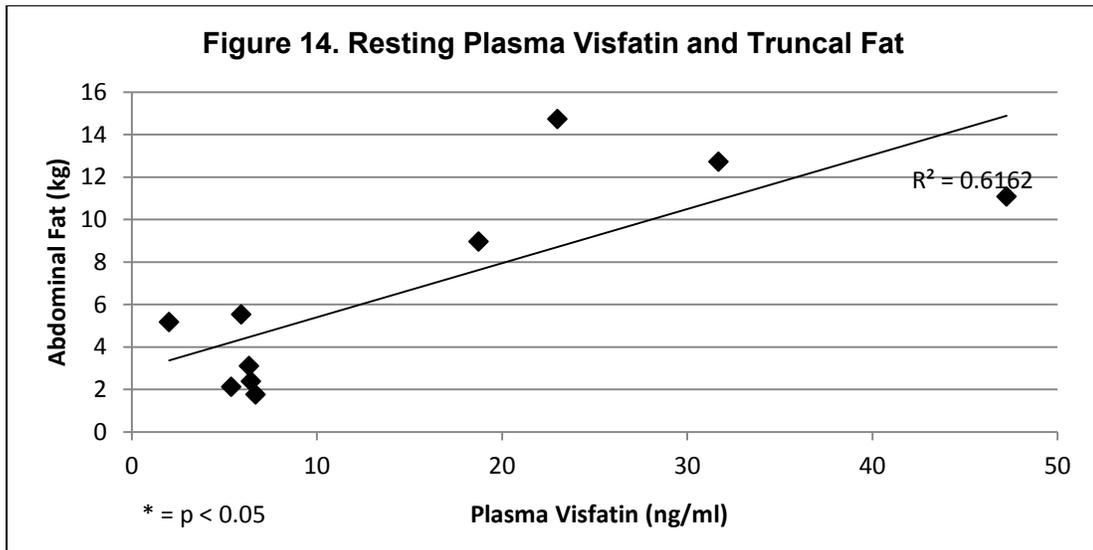


Differences in plasma insulin between treatments were further analyzed by determining relative area under the curve. A paired t-test revealed a significantly lower area during the placebo treatment when compared to the carbohydrate supplement ( $p = 0.019$ ; Figure 13).



### Visfatin and Adipose Tissue

To determine the relationship between adipose tissue and plasma visfatin, resting plasma visfatin values were correlated to body fat percentage or truncal adiposity (as determined by trunk fat during a DXA scan). Visfatin and body fat were not significantly correlated ( $p = 0.317$ ,  $r = 0.2135$ ; data not shown), but truncal fat and plasma visfatin were significantly correlated ( $p = 0.043$ ,  $r = 0.6162$ ; Figure 14).



### Visfatin and Blood Glucose

To further examine the relationship between blood glucose and plasma visfatin, visfatin values were correlated with blood glucose values across all time points (Figure 15). Mean values from each time point were correlated with mean glucose values from each time point. No significant correlation was observed between blood glucose and plasma visfatin.

Additionally, mean visfatin values were correlated with mean plasma insulin values (Figure 16). Mean values from each time point were correlated with mean insulin values from each time point, and no significant relationship was shown between insulin and visfatin. Finally, resting plasma visfatin was correlated with insulin resistance (assessed via HOMA-IR) and no significant correlation was found ( $r = 0.0047$ ) (data not shown).

Figure 15. Plasma Visfatin and Blood Glucose

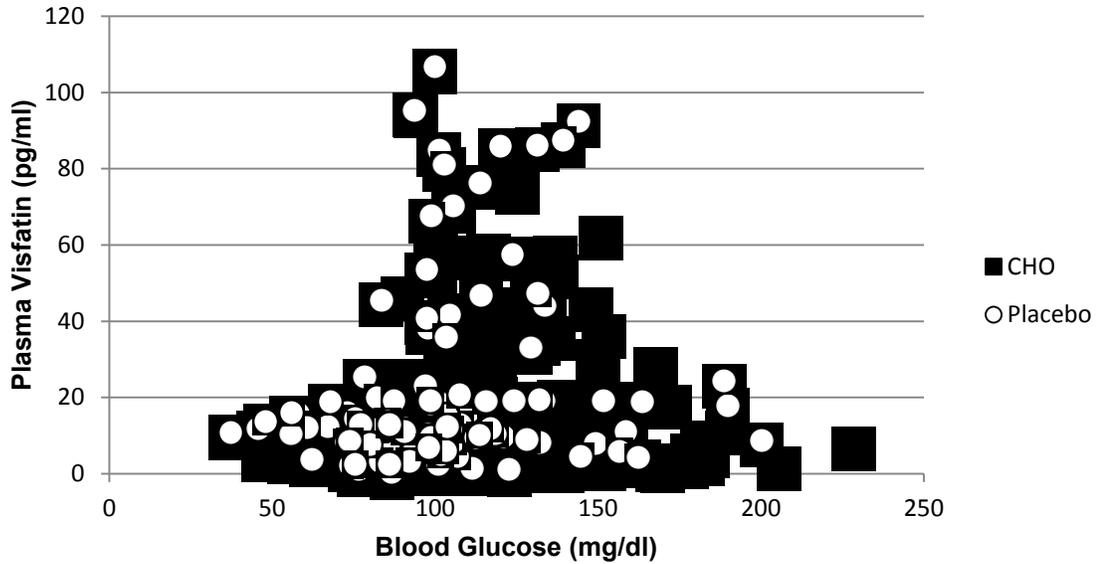
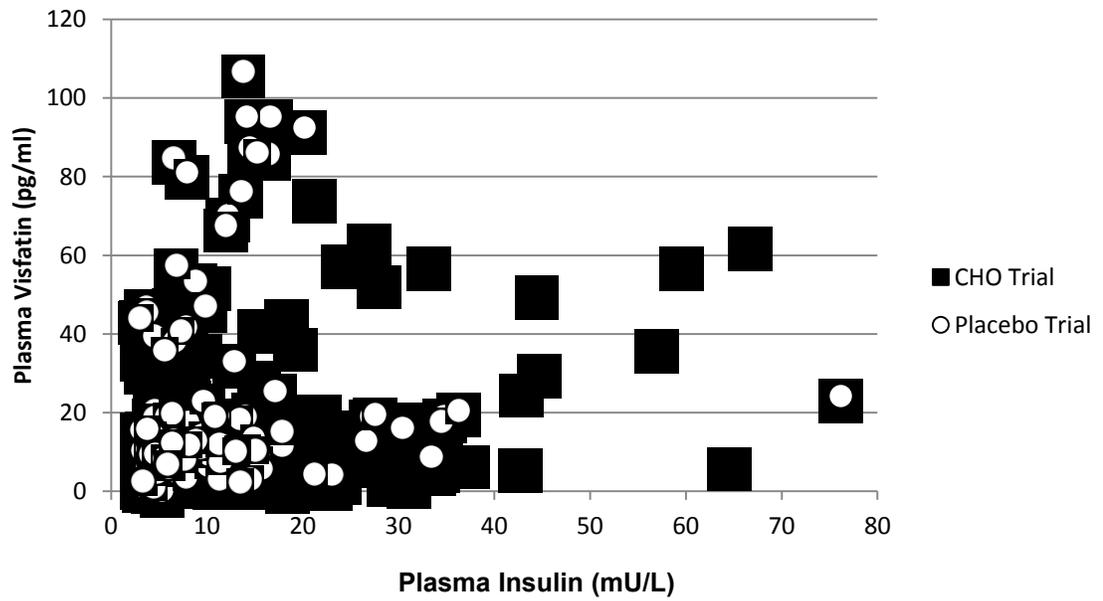


Figure 16. Plasma Visfatin and Insulin



## CHAPTER V

### DISCUSSION

The primary focus of this study was to determine the effects of carbohydrate supplementation and high-intensity exercise on plasma visfatin. The major finding of the current study is that plasma visfatin did not significantly change after carbohydrate supplementation or high-intensity exercise. To our knowledge, this is the first study to examine the effect of high-intensity exercise and a carbohydrate supplement on plasma visfatin. As expected, plasma insulin and glucose were elevated after carbohydrate supplementation. Minor increases were seen in blood glucose and insulin towards the end of exercise during the placebo trials. The glucose and insulin results are similar to other studies which have examined plasma insulin and blood glucose after carbohydrate supplementation in trained cyclists (Bacurau, Bassit et al. 2002; Lancaster, Jentjens et al. 2003). We hypothesized that high-intensity exercise would increase plasma visfatin, and that this increase would be augmented by carbohydrate supplementation. Based on the results, both hypotheses were rejected.

High-intensity exercise did not illicit a significant change in plasma visfatin in the current study. This is in contrast to some previous studies (Jurimae, Ramson et al. 2009; Ghanbari-Niaki, Saghebjoos et al. 2010), but supports others (Frydelund-Larsen, Akerstrom et al. 2007). The reason for the lack of statistically significant differences found in this study is likely multi-factorial. Studies that have reported changes in visfatin in healthy populations have used different exercise treatments. Jurimae et al. (2009)

found that 120 minutes of rowing done at 88% of heart rate max led to a decrease in plasma visfatin 30 minutes after exercise, but not immediately after. This decrease was accompanied by a decrease in insulin immediately after and 30 minutes post exercise. Blood glucose did not significantly change. The authors suggested that this decrease in visfatin was partially due to the disruption in metabolic homeostasis elicited by 120 minutes of moderate intensity exercise. It is possible that exercise-induced alterations in visfatin require some disruption of metabolic homeostasis related to; longer durations of exercise, alterations in the fuel mix, or changes in the hormonal milieu that drive changes in insulin. With 120 minutes of submaximal exercise, it is likely that blood glucose was utilized as a fuel at some point. Since glucose never decreased, the authors further concluded that visfatin and insulin were both inhibited in an attempt to maintain blood glucose. A second possibility is that 120 minutes of exercise enhanced glucose uptake through an insulin-independent mechanism which allowed for plasma visfatin to decrease since glucose uptake was already enhanced by exercise. Since blood glucose was not altered and glucose uptake was not assessed, this is speculation. It is possible that the exercise protocol utilized in the current study was not long enough to cause significant inhibition of insulin since insulin was not significantly decreased below resting values during or after exercise. This lack of insulin inhibition may have led to the lack of a significant visfatin alteration. If visfatin is primarily involved in glucose homeostasis, then it would be expected to be altered in a manner similar to insulin. In the current study, visfatin did not change in concert with either glucose or insulin in response to exercise or carbohydrate supplementation, suggesting it is not acting primarily as an insulin-mimetic.

Ghanbari-Niaki (2010) et al. found an increase in plasma visfatin after a series of 35 m treadmill sprints. Insulin, blood glucose, and HOMA-IR were also elevated immediately after the sprint exercise. The authors concluded that this may be due to the transient increase in insulin resistance seen with high-intensity exercise. High-intensity exercise has been shown to cause a transient decrease in insulin sensitivity partially due to elevated catecholamines and growth hormone levels (MacDougall, Ward et al. 1977; Felsing, Brasel et al. 1992; Zouhal, Vincent et al. 2009). The authors suggested that visfatin is elevated after high intensity exercise in an attempt to enhance glucose uptake by the muscle in a state of transient insulin resistance. The exercise protocol utilized in the current study was a lower intensity than used during this previous study. Seeing as no change in insulin sensitivity was seen (as assessed by HOMA-IR), it is likely that the intensity utilized was not high enough to elicit a change in insulin sensitivity or a resulting change in plasma visfatin.

Frydelund et al. (2006) found results somewhat similar to the current study. In this study, healthy young men were also used as subjects. Subjects exercised for 3 hours at 60% of  $VO_2$  max. Plasma visfatin did not change, but plasma insulin decreased with long duration exercise. Furthermore, they found visfatin mRNA in adipose tissue was significantly elevated. A similar elevation was not seen in visfatin mRNA in skeletal muscle. The authors concluded that visfatin does not play a cellular role regarding glucose uptake, but may instead have more of an enzymatic role in adipose tissue during exercise. As previously stated, visfatin has also been referred to as NAMPT for its enzymatic action in the NAD-salvage pathway. During prolonged exercise, visfatin mRNA may be increased to catalyze the conversion of nicotinamide to NMN within the tissue, resulting in greater NADH concentration and enhanced oxidative

phosphorylation. As mRNA in active muscle was not elevated, this seems less likely to be a plausible explanation in this case. Furthermore, visfatin mRNA was at its highest level 1.5 hours after exercise but plasma visfatin was never altered. These mRNA findings may be more indicative of visfatin's role as a proinflammatory cytokine than its enzymatic role. Since the final blood draw was 90 minutes after exercise, it is possible that plasma visfatin was elevated after this as part of an inflammatory response.

The lack of plasma changes (in the current study as well as the Frydelund et al study) suggests that visfatin may have autocrine actions rather than endocrine actions. Perhaps the reason that plasma visfatin changes have been so varied is that any metabolic action of visfatin takes place in an autocrine fashion. Since multiple studies have shown visfatin to enhance glucose uptake in vitro (Fukuhara, Matsuda et al. 2005; Xie, Tang et al. 2007), and it is not fully understood what tissues preferentially secrete visfatin, any metabolic effect visfatin has may be strictly within a cell without being secreted into the blood. Because it lacks a typical signal sequence for secretion, visfatin was originally thought to be only present in plasma due to cell lysis or death (Hug and Lodish 2005). Visfatin has since been shown to be secreted through a non-classical pathway (Revollo, Korner et al. 2007). This same study by Revollo et al (2007) only examined visfatin secretion from adipocytes. The autocrine action of visfatin may provide a possible explanation for the lack of significant findings in the current study. Visfatin's enzymatic action in the NAD salvage pathway could partially explain why exogenous visfatin has been shown to increase glucose uptake. Increasing visfatin would lead to an increase in available NAD. It could be speculated that increasing the availability of NAD may increase the glycolytic rate within a cell. As the glycolytic rate is increased, glucose uptake would also be increased. This possible affect on glycolytic

rate has not been shown, but it may provide an explanation for why exogenous visfatin has been shown to increase glucose uptake. Furthermore, it may help to explain why plasma visfatin responds completely differently in obese populations than it does in healthy populations.

Finally, it is possible that protein expression of visfatin in the muscle or adipose tissue was altered by treatment in the current study, but we did not assess either mRNA or protein content and therefore, we can't make conclusive comments about this. Furthermore, as Revollo et al (2007) have suggested, visfatin may only be secreted from specific cells. As no study has examined the response of visfatin in human skeletal muscle to exercise, its response is unknown. Further research is needed to determine if the exercise-induced visfatin response is largely autocrine.

The aforementioned studies showed that plasma visfatin can be either increased or decreased with exercise. Based on this limited research, the potential metabolic action of visfatin may be more dependent on intensity and substrate utilization than anything else. In the current study, the lack of change in visfatin may be partially due to the intensity utilized. The intensity may have not have been high enough to cause a transient insulin resistance and the theorized resulting increase in plasma visfatin. Conversely, the decrease in plasma visfatin after 120 minutes of exercise shown in other studies (Jurimae, Ramson et al. 2009) may have been due to the duration of exercise accomplished and the concomitant decrease in insulin release seen with long-duration exercise. In the current study, subjects exercised for a total of 45 minutes. To summarize, it is possible that the protocol used in the current study may not have been long enough to elicit a decrease in plasma visfatin due to a decrease in blood glucose

and not intense enough to cause an increase in plasma visfatin due to transient insulin resistance.

Other studies that have shown changes in plasma visfatin have utilized subjects where altered insulin function is present, such as type I or type II diabetics, or those who are insulin resistant. In addition, most of these studies have used chronic exercise training as the treatment. All of these studies showed decreases in plasma visfatin after training (Haider, Pleiner et al. 2006; Choi, Kim et al. 2007; Brema, Hatunic et al. 2008; Haus, Solomon et al. 2009). The overall conclusion drawn from these studies is that visfatin may only play a metabolic role when insulin function is already impaired. With exercise training, insulin sensitivity is enhanced, and plasma visfatin may play less of a role which could lead to a decrease in plasma levels. While this conclusion may be correct, it cannot be confirmed by the current study as subjects were apparently healthy, non-diabetic, trained, and the study was acute. Furthermore, one study has shown visfatin to increase insulin release from the pancreas in rats (Revollo, Korner et al. 2007). It could be hypothesized that the effect on pancreatic insulin release may also be more robust in obese individuals (similar to other visfatin related effects) compared to healthy individuals, but the current study did not assess pancreatic insulin release and therefore, we can't make any conclusions related to the effect of visfatin on the pancreas.

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The second aim of this study was to determine the effect of carbohydrate supplementation on plasma visfatin. Ingestion of a glucose supplement prior to exercise had no effect on plasma visfatin, or on its response to exercise. While research is minimal and varied regarding the response of visfatin to carbohydrate intake (Marcinkowska, Lewandowski et al. 2007; Zhaoxia, Ying et al. 2012), no study has examined the effect of glucose intake on plasma visfatin in healthy individuals. In the current study, glucose ingestion led to a significant increase in both plasma insulin and glucose with no change in plasma visfatin. According to these data, the second hypothesis is rejected. Again, if visfatin plays a role in blood glucose homeostasis, then it would be expected to be altered with carbohydrate ingestion.

Carbohydrate supplementation caused an elevation in plasma glucose and insulin which lasted through the first half of exercise when compared to the placebo trial. It can be assumed that the carbohydrate supplement caused an alteration in

macronutrient fuel utilization during exercise, although we did not assess changes in R values during exercise. The increased availability of glucose likely lead to an increased reliance on blood glucose as a fuel for exercise (as blood glucose was elevated prior to exercise during the CHO trial) (Pirnay, Lacroix et al. 1977; Jenkins, Hutchins et al. 1994). Blood glucose returned to baseline halfway through exercise. This clearing of blood glucose was likely due to an increase in glucose uptake by active muscles during exercise (through both insulin-dependent and independent mechanisms). This glucose response is typical of fit individuals after a carbohydrate supplement prior to exercise (Jeukendrup, Brouns et al. 1997; Pedersen, Lessard et al. 2008). As plasma visfatin did not change, it can be concluded that plasma visfatin did not facilitate the clearing of blood glucose and did not improve glucose uptake in the current study.

The initial increase in insulin in the CHO trial is due to the elevated blood glucose after carbohydrate ingestion. The continued elevation in insulin may be due to an increase in transient insulin resistance in response to elevated growth hormone and catecholamines seen with high-intensity exercise (MacDougall, Ward et al. 1977; Felsing, Brasel et al. 1992; Jurimae, Ramson et al. 2009; Ghanbari-Niaki, Saghebjo et al. 2010). These previous studies have shown blood glucose and insulin to be elevated towards the end of high-intensity exercise in healthy populations. While not statistically significant, blood glucose did slightly increase 30 minutes after exercise. This may be due to an increase in gluconeogenesis and glucose output from the liver as a result of elevated catecholamines and an attempt to replenish muscle glycogen.

To summarize, visfatin has been shown to have three actions; enzymatic action in the NAD-salvage pathway, inflammatory effects as a pre-B cell colony enhancing

factor, and its role in insulin signaling/glucose homeostasis. The lack of significant findings in the current study may be partially explained by visfatin's enzymatic action. Visfatin catalyzes the conversion of nicotinamide to nicotinic acid mononucleotide (NMN) in the NAD salvage pathway (Figure 1). During exercise, NAD turnover rate is increased due to an increase in glycolytic rate and concomitant increase in NADH production. Visfatin may be enhanced within the muscle in order to catalyze this increase in NAD synthesis. This is supported by previous research which has shown visfatin to increase NAD production and the activity of sirtuin proteins (Revollo, Grimm et al. 2004). Sirtuin proteins respond to alterations in energy balance and NAD turnover rate. The enzymatic activity of visfatin may also explain the results of previous studies which have shown exogenous visfatin to enhance glucose uptake *in vivo* and *in vitro* (Fukuhara, Matsuda et al. 2005; Xie, Tang et al. 2007; Song, Lee et al. 2008). An increase in the availability of visfatin may increase NAD synthesis which would increase the glycolytic rate. This increase in the glycolytic rate may increase the breakdown of cellular glycogen and increase glucose uptake by mechanisms independent of insulin. The timeline for this is still unclear.

The elevation in visfatin seen with type II diabetes and obesity may be due to visfatin's proinflammatory action. Visfatin was originally discovered as an inflammatory cytokine expressed in the presence of pokeweed mitogen (Samal, Sun et al. 1994). Additionally, it has been suggested to influence the NF- $\kappa$ B pathway (Tilg and Moschen 2008). Its potential interaction with the NF- $\kappa$ B pathway is another characteristic it shares with the sirtuin proteins. Visfatin has been shown to be elevated in states of obesity-induced inflammation as well as other states of inflammation along with the sirtuin proteins and NF- $\kappa$ B (Alexandraki, Piperi et al. 2006; Tilg and Moschen 2008; Li, Yang et

al. 2009). The interaction of these substances is not fully understood, but it is possible that the elevated visfatin levels seen in type II diabetics and obese individuals may partially be a result of chronic inflammation and not just due to an alteration in glucose homeostasis or insulin sensitivity.

In addition to the lack of a significant change with treatment, plasma visfatin varied greatly between subjects in the current study. Resting plasma visfatin levels ranged from 3.67 ng/ml to 84.83 ng/ml. This variation is consistent with other studies which have reported mean resting visfatin values from 6 ng/ml to 55.8 ng/ml in healthy individuals and values up to 142 ng/ml in obese individuals (Ghanbari-Niaki, Saghebjoor et al. 2010; Jorge, de Oliveira et al. 2011; Jurimae, Gruodyte et al. 2011). The reason for this large variability in resting values is not fully understood. In the current study, plasma visfatin values were not significantly correlated to blood glucose, insulin or body fat percentage, but were significantly correlated with truncal adiposity. The third specific aim of this study was to determine the relationship between truncal fat and plasma visfatin. We hypothesized (and confirmed) that plasma visfatin would be positively correlated with truncal fat in healthy males. Previous studies have shown there to be a positive relationship between truncal fat and abdominal fat (Parikh, Joshi et al. 2007). This relationship has between abdominal fat and plasma visfatin has been shown in obese populations in previous studies, but to our knowledge, this is the first study to establish such a correlation in healthy non-obese individuals, and may partially explain the large variation in visfatin levels at rest. The variation in the current study is likely due to this relation with truncal fat and not likely due to variations in insulin sensitivity between subjects as suggested in previous research (Ghanbari-Niaki, Saghebjoor et al. 2010; Jurimae, Gruodyte et al. 2011), considering all subjects were apparently healthy,

and plasma visfatin was not correlated to blood glucose, insulin, or insulin resistance (HOMA-IR) at any time point.

All subjects filled out health-history and physical activity questionnaires (appendix A and B). One subject reported having a family history of type II diabetes. Fasting blood glucose levels for this subject were elevated (mean of 130 mg/dl) compared to other subjects. One other subject had an elevated mean fasting glucose value (107 mg /dl) without reporting any family history of T2D. Fasting visfatin levels of both subjects were less than one standard deviation from the group mean. Furthermore, after statistical analysis of insulin and visfatin with resting blood glucose as a cofactor, the results did not differ from what has been presented.

Ten subjects were tested in the study as determined by a power analysis done prior to the study and based on previous published data. The observed power of the main effect (visfatin response) in the current study was 0.812. While the desired power of 0.80 was reached, more subjects may have increased the power and the likelihood of significant results.

The current study was an attempt to clarify the role that visfatin has regarding glucose metabolism. As much control was employed as possible to eliminate confounding variables from the current study. There were, however, some limitations. First, training status varied from subject to subject. Subjects were eliminated if they did not train at a high-intensity at least twice per week and have a body fat percentage below 20 (assessed via 7-site skinfold). While that did limit the subject pool to trained individuals, some subjects were more highly trained than others. Another limitation is the mode with which most subjects were trained. When possible, subjects who cycled

regularly were used, but some subjects (n = 4) had very little experience cycling. As the mode of exercise was cycling, inexperienced subjects may have been somewhat limited by their cycling ability. Furthermore, some subjects (n = 2) had elevated mean fasted blood glucose. We expected active individuals to have normal fasting blood glucose, so we did not use elevated fasting blood glucose as one of the exclusion criteria, but we recognize that this may have influenced our results. Also, carbohydrate supplementation was not based on body weight. There was not a vast difference in body weight between subjects ( $78.78 \text{ kg} \pm 9.10$ ) or grams of carbohydrate per kg of body weight ( $641 \text{ mg} \pm 68 \text{ mg}$ ) and plasma visfatin was not correlated to body weight. The dosage of carbohydrate was based on a previous study which found 50g of carbohydrates elevated blood glucose in healthy individuals (Kraemer, Francois et al. 2011). While supplementation was not based on body weight, blood glucose was significantly increased beyond resting levels in all individuals after supplementation. As the purpose of this study was to assess plasma visfatin changes in response to blood glucose changes, the carbohydrate supplement used should have been an adequate stimulus. Finally, due to budget constraints, a true control (no exercise & no supplementation) was not used in this study. The ideal study design would have used more trials to test various combinations [no exercise & no supplementation; no exercise & CHO supplementation; no exercise & placebo; exercise & CHO supplementation and exercise & placebo].

Future research is needed to determine the extent to which visfatin acts on glucose homeostasis. There are still very few studies which examine plasma visfatin in healthy individuals. Another possible direction that could be used to ascertain the role of visfatin is to complete glucose or insulin clamping studies. These types of studies could

help to determine visfatin's action in states of altered insulin action and would provide another method to manipulate blood glucose. Also, repeating this study in a hyperglycemic population may help to further determine the action of visfatin on blood glucose. Previous studies using hyperglycemic individuals as subjects have shown significant roles for visfatin (refs), the current results could be compared to hyperglycemic individuals to determine how visfatin acts when blood glucose is manipulated through exercise and supplementation in states of decreased insulin sensitivity. Furthermore, multiple studies (including this one) have found resting visfatin levels to be greatly varied between individuals. The reason for this variation can only be partially explained by truncal adiposity and requires further investigation. Very little is known regarding the release or mechanism of action of visfatin, or its interaction with other hormones and compounds in the majority of tissues. Further research is needed to determine which of visfatin's actions is primary and if its actions vary between tissues.

Finally, visfatin's potential autocrine actions have not been investigated. The present study was an attempt to determine the response of plasma visfatin to alterations in blood glucose. No relationship between the two was found. Previous studies which have investigated the response of plasma visfatin to exercise or carbohydrate ingestion have been minimal and the results of been varied. In order to fully understand the role visfatin may play in metabolism, it is important to assess its action within the cell.

In summary, this study showed that high-intensity intermittent exercise had no effect on the plasma visfatin levels of trained healthy males. Furthermore, carbohydrate supplementation had no effect on resting or exercise plasma visfatin levels. No relationship was shown between plasma visfatin and blood glucose, plasma insulin, or

body fat percentage. A significant correlation, however, was found between plasma visfatin and total truncal fat.

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APPENDIX A

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q)

Subject: \_\_\_\_\_

ID: \_\_\_\_\_

Please circle Yes or No for each question. If Yes please explain.

**Health History**

Do you have any musculoskeletal illnesses or injuries that would restrict your participation in a submaximal exercise bout (as performed on a treadmill)?

YES

NO

If yes, please describe. \_\_\_\_\_

Have you ever been diagnosed with cardiovascular disorders (heart problems, high blood pressure, high cholesterol, abnormal heart rhythms, etc.)?

Family History

YES

NO

Cigarette smoking

YES

NO

Hypertension

YES

NO

Hypercholesterolemia

YES

NO

Impaired fasting glucose

YES

NO

Obesity

YES

NO

Sedentary lifestyle

YES

NO

High serum LDL cholesterol

YES

NO

If yes, please describe. \_\_\_\_\_

Have you ever been diagnosed with any metabolic disorders (diabetes, etc.)?

YES

NO

If yes, please describe. \_\_\_\_\_

\_\_\_\_\_

Could you currently be pregnant?

YES

NO

Please list any major illnesses, hospitalizations, or surgical procedures within the last two years.

\_\_\_\_\_

\_\_\_\_\_

**Drug/Supplement Usage**

Are you a *current* smoker or user of tobacco?

YES

NO

Have you ever smoked in the past?

YES

NO

If yes, please describe history. \_\_\_\_\_

\_\_\_\_\_

Are you currently taking any medication(s)?

YES

NO

If yes, please list name of medication(s), reason for usage and length of administration.

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Are you currently taking any nutritional/vitamin supplements?

YES

NO

If yes, please list name of supplement, reason for usage and length of administration.

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Do you have any joint pain?                      YES                      NO

Have you experienced shortness of breath at rest or during exercise? YES                      NO

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---

Have you had a large change in weight over the past 3 months? YES                      NO

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Have you seen a change in your health status in the past 3 months? YES                      NO

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## APPENDIX B

### AHA/ACSM HEALTH/FITNESS FACILITY PRE-SCREENING

Assess your health needs by marking all true statements.

#### History

You have had:

- a heart attack
- heart surgery
- cardiac catheterization
- coronary angioplasty (PTCA)
- pacemaker/implantable cardiac defibrillator/rhythm disturbance
- heart valve disease
- heart failure
- heart transplantation
- congenital heart disease

#### Symptoms

- You experience chest pain with exertion.
- You experience unreasonable breathlessness.
- You experience dizziness, fainting, blackouts.
- You take heart medications.

#### Other health issues

- You have musculoskeletal problems.
- You take prescription medication(s).
- You may be pregnant.

**If you marked any of the statements in this section, consult your healthcare provider before engaging in exercise. You may need to use a facility with a medically qualified staff.**

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Cardiovascular Risk Factors

- You are a man older than 45 years.
- You are a woman older than 55 years.
- You smoke.
- Your blood pressure is > 140/90.
- You take blood pressure medication.
- Your blood cholesterol level is > 240 mg/dl.
- You have a close blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister).
- You are physically inactive (ie, you get < 30 minutes of physical activity on at least 3 days per week).

**If you marked 2 or more of the statements in this section, consult your healthcare provider before engaging in exercise. You might benefit by using a facility with a professionally qualified exercise staff to guide your exercise program.**

---

- None of the above is true.

**You should be able to exercise safely without consulting your healthcare provider in almost any facility that meets your exercise program needs.**

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Please print, complete and bring this questionnaire to your physician if you have any further questions.

APPENDIX C  
FITNESS ACTIVITY

Please describe your current participation in the following types of exercise:

1. *Aerobic (aerobic classes, walking, jogging, stair climbing, cycling, etc.)*

Frequency (# of days per week): \_\_\_\_\_

Duration (time spent per session on avg.): \_\_\_\_\_ minutes

Intensity (difficulty level):            light      somewhat hard      hard      very hard

If you know your HR responses please list it here. \_\_\_\_\_

---

How long have you been participating in aerobic activity as described above?

Less than three months      3-6 months      6-12 months      greater than 12 months

Type of activity: \_\_\_\_\_

Additional comments: \_\_\_\_\_

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2. *Anaerobic (weight training, sprinting, etc.)*

Frequency (# of days per week): \_\_\_\_\_

Duration (time spent per session): \_\_\_\_\_ minutes

Intensity (difficulty level):            light      somewhat hard      hard      very hard

How long have you been participating in anaerobic activity as described above?

Less than three months      3-6 months      6-12 months      greater than 12 months

Type of activity: \_\_\_\_\_

Additional comments: \_\_\_\_\_

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3. *Organized or Recreational sports or activities*

Type of sport(s): \_\_\_\_\_

Frequency (# of days per week): \_\_\_\_\_

Duration (time spent per session): \_\_\_\_\_ minutes

Intensity (difficulty level):      light      somewhat hard      hard      very hard

How long have you been participating in sports activity as described above?

Less than three months      3-6 months      6-12 months      greater than 12 months

Additional comments: \_\_\_\_\_

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