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By: Paul F. Mellick, Bryan J. Feger, Douglas J. Oberlin, Paul G. Davis, and Laurie Wideman

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Abstract:

The purpose of the study was to examine the effect of high-intensity exercise and carbohydrate supplementation (CHO) on plasma visfatin. On 2 separate days, 10 sprint-trained males (age = 26.4 ± 5.3 yr; Ht = 1.77 ± 0.03 m; Wt = 78.78 ± 9.10 kg; BF% = $13.96 \pm 7.28\%$) completed 4, 3-min bouts of cycling at 50% mean anaerobic power, with 6 min of rest between bouts. On CHO day, subjects ingested 50g of CHO 30 min before exercise. On control day, subjects ingested a sugar-free drink (CON) 30 min before exercise. Blood was drawn before supplementation, 15 min before exercise, before and after each exercise bout, and 15 and 30 min post exercise. Visfatin, glucose, and insulin were determined. Truncal fat was assessed by dual energy x-ray. 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 significantly elevated after CHO ingestion and remained elevated throughout the first half of exercise. Baseline visfatin was significantly correlated with truncal fat ($r^2 = 0.7782$, $p < 0.05$). Visfatin was correlated to truncal fat in sprint-trained males, but was not altered by exercise or CHO supplementation.

Keywords: Visfatin | glucose | exercise | carbohydrate supplementation | insulin

Article:

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Research article

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Abstract

The purpose of the study was to examine the effect of high-intensity exercise and carbohydrate supplementation (CHO) on plasma visfatin. On 2 separate days, 10 sprint-trained males (age = 26.4 ± 5.3 yr; Ht = 1.77 ± 0.03 m; Wt = 78.78 ± 9.10 kg; BF% = 13.96 ± 7.28%) completed 4, 3-min bouts of cycling at 50% mean anaerobic power, with 6 min of rest between bouts. On CHO day, subjects ingested 50g of CHO 30 min before exercise. On control day, subjects ingested a sugar-free drink (CON) 30 min before exercise. Blood was drawn before supplementation, 15 min before exercise, before and after each exercise bout, and 15 and 30 min post exercise. Visfatin, glucose, and insulin were determined. Truncal fat was assessed by dual energy x-ray. 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 significantly elevated after CHO ingestion and remained elevated throughout the first half of exercise. Baseline visfatin was significantly correlated with truncal fat (r^2 = 0.7782, p < 0.05). Visfatin was correlated to truncal fat in sprint-trained males, but was not altered by exercise or CHO supplementation.

Key words: Visfatin, glucose, exercise, carbohydrate supplementation, insulin.

Introduction

Visfatin is a protein preferentially expressed in visceral fat that has been implicated in multiple cellular functions (Rongvaux et al. 2002; Samal et al. 1994; Xie et al. 2007). These cellular functions include inflammation, regulation of nicotinamide adenine dinucleotide (NAD) biosynthesis, and glucose metabolism. Recently, the role of visfatin in glucose metabolism has generated considerable interest since the underlying mechanism related to the influence of visfatin on glucose uptake has yet to be identified. One popular proposed mechanism, displayed in hyperglycemic mice and human osteoblasts, is the visfatin-mediated increase in glucose uptake via increasing tyrosine phosphorylation of the insulin receptor, insulin receptor substrates 1 and 2 (IRS-1, IRS-2), and phosphatidylinositol 3-kinase (PI3K) (Xie et al. 2007). This mechanism may contribute to the restoration of insulin pathway sensitivity observed in hyperglycemic states (Choi et al. 2007; Fukuhara et al. 2005; Haider et al. 2006). Moreover, visfatin-mediated glucose uptake was observed to be dose-dependent in human mesangial cells. This effect of

visfatin may be a result of visfatin's ability to noncompetitively bind to the insulin receptor (Song et al. 2008). While this evidence suggests that visfatin influences glucose uptake on an intracellular level (possibly through its role in the NAD biosynthetic pathway), few studies have examined the extracellular relationship between plasma visfatin and glucose uptake.

The interaction between plasma visfatin and extracellular glucose regulation during exercise is not fully understood. Following chronic aerobic exercise training, resting blood glucose decreases, which is paralleled by plasma visfatin levels (Brema et al. 2008; Choi et al. 2007; Haider et al. 2006; Haus et al. 2009; Jorge et al. 2011). Correspondingly, resting plasma visfatin levels are above acceptable levels in hyperglycemic individuals (Chang et al. 2011). These data suggest that in aerobic training studies completed in humans, plasma visfatin may be altering glucose regulation in a manner similar to insulin, since the plasma levels of both hormones respond to glucose similarly (Fukuhara et al. 2005; Revollo et al. 2007). The effect of visfatin on glucose regulation in acute stress is also unclear, due to conflicting published results (Frydelund et al. 2007; Ghanbari-Niaki et al. 2010; Jurimae et al. 2009). In elite rowers, 2 hr of rowing performed at 80% of heart rate reserve resulted in significant decreases in plasma visfatin and insulin, despite no significant change in blood glucose (Jurimae et al. 2009). Conversely, visfatin was elevated more than two-fold after one exercise session including 7 bouts of 35 m sprint runs, and this elevation was mirrored by blood lactate, plasma insulin and blood glucose (Ghanbari-Niaki et al. 2010). Finally, in healthy subjects that cycled for 3 hr at 60% of VO_2 max, there was no significant change in plasma visfatin, despite a significant decrease in plasma insulin and blood glucose (Frydelund-Larsen et al. 2007).These seemingly contradictory results are likely due to the varying exercise protocols, as well as the varying subject characteristics.

Although the exercise literature is murky, carbohydrate intake studies can be used to provide some clarity in the response pattern of plasma visfatin compared to changing plasma glucose concentrations. Data from two carbohydrate intake studies in individuals with impaired insulin function suggest that plasma visfatin augments glucose uptake in a manner independent of insulin (Park et al. 2013; Rezvan et al. 2012). Research regarding carbohydrate intake and visfatin is limited, but available data suggest that glucose intake may suppress visfatin release;

however, the exact mechanism is still unclear (Bala et al. 2011). To our knowledge, no study has controlled for both carbohydrate supplementation and exercise in order to assess the relationship between visfatin, insulin, and blood glucose. In order to gain a better understanding of how plasma visfatin may affect glucose uptake, it is necessary to examine it in individuals without impaired insulin function. Therefore, the purpose of this study was to further investigate the effect of acute exercise and carbohydrate supplementation on plasma visfatin in an effort to further understand any role that visfatin may have in blood glucose regulation.

Methods

The University of North Carolina at Greensboro Institutional Review Board approved all methods. Written informed consent was received from all subjects, and a screening session was completed prior to the exercise sessions. During the screening session, abdominal circumference and BMI were assessed, and a Wingate test was performed to determine mean power (MP). Resistance during the Wingate test was 7.5% of body weight. Mean power was calculated as MP (W) = load (Kg on flywheel) x average revolutions x 11.765. Wingate tests have been used in previous studies to determine workload for a high-intensity interval exercise (Burgomaster et al. 2008; Franchini et al. 2016; Gibala et al. 2014) Resting heart rate (HR), blood pressure, medical history, and exercise history were also assessed. During the screening session, body composition and body fat distribution were determined by dual energy x-ray absorptiometry (DXA; Prodigy Advanced, GE Lunar). Truncal fat was determined by DXA analysis and defined 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 et al. 2010; Parikh et al. 2007). A 3-day dietary record was given to all subjects, and was completed for the 3 days prior to each exercise session. Dietary records were analyzed for total caloric intake and macronutrient percentage using the My Fitness Pal software (MyFitnessPal, LLC, San Francisco, CA).

After initially screening 12 individuals, 10 sprint-trained males between the ages of 18 and 30 with less than 20% body fat qualified for the study. A group of 10 subjects was determined via power analysis to be appropriate to yield a power of 0.80 with an alpha value of 0.05. 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 cycling. The majority of subjects (n = 6) cycled regularly, four of whom cycled at least 100 miles per week. Furthermore, mean peak power for all subjects was 1068.6 ± 79.3 watts (W), which ranks above the 95th percentile (Maud and Shultz 1989).

Subjects reported to the lab two times after overnight fasts. An indwelling intravenous (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 taken 30 min prior to exercise. Immediately after the first blood draw, a supplement of either 50 g of carbohydrates (236 ml of Gatorade G01 Prime Solution; 0g of protein, 0g of fat) or 236 ml of sugar-free Kool-Aid was administered as the carbohydrate supplementation (CHO) or the placebo control (CON), respectively. The order of supplement for each subject was determined by a coin flip to maintain randomness. Exercise began with a 6-min warm-up at a standardized intensity of 50 W. After the warm-up, each subject completed four 3-min exercise bouts at 50% of MP, separated by 6 min of active recovery at an intensity of 50 W. This timing and intensity was chosen based on a pilot study, as it elicited the necessary alterations in blood glucose (unpublished). Subjects were given water *ad libitum*, and water intake was not recorded. Blood draws were taken immediately prior to and immediately after each high-intensity interval. The final draws were taken 15 min and 30 min post exercise. These time points were chosen since exercise-induced changes in visfatin were shown to be reduced to baseline levels 30 min post exercise (Jurimae et al. 2009). Finally, in order for some subjects to be able to complete each bout, resistance was adjusted minimally; however, to control for any difference between exercise sessions, total work was calculated for each bout. Also, heart rate was recorded at each blood draw to further assess work. A 10-ml blood sample was obtained at each collection time point.

Blood was collected into EDTA-treated tubes, centrifuged at 3,000 rpm at 4 °C to separate plasma, and frozen at -80 °C until analysis. All blood variables were analyzed in duplicate. Plasma visfatin concentration was determined using a human visfatin enzyme-linked immunoassay (ELISA) (Adipobioscience, Santa Clara, CA). The sensitivity for this assay was 20-30 pg/ml, and the intra- and inter-assay CV were both less than 10%. Plasma insulin concentration was determined using a human ELISA kit (Mercodia, Winston-Salem, NC). The sensitivity for this assay was 1 mU/L, and the intra- and inter-assay CV were both less than 5%. Finally, blood glucose concentration was determined using a human ELISA kit (Wako Diagnostics, Richmond, VA). The sensitivity of this assay was 0.7 mg/dl, and the intra- and inter-assay CV were both less than 5%. Blood lactate concentrations were analyzed using a modified version of the Hohorst enzymatic assay (Sigma Aldrich, St. Louis, MO) (Hohorst 1957).

Mean caloric intake, exercise workload, and HR were analyzed using a paired samples t-test for subjects between conditions (CHO and CON). Blood variables were analyzed using a 2 (CHO and CON) x 12 (time point) multivariate analysis of variance (MANOVA). When a main effect was found, post hoc testing was performed using least squared differences. Blood variables were also compared between groups by determining relative area under the curve (AUC) using the trapezoidal integration method and analyzing AUC with a paired samples t-test. Finally, the relationship between plasma visfatin and body fat were correlated using a Pearson Product-Moment correlation coefficient. All data were analyzed using SPSS statistical package (Version 21, IBM, Minneapolis, MN) with statistical significance set at an alpha level of ≤ 0.05. All results are presented as mean

± standard deviation unless otherwise noted.

Results

All subjects were of healthy weight based on both body fat percentage (13.96 ± 7.28 %) and BMI (24.19 ± 2.82 kg·m^{-2}). Furthermore, there were no statistically significant differences in total mean caloric intake (2398 ± 241 vs 2418 ± 303 kcal·day^{-1}; p = 0.897) or in carbohydrate intake (1255.1 ± 241.2 vs 1305.4 ± 198.2 kcal; p = 0.749), protein intake (667 ± 86 vs 709 ± 70 kcal; p = 0.871)), or fat intake (545 ± 103 vs 487 ± 189 kcal; p = 0.782) for the 3 days prior to either the CHO or CON trial, respectively. Insulin sensitivity was determined via homeostatic model assessment for insulin resistance and no significant difference was observed between CHO and CON (p = 0.721). Baseline plasma glucose (5.22 ± 0.75 vs 5.04 ± 1.03 mmol·l^{-1}; p = 0.2367 CHO vs CON, respectively), plasma insulin (5.14 ± 1.89 vs 5.80 ± 4.12 mU·l^{-1}; p = 0.5732 CHO vs CON, respectively), and plasma visfatin (15.35 ± 14.74 vs 22.67 ± 25.46 pg·ml^{-1}; p = 0.3878 CHO vs CON, respectively) were not significantly different prior to carbohydrate or placebo supplementation.

Mean power from the Wingate test for all subjects was 633.8 ± 94.52 W, yielding a mean workload of 317.4 ± 47.3 W for each exercise bout. In order for some subjects to complete each 3-minute bout, resistance was minimally decreased, but the mean workload between trials was not significantly different (293.5 ± 7.5 vs 292.6 ± 10.5 W, p = 0.787). Furthermore, HR was not significantly different between trials at any time point (p > 0.05 between each time point). Total work done and exercise heart rates are presented in Table 1. Finally, to control for blood volume, hematocrit was assessed before, halfway through, and after each exercise session, and no significant differences were found (p > 0.05 at each time point).
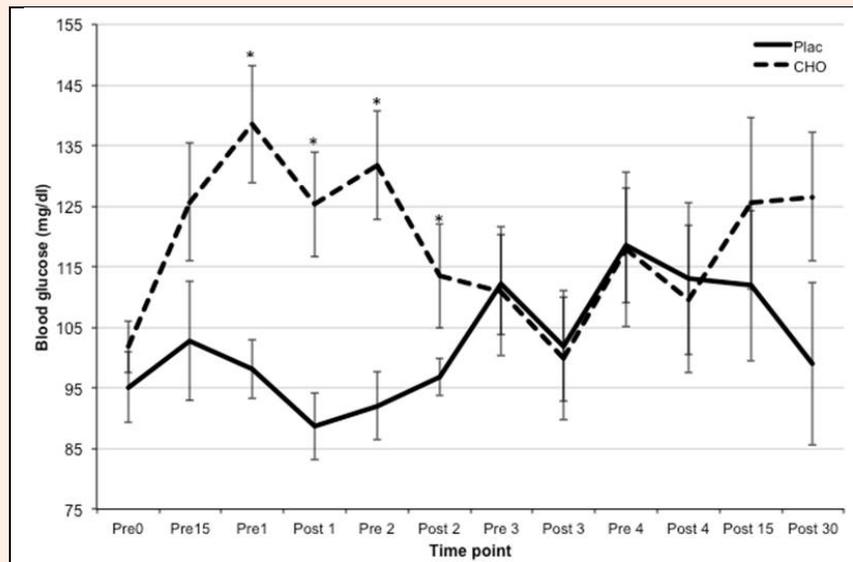
Fasted blood glucose was significantly higher immediately prior to the first bout of exercise in the CHO group but not the CON group when compared to the baseline value (138.57 ± 30.71 vs 98.10 ± 15.23, p < 0.05). Blood glucose was significantly increased 30 min after exercise and remained significantly elevated through the second exercise bout in the CHO group compared to the CON group (Figure 1). This increase in blood glucose was accompanied by an increase in plasma insulin at the same time point in the CHO group compared to the CON group, and continued for the same amount of time as the rise in glucose (Figure 2). Plasma visfatin was not significantly different between trials at any time point and did not differ across time points within either trial (Figure 3). Finally, plasma visfatin was not significantly correlated to blood glucose (r^2 = 0.0047, p = 0.626) or plasma insulin (r^2 = 0.0578, p = 0.554). Plasma visfatin was, however, significantly correlated to truncal fat (r^2 = 0.7782, p < 0.05). Lastly, blood lactate was significantly elevated after the first bout of exercise (p > 0.05) in both trials and remained elevated for the remainder of exercise with no further increase or difference between trials (Figure 4).

**Table 1.** Exercise workload and heart rate (HR). Data are means (±SD).

	Predicted Workload (W)	CHO Trial Workload (W)	Placebo Trial Workload (W)	CHO Trial HR	Placebo Trial HR
Bout 1	317.4 (47.3)	303.3 (44.0)	302.6 (41.6)	154 (23)	159 (22)
Bout 2	317.4 (47.3)	294.5 (46.3)	300.3 (50.0)	160 (26)	163 (29)
Bout 3	317.4 (47.3)	290.7 (45.9)	286.2 (50.2)	170 (17)	162 (33)
Bout 4	317.4 (47.3)	285.4 (51.4)	281.1 (54.5)	172 (30)	170 (29)
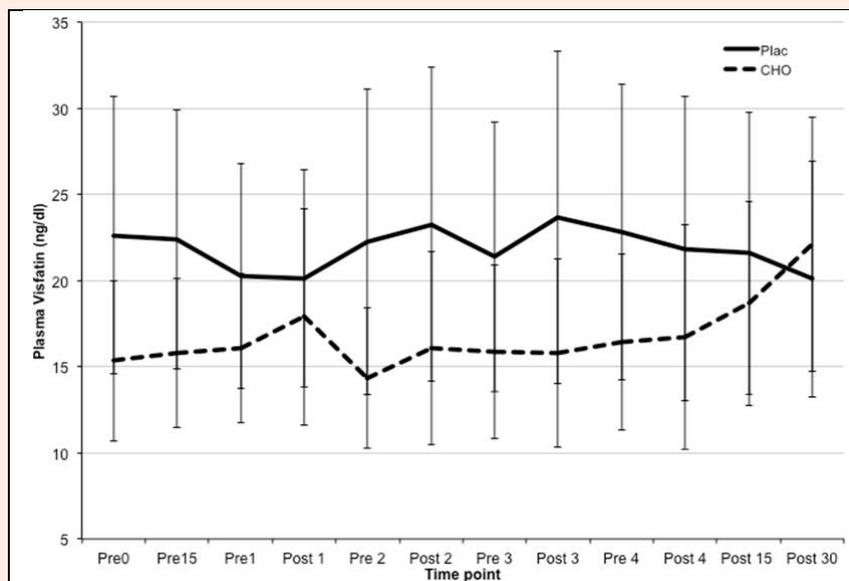
Figure 1. Plasma visfatin before and after each exercise bout.

Figure 2. Blood glucose before and after exercise bout. * Significantly higher than corresponding time point in placebo trial (p < 0.05).
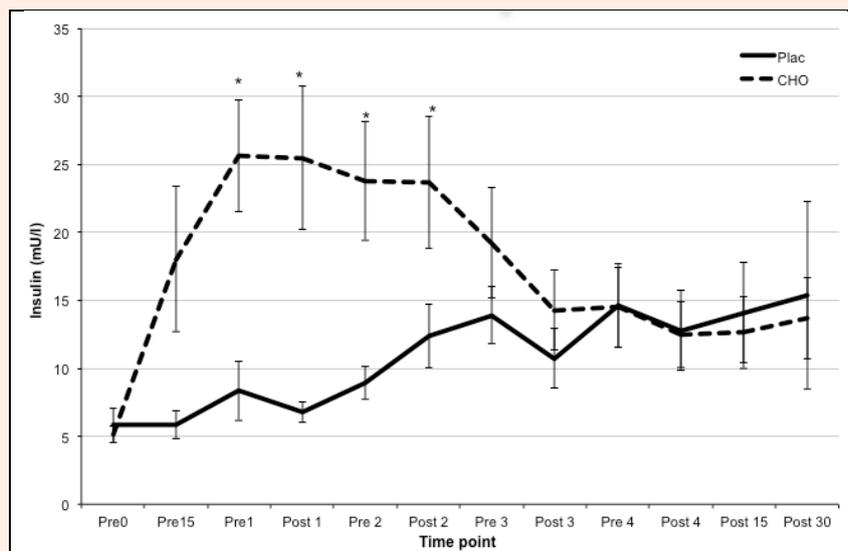

Figure 3. Plasma insulin before and after exercise bout. * Significantly higher than corresponding time point in placebo trial (p < 0.05).

Discussion

Previous literature suggests that visfatin may play a role in glucose metabolism, specifically by acting as an insulin mimetic. For example, plasma visfatin levels mirror changes in blood glucose following aerobic exercise, and in hyperglycemic, insulin-imparied subjects, plasma visfatin independently augments glucose uptake (Brema et al. 2008; Choi et al. 2007; Haider et al. 2006; Haus et al. 2009; Jorge et al. 2011; Park et al. 2013; Rezvan et al. 2012). Unfortunately, this small amount of existing data is not complete. Therefore, the purpose of this study was to assess plasma visfatin response to high-intensity exercise and carbohydrate supplementation-induced alterations in normoglycemic, insulin-sensitive subjects. The major finding of this study was that visfatin was not af-

fected by changes in plasma glucose due to either carbohydrate supplementation or high-intensity exercise. In contrast, after carbohydrate supplementation, plasma glucose and insulin significantly increased compared to placebo, and these increases were maintained through the first two bouts of exercise relative to placebo. Here, this maintained increase in plasma glucose most likely was due to carbohydrate supplementation and, in turn, an alteration in fuel availability during exercise (Jenkins et al. 1994; Pirnay et al. 1977). After the second bout of exercise, plasma glucose returned to baseline. This clearing of glucose was likely due to increased glucose uptake by active skeletal muscle through both insulin-dependent and independent mechanisms. Moreover, this glucose response is typical of exercise-trained individuals after receiving a carbohydrate supplement prior to exercise
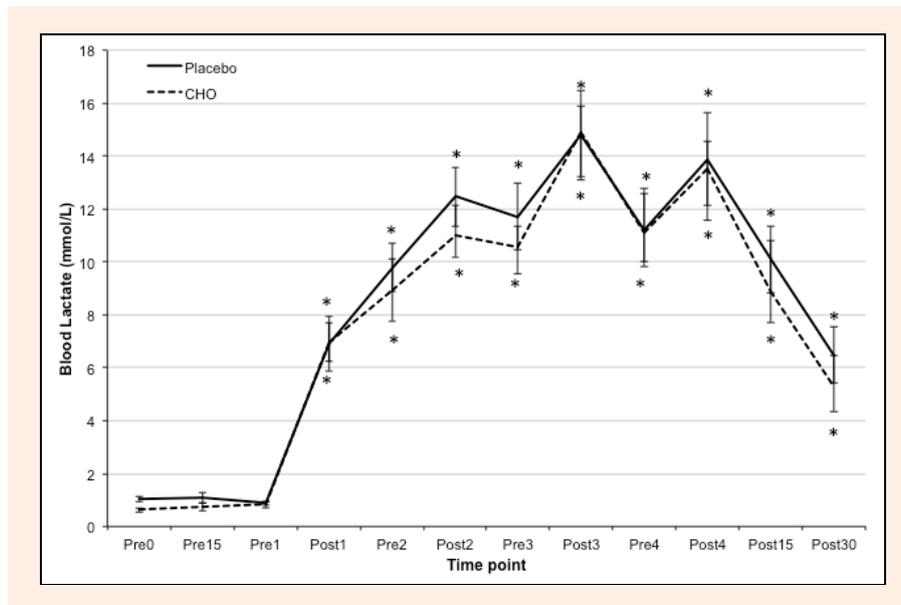
Figure 4. Blood lactate before and after exercise bout. * Significantly higher than pre-0, Pre-15 and Pre-1 values (p < 0.05).

(Jenkins et al. 1994; Jeukendrup et al. 1997; Pedersen et al. 2008). Furthermore, an increase in blood lactate was seen throughout exercise, which likely is partially due to the high-intensity exercise-mediated increase in glycolytic rate within skeletal muscle. Despite the increase and clearance of blood glucose, plasma visfatin did not change. Conversely, insulin responded in a manner similar to plasma glucose, significantly increasing after supplementation and remaining elevated halfway through the exercise trial (compared to placebo). The increase in insulin secretion is likely due to two factors: 1) an increase in blood glucose due to carbohydrate ingestion; and 2) an increase in transient insulin resistance often seen with high-intensity exercise (Ghanbari-Niaki et al. 2010). During high-intensity exercise, insulin resistance often occurs partially due to inhibition of insulin signaling by increased levels of catecholamines, growth hormone and cortisol (Felsing et al. 1992; Ghanbari-Niaki et al. 2010; Jurimae et al. 2009; MacDougall et al. 1977). Although insulin resistance, GH, and cortisol were not assessed in the current study, these data support that the subjects used in the current study were metabolically healthy, exercise trained, and thus, a good cohort in which to examine plasma visfatin as an insulin mimetic.

As a speculative insulin mimetic, visfatin should follow plasma insulin dynamics. In the current study, plasma visfatin was not significantly changed during either high-intensity exercise condition, although plasma glucose, insulin, and blood lactate were altered by carbohydrate supplementation and/or exercise. The only other high-intensity exercise study to investigate plasma visfatin showed that plasma visfatin was increased after 7 sets of 35 m sprints performed on a treadmill in healthy males (Ghanbari-Niaki et al. 2010). This increase in visfatin followed the post-exercise increase in insulin and glucose. The authors suggested that the increase in visfatin was a visfatin-mediated response to increase glucose uptake in the face of decreased insulin sensitivity seen with high-

intensity exercise. While the current study also utilized a high-intensity model, the duration of exercise bouts was significantly longer (3 min compared to 1 min), as was the rest time between bouts (6 min compared to 1 min).

Since plasma visfatin was not different immediately before or after any of the exercise sessions, one can assume that plasma visfatin did not play a significant role in glycolytic function in healthy individuals despite alterations in exercise metabolism. The reason for this lack of change in visfatin, again, may be due to a lower intensity of exercise or shorter rest periods compared to previous studies that used higher intensity exercise as a treatment (Ghanbari-Niaki et al. 2010). If visfatin does potentially mediate glycolytic function, exercise done at this intensity may have been low enough to allow for reliance on other substrates. Furthermore, if visfatin acts to increase glucose uptake when insulin function is impaired, as previously suggested, there likely was not the same transient insulin resistance seen with activity in the current study that has been seen in previous studies (Fukuhara et al. 2005; Ghanbari-Niaki et al. 2010).

The visfatin response to exercise is different in moderate endurance exercise. For example, Jurimae et al. showed plasma visfatin decreased 30 min after 2 hr of rowing, which was considered to be due to increased uptake of plasma visfatin by subcutaneous adipose tissue (2009). While it is well known that subcutaneous adipose tissue is actively involved in visfatin production and secretion, the evidence for visfatin uptake into adipose tissue is lacking. Moderate intensity exercise initiates an increase in subcutaneous adipose tissue visfatin mRNA; however, increases in plasma visfatin do not follow (Frydelund-Larsen et al. 2007), suggesting that while mRNA transcripts for visfatin are increased intracellularly, the translation to protein is not occurring at the same level or that any visfatin produced is not being released into the systemic circulation. Arguably, the increase in subcutaneous mRNA could have a local autocrine and/or

paracrine effect that may enhance insulin sensitivity after exercise. While previous studies suggest that visfatin may play a role in plasma glucose regulation during high-intensity running sprints and moderate-intensity endurance exercise, the present study suggests otherwise.

Another potential interaction between visfatin and glucose regulation could be its effect on metabolism as an enzyme in the NAD salvage pathway. Some studies have shown that enhancing caloric restriction and chronic exercise both lead to increases in mitochondrial respiration partially by increasing NAD availability through increased activation of visfatin (as NAMPT) both *in vivo* and in cell culture (Revollo et al. 2004; Rongvaux et al. 2008; Yang et al. 2007; Hsu et al. 2009). Frederick et al. recently showed that enhancing NAD synthesis specifically within skeletal muscle did not lead to any changes in oxidative metabolism in healthy subjects (2015). Those results suggest that the role of visfatin (as either a hormone or an enzyme) may likely be important in maintaining mitochondrial respiration as well as glucose intake in individuals for whom glucose uptake is impaired, but not in a healthy population.

The acute effect of carbohydrate intake on plasma visfatin is not clear. Bala et al. (2011), showed plasma visfatin to be significantly decreased after ingestion of carbohydrate and this decrease was more pronounced in individuals with a higher BMI. The subjects in this study, however, were not screened for obesity or hyperglycemia. Alexiadou et al., on the other hand, found plasma visfatin to be unchanged with glucose intake in type I diabetics (2012). Lastly, Zhaoxia et al. found visfatin levels to increase in gestationally diabetic women after an oral glucose tolerance. These results again suggest that visfatin may mediate glucose uptake when insulin function is impaired (Zhaoxia et al. 2012). The reason for the discrepancy in previous studies is likely due to the heterogeneity of subjects used in the different studies.

In the current study, plasma visfatin was unchanged by carbohydrate intake. Because the subjects used in this study were insulin sensitive, plasma visfatin does not appear to be affected by alterations in plasma glucose in healthy young men. To our knowledge, this is the first study to examine the effect of carbohydrate intake on plasma visfatin in trained, healthy young males. Although no change in plasma visfatin response was observed in the current study, a cellular visfatin response cannot be excluded. In fact, multiple studies have shown that alterations in cellular visfatin protein levels enhance glucose uptake, including skeletal muscle (Fukuhara et al. 2005; Revollo et al. 2007; Song et al. 2008; Xie et al. 2007). Because no cellular analyses were performed in the current study, it is not possible to say if visfatin content and/or activity were higher or lower in skeletal muscle after carbohydrate ingestion or high-intensity exercise. This potential relationship should be further assessed, since this mechanism holds promise as a therapeutic target for insulin resistance-based pharmaceuticals.

Because visfatin can be secreted from adipose tissue, the correlation between truncal fat and plasma visfatin was also examined in the current study. While previous studies have not assessed truncal fat, the relationship between visceral fat and plasma visfatin has been debated. Fukuhara et al. (2005) showed a direct correlation between visceral fat and plasma visfatin, while Chang et al. (2010) found no correlation. Although the study by Fukuhara et al. (2005) was later retracted for reasons unrelated to this part of the study, the authors stood by their findings related to visceral fat and visfatin. In the current study, a significant correlation was found between truncal fat and plasma visfatin (r = 0.7782, p < 0.05). Visceral fat was not directly assessed, but truncal fat has been shown to be a direct indicator of visceral fat (Dwimartutie et al. 2010; Parikh et al. 2007). Although all subjects had a body fat percentage below 20, this direct correlation may partially explain the large standard deviation seen in the current study (resting visfatin: 11.80 ± 10.15). Furthermore, this is the first study to show a significant correlation between plasma visfatin and truncal fat in healthy subjects.

Every attempt was made to control confounding variables as much as possible in the current study. There were, however, some limitations to this study. First, not all subjects were cyclists. Subjects were excluded if they did not exercise at a high-intensity at least three times per week. This led to a well-trained subject pool, but some subjects (n = 6) were experienced cyclists (cycling at least 100 miles/week). As the mode of exercise was a cycle ergometer, this may have altered the exercise response in some subjects. Also, carbohydrate supplementation was not based on body weight, although there was not a significant difference regarding grams of carbohydrate per kg of body weight (641 mg ± 68 mg) between subjects. The dosage of carbohydrate was based on a previous study, which found 50 g of carbohydrates elevated blood glucose in healthy individuals (Kraemer et al. 2011), as well as an unpublished pilot study. While supplementation was not based on body weight, blood glucose was significantly increased (33.12 ± 1.24 %) beyond resting levels in all individuals after carbohydrate supplementation. As the purpose of this study was to assess plasma visfatin changes in response to blood glucose changes, the carbohydrate supplement used was an adequate stimulus. Despite these limitations, the present study shows that in healthy young men, plasma visfatin is not affected by carbohydrate supplementation or acute intermittent, high-intensity exercise. Future studies are needed to determine the relationship of visfatin and blood glucose regulation on an intracellular level as well as visfatin's relationship to other hormones associated with blood glucose regulation during exercise.

Conclusion

The present study demonstrates that no significant relationship exists between plasma glucose and plasma visfatin in young, exercise-trained, insulin-sensitive males, and that visfatin likely does not mediate glucose uptake since plasma visfatin did not change despite changes in blood glucose, plasma insulin, or lactate. The lack of effect in this population suggests the possibility that plasma visfatin may only be an effective contributor to glucose control when insulin function is impaired or possibly through its

enzymatic regulation of NAD salvage. Still, future research is needed to further examine the basic biological role of visfatin in the human.

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Key points

- Plasma visfatin was not affected by exercise or carbohydrate supplementation.
- Plasma visfatin was significantly correlated to abdominal fat.
- Plasma visfatin did not follow a similar pattern to blood glucose or plasma insulin as has been shown in previous studies.

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