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The Effect of an Acute Aerobic Bout on Time Course Alterations of Visceral Adipose Tissue Gene Expression in Diabetic Rats

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Abstract

Introduction: Diabetes mellitus is a group of metabolic disorders that are characterized by a decline in insulin secretion, insulin malfunction or both. Visfatin is a recently discovered protein that is expressed and secreted in visceral fat tissue. Elevated plasma visfatin levels have been reported in humans with type 2 diabetes mellitus. The purpose of this study was to investigate the effect of a single bout of aerobic exercise on visceral adipose tissue visfatin gene expression in a diabetic rat model.

Material and Methods: Diabetes was induced by streptozotocin (50 mg/kg) injection in a single dose administered to twenty male Wistar rats (6 – 8 weeks old, 165 ±5 grams weight). The rats were randomly divided into four groups, each comprising five rats; one control group and three exercise groups. The acute aerobic exercise bout required the three exercise groups to complete a single session of running for 50 minutes on a treadmill at a velocity of20m.min^{-1}. Following the treadmill run, one of the exercise groups were anesthetized immediately after the exercise, the second one 4h -after exercise and the last group were anesthetized 24h post exercise. Tissue samples were collected immediately after the animals were anesthetized. To determine the relative levels of gene expression, semi-quantitative RT-PCR was performed.

Results: The results showed a significant increase in the expression of visfatin in the 4h and 24h post exercise groups compared with the control group ($P<0.05$).

Discussion and Conclusion: In summary, exercise can improve the visceral fat tissue levels of visfatin mRNA expression. The increase in the visceral fat tissue visfatin mRNA suggests that visfatin in visceral fat tissue could possibly play an important role in glucose metabolism and act as a paracrine messenger.

Key words: Aerobic exercise, Type 2 diabetes, Visfatin

Introduction

A correlation between fat accumulation in visceral adiopose tissue,insulin resistance, obesity, and cardiovascular disease is well documented. The association between accumulation of visceral adipose tissue (VAT) and insulin resistance is well established in obesity and type 2 diabetes, while both visceral fat and insulin resistance are strongly associated with increased risk of cardiovascular disease[1-3].Visfatin/PBEF has recently been identified as a protein highly expressed in VAT compared with subcutaneous adipose tissue (SAT)[4]. Visfatin, has been previously known as a pre–B-cell colony–enhancing factor (PBEF), also having a function in the immune system, where it is described as a growth factor for early B-cells[5]. Visfatin/PBEF binds with and activates the insulin receptor in different insulin-sensitive cells in vitro. Also in mice treated with recombinant visfatin/PBEF, it elicited insulin-like effects in vivo[6].

Haider et al [7] reported an increased circulating visfatin in response to thiazolidinedione treatment, which was abolished by acute elevation of free fatty acids, further demonstrating visfatin's response to nutrient availability. Recent reports now suggest that visfatin is an enzyme (Nampt) that is responsible for both intracellular and extracellular NAD biosynthesis and that the product of the Nampt enzyme, nicotinamide mononucleotide (NMN), may provide a mechanism for NAD-stimulated insulin secretion in isolated pancreatic islets. Collectively, these data suggest that visfatin mediates glucose homeostasis and exerts a potential anti-diabetic effect through a nutrient-sensing mechanism that may modulate

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pancreatic A-cell function [8].

Exercise also has a profound effect on nutrient balance and improves insulin sensitivity. As a result, exercise is widely advocated as a first-line treatment in the control of obesity, insulin resistance, and type 2 diabetes. It has recently been reported that VAT loss after aerobic exercise training improves glucose metabolism and is associated with the reversal of insulin resistance in older obese men and women[9]. Thus, it seems likely that visfatin may respond to exercise training, however studies examining the effects of exercise on circulating visfatin are limited [10-13]. Furthermore we are unaware of any published studies that have focused on the time course of the gene expression in visceral adipose tissue following a single bout of exercise.

Given that regular exercise enhances insulin sensitivity, and that visfatin enhances glucose uptake in adipocytes, we hypothesize that exercise regulates visfatin in visceral adipose tissue. In the present study, we investigated the effect of a single session of aerobic exercise on visfatin mRNA levels in rat visceral adipose tissue[12].

Material and Methods

Animals

All experiments involving the animals were conducted according to the policy of Iranian Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes, and the protocol was approved by the Ethics Committee of the School of Medicine Sciences, Tarbiat Modares University (TMU), Tehran, Iran. Twenty Wistar male rats (8 weeks old, 165 +5grams weight) were used for this study. Animals were obtained from Pasteur's Institute (Amol, Iran) and were housed in the Central–Animal House, in the Faculty of Biology of Mazandaran University. 5 animals were housed per 46-L volume cage. Light was controlled on a 12:12 h light–dark cycle. Temperature was 22 ± 1.4 C and humidity was 55.6 ± 4.0%. Animals were fed a rodent pellet diet ad libitum and had free access to water. Animals were randomly assigned into four groups of five animals each. A control group remained sedentary (Con) while the other three groups undertook a single bout of 50 minutes treadmill run at a velocity of20 $m.min^{-1}$.

Exercise training protocol

The treadmill exercise began with familiarization of rats with the apparatus for a week by placing them on the motorized-driven treadmill (Iranian Model, 10 lanes,Ghanbari-Niaki, Physical Education & Sports Sciences Department of Mazandaran University, Babolsar, Iran).The three exercise groups performed a single exercise session that included a 10-min light warm-up, followed by a 50-min run on the treadmill at 20 $m.min^{-1}$ speed. The duration of the entire session was 60 min. This condition corresponded to an intensity of approximately 50-55% of maximal oxygen consumption[15]. One exercise group were anesthetized immediately after exercise, the other group 4hrs, and the last group 24 hours post-exerciser., Rats were anesthetized intraperitoneally with a mixture of Ketamine (30–50 mg/kg bw, ip) and Xylazine (3–5 mg/kg bw, ip), and a part of the visceral tissue were excised, washed in ice-cold saline, and was immediately frozen in liquid nitrogen for the extraction of visfatin mRNA. All frozen adipose tissue pieces were stored at -80 ^{O}C before the analysis was performed. Blood samples were collected directly from the heart, in test tubes containing EDTA. Then they were centrifuged, frozen and stored at -80 ^{O}C until the biochemical analysis was performed.

visfatin expression

The quick-frozen part of visceral adipose tissue was powdered with cold mortar and pestle, and approximately 50 mg of the mixture was used for the isolation of RNA. Total RNA was extracted by the guanidine thiocyanate method [16] and mRNA was purified using mRNA Isolation Kit (Roche, Germany) according to the manufacturer's instruction. Two-hundred nanogram of mRNA was used for the synthesis of first strand cDNA in a 20 volumeusing oligo (dT) primer in the first-strand synthesis kit (Fermentase, Germany). Relative expression levels of visfatin mRNA in the adipose tissue, were determined using a semi-quantitative PCR method. The following primers were used to amplify rat visfatin and b-actin (as an internal control) cDNA:

For-vis 5`-ACCAACTGGATTGAGACTATTC-3`,
Rev-vis P: 5`-GACTCCTCTGTAGCCGAAG-3`
Forward-beta-act: 5`-
TTGTAACCAACTGGGACCCCCGATATG-3` (27 bp),
Reverse-beta-act:5`-
CGCTCTTGCCGATAGTGATG-3` (20 bp)

Visfatin cDNA was amplified giving a 237-bp product. PCR was formed for 35 cycles of denaturation 94 C for 30 s, annealing of 55.5 C for 30

s and extension at 72 C for 50 s. Reactions were set up using a twofold serial dilution of cDNA template to assess the best dilution of template in PCR. cDNA Template was standardized by the amplification of a 315-bp internal control of b-actin, a house keeping gene. All the reactions were repeated at least three times to ensure repeatability. All PCR products were electrophoresed on an agarose gel and bands visualized by ethidium bromide staining and were quantified by a computer integrated densiometry (Kodak, CT). Levels of mRNA were expressed as a ratio of signal intensity for the b-actin gene. Lipids and lipoproteins Plasma high density lipoprotein cholesterol (HDL) were determined by direct Immuno method (HDL-C Immuno FS, Pars Azmoun, Tehran, Iran), the Intra-assay coefficient of variation and sensitivity of the method were 1.2% and 0.03 mmol/L respectively. Plasma total triglyceride (TG) was determined by enzymatic (GPO, Glycerol-3-Phosphate Oxidase) colorimetric method (Pars Azmoun, Tehran, Iran), the Intraassay coefficient of variation and sensitivity of the method were 2.2% and 1 mg/dL, respectively. Plasma total cholesterol (TC) was determined by enzymatic (CHOD-PAP, Cholesterol Oxidase-Amino Antipyrine) colorimetric method (Pars Azmoun, Tehran, Iran), the Intra-assay coefficient of variation and sensitivity of the method were 1.9% and 0.08 mmol/L, respectively. The procedure of Friedewald et al[17]was used to estimate low-density lipoprotein cholesterol (LDL-C). All results are expressed as means ± SEM. All variables were compared using a one-way ANOVA. All statistical analyses were performed using SPSS (Version 19). All P values <0.05 were considered significant.

Results

Significant differences were found between different groups and time points (P<0.05). Using a suitable post hoc test revealed a higher and significant visfatin gene expression in visceral adipose tissue 4 and 24 hours after the accomplishment of an acute aerobic exercise bout (Pvisceral adipose tissue visfatin mRNA increased in response to exercise compared with control group (Fig. 1)). A significant increase in visfatin mRNA was observed at two time points in the exercise group, 4 h, and 24 h (Post-exercise), which were also significantly different from the control group (*P*< 0.05)(Fig. 2). Visfatin concentration in adipose tissue also increased following exercise (Table.1) but plasma visfatin decreased significantly in the training group compared to the control group (Table.1).

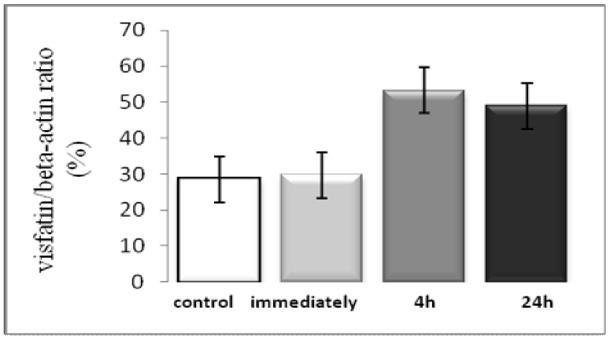

Figure 1: semi-quantitative RT-PCR of visfatin gene expression in visceral adipose tissue in rats

Table 1: Plasma levels of visfatin, Liver Glycogen, plasma NEFA, HDL, LDL, Cholesterol, Triglycerides, Glucose, Adipose tissue visfatin concentration in response to exercise and control groups

Avisfatin (ng/mg)	Pvisfatin (ng/mg)	Glucose (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	plasma NEFA (mg/dl)	Liver Glycogen (mg/g)	Group	
1062.12	31.28	184.62	122.75	133.63	91.30	26.06	4.49	.31	Mean	DC
117.44	20.97	28.22	40.64	7.90	11.41	6.28	.99	.092	SD	
1240.62	10.63	175.13	86.75	130.75	81.05	36.32	6.30	.23	Mean	DTa
124.17	5.09	16.47	17.36	19.46	21.94	7.51	1.51	.026	SD	
1290.00	14.50	184.88	106.50	133.38	91.62	28.06	5.06	.26	Mean	DTb
174.87	8.33	26.79	57.24	14.62	17.04	6.73	2.26	.039	SD	
1050.75	16.26	204.75	111.88	129.63	81.87	29.02	5.34	.29	Mean	DTc
241.05	12.51	35.22	34.65	10.26	15.92	4.23	1.10	.046	SD	

DC: diabetes control, DTa: immediately after exercise, DTb: 4h after exercise and DTc: 24h after exercise

Discussion and Conclusion

In the past decade, many papers have shown that increased secretion of cytokines from fat tissue, may be responsible for Proinfalmmatory starting position that leads to the development of insulin resistance. These observations, in an effort to reduce or stop the increasing prevalence of type 2 diabetes on obesity as the primary intervention, have been proposed.[18].

The data show that endogenous visfatin is involved in regulating glucose homeostasis. Although visfatin affinity with the insulin-receptor is similar to insulin, but fasting plasma visfatin level is about 10 percent, and, when full, about 3 percent less than that of insulin. Thus the contribution of endogenous visfatin in maintaining euglycemia is less than insulin.. Increased visfatin synthesis, associated with obesity and type 2 diabetes, may be a compensatory response to maintain the normoglycemia[19].

The main finding of the current study was that visfatin gene expression increased 4h after exercise training, and partly decreased 24h post-exercise. Chen and colleagues (2007) investigated visfatin levels in Chinese society and found a positive relationship between visfatin and diabetes type 2. The data suggest that visfatin is important for normal insulin secretion, but the relationship between visfatin and the risk of developing type 2 diabetes is still questionable. Therefore, visfatin may be a compensatory mechanism or a part of the pathophysiology of diabetes[20].

After Fukuhara et al (2005) showed the role of visfatin in glucose metabolism, other researchers investigated the association of type 2 diabetes, with insulin secretion and sensitivity as well as its relationship with other adipocytokines like adiponectin. Revello et al (2007) showed that visfatin is an essential enzyme in the production of NAD. Since the pancreas contains very low levels of intracellular visfatin, these researchers showed that maintaining high levels of circulating NMN by extracellular visfatin should be critical for beta cell normal function[8]. Horden et al (2012) also showed that visfatin can regulate insulin secretion, insulin receptor phosphorylation and expression of several genes associated with beta cell function in mice[21].

The regulation of blood glucose is controlled by a large number of endocrine factors, including insulin, glucagon, and other hormones. The continuing discoveries of adipocyte-derived cytokines, including leptin, adiponectin, resistin, and IL-6 have shed light on the cause of type 2 diabetes. These adipokines provide an extensive network in which each part communicates with other adipocytes and remotely with other tissues and organs. The network is involved in multiple biological functions, including insulin resistance, lipid metabolism, and energy homeostasis[22].

Evidence suggests that visfatin is an adipokine that exerts insulin-like action. Visfatin is able to mimic insulin function and lower plasma concentrations of glucose through binding to the insulin receptors[23]. Other studies found that the serum concentrations of visfatin increased in diabetic patients, suggesting that visfatin may act as a compensatory factor in glucose metabolism[24].

Acute intravascular induced visfatin reduces plasma glucose levels but renders no effect on insulin. visfatin directly affects hypoglycemia but not through insulin secretion stimulation[4]. Furthermore, visfatin stimulates preadiposities differentiation to mature cells and accelerates triglyceride synthesis from glucose. In humans, plasma visfatin is associated with body fat percent, BMI and visceral fat tissue visfatin mRNA levels, but not with visceral fat mass or waist to hip ratio[25].

Visfatin may be involved in improving insulin sensitivity[23]. Contrary to previous findings, mRNA levels of visfatin in visceral fat tissue, after correction of BMI, was not associated with indices of insulin resistance[26]. Some studies reported that circulating visfatin increased in obesity and diabetes type 2, however some of them do not show any correlation with obesity and metabolic variables. The practice has been successful (effective) in reducing levels of visfatin and improved insulin sensitivity[23].

As previously mentioned, the present study is the first published research that has studied visceral adipose tissue visfatin gene expression in response to a single exercise session in diabetic subjects (animal and human). The findings of this study showed a significant increased in the gene expression of visfatin in response to aerobic activity in diabetic rats 4 hrs and 24 hrs after exercise.

Fukuhara et al (2005) demonstrated the relationship between visfatin plasma levels, visfatin mRNA in visceral fat tissue and acute exercise [4]. It is unclear,

whether visfatin is secreted into the circulatory system, because the primary amino acid sequence of visfatin does not contain a single peptide[5]. Previous reports showed that visfatin was primarily located in the cell nucleus and cytoplasm and questioned an endocrine role of visfatin.

In the present study, the tendency towards a decrease in plasma visfatin levels in the exercise group compared to the control group is consistent with the above report. However, exercise-induced changes in mRNA levels of visceral fat tissue were not consistent with the changes in plasma visfatin levels. Because the effects of a session of aerobic exercise on visfatin gene expression in human and animal samples have not been undertaken so far, data is very limited in this regard. In this study we postulate that the role of visfatin in response to acute exercise is related to a paracrine messenger rather than a consequence of endocrine effects.

Recent studies (27, 28) have shown that visfatin was involved in inflammatory processes and was an important part of atherosclerosis. Plasma visfatin levels positively correlated with serum levels of IL-6 and CRP, which indicates that circulating visfatin may show inflammation status[28]. Furthermore it is shown that visfatin plays an important role in NAD biosynthesis pathway. Recent studies have also shown that visfatin may be involved in mild inflammation [29].

In the present study, plasma visfatin levels decreased significantly in all exercise groups compared to the control group. According to the data by Kasapis and colleagues (2005), a training session stimulates increased release of Proinflammational cytokines leukocytes-dependent and increased C-reactive protein plasma concentration. This proinflammatory response to acute exercise is associated with a sudden increase in oxidative stress and the subsequent inflammation adaptations mechanisms[30]. Talebi et al (2012) in a study with a similar protocol to the present study, have investigated the effect of aerobic training on gene expression and plasma concentration of Lipokalin-2, which showed a significant reduction in Lipocalin-2. Lipocalin-2 increases in response to inflammatory stimuli[27] and there is strong positive correlation between lipokalin-2 and serum CRP[32]. In this regard Staels et al (2005) showed that plasma CRP and lipokalin-2 levels increased in obese people and that relative changes in lipokalin-2 concentrations mildly correlated with the changes in CRP concentration[33]. Probably this timeline of diabetes and training intensity did not lead to an adequate inflammation level for the plasma visfatin levels to change.

The present study also shows a glucose decrease in the exercise group compared to the control group, but these differences were not significant. Fukuhara et al (2005) linked visfatin with glucose metabolism and obesity through its abilities (activating insulin receptor, increasing glucose consumption) for the first time[4]. However, other researchers showed that the relationship between glucose and visfatin with obesity related variables could be due to its role as a biosynthetic enzyme Nampt and NAD involvement in transcription regulation. Regardless of the mechanism, the researcher investigated the role of visfatin and visfatin gene polymorphism in glucose-and obesity-related conditions and visfatin response to pharmacologic interventions to reduce weight[4, 34]. It has been indicated that aerobic exercise can be a suitable way of improving health, for example glucose tolerance and insulin sensitivity[35].

Given the contradictory results, the effect of exercise on visfatin's exact mechanism is not well known. However, it is likely that changes in plasma visfatin in terms of its tissue expression is influenced by several factors such as decrease or increase in blood glucose, insulin, weight loss, body mass index, caloric restriction and fat-rich food. In contrast, in the researches that reported visfatin decrease, lower plasma glucose and insulin levels, weight loss and body mass index were noted [11, 37-39].

Studies show that human fatty tissues are not only energy storage organs, but also endocrine organs that secrete Resistin and adipokines such as adiponectin, and leptin. It has been proved that obesity, especially visceral obesity, is common in insulin resistance and in type 2 diabetes. Visfatin affects insulin signaling and could link to insulin receptors. It has been shown that the visfatin expression affects plasma glucose and lipid concentrations [35]. However further studies are needed to clarify molecular mechanisms through which plasma visfatin affects glucose and lipid metabolism. .

The present study also demonstrated a significant increase in HDL and NEFA immediately after exercise. Recently, the relationship between serum visfatin and HDL has been reported in Indian subjects, but such a relationship was not found in

Caucasian subjects[40]. The relationship between visfatin and lipid profiles can be explained through the cytosol function of visfatin as phosphoribosyltransferase (NAMPT). Observations have shown that maintaining low plasma triglycerides and high HDL may be associated with NAD metabolism. Also the relationship between visfatin levels and lipid profiles indicated that visfatin can act as an interface between these two processes. The positive correlation between visfatin and HDL cholesterol and its negative correlation with , triglyceride levels shows that circulating visfatin, is a useful marker of lipid metabolism that is associated with NAD metabolism[41]. Further studies in this regard may help us understand the physiological role of circulating visfatin.

Conclusion

In summary, this study showed that one acute exercise session on a treadmill improves the mRNA levels of visfatin in visceral fat tissue. These findings suggest that visfatin in visceral fat tissue may have of a metabolic role in the recovery period after exercise and may also be involved in inflammatory processes induced by exercise. The increase in visceral fat tissue mRNA expression was not associated with elevated plasma visfatin levels, and visceral fat tissue, and this shows that visfatin may act as an endocrine Paracrin. However, considering the conflicting results further research is needed in this regard.

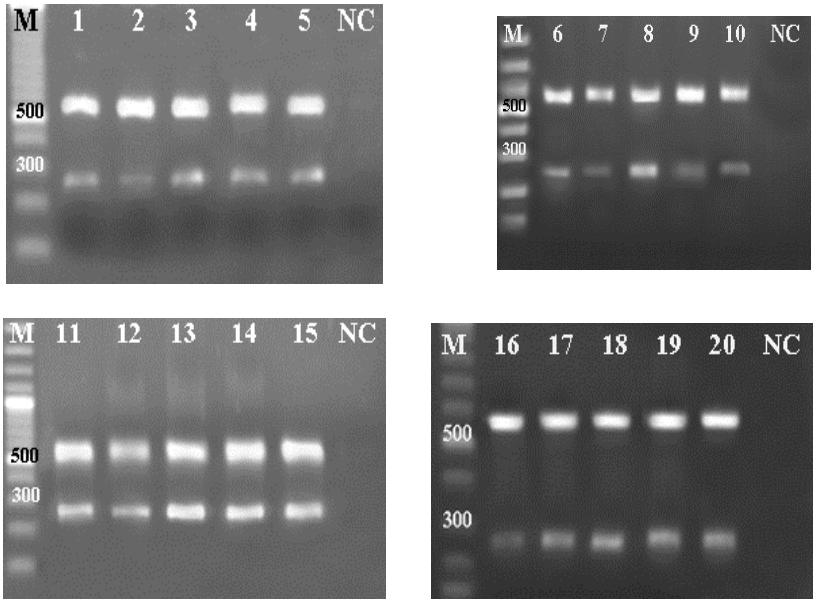

Figure 2: Results for semi quantitative reverse-transcription PCR of visfatin mRNA in visceral adipose tissue in rats

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