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VASPIN, ADIPONECTIN AND LEPTIN LEVELS IN TYPE 1 DIABETIC RATS INDUCED BY STREPTOZOTOCIN

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Abstract

Background. Adiponectin, vaspin and leptin are only a few of these numerous adipocytokines. Little is known about the behavior of adipocytokines and how adipose tissue metabolism is affected in this Type 1 DM model. In this study we investigated the serum levels of adiponectin, leptin, vaspin in streptozotocin(STZ) induced diabetic rats.

Material and methods. Twelve Spraque Dawley albino rats were included in the study. The animals were divided into two groups. The first group was diabetic (D) (n: 6) and 60mg / kg STZ was administered intraperitoneally (i.p.) to these rats. The second group was the non-diabetic control (ND) group (n: 6). All the animals were euthanized by cervical dislocation. Quantification of vaspin, Adiponectin, leptin in serum was performed using the ELISA kit.

Results. Adiponectin, vaspin levels of diabetic group were found to be statistically lower than of control group ($p<0.05$). Leptin levels were significantly higher in the diabetic group ($P<0.05$).

Conclusion. There is a need for new researches that can explain the relationship between Vaspin, Leptin and Adiponectin and Type 1 diabetes. New studies in this area will open new horizons for the identification of new biomarkers in the diagnosis and treatment of Type 1 diabetes.

Keywords: Vaspin, Adiponectin, Leptin, Type 1 diabetes, Streptozotocin.

INTRODUCTION

Although the adipose tissue was only considered as a fat deposition site previously, it is now considered to be an endocrine organ that secretes a large number of cytokines that have been implicated in insulin sensitivity, inflammation, coagulation, and atherosclerosis in recent years (1). Numerous cytokine secretions by adipose tissue have been found, such as Leptin, Resistin, Adiponectin, Vaspin, Chemerin, Apelin, Visfatin, Hepcidin, Adipsin, Omentin, TNF-α, IL-6, IL-8, Monocyto chemoattractant protein (MCP-1), Prostaglandin I2 (PI2), Prostaglandin F2α (PG2F2α), Nerve Growth Factor (NGF), Plasminogen activator inhibitor-1(PAI-1), acylation-stimulating protein (ASP) and insulin-like growth factor (2). Adiponectin, Vaspin and Leptin are only a few of these numerous adipocytokines.

Vaspin is a member of the serine protease inhibitor (serpin) family, first described in the visceral adipose tissue of diabetic rats (3). Diabetic or insulin-resistant subjects were found to have higher vaspin levels than low-fat normal subjects (4). It has also been claimed in recent studies that vaspin has a modulatory role on glucose metabolism (5). But its mechanism is not clear. It is thought that this modulatory role of vaspin may be associated with the regulation between the decrease in pancreatic insulin secretion and the changes in insulin sensitivity in peripheral tissues (KC, muscle, etc.) (6,7). It is also emphasized in some studies that worsening diabetes results in weight loss and decreased vaspin expression, which is normalized with the treatment of insulin or pioglitazone. It is also thought that vaspin may have an insulin sensitizing effect on white fat tissue (5). All these findings may lead to the development of new treatments in the future with the use of vaspin analogs or antagonists to increase insulin sensitivity in the metabolic syndrome (8).

Leptin regulates energy homeostasis and informs the hypothalamus about body fat tissue. Leptin also participates in glucose metabolism by reducing glucogenolysis in the muscle, liver and fat cells while

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increasing glyconeogenesis (9). Depending on the state of energy in the body, it is synthesized and secreted in fat cells (10). Leptin is also effective on different tissues and systems such as body lipid metabolism, hematopoiesis, pancreatic beta cell function (11-13). Leptin reduces intracellular lipid levels in the skeletal muscle, liver and pancreatic beta cells by enhancing insulin sensitivity (14). Many experimental studies have shown that the role of glucose on leptin release from adipocytes is very important (15).

Adiponectin is a collagen-like plasma protein synthesized by adipose tissue (16). In clinical studies, it has been determined that adiponectin level is low in obesity, type 2 DM and coronarian patients (17-20).

Diabetes induction with streptozotocin (STZ) is a widely used experimental model for studying the pathophysiology of diabetes mellitus. It is known that STZ causes diabetes mellitus by damaging the pancreas and the liver in rats (21, 22). This experimental model is a type 1 DM model. Little is known about the behavior of adipocytokines and how adipose tissue metabolism is affected in this DM model. Indeed, there are few reports that show the behavior of adipokines, specifically vaspin, adiponectin and leptin, in experimental diabetes models. A better understanding of the relationship between adipocytokines and DM can provide new information about the physiopathology of diabetes. This new information can lead to new and different treatment methods in diabetes. Therefore, in this study we investigated the serum levels of adiponectin, leptin, vaspin in streptozosin (STZ) induced diabetic rats.

MATERIAL AND METHODS

Animals

Sprague Dawley albino male rats (250 gr) were housed in a room at constant temperature of 22°C, with 12-h light/dark cycles and fed on standard pellet chow and water *ad libitum*. The animals were obtained from the laboratory Animal Care Division of the Health Science of University.

Experimental procedure

Twelve Spraque Dawley albino male rats (250-300 g) were included in the study. The animals were randomly divided into two groups. The first group was diabetic (D) (n: 6) and 60mg / kg STZ was administered intraperitoneally (i.p.) to these rats. Forty eight hours after STZ injection, blood glucose levels were measured and rats having more than 200 mg/dL were considered as diabetic. The second group was the non-diabetic control (ND) group (n: 6) and no treatment was performed.

At the end of the 6 week experimental period, all the animals were euthanized by cervical dislocation. The blood was immediately collected and centrifuged and aliquots of the serum were frozen at -80°C for subsequent analyses. Levels of vaspin, adiponectin and leptin were measured in these serums. The liver, pancreas and adipose tissue depots were removed immediately using the anatomical localization of the organ previously described.

Determination of vaspin, adiponectin, leptin

Quantification of vaspin, adiponectin, leptin in serum was performed using the enzyme linked immunosorbent assay (ELISA) kit. Adiponectin levels were analyzed using DRG® Adiponectin (Rat) ELISA (EIA-4570). The limit of sensitivity of this assay is 0.155 ng/mL Rat Adiponectin (20 µL sample size). This assay is specific for rats. Leptin levels were analyzed using DRG® Leptin (Rat) ELISA (EIA-4607). The limit of sensitivity of this assay is 0.04 ng/mL using a 10 µL sample size. This assay is specific for rats. Vaspin levels were analyzed using AVISCERA BIOSCIENCE Vaspin (rat) ELISA (SK00560-03) kits. The limit of sensitivity of this assay is 0.097 ng/mL using 50 µL sample size. All the used kits followed the producer's recommendations and the results were expressed in pg/mL or ng/mL.

Histopathological evaluations

Tissue specimens were fixed in 10% formaldehyde and then embedded in paraffin blocks. Paraffin sections (4-5µm) were stained with hematoxylin &eosin (H&E), examined and photographed under a photomicroscope (Olympus BX51, Tokyo, Japan) by the histologist in a blind manner.

Statistical analysis

SPSS 15.0 Statistics Software and InStat3 GraphPad Software were used for all statistical analyses. The Mann-Whitney Test was used to compare nonparametric data in independent groups with more than two groups. Pearson correlation analysis was used for correlation analysis of parametric data and Spearman correlation analysis for nonparametric data.

RESULTS

Body weight and blood glucose level

The body weight and blood glucose levels were measured at the initial and 48 hours after STZ injection (Table 1). The body weight and the blood

glucose levels were similar at the study baseline among the groups. 48 hours after STZ injection, animals had a significant weight loss compared to age-matched controls and the average blood glucose levels were significantly increased; 6 weeks after STZ injection, the average blood glucose levels were significantly increased (Table 1).

Serum levels of adipokines

- Comparison of groups

Adiponectin and vaspin levels of diabetic group were found to be statistically lower than of control group ($p<0.05$)(Figs 1,2). However, Leptin levels were significantly higher in the diabetic group than those in the normal group ($P<0.05$)(Fig. 3, Table 2).

- Correlation studies

There was a moderate negative correlation between adiponectin and leptin levels in the entire experimental study group (Spearman r=0.5607 P=0.0297) (Fig. 4).

There was a moderate positive correlation between adiponectin levels and vaspin levels in the entire experimental study group (Non-DM and DM rats) (Spearman r= 0.5893 P=0.0208)(Fig. 5).

Histology of pancreatic tissue

The histological micrograph analyses of pancreatic tissues are presented in Figure 6. For the non-diabetic group: a normal lobular architecture in the pancreatic tissue (Fig. 6a) was observed. For the diabetic group: shows a reduced area and distorted forms of islets of Langerhans, as well as the presence of inflammatory cells (Fig. 6b).

DISCUSSION

In this study we examined the status of adipocytokines in type 1 DM in STZ-induced diabetic

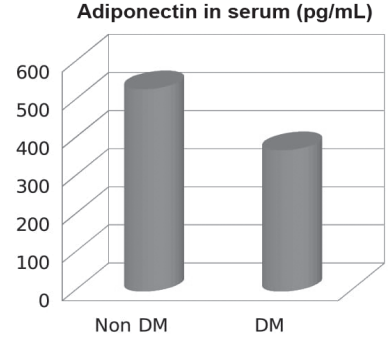

Figure 1. Adiponectin in serum levels of diabetic group were found to be statistically lower than of control group ($p<0.05$).

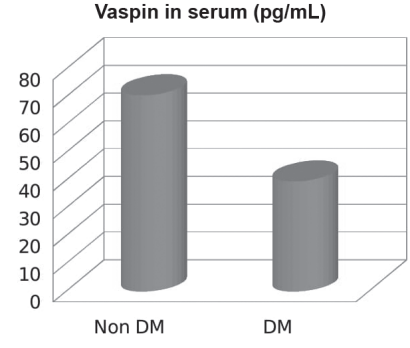

Figure 2. Vaspin in serum levels of diabetic group were found to be statistically lower than of control group ($p<0.05$).

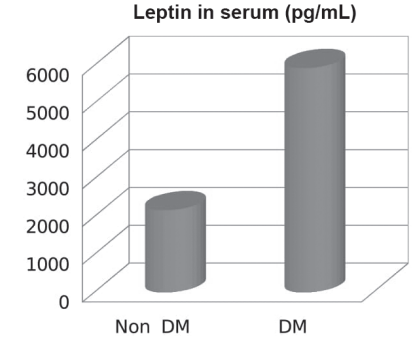

Figure 3. Leptin in serum levels were significantly higher in the diabetic group than those in the normal group ($P<0.05$).

Table 1. Body weight and blood glucose levels in DM and Non-DM groups

Animal Groups	Body Weight (g)		Blood Glucose (mg/dL)		
	Initial	48 h later	Initial	48 h later	6 weeks later
DM	290.8±14.63	239.6±13.97	94.1±5.57	315.1±21.03[a]	425.2±32.08
Non-DM	288.1± 12.45	290.1±12.41	90.2± 5.36	98.2+14.02	96.2+13.05

Values are expressed as means ± standard errors of mean (SEM) (n=6 in each group). [a,] $p<0.001$ compared to I^0.

Table 2. Characteristics of Rats and analysis results according to groups

	All Rats	Non-Diabetic Group	Diabetic Rat Group	*P Values
		Mean±SD Median(Min-Max)		
N	12	6	6	-
Adiponectin, ng/mL	0.44±0.14 0.40 (0.21-0.74)	0.53±0.15 0.49 (0.36-0.74)	0.37±0.07 0.37 (0.21-0.46)	[a]0.0496
Leptin, ng/mL	4.42±3.67 5.01 (0.31-15.18)	2.17±1.71 1.68 (0.31-5.73)	5.93±3.65 5.42 (1.30-15.18)	[a]0.0256
Vaspin, pg/mL	52.0±31.6 47.4(11.7-108.5)	70.6±21.4 69.9(38.3-108.5)	39.6±29.4 28.0(11.7-103.3)	[a]0.0496

*Comparison between Non-Diabetic group and diabetic rat group, [a]Mann-Whitney Test. SD: Standard Deviation, Min-Max: Minimum-Maximum.

rats. We found that adiponectin and vaspin levels were lower in diabetics and leptin levels were higher. We also found a negative correlation between adiponectin and leptin, and a positive correlation between vaspin and adiponectin.

STZ is a drug widely used to induce experimental diabetes mellitus in animals. Its diabetogenic action occurs by the irreversible damage to pancreatic beta cell resulting in the loss of cellular functionality to produce and release insulin (23-25). Thus, the reduction in size of islets, resembling the findings in type 1 diabetic animals was observed due to toxic and harmful action of STZ on the pancreatic beta cells and was also observed in this study (Fig. 6).

Although the adipose tissue was only considered as a fat deposition site previously, it is now considered to be an endocrine organ that secretes a large number of cytokines that have been implicated in insulin sensitivity, inflammation, coagulation, and atherosclerosis in recent years (1). Numerous cytokine secretions by adipose tissue have been found, such as Leptin, Resistin, Adiponectin, Vaspin, Chemerin, Apelin, Visfatin, Hepcidin, Adipsin, Omentin, TNF-α, IL-6, IL-8, Monocyto chemoattractant protein (MCP-1), Prostaglandin I2 (PI2), Prostaglandin F2α (PG2F2α), Nerve Growth Factor (NGF), Plasminogen activator inhibitor-1 (PAI-1), acylation - stimulating protein (ASP) and insulin-like growth factor (2). Adiponectin, Vaspin and Leptin are only a few of these numerous adipocytokines.

Vaspin is a member of the serine protease inhibitor (serpin) family, first described in the visceral adipose tissue of diabetic rats (3). Persistent diabetic or insulin-resistant subjects were found to have higher

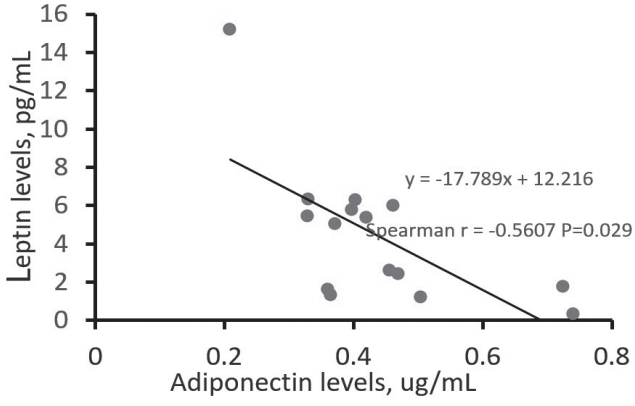

Figure 4. A correlation graph showing the relationship between serum adiponectin and leptin levels in the non-DM and DM rats at a moderate negative level (Spearman r ¬= 0.5607 P=0.0297).

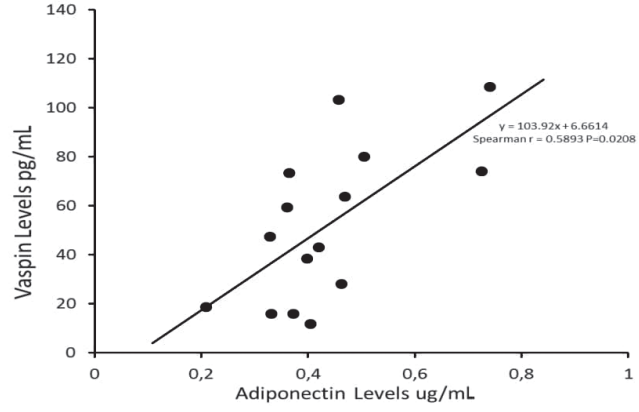

Figure 5. A correlation graph showing the relationship between serum adiponectin and vaspin levels in the non-DM and DM rats at a moderate positive level (Spearman r = 0.5893 P=0.0208).

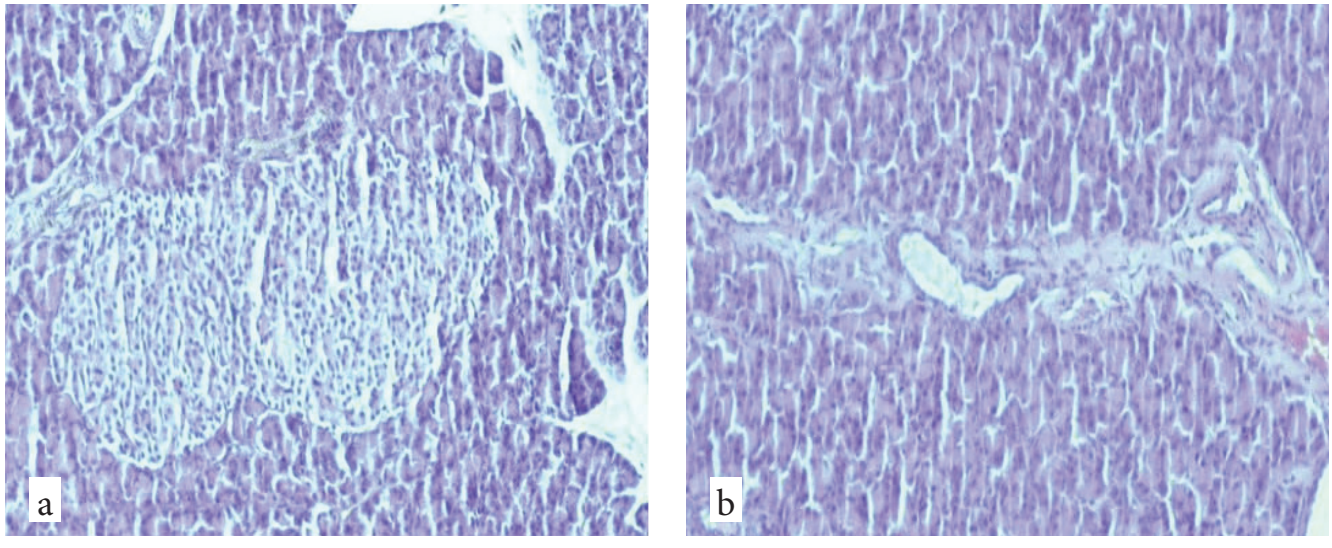

Figure 6. Photomicrograph of rat pancreatic tissue. (a) Non-diabetic group shows a normal lobular architecture, islets of Langerhans surrounded by the pancreatic acini, (b) Diabetic group shows a reduced area and distorted forms of islets of Langerhans, as well as the presence of inflammatory cells (H&E Bar 20µm).

vaspin levels than low-fat normal subjects (4). It is known that vaspin is expressed in adipocytes in visceral white fat tissue in rats and improves insulin sensitivity (26). After high-calorie diet in Wistar rats, vaspin has been shown to induce obesity and insulin resistance as expressed from visceral adipose tissue (27). Again Wada (5) showed that vaspin administration in rats fed on a high fat diet increased insulin sensitivity. Furthermore, Hida *et al.* (3) showed an improvement in insulin sensitivity and glucose tolerance after vaspin administration in rats. In recent studies, it has been claimed that vaspin has a modulatory role on glucose metabolism. But its mechanism is not clear. It is claimed that this modulatory role of vaspin may be due to the regulation of the relationship between the decrease in insulin secretion due to pancreatic progressive damage and changes in insulin sensitivity on peripheral tissues (KC, muscle, etc.)(6). Considering all these studies, it is seen that vaspin is important in physiopathology of diabetes and vaspin levels are lower in diabetics. We also found that vaspin levels were lower in type 1 diabetics. These findings may lead to the development of new treatments for the future use of vaspin analogs or antagonists in the treatment of diabetes.

Adiponectin is a polypeptide of 30kDa in size synthesized by adipose tissue and is a collagen-like plasma protein (16). It is thought that there may be a relationship between vaspin and serum adiponectin levels. Hida *et al.* showed that administration of recombinant vaspin increased adiponectin concentrations. Adiponectin and vaspin are associated with increased insulin sensitivity and reduced glucose intolerance in diabetes. Both are reduced in type 2 diabetes (28). Studies have shown that adiponectin has an effect on insulin sensitivity in diabetic animals (29). It has been determined that adiponectin level is low in obesity, type 2 DM and coronary artery patients (17, 18). In our study, we found that adiponectin levels were lower in type 1 diabetic patients, and there was a positive correlation between vaspin and adiponectin levels.

Leptin is a peptide protein consisting of 167 amino acids weighing 16 kDa. It is a cytokine that is secreted from adipocytes and affects the hypothalamus with negative feedback. It increases energy expenditure by suppressing food intake. Therefore leptin regulates energy homeostasis and informs the hypothalamus about body fat tissue. Leptin also participates in glucose metabolism by increasing gluconeogenesis in the muscle, liver and fat cells, while reducing glucogenolysis (9). Depending on the state of energy in the body, it is synthesized and secreted in fat cells (10). Leptin is also effective on different tissues and systems such as body lipid metabolism, hematopoiesis, pancreatic beta cell function (11-13). The most important function of leptin is to keep the amount of fat in the body constant. Leptin reduces intracellular lipid levels in the skeletal muscle, liver and pancreatic beta cells by enhancing insulin sensitivity (14). Many experimental studies have shown that the role of glucose on leptin release from adipocytes is very important (15).

Leptin is an ob gene product and produced in adipocytes as a factor of toughness. Leptin deficiency increases the hypothalamic level of the hypothalamic neuropeptide Y. It has been shown that when flare recombinant leptin is administered, it plays a role in energy expenditure and regulation of food intake. *In vivo* studies have shown that leptin enhances glucose metabolism and insulin sensitivity, particularly through hypothalamus in normal and obese rodents. In addition, it has been reported that leptin is an antidiabetic agent in diabetic mice, which are lipoatrophic and insulin deficient (30). Besides, some studies have reported that leptin activates ATP-sensitive K channels in B cells, suppressing insulin secretion and thus affecting insulin secretion. Leptin is also thought to have a negative feedback effect on insulin secretion (31). We also found that leptin levels in Type 1 diabetic rats were higher in our study. Decreased pancreatic insulin secretion, which has been destroyed by STZ, due to the impaired negative feedback control, leptin levels may have increased.

To date, the effect of these adipocytokines on diabetes mellitus physiopathology has generally been explained on insulin resistance, obesity and Type 2 DM. However, as we found in our study, the serum levels of these adipocytokines change in people with type 1 DM. Therefore, it should be considered that there may be common points between adipocytokines and diabetes mellitus physiopathology that can be explained by other mechanisms besides insulin resistance and obesity. New research is needed to explain the relationship between Vaspin, Leptin, Adiponectin and Type 1 DM. New studies on this subject will open new horizons for the detection of new biomarkers in the diagnosis and treatment of DM.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgement

There is not funding to report for this study.

Ethical approval

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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