The Relationship Between Plasma Adipsin, Adiponectin, Vaspin, Visfatin, and Leptin Levels with Glucose Metabolism and Diabetes Parameters

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Abstract

Introduction: Recently, interesting adipokines have been described that affect glucose metabolism and insulin resistance. However, the relationships between these adipokines and their importance in the pathogenesis of diabetes are contradictory. The aim of the present study was to investigate the relationship between plasma adipsin, adiponectin, vaspin, visfatin, and leptin levels in diabetic rats with liver enzymes related to glucose metabolism and diabetes parameters.

Methods: Wistar albino rats were divided into three groups: control, diabetic control, and treatment groups, each with 7 of them. One group of diabetic rats was orally treated with 200 mg/kg/day metformin, and the other diabetic group received placebo. After 6 weeks, the animals were sacrificed by cardiac puncture. Glucose, insulin, glycosylated hemoglobin (HbA1C), adipsin, adiponectin, visfatin, vaspin, and leptin levels in blood were measured by enzyme-linked immunosorbent assay (ELISA), and insulin resistance was determined. In liver homogenates, glucose-6-phosphate dehydrogenase (G6PD), glucose-6-phosphatase (GPase), glucokinase, pyruvate kinase (PK), and fructose-1.6-diphosphatase (F1.6DP) activities were measured by ELISA.

Results: Dietary food intake and water consumption, fasting blood glucose and HbA1c levels, and insulin resistance of diabetic rats were higher than those of the control and treatment groups. Plasma adipsin, adiponectin, vaspin, and leptin levels significantly decreased, and visfatin level increased in diabetic rats. G6PD, glucokinase, and PK activities decreased, whereas G6Pase and F1.6DP activities increased in diabetic rats. There were a negative correlation between plasma adipsin and adiponectin levels and insulin resistance and a positive correlation between visfatin level and insulin resistance in diabetic rats. In addition, adipsin, adiponectin, and leptin levels were positively correlated with each other and with some liver enzymes.

Discussion and Conclusion: In the diabetic rat model, body weight, glucose metabolism enzymes, insulin sensitivity, and changes in plasma adipsin, adiponectin, vaspin, visfatin, and leptin levels indicate that these adipokines play an important role in the glucose metabolism, insulin resistance, and pathogenesis of diabetes.

Keywords: Adipokines; diabetes; metformin; rat.

Currently, high-calorie diet, fast food dietary habits, and sedentary lifestyle have increased health problems, such as obesity and diabetes. Diabetes, defined as the epidemic of the 21st century by the World Health Organization, disrupts the quality of life by acute and chronic complications. As the cost of treatment is high, it constitutes a significant burden on the economy of underdeveloped and developing countries. In addition, current medical treatment methods do not fully meet the expectations of patients’ recovery [1–3]. Various theories about the pathogenesis of diabetes have been proposed. However, the pathophysiological mechanisms related to the development and pro-
gression of diabetes have not yet been fully elucidated. In
the last 30 years, attention has been focused on the role
of adipose tissue in inflammatory and metabolic diseases,
such as obesity, metabolic syndrome, insulin resistance,
and diabetes [4–6].

There are two different adipose tissues in mammalian or-
ganisms. Brown adipose tissue with abundant mitochon-
dria is specialized in heat production and thermogenesis.
The functional capacity of subcutaneous and visceral adi-
pose tissues is wider [6]. As the largest endocrine organ
of the body, it has been found to secrete a large number
of bioactive molecules called adipokines. These bioactive
substances play a role in many important physiological
processes, such as body weight, appetite, glucose, and
lipid metabolism and regulation of reproduction, fibrinol-
sis, angiogenesis, vascular tone control, blood coagulation,
and immunity [7, 8]. The functional impairment of adipose
tissue leads to the disruption of the production and se-
cretion of adipokines, and many diseases and its complica-
tions occur [8, 9]. Experimental and clinical evidence has
shown that adipokines can be used as biomarkers to diag-
nose metabolic diseases [9, 10]. It has been suggested that
some adipokines worsen diabetes, whereas others show
insulin-like effects, stimulate glucose uptake in the target
cells, and reduce insulin resistance [11–18].

Adipsin is the first adipokine defined in 1987. It is then re-
ferred to as complement factor D. Serum adipsin levels are
lower in patients with diabetes with β cell failure. Adipsin
therapy in diabetic rats for only a few days has shown signif-
icant antidiabetic effects. Therefore, it has been suggested
that adipsin is extremely important in maintaining the func-
tioning of the insulin-secreting pancreatic β cell [11–13].

Adiponectin is discovered in 1994 as a new protein se-
creted into the blood from adipose tissue [14]. It increases
glycine uptake and oxidation of fatty acids in target cells,
as well as inhibits hepatic gluconeogenesis. Serum level
has been found to be high in patients with diabetes and
coronary heart failure. However, some researchers have re-
ported that plasma adiponectin level is low in patients with
diabetes [15–21].

Vaspin is first found as a new adipokine in the visceral adi-
pose tissue of insulin-resistant obese rats used as a model
for type 2 diabetes. It has been reported that insulin sensi-
tivity is increased in obese mice treated with recombinant
vaspin [22–24]. Although serum level has been shown to
correlate with body fat mass and insulin resistance, vaspin
levels have not changed in male and female patients with
type 2 diabetes [25].

Vaspin is a adipokine mainly secreted from visceral adi-
pose tissue and acts on the insulin receptor by binding to a
different site from where insulin is bound. It has the ability
to mimic the effects of insulin and plays an important role
in insulin secretion in pancreatic β cells [26, 27]. Plasma vis-
fatin levels have been reported to be high in patients with
type 2 diabetes [28–31]. Although visfatin plays an impor-
tant role in the regulation of insulin secretion, its effects on
diabetes risk or on the progression of diabetes have not yet
been fully understood [32].

Leptin is an important adipokine secreted mainly from
subcutaneous adipose tissue and is known as appetite
and satiety-regulating hormone [33]. It inhibits insulin and
glucagon secretion in the pancreas in addition to increases
blood circulation and fatty acid oxidation in skeletal mus-
cles and improves insulin sensitivity [34]. However, there
are contradictions between the results of plasma leptin
concentration in patients with type 2 diabetes. Some re-
searchers have reported that leptin levels decrease in pa-
ients with type 2 diabetes, whereas others have shown an
increase or no difference [35–37].

Since adipose tissue is also an important integrator of glu-
cose metabolism, it is significant to understand its role in
the pathogenesis of metabolic diseases, such as obesity
diabetes. Therefore, the information to be obtained
may provide a promising technique for a more rational
treatment of certain metabolic diseases. The aim of the
present study was to determine the changes in plasma
adipsin, adiponectin, vaspin, visfatin, and leptin levels in
diabetic rats and to investigate the relationship between
these adipokines and glucose metabolism and diabetes
parameters. In addition, we aimed to collect information
on new therapeutic strategies to prevent the development
and progression of diabetes.

Materials and Methods
This research was conducted at the Science Technology
Application and Research Center Laboratories of University.
The study was approved by the University Animal Research
Ethics Committee (Date: 06/12/2017, No: 2017/1417).

Animals
A total of 28 adult male Wistar albino rats weighing 260–
300 g were obtained from the Health Sciences Research
and Application Center of our university. The animals were
kept in stainless steel cages with 12-hour light:12-hour
dark rhythm, 22±2 °C room temperature, and 55%–60%
humidity and fed with standard rat chow and water.
Chemicals, Drugs, and Measurement Kits

The chemicals (diethyl ether and streptozotocin) used in this research were purchased from Sigma Aldrich (St. Louis, MO, USA). The drug (metformin) was purchased from Bilim Pharmaceuticals (Istanbul, Turkey). Enzyme-linked immunosorbent assay (ELISA) kits used in the measurement of glucose-6-phosphate dehydrogenase (G6PD), glucose-6-phosphatase (G6P-ase), glucokinase, pyruvate kinase (PK), and fructose-1,6-diphosphatase (F1,6DP) activities were purchased from BioVision (CA, USA). Glucose measurement kit was commercially obtained from Sigma Aldrich. Insulin, glycosylated hemoglobin (HbA1c), adipinsin, adiponectin, vispin, visfatin, and leptin ELISA measurement kits were commercially obtained from MyBioSource Inc. (San Diego, CA, USA).

Tools and Equipment

In the present study, ELISA reader (Multiskan GO; Thermo Scientific, MA, USA), air-cooled microcentrifuge (SL16R; Thermo Scientific, Germany), blood glucose meter and strips (Contour TS; Bayer, Tarrytown, NY, USA), electronic weighing (Sartorius Basic; Göttingen, Germany), precision balance (Precisa XB 220A; Dietikon, Switzerland), homogenizer (IKA Labortechnik Ultra-Turrax T25; Staufen, Germany), deep freezer (Vestel, Turkey), surgical set (Kruuse, Langeskov, Denmark), and automatic pipettes were used.

Diabetic Rat Model

Body weight of all animals and fasting blood glucose levels were measured before the start of the study. To induce diabetes, streptozotocin (55 mg/kg) prepared in citrate buffer (0.1 M, pH 4.5) was injected intraperitoneally (IP) in a single dose. The control group was treated only with placebo (citrate buffer) IP. Fasting blood glucose was measured in blood samples obtained from the tail veins of rats 48 h after streptozotocin injection. Rats with fasting blood glucose levels of ≥300 mg/dl were included in the diabetic group.

Experimental Groups

Wistar albino rats were divided into three groups, each with seven animals, without any significant difference in body weight:

1. Control group: This group was fed with water and standard laboratory rat chow for 6 weeks without restriction.
2. Diabetic control group: Seven diabetic rats in this group were fed as in the control group and did not receive any treatment.
3. Metformin group: Seven diabetic rats were treated intragastrically with metformin 200 mg/kg/day for 6 weeks. Metformin dose was adjusted every week according to changes in animals’ body weights.

Finalizing the Research

After 6 weeks, after fasting for 12 h, the anterior abdominal wall was opened with an incision under ether anesthesia. Their livers were removed, washed with 0.9% cold saline, and stored at −80 °C until the measurement day to determine the levels of glucose metabolism-related enzymes.

Diabetes Parameters

Fasting blood glucose was evaluated photometrically by the enzymatic kit obtained from Sigma Aldrich. Serum insulin and HbA1c levels were estimated using rat ELISA assay kits. Insulin resistance was calculated as homeostatic model assessment of insulin resistance (HOMA-IR). HOMA is a method for assessing β cell function and IR from basal (fasting) glucose and insulin or C-peptide concentrations. The formula is calculated as follows: HOMA-IR=[fasting glucose (mmol/l)×fasting insulin (μU/l)]/22.5.

Evaluation of Plasma Levels of Selected Adipokines

The levels of plasma adipsin, adiponectin, vispin, visfatin, and leptin were measured by immunoenzymatic methods using specific ELISA kits (MyBioSource).

Preparation of Liver Homogenates and Determination of Enzyme Activities

To prepare the homogenate, 9 ml of phosphate buffered saline was added to 1 g of liver tissue and was crushed with a cooled mechanical homogenizer at 1200 rpm for 5 min. After centrifugation, supernatants were obtained to determine enzyme activities. To evaluate the supernatant protein content, we preferred to use the Lowry method as modified by Hartree [38]. G6PD, G6P-ase, glucokinase, PK, and F1,6DP activities in the supernatants were measured by appropriate rat ELISA kits at the University Science and Technology Application and Research Center Laboratory.

Statistical Analysis

Statistical evaluations of the data were performed using SPSS version 20.0 package program (SPSS Inc., Chicago, IL, USA). All results were expressed as mean±standard deviation. Since the number of data in each group was <30,
the findings were evaluated by non-parametric statistical analysis methods. Mann–Whitney U test was used for the analysis of independent groups with two groups. Kruskal–Wallis ANOVA test was used in the analysis of independent groups with more than two groups for non-parametric tests. Wilcoxon matched pairs test was used in the analysis of dependent groups with two groups. Spearman’s non-parametric correlation test was used to examine the relationship between the parameters. A p value <0.05 was considered significant.

Results

Body Weight Changes, Food, and Water Consumption

The increase in food and water consumption in normal rats was accompanied by an increase of 24.81% in body weight at the end of week 3. In diabetic rats, the increase in food intake starting on week 1 continued to increase further toward the end of the experiment. However, the body weight of diabetic rats decreased by 5.42% on week 1, 12.27% on week 2, and 23.42% on week 3. Metformin treatment significantly reduced food and water consumption in diabetic rats with adverse appetite-reducing effects. However, the increase in body weight of animals remained well below healthy controls (Tables 1, 2).

Glycemic Parameters

At the end of week 6, the average fasting blood glucose level increased approximately fourfold in diabetic rats compared with healthy control values. Metformin treatment provided an improvement of approximately 64% in blood glucose levels. Plasma insulin levels in diabetic rats decreased by 45% compared with the control value, whereas HbA1c and insulin resistance (HOMA-IR) increased by approximately 100% and were twice the normal value. Metformin treatment partially improved the plasma insulin levels of diabetic rats. In addition, a significant improvement in HbA1c was observed in metformin-treated rats, and there was a slight decrease in insulin resistance (Table 3).

Plasma Levels of Adipokines

As shown in Table 4, plasma adipins, adiponectin, vaspin, and leptin levels were significantly decreased in diabetic rats compared with control values, whereas visfatin levels increased. Treatment with metformin for 6 weeks significantly decreased the visfatin plasma level of diabetic rats compared with untreated animals, whereas other adipokines, such as adipin, adiponectin, vaspin, and leptin, significantly increased plasma levels.
Liver Enzymes

G6P-ase and F1,6DP levels increased significantly, whereas liver G6PD, glucokinase, and PK levels decreased in diabetic rats. Significant improvements were found in liver enzymes that affect glucose metabolism in diabetic rats treated with metformin. Metformin treatment significantly inhibited both low G6PD, glucokinase, and PK levels and high G6P and F1,6DP levels (Table 5).

Relationships Between Adipokines and Glucose Metabolism and Diabetes

We have tested the relationships between plasma adipsin, adiponectin, vaspin, visfatin, and leptin levels with each other and with glucose metabolism enzymes and HOMA-IR (as an index of insulin resistance). There was no correlation between the plasma adipokine levels of control rats. In contrast, plasma adiponectin and leptin levels were correlated in diabetic rats (Fig. 1). A similar relationship was also found between plasma adipsin and leptin levels in diabetic rats treated with metformin (Fig. 2). Some adipokines are associated with insulin resistance (plasma fasting glucose and insulin levels). Plasma adipsin and adiponectin levels were negatively correlated with insulin resistance in diabetic rats.

<table>
<thead>
<tr>
<th>Table 4. Plasma adipsin, adiponectin, vaspin, visfatin, and leptin levels in control, diabetic, and metformin-treated diabetic rats</th>
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<tbody>
<tr>
<td><strong>Parameters</strong></td>
</tr>
<tr>
<td>Adipsin (ng/ml)</td>
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<tr>
<td>Adiponectin (mg/l)</td>
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<tr>
<td>Vaspin (ng/l)</td>
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<td>Visfatin (ng/ml)</td>
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<td>Leptin (pg/ml)</td>
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All data are presented as mean±SEM of the indicated number of rats (n=7); <sup>a</sup>p<0.01, <sup>b</sup>p<0.05, compared with the control group; <sup>c</sup>p<0.01, <sup>d</sup>p<0.05, compared with the diabetic group.

<table>
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<th>Table 5. Liver enzymes in normal, diabetic, and metformin-treated rats</th>
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<tr>
<td><strong>Parameters</strong></td>
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<td>G6PD (ng/g protein)</td>
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<td>G6P-ase (ng/g protein)</td>
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<td>Glucokinase (μg/mg protein)</td>
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<td>PK (μg/mg protein)</td>
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<tr>
<td>F1,6DP (μmol/g protein)</td>
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</table>

All data are presented as mean±SEM of the indicated number of rats (n=7); <sup>a</sup>p<0.001, <sup>b</sup>p<0.05, compared with the control group; <sup>c</sup>p<0.01, <sup>d</sup>p<0.05, compared with the diabetic group; G6PD, glucose-6-phosphate dehydrogenase; G6P-ase, glucose-6-phosphatase; PK, pyruvate kinase; F1,6DP, fructose-1,6-diphosphatase.

Figure 1. Correlation between plasma adiponectin and leptin level in diabetic rats. R²=0.416, p<0.05.

Figure 2. Correlation between plasma adipsin and leptin level in diabetic rats treated with metformin. R²=0.603, p<0.05.
In addition, the level of plasma visfatin is related to the enzyme of glucose metabolism in the liver of control and diabetic rats. There was a negative correlation between plasma visfatin levels and liver G6P and F1.6DP in control rats, but there was a positive correlation in diabetic rats (Figs. 5, 6). The correlation between plasma visfatin and liver G6P levels in control and diabetic rats was not shown in the figure. Correlation coefficients and correlation p values are presented below. The correlation coefficients between plasma visfatin and liver G6P in control and diabetic animals were $r=-0.792$, $p<0.05$ and $r=0.821$, $p<0.05$, respectively.

**Discussion**

For many years, fat tissue was believed to be a highly passive lipid accumulation. We have learned that currently, as
a very important endocrine organ, it can synthesize and secrete bioactive molecules called adipokines associated with carbohydrate and lipid metabolism. The list of adipokines is expanding gradually with the great interest and effort of the researchers. Adipokines have autocrine, paracrine, and endocrine effects that regulate insulin sensitivity and many physiological processes. Some adipokines are responsible for the pathogenesis of many metabolic diseases, such as diabetes and its complications [5–9]. Adipokines, which are generally proinflammatory, is believed to induce the development and complications of diabetes, whereas anti-inflammatory adipokines are estimated to have beneficial or preventive effects on diabetes [7–9, 13, 17–19]. Adipsin, adiponectin, visfatin, and leptin are known as adipokines, which increase insulin sensitivity and reduce adiponectin, vaspin, visfatin, and leptin are known as adipokines, which increase insulin sensitivity and reduce glucose intolerance [20–24, 31–34]. However, some experimental results have shown that these adipokines have no beneficial effect on glucose metabolism and insulin resistance [8]. In the present study, we found that the plasma adipsin, adiponectin, visfatin, and leptin levels of diabetic rats were lower than those of control and metformin-treated diabetic rats. Many studies have demonstrated that these adipokines have direct or indirect anti-inflammatory and antidiabetic effects, and their plasma levels are low in individuals with type 2 diabetes [19, 22, 37]. Although our findings support the literature knowledge, it is in contrast with the results of some studies. There is no complete agreement between the results of experimental and clinical trials in this subject [25, 35].

We observed significant improvements in plasma adipsin, adiponectin, visfatin, and leptin levels in diabetic rats treated with metformin. Even in previous studies, metformin showed similar effects. The mechanism associated with the role of metformin in the regulation of plasma levels of adipokines with antidiabetic activity is not yet fully understood. It may be speculated that metformin stimulates the secretion of anti-inflammatory and antidiabetic adipokines from adipose tissue. Visfatin is one of the most studied adipokines. It has been proven by many studies that it mimics the effects of insulin. It is surprising that this adipokine plasma level is significantly high in diabetic rats [32]. We also found that plasma visfatin levels were increased in untreated and metformin-treated diabetic rats. Plasma visfatin levels were generally found to be high not only in experimental diabetic animal models but also in humans with type 2 diabetes [28].

G6PD, G6P, F1.6DP, glucokinase, and PK are key enzymes in the liver that regulate glucose metabolism. It is known that the enzyme activity responsible for glycolysis and gluconeogenesis changed in the liver of diabetic rats. We also found that G6PD, glucokinase, and PK levels were decreased, and G6P and F1.6DP levels increased in untreated diabetic rats. Thus, in diabetic rats, on the other hand, an increase in gluconeogenesis in the liver, a condition that limits the consumption of glucose in the liver and in peripheral tissues, has contributed significantly to hyperglycemia. Our results showed that there was a positive correlation between liver G6P levels in diabetic rats despite a negative correlation in control rats. Visfatin increases glucose uptake in peripheral tissues and inhibits glucose release from the liver to the blood [8, 32]. Although plasma visfatin levels were significantly increased in diabetic rats, liver enzymes responsible for gluconeogenesis remained elevated. Could this be a paradox about the insulin mimicked effect of visfatin? Or could insulin resistance, as well as visfatin resistance, occur in diabetic rats? Some researchers have reported that leptin resistance develops in some obese rats [8, 33]. In light of this knowledge, it can be considered that any resistance to other adipokines may be in pathological conditions or diseases. It is important to clarify visfatin resistance with regard to the pathogenesis of diabetes.

Recently, the presence of subclinical chronic inflammation in the adipose tissue of individuals with type 2 diabetes and the change in adipocyte metabolism are assumed to play an important role in the pathogenesis of the disease [39]. Impairment in adipose tissue metabolism, particularly a decrease in anti-inflammatory adipokine production or an increase in inflammatory adipokine synthesis, may be one of the major pathophysiological mechanisms of diabetes. Our data show that the plasma levels of adipokines are altered in an experimental diabetic rat model. Adipsin, adiponectin, visfatin, and leptin levels decrease, whereas visfatin levels increase. An increase in visfatin level may be important in limiting diabetes. However, the increase in plasma visfatin did not fully compensate for the attenuation of the effect of insulin sensitizer adipsin, adiponectin, visfatin, vaspin, and leptin. According to the results of the present study, there is a relationship between plasma adiponectin and visfatin levels with insulin sensitivity and the key enzymes of gluconeogenesis in diabetic rats. In conclusion, the present study showed that some anti-inflammatory adipokines have beneficial effects on insulin sensitivity and glucose metabolism in diabetic rat plasma. Thus, agents capable of increasing the secretion of anti-inflammatory adipokines or reducing the secretion of inflammatory adipokines may be included in new treatment protocols if they have proven useful by adequate clinical trials.

**Ethics Committee Approval:** This study was performed in the laboratories of the University Science and Technology Application and Research Center after approval from the local ethics committee (Approval Number: 2017/05).
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