

*Full Length Research Paper*

# Effect of visfatin on blood glucose and serum lipids in normal and streptozotocin induced diabetic Rats

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**Visfatin is a novel adipokine showing altered plasma levels in different disorders, for example type-2 diabetes mellitus, obesity fetal growth retardation and gestational diabetes mellitus. We found that no study demonstrated the effect of visfatin on blood glucose and serum lipids in rats. Therefore, we investigated the effect of visfatin on blood glucose and serum lipids in rats. We found that visfatin produced an inhibitory effect on blood glucose level in a dose dependent manner and it caused further reduction in blood glucose level when combined with oral hypoglycemic drugs. We also found that visfatin produced a significant inhibitory effect on triglycerides, LDL and VLDL. These data are the first to show that visfatin can inhibit blood glucose in a dose dependent manner and the first to find an inhibitory effect on some serum lipids.**

**Key words:** Visfatin, glucose, diabetic rats, oral hypoglycemic, obesity, adipose tissue.

## INTRODUCTION

Adipose tissue secretes a variety of bioactive compounds into the circulation that can have profound effects on metabolism (Guerre-Millo, 2002; Cekmez et al., 2013). Visfatin (nicotinamide phosphoribosyltransferase (Nampt) enzyme) is an adipokine secreted by adipose tissue and involved in the biosynthesis of nicotinamide adenine dinucleotide as it catalyzes the condensation of nicotinamide with 5-phosphoribosyl-1-pyrophosphate to yield nicotinamide mononucleotide (Preiss J and Handler, 1957; Fukuhara et al., 2005).

Fukuhara et al. (2005) was the first who identified the potential role of visfatin as an insulin mimetic and they found that visfatin stimulated insulin receptor in a different way compared with insulin. Moreover, visfatin stimulated the accumulation of triglycerides in pre-adipocytes similarly to cells treated with insulin (Fukuhara et al., 2005).

However, the concept that visfatin mediates its effects via activation of the insulin receptors has been supported by (Dahl et al., 2007). It was also found that visfatin has insulin like effects on human osteoblasts and induced the same responses as insulin at a 10-fold lower molar concentration (Xie et al., 2007). This was reported also by Moschen et al. (2007) who found a significant approx.

2-fold up-regulation of glucose uptake in human adipocytes in vitro at a visfatin concentration of 100 nmol/l.

Circulating visfatin levels were shown to be strongly correlated with the amount of visceral adipose tissue (VAT) (Fukuhara et al., 2005); however, there was only a weak correlation between plasma visfatin concentrations and the amount of abdominal subcutaneous fat. Another study by Berndt et al. (2005) showed that plasma visfatin concentrations correlated positively and significantly with body mass index and percentage body fat, as well as visfatin mRNA expression in VAT.

However, they found a negative correlation between circulating visfatin levels and mRNA expression in subcutaneous fat. In contrast with the study Fukuhara et al. (2005); Berndt et al. (2005), showed that Plasma visfatin concentrations were not associated with visceral fat mass and plasma visfatin levels did not correlate with fasting plasma insulin and glucose concentrations.

Varma et al. (2007), found that subcutaneous adipose tissue visfatin is highly expressed in lean and insulin sensitive subjects and is attenuated in subjects with high intramyocellular lipids and low insulin sensitivity. They also found that visceral adipose tissue visfatin and subcutaneous adipose tissue visfatin are regulated oppositely with body mass index.

Dogru et al. (2007) and Pagano et al. (2006) found no correlation between circulating visfatin on one hand and BMI, insulin, glucose and lipid levels on the other hand and they found that visfatin levels increased significantly in the diabetics compared with controls. However, Pagano et al. (2006) found that circulating levels of visfatin were decreased by approx. 50% in obese patients compared with controls and a negative correlation was found between circulating visfatin levels and BMI in obese patients.

Hammarstedt et al. (2006) reported a 2-fold increase in plasma visfatin concentrations in individuals with T2DM compared with healthy controls and circulating visfatin concentrations did not correlate with the amount of visceral fat in the diabetic group. And this was found by other studies in patients with T2DM showed that visfatin plasma levels were significantly increased in T2DM compared with controls (Chen et al., 2006; Haider et al., 2006 and Lopez-Bermejo et al., 2006).

Interestingly, Lopez-Bermejo et al. (2006) found that visfatin levels were significantly increased in patients with long standing T1DM (Type 1 diabetes mellitus) compared with subjects with T2DM or non-diabetic subjects and in non-diabetic men, serum visfatin levels correlated significantly with fasting insulin and insulin sensitivity.

Krzyzanowska et al. (2006) found that plasma visfatin concentrations were significantly increased in women with Gestational Diabetes Mellitus (GDM); however, there was no correlation between visfatin concentrations and fasting plasma glucose, plasma insulin and BMI. Similarly, Lewandowski et al. (2007) found that visfatin serum levels were elevated in GDM but in contrast to Krzyzanowska et al., (2006) they found that visfatin serum levels significantly correlated with fasting plasma insulin. However, Chan et al. (2006) found - in contrast with the other two studies (Krzyzanowska et al., 2006; Lewandowski et al., 2007) that visfatin levels were decreased by approx. 25% in GDM compared with controls.

For all this controversy about the role of visfatin, our aim of this study is to search the direct effect of visfatin on blood glucose level in normal and streptozotocin induced diabetic rats. We also examined the effect of visfatin in combination with oral hypoglycemic drugs. Also we examined the effect of visfatin on serum lipids to demonstrate the exact relationship between adipose tissue and blood glucose.

## MATERIAL AND METHODS

Recombinant human visfatin was purchased from Alexis Biochemical Lausen, Switzerland. A total number of 48 Male albino rats average weight (150-200 gm) were obtained from the Laboratory Animal Research Unit of

College of Agriculture, Zagazig University, Egypt. Glibenclamide and metformin were obtained from sigma chemical co. and dissolved in dimethyl sulfoxide and distilled water, respectively before they were administered.

## Methods

All experiments were performed in accordance with the Institutional guidelines for the Care and Use of Animals for Scientific Purposes and in accordance with the recommendations from Helsinki Declaration. Animals were housed at  $25 \pm 2^\circ\text{C}$  under 12 hour cycles of dark and light and were allowed standard food and water. Dose response experiments were performed using doses of visfatin 1, 10, 100 nM to determine the effect of visfatin on normal blood glucose then we chose 10 nM as submaximal dose to compare the effect of visfatin with oral hypoglycemic drugs.

The rats were fasted for 18 hours before induction of diabetes. Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (60 mg/kg) dissolved in 0.1 M citrate buffer (pH 4.5) (Erejuwa et al., 2011). Another group of rats (control) were injected with the same volume of citrate buffer. Streptozotocin (STZ)-injected rats exhibited symptoms of diabetes mellitus such as polyuria, polydipsia, polyphagia, and weight loss after 48 hours post STZ administration. Two days after the injection of STZ, fasting blood glucose concentration was measured with an Accu-Chek glucometer (Roche, Germany) using blood samples from the tail vein. Animals with blood glucose concentrations  $\geq 14$  mMol / L (250mg / dl) were considered diabetic and used in this study. Subsequently, fasting blood glucose was measured weekly in each rat. Once each morning (9-10 a.m.) the rats were injected intravenously with visfatin (10nM) dissolved in normal saline Lim et al., (2008), or intraperitoneal injection with glibenclamide and/or metformin for four weeks (Erejuwa et al., 2011).

## Animals were treated for 4 weeks as follows

**1<sup>st</sup> group (6 rats):** non diabetic control group are given distilled water.

**2<sup>nd</sup> group (6 rats):** non diabetic given visfatin in dose 10 nM.

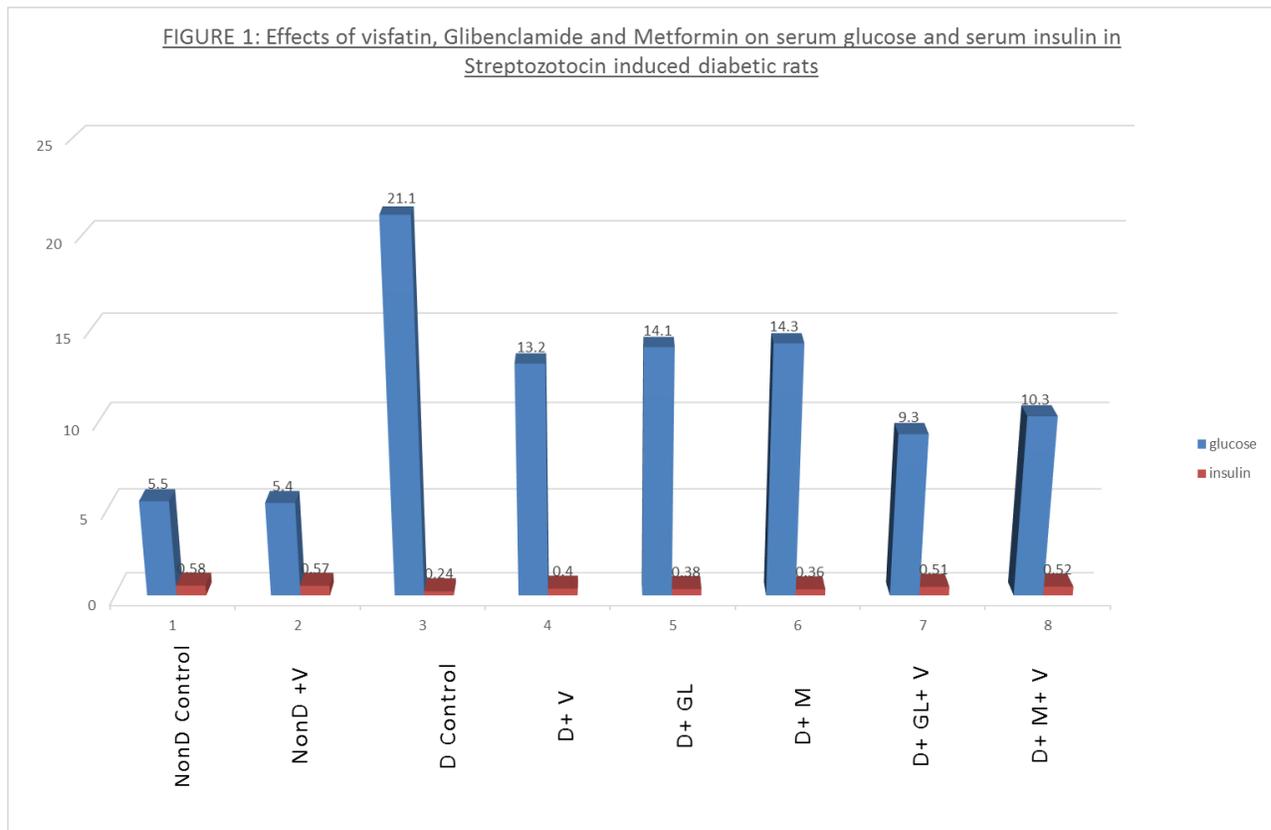
**3<sup>rd</sup> group (6 rats):** diabetic control given distilled water (0.5 ml).

**4<sup>th</sup> group (6 rats):** diabetic given visfatin in dose 10 nM.

**5<sup>th</sup> group (6 rats):** diabetic given glibenclamide (0.6 mg/kg/body weight) (Erejuwa et al., 2011).

**6<sup>th</sup> group (6 rats):** diabetic given metformin (100 mg/kg/body weight) (Erejuwa et al., 2011).

**7<sup>th</sup> group (6 rats):** diabetic given visfatin (10 nM) with gli-

**Figure 1.** Summarizes the results of serum glucose and serum insulin in different groups of animals

benclamide.

**8<sup>th</sup> group (6 rats):** diabetic given visfatin (10 nM) with metformin.

At the end of the treatment period, the animals were fasted for 16 hours and sacrificed by decapitation. Blood samples were collected in centrifuge tubes without anticoagulants and allowed to clot. The clotted blood was then centrifuged at 3000 x g for 20 min. Serum was separated and then quickly stored at -80°C for biochemical analyses.

### Biochemical Analyses

Serum glucose was determined by the glucose oxidase method as described by Barham D and Trinder P, (1972) using Stanbio Laboratory USA Kits. Serum insulin was determined using a rat insulin enzyme-linked immunosorbent assay kit (Crystal Chem, Chicago, IL) with rat antibody. Serum high density lipoprotein (HDL) was estimated by the method of (Warnick et al., 1985). Total cholesterol (CH) and triglycerides (TG) were estimated by the methods of (Siedel et al., 1983; Foster and Dunn, 1973) respectively. Low density lipoprotein

(LDL) and very low density lipoprotein (VLDL) were calculated by (Friedwald formula, 1972).

### Statistical analysis

Statistical analysis was carried out using SPSS version 12. The data are expressed as mean  $\pm$  SEM. Groups were compared by the Kruskal-Wallis H test followed by Mann-Whitney U test to identify significance of difference between two groups. *P* value < 0.05 was considered statistically significant.

### RESULTS

Figure 1: Summarizes the results of serum glucose and serum insulin in different groups of animals at the end of the four-week treatment period. The serum glucose concentrations of the diabetic control rats were significantly higher ( $21.1 \pm 0.9$  mmol/L) than those of the non-diabetic control rats ( $5.5 \pm 0.3$  mmol/L). Treatment with glibenclamide, metformin, or Visfatin significantly decreased the glucose levels ( $14.1 \pm 2.4$ ,  $14.3 \pm 2.5$  or  $13.2$

**Table 1.** Show the effect of Visfatin, Glibenclamide and/or Metformin on serum lipids (mg/dl).

| G  |                | TG              | CH             | HDL          | LDL          | VLDL           |
|----|----------------|-----------------|----------------|--------------|--------------|----------------|
| 1. | NON D, CONTROL | 0.43 ± 0.03     | 1.48 ± 0.14    | 0.81 ± 0.07  | 0.42 ± 0.08  | 0.23 ± 0.01    |
| 2. | Non D + V      | 0.48 ± 0.02     | 1.60 ± 0.11    | 0.87 ± 0.02  | 0.51 ± 0.08  | 0.23 ± 0.01    |
| 3. | D, CONTROL     | 0.68 ± 0.21     | 1.49 ± 0.08    | 0.77 ± 0.09  | 0.41 ± 0.03  | 0.32 ± 0.11    |
| 4. | D+ V           | 0.24 ± 0.04*#   | 1.41 ± 0.12    | 0.91 ± 0.09# | 0.39 ± 0.04  | 0.16 ± 0.03#   |
| 5. | D+ GL          | 0.30 ± 0.08#    | 1.06 ± 0.08*## | 0.66 ± 0.09  | 0.31 ± 0.03  | 0.17 ± 0.04#   |
| 6. | D+ M           | 0.20 ± 0.04*#   | 1.13 ± 0.07    | 0.82 ± 0.11  | 0.36 ± 0.03  | 0.15 ± 0.02*#  |
| 7. | D+ GL+ V       | 0.27 ± 0.18**#  | 1.14 ± 0.15#   | 0.87 ± 0.08  | 0.41 ± 0.03  | 0.13 ± 0.08**# |
| 8. | D+ M+ V        | 0.16 ± 0.03**## | 0.88 ± 0.15*#  | 0.69 ± 0.11  | 0.26 ± 0.03# | 0.06 ± 0.01**# |

D: Diabetic, V: Visfatin, GL: Glibenclamide, M: Metformin.

\* = significant (P < 0.05) compared to nondiabetic \*\* = highly significant (P < 0.01) compared to nondiabetic

# = significant (P < 0.05) compared to diabetic ## = highly significant (P < 0.01) compared to diabetic

± 3.2 mmol/L, respectively) in diabetic rats. When visfatin was added to glibenclamide or metformin, further reduction in glucose concentrations were found (9.3 ± 2.4 or 10.3 ± 2.5 mmol/L, respectively) in diabetic rats. The diabetic control rats had significantly (p < 0.01) reduced insulin level (0.24 ± 0.02 ng/ml) compared to non-diabetic rats (0.58 ± 0.11 ng/ml).

Treatment of diabetic rats with Visfatin, glibenclamide, metformin (0.40±0.06, 0.38±0.05 and 0.36±0.03 respectively) produced a significant increase in insulin levels compared to diabetic controls.

Also combinations of visfatin with Glibenclamide and metformin produced a further significant increase in insulin levels compared to diabetic controls (0.31±0.02 and 0.32±0.03 respectively).

Table (1) Shows the serum levels of triglycerides, total cholesterol, HDL, LDL and VLDL of control and streptozotocin-induced diabetic rats. Insignificantly increased levels of TG and VLDL (0.48 ± 0.02 and 0.23 ± 0.01 respectively) with no much change in total cholesterol, HDL and LDL (1.60 ± 0.11, 0.87 ± 0.02 and 0.51 ± 0.08 respectively) levels were observed in diabetic control rats compared to non-diabetic rats (TG: 0.43 ± 0.03, VLDL: 0.23 ± 0.01, CH: 1.48 ± 0.14, HDL: 0.81 ± 0.07, LDL: 0.42 ± 0.08).

Administration of Visfatin, glibenclamide or metformin significantly (p < 0.05) decreased the levels of TG and VLDL in diabetic rats (0.24 ± 0.04 and 0.16 ± 0.03 respectively) compared to diabetic control rats (0.68 ± 0.21, 0.32 ± 0.11). Besides, combination of glibenclamide or metformin with Visfatin further reduced the levels of TG and VLDL in diabetic rats (TG: 0.30 ± 0.08, 0.16 ± 0.03, VLDL: 0.13 ± 0.08, 0.06 ± 0.01). Glibenclamide, glibenclamide with Visfatin or metformin with visfatin significantly decreased total cholesterol in diabetic rats (1.06 ± 0.08, 1.14 ± 0.15 and 0.88 ± 0.15).

## DISCUSSION

Visfatin is an adipokine secreted by adipose tissue and act as a regulator of fat metabolism (Hosseinzadeh et al., 2012). Its effect on blood glucose and its role in obesity is still in great controversy. In our study, we found that visfatin insignificantly decreased blood glucose level in normal nondiabetic rats (figure 1). However, we found that visfatin produced a significant decrease in blood glucose level in diabetic rats. When visfatin was added to glibenclamide or metformin, further significant reduction in glucose concentrations was found in diabetic rats. These findings were in agreement with Fukuhara et al. (2005) who found that visfatin had a glucose-lowering effect and when they injected recombinant visfatin to mice intravenously resulted in a statistically significant fall in plasma glucose levels within 30 min. Also our finding was supported by Moschen et al. (2007) who found a significant approx. 2-fold up-regulation of glucose uptake in human adipocytes in vitro at a visfatin concentration of 100 nmol/l. Our results also in agree with Fukuhara et al. (2005) in that visfatin significantly reduced plasma glucose concentrations in insulin-resistant obese mice and this effect was similar to that induced by insulin injection and they reported that similar results were seen with streptozotocin (STZ)-treated insulin-deficient mice.

We also found that treatment of diabetic rats with Visfatin, glibenclamide, metformin produced a significant increase in insulin levels compared to diabetic controls. Our findings were supported by Dahl et al. (2007) who confirmed that visfatin mediates its effects via activation of the insulin receptors and we also in agree with Xie et al., (2007) who found that visfatin has insulin like effects on human osteoblasts and induced the same responses as insulin at a 10-fold lower molar concentration. However, our findings in controversy with Fukuhara et al.

**Table 2.** Show the effect of different doses of visfatin on blood glucose (mMol/l) level in normal and streptozotocin induced diabetic rats.

|    |      | Control    | Visfatin 1nM | Visfatin 10nM | Visfatin 100nM |
|----|------|------------|--------------|---------------|----------------|
| 1- | NonD | 5.5±0.3    | 5.4±0.2      | 5.2±0.2       | 5.0±0.1        |
| 2- | D+ V | 21.1±0.9** | 15.2±2.8#    | 13.2±3.2#     | 12.2±3.1#      |

NonD: NonDiabetic. D+V: Diabetic + Visfatin. \* = significant (P < 0.05) compared to nondiabetic

\*\* = highly significant (P < 0.01) compared to nondiabetic # = significant (P < 0.05) compared to diabetic

## = highly significant (P < 0.01) compared to diabetic

(2005) who found that visfatin stimulated insulin receptor in a different way compared with insulin and they reported that this effect was not due to changes in plasma insulin levels. Table 2

This study also demonstrated that combinations of visfatin with Glibenclamide and metformin produced a further significant increase in insulin levels compared to diabetic controls. Our finding in agree with Lopez-Bermejo et al. (2006) who found that serum visfatin levels correlated significantly with fasting insulin and insulin sensitivity.

In the present study, we also found that administration of visfatin, glibenclamide or metformin significantly ( $p < 0.05$ ) decreased the levels of TG and VLDL in diabetic rats compared to diabetic control rats. Our finding was found to be in agree with Fukuhara et al, (2005) who found that visfatin stimulated the accumulation of triglycerides in pre-adipocytes similarly to cells treated with insulin.

Also our findings were supported by Sun et al. (2007) who found that serum visfatin concentrations at baseline were positively correlated with serum triacylglycerols. However our results in controversy with Dogru et al. (2007) and Pagano et al. (2006) who found no correlation between circulating visfatin on one hand and BMI, insulin, glucose and lipid levels on the other hand.

However they found that visfatin levels increased significantly in the diabetics compared with controls and this finding support our finding in that visfatin reduced significantly serum levels of VLDL, cholesterol and triglycerides in diabetic rats and visfatin here may act as a counterregulator to serum lipids.

Our results also in controversy with Pagano et al. (2006) in that circulating levels of visfatin were decreased by approx. 50% in obese patients compared with controls and a negative correlation was found between circulating visfatin levels and BMI in obese patients. However our results were strongly supported by Hammarstedt et al. (2006) who reported a 2-fold increase in plasma visfatin concentrations in individuals with T2DM compared with healthy controls.

This was also found by other studies in patients with T2DM showed that visfatin plasma levels were significantly increased in T2DM compared with controls (Chen et al., 2006; Haider et al., 2006; Lopez-Bermejo et al., 2006).

This controversy can be explained by our suggestion that plasma visfatin is increased in diabetics in a trial to counteract elevated blood glucose as well as serum lipids but the plasma level of visfatin in normal subjects is so small that no effect of visfatin on serum biochemical parameters and this explanation is supported by Lopez-Bermejo et al., (2006) who found that visfatin levels were significantly increased in patients with long-standing T1DM (Type 1 diabetes mellitus) compared with subjects with T2DM or non-diabetic subjects and in non-diabetic men.

Also, we found that combination of glibenclamide or metformin with Visfatin further reduced the levels of TG and VLDL in diabetic rats.

Also, Glibenclamide, glibenclamide with visfatin or metformin with visfatin significantly decreased total cholesterol in diabetic rats. Our findings were in agree with Fukuhara et al. (2005) who found that circulating visfatin levels were shown to be strongly correlated with the amount of visceral adipose tissue (VAT) but they also found a weak correlation between plasma visfatin concentrations and the amount of abdominal subcutaneous fat.

Our findings confirmed that visfatin decreases some serum lipids, so elevated visceral fat secretes more visfatin which in turn decreases serum lipids to prevent hyperlipidemia and this explanation was supported by Zahorska-Markiewicz et al., (2007) who found that serum concentration of visfatin was significantly higher in obese women when compared to controls and they concluded that the observed increase of visfatin in obesity may be a counter regulation preventing further glucose increase. Our explanation was also supported by Jin et al. (2008) who found that serum visfatin levels were significantly higher in obese subjects than in non-obese subjects.

Our study was also supported by Berndt et al. (2005) who showed that plasma visfatin concentrations correlated positively and significantly with body mass index and percentage body fat, as well as visfatin mRNA expression in VAT.

However our findings were in controversy with Berndt et al. (2005) in that plasma visfatin levels did not correlate with fasting plasma insulin and glucose concentrations.

However this controversy may be attributed to species differences where the findings of Berndt et al., (2005) were in humans but our study was carried out on rats.

## CONCLUSION

Our study demonstrated that visfatin has a significant blood glucose lowering effect on blood glucose level in diabetic rats and insignificant lowering effect on normal blood glucose level. Also our study found - for the first time - that visfatin has a significant lowering effect on some serum lipids in diabetic rats. We suggested that visfatin act as a counter regulator to elevated serum levels of glucose, VLDL and triglycerides. Further studies should be done on other animals to confirm this inhibitory effect on serum lipids.

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