Iran Red Crescent Med J. 2016 March ; 18(3): e23814. doi: 10.5812/ircmj.23814

Published online 2016 March 2. **Research Article**

Effect of Metformin, Acarbose and Their Combination on the Serum Visfatin Level in Nicotinamide/Streptozocin-Induced Type 2 Diabetic Rats

Zahra Salemi,[1,2,*] Elham Rafie,[2] Mohamad Taghi Goodarzi,[3] and Mohamad ali Ghaffari[4]

[1]Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, IR Iran
[2]Department of Biochemistry, Arak University of Medical Sciences, Arak, IR Iran
[3]Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran
[4]Department of Biochemistry, Jundishapur University of Medical Sciences, Ahvaz, IR Iran

*Corresponding Author: Zahra Salemi, Department of Biochemistry, Arak University of Medical Sciences, Arak, IR Iran. Tel: +98-9183645842, Fax: +98-8634173529, E-mail: Dr.zsalemi@arakmu.ac.ir

Received 2014 September 23; **Revised** 2014 October 15; **Accepted** 2014 November 11.

Abstract

Background: Diabetes mellitus is a chronic metabolic disease with life-threatening complications. Metformin and acarbose are two oral antidiabetic drugs.

Objectives: This experimental study was designed and carried out at the Arak University of Medical Sciences in Arak, Iran, to investigate the effects of these drugs (both alone and in combination) on glycemic control, lipid profile, and serum visfatin levels in nicotinamide/streptozotocin type 2 diabetic rats.

Materials and Methods: Type 2 diabetes was induced in 30 male Wistar rats by the administration of streptozotocin (STZ) (60 mg/kg body weight) intraperitoneally (IP) 15 minutes after the IP administration of nicotinamide (110 mg/kg body weight). After one week, the diabetic rats were randomly divided into four groups. Three diabetic groups were treated with 150 mg/kg/day of metformin, acarbose (40 mg/100 g of diet), or a combination of the two for six weeks, respectively. Biochemical parameters, including fasting blood glucose, glycated hemoglobin, lipid profile, insulin, and visfatin were assessed and compared with those of the control diabetic group.

Results: The data showed metformin, acarbose, and acarbose + metformin downregulated visfatin levels in diabetic rats, but only the reduction in metformin-treated rats was significant (162 ± 21.7, 195.66 ± 6.45 (ng/l), P = 0.001). Fasting blood glucose and glycated hemoglobin decreased significantly in all treated rats, specifically in the treated group that received the two drugs in combination. The serum insulin level was also reduced in all treated groups, and it was significant in the acarbose ($P < 0.05$) and the combination therapy groups ($P < 0.05$). The lipid profile improved in all treated groups.

Conclusions: Compared with acarbose or metformin monotherapy, the addition of acarbose to metformin had superior antihyperglycemia efficacy and provided an efficacious and safe alternative for the treatment of type 2 diabetic rats. Acarbose/metformin reduced the fasting blood glucose and glycated hemoglobin without significant changes in serum visfatin levels.

Keywords: Acarbose, Metformin, Diabetes Mellitus Type 2, Rats, Visfatin

1. Background

Metabolic syndrome is a cluster of several metabolic abnormalities, including central obesity, insulin resistance, hypertension, dyslipidemia, and hyperglycemia, that has become a major public health challenge (1). DM is the most common metabolic disorder worldwide and is a major risk factor for cardiovascular disease (CVD). It is estimated that the incidence of diabetes will be 366 million by the year 2030 (2). Many risk factors for CVD, including hyperglycemia, abnormal lipid profiles, and alterations in inflammatory mediators, are carried by type two diabetes mellitus (T2DM) patients (3, 4). The most important risks for the development of insulin resistance and T2DM are obesity and excess adiposity (5).

White adipose tissue is not only a site of triglyceride and energy storage but is also an active endocrine organ that secretes many biologically active mediators, referred to as "adipokines," and is an active participant in energy homeostasis and physiological functions, such as immunity and inflammation (6, 7). As a new adipocytokine, visfatin is associated with a wide range of biologic effects, including glucose and lipid metabolism, and has been implicated in the pathogenesis of diabetes and obesity. It was previously described as a pre-beta cell colony- enhancing factor, which is abundantly expressed in visceral adipose tissue. Numerous studies have indicated that visfatin plays an important role in glucose homeostasis (8). Visfatin has insulin mimetic effects; it binds to the insulin receptor at a different binding site than insulin and activates it. Therefore, visfatin is an attractive target molecule that is also non-competitive with insulin and could offer a new approach in the pharmacotherapy of insulin-resistant conditions (9).

Copyright © 2016, Iranian Red Crescent Medical Journal. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/) which permits copy and redistribute the material just in noncommercial usages, provided the original work is properly cited.

Archive of SID

www.SID.ir

Several clinical studies have reported that higher plasma visfatin levels are associated with a higher body mass index (BMI) and more body fat (10), T2DM (11), obesity (8), and dyslipidemia (12). Recently, the relationships between visfatin and metabolic disorders, such as insulin resistance and dyslipidemia, have been studied in humans, but many aspects of these relations are still unknown. In addition to the beneficial effects of visfatin on glucose homeostasis, visfatin is speculated to provide a compensatory mechanism in response to hyperglycemia in the condition of insulin resistance. One study showed increased levels of circulating visfatin (8), while another study confirmed reduced plasma visfatin levels in obesity (13). Paradoxically, in humans, both weight reduction (8) and over-nutrition down regulated the circulating visfatin concentrations (14). In various models of obesity, controversial findings related to visfatin levels, including increased (15), unchanged (16), or decreased levels (17), have been reported.

Metformin, a biguanide, is generally considered the first-choice oral medication in T2DM due to its antihyperglycemic efficacy, favorable effect on body weight, low risk of hypoglycemia, and low cost. If metformin monotherapy fails to attain sufficient glycemic control, current guidelines recommend adding another complementary pharmacotherapeutic agent (18, 19). In addition to biguanides, alpha-glucosidase inhibitors are another potentially beneficial class of oral medications with respect to body weight and cardiovascular parameters (18, 19). Acarbose is a complex oligosaccharide that binds competitively to the α-glucosidases at the brush border of the small intestine, thus delaying the breakdown of sucrose and starch and the absorption of glucose and fructose. This drug has proven efficacious in reducing post-prandial increases in glucose and insulin (20); several studies have indicated that acarbose improved glycemic control in obese hypertensive participants with glucose tolerance (21) or overt diabetes (22). However, the effect of acarbose on overall insulin sensitivity has been observed in obese and glucose-tolerant patients as well as in elderly and obese T2DM patients, but no changes have been reported in most studies with diabetic patients (23).

2. Objectives

The focus of this study was to investigate the effects of metformin and acarbose alone and in combination on visfatin levels, glycemic control, and lipid profile in a TD2M rat model.

3. Materials and Methods

3.1. Chemicals and Reagents

Streptozotocin (STZ) and nicotinamide were purchased from Sigma-Aldrich (USA). All other chemicals used in this study were analytical grade and were obtained from Merck (Germany). Serum concentrations of fasting blood glucose (FBG), triglycerides (TG), total cholesterol, and high-density lipoprotein cholesterol (HDL-C) were measured enzymatically using commercial kits (Pars Azemoon, Tehran, Iran) with the aid of a spectrophotometer (JENWAY 6505 Europe Union). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula:

$$\text{LDL} - C = \text{Total} \quad \text{Cholestrol} - \left[\text{HDL} - C + \left(\frac{\text{TC}}{5}\right)\right] \quad (1)$$

Glycosylated hemoglobin (HbA1c) was estimated with the method of cation exchange chromatography using a Biosystems kit (Barcelona, Spain). The serum visfatin level was determined by commercial enzyme immunoassay kits (Bioassay Technology Laboratory, Shanghai, China), and fasting insulin was detected using a commercial enzyme immunoassay kit (MERCODIA, Sweden) with the aid of an enzyme-linked immunosorbent assay (ELISA) reader Bio Tek ELX800TM (USA).

3.2. Animals

Thirty male Wistar rats (260 - 300 g) were purchased from the central animal house at Tehran University of Medical Sciences and maintained in an air-conditioned room (25 ± 1°C) and at a constant humidity (55 ± 5%). All rats were provided with a commercially available normal rat diet (which contained 60% carbohydrate (w/w), 2% fat (w/w), 17.5% protein (w/w), 8% fiber (w/w)), and water ad libitum. The experiments were performed in accordance with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines. The study protocols were approved by the Institutional Animal Ethics Committee of Arak University of Medical Sciences in Arak, Iran (code: 92-151-1, date: November 18, 2013).

3.3. Induction of Type 2 Diabetes

Type 2 diabetes was induced in male Wistar rats by a single intraperitoneal (IP) injection of 60 mg/kg bw STZ dissolved in a citrate buffer (pH 4.5) 15 minutes after the IP administration of 110 mg/kg bw of nicotinamide (dissolved in normal saline). The respective control rats were given a vehicle citrate buffer and normal saline (24). Hyperglycemia was confirmed by elevated fasting glucose levels determined 72 hours after STZ or vehicle injection. Rats with an FBG of more than 126 mg/dl were considered diabetic (25).

3.4. Experimental Design

In this experimental study, 30 male Wistar rats were randomly divided (simple randomization) into five groups (with six rats in each group). One of these groups was randomly selected as the control (Group 1), and T2DM was induced in the four other groups with the administration of 60 mg/kg of body weight STZ IP after a nicotinamide injec-

Archive of SID

www.SID.ir

tion. One of the diabetic rat groups was selected randomly as the diabetic control (Group 2), and the three other groups were treated with antidiabetic drugs. Throughout the treatment period, the rats were maintained in single cages. The studied groups were as follows:

- Group 1: Control rats: received water and feed ad libitum.
- Group 2: Diabetic control rats: received water and feed ad libitum.
- Group 3: Metformin-treated diabetic rats: received 150 mg/kg/day of metformin in their drinking water for 6 weeks.
- Group 4: Acarbose-treated diabetic rats: received 40 mg/100 g chow/day of acarbose for 6 weeks.
- Group 5: Metformin- and acarbose-treated diabetic rats: received metformin for 2 weeks and then a combination of both for 4 weeks.

3.5. Acarbose Treatment

The daily food intake for each rat was determined over a period of one week before initiating the acarbose treatment as the daily difference between the amount of food provided and what remained. This measurement allows for the assessment of the actual dose each rat received. The mean acarbose doses were 15 ± 0.5 mg/kg bw/day for each animal.

The initial and final FBG levels of all groups were recorded after 6 weeks. In a fasting condition, the animals were anesthetized using ketamine (75 mg/kg bw) and xylazine (10 mg/kg bw) IP. Blood samples were collected by cardiac puncture, and the serum was separated immediately.

3.6. Statistical Analysis

All data were expressed as mean ± standard error of three replicates for six rats in each group. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 19 (SPSS Inc., Chicago, IL, USA). The normality assumption was checked using a one-sample Kolmogorov-Smirnov test. One-way analysis of variance was applied to determine the differences between the results of the studied groups. Post hoc test was used to compare the data. Values of $P < 0.05$ were considered statistically significant.

4. Results

Table 1 presents the effect of metformin, acarbose, and a combination of metformin and acarbose on changes in fasting blood glucose levels and HbA1c in type 2 diabetic rats. The serum glucose level was measured in both the normal and diabetic rats on day 0 and day 42 following the administration of antidiabetic drugs. Three days after the STZ injection, the diabetic rats showed a significant increase in their fasting blood glucose compared to the control group ($P = 0.0001$). The administration of metformin, acarbose, and a combination of the two drugs for 6 weeks revealed a highly significant decrease in the FBG compared to the untreated diabetic rats ($P = 0.0001$).

The HbA1c increased significantly in diabetic rats ($P = 0.0001$); after treatment with all methods, the values were brought towards normal levels.

The effects of antidiabetic drug administration on the lipid profiles are shown in Table 2. Serum TG levels decreased in all treated groups, and this difference was significant in all groups when compared to untreated diabetic rats ($P = 0.0001$). This reduction was higher in the group that received a combination of metformin and acarbose. Additionally, in this same group, the total cholesterol was significantly decreased ($P = 0.001$). Serum HDL-C was also significantly increased in diabetic rats treated with metformin ($P = 0.0001$), while the LDL-C significantly decreased in diabetic rats treated with acarbose alone ($P = 0.0001$).

Table 3 shows the effect of antidiabetic drugs on changes in the visfatin and insulin concentrations in both normal and diabetic rats. Serum visfatin levels were found to be significantly elevated in diabetic rats ($P = 0.0001$). The mean serum level of visfatin in all treated groups receiving antidiabetic drugs was decreased when compared to untreated diabetic rats, but metformin alone also decreased the serum visfatin levels significantly ($P = 0.001$).

The serum insulin levels decreased in diabetic rats ($P = 0.0001$), and this reduction continued after treatment. The difference was significant in both the acarbose-treated group ($P = 0.020$) and the combined treatment group ($P = 0.037$).

Table 1. The Effect of the Oral Administration of Metformin, Acarbose, and Their Combination on Fasting Blood Glucose Levels and Glycated Hemoglobin in Normal and Nicotinamide/Streptozotocin-induced Type 2 Diabetic Rats[a,b]

Group	Serum Glucose Levels, mg/dL		HbA1c
	Day 0	Day 42	
Normal	69.3 ± 3.90	77.1 ± 3.90	4.46 ± 0.27
Diabetic control	269.8 ± 22.0[c]	295.8 ± 27.0[c]	7.25 ± 0.79[c]
Diabetic + metformin	307.0 ± 18.8[c]	105.8 ± 11.3[c,d]	4.91 ± 0.44[d]
Diabetic + acarbose	277.1 ± 19.0[c]	89.1 ± 6.3[d]	4.21 ± 0.17[d]
Diabetic + metformin and acarbose	258.6 ± 32.0[c]	68.3 ± 2.9[d]	3.80 ± 0.32[d]

[a] Each value is the mean ± SD of the six rats in each group.
[b] A1c, diabetic control group compared with all other groups after day 42 ($P = 0.0001$); FBS, diabetic control group compared with all other groups after day 42 ($P = 0.0001$).
[c] $P < 0.05$ as compared with normal rats.
[d] $P < 0.05$ in comparison with diabetic rats.

Archive of SID
www.SID.ir

Table 2. Effect of Oral Administration of Metformin, Acarbose, and Their Combination on Serum Lipids in Normal and Nicotinamide/Streptozotocin-induced Type 2 Diabetic Rats[a,b]

Groups	Total Cholesterol	Triglyceride	LDL Cholesterol	HDL Cholesterol
Normal	128.2 ± 8.6[c]	66.4 ± 4.6[c]	76.2 ± 12.5	34.6 ± 3.9
Diabetic control	109.0 ± 3.0	96.2 ± 11.5[d]	58.2 ± 2.6[d]	29.2 ± 8.3
Diabetic + metformin	120.0 ± 12.5[d]	63.0 ± 15.18[c]	66.6 ± 3.5	42.6 ± 2.8[d,c]
Diabetic + acarbose	87.6 ± 11.1[d,c]	59.6 ± 9.4[c]	40.2 ± 5.5[d,c]	24.4 ± 1.5[d]
Diabetic + metformin and acarbose	83.4 ± 13.7[d,c]	50.6 ± 4.3[c]	47.8 ± 4.7[d]	27.2 ± 4.0[d]

[a]Each value represents the mean ± SD of the six rats in each group; the unit for all values is mg/dl.
[b]Total Cholesterol (TC): The diabetic control group compared with the normal group (P = 0.013), the diabetic control group compared with the acarbose-treated group (P = 0.005), and the diabetic control group compared with the two-drug combination group (P = 0.001); Triglyceride (TG): The diabetic control group compared with all groups (P = 0.0001); LDL: The diabetic control group compared with the normal group (P = 0.0001), the diabetic control group compared with the acarbose-treated group (P = 0.0001), and the diabetic control group compared with the two-drug combination group (P = 0.046); HDL: The diabetic control group compared with the normal group (P = 0.013), the diabetic control group compared with metformin-treated group (P = 0.0001), and the metformin-treated group compared with all groups (P = 0.0001).
[c]P < 0.05 in comparison with diabetic rats.
[d]P < 0.05 compared with normal rats.

Table 3. The Effect of Oral Administration of Metformin, Acarbose, and Their Combination on Serum Visfatin and Insulin Levels in Normal and Nicotinamide/Streptozotocin-induced Type 2 Diabetic Rats[a,b]

Groups	Visfatin, ng	Insulin, μg
Normal	154.4 ± 11.9[c]	0. 75 ± 0.1[c]
Diabetic control	195.6 ± 6.4[d]	0.37 ± 0.07[d]
Diabetic + metformin	162.7 ± 21.2[c]	0.31 ± 0.12[d]
Diabetic + acarbose	183.5 ± 11.3[d]	0.22 ± 0.014[d,c]
Diabetic + metformin and acarbose	180.1 ± 5.1[d]	0.24 ± 0.013[d,c]

[a]Each value is mean ± SD of the six rats in each group.
[b]Visfatin: The diabetic control group compared with the normal group (P = 0.0001), the diabetic control group compared with the metformin-treated group (P = 0.001), and the metformin-treated group compared with the acarbose-treated group (P = 0.047) Insulin: The diabetic control group compared with the normal group (P = 0.0001), the diabetic control group compared with the acarbose-treated group (P = 0.020), the diabetic control group compared with the two-drug combination group (P = 0.037), and the normal group compared with all groups (P = 0.0001).
[c]P < 0.05 in comparison with diabetic rats.
[d]P < 0.05 compared with normal rats.

5. Discussion

Experimental models are necessary to better understand the pathogenesis, genetic environmental factors, and biological complications that are involved in T2DM, and also to better examine the various therapeutic agents available. The association between nicotinamide and STZ is being increasingly used to induce diabetes mellitus in experimental animals that is similar to human T2DM (26). In this combination, nicotinamide protects the beta cells of the pancreas, improves their regeneration, and controls blood glucose (27), whereas STZ has a toxic effect and promotes the increased formation of free radicals that fragment the DNA of pancreatic beta cells (28). This DNA damage is followed by activation of nuclear enzymes, which results in reduced insulin synthesis in beta cells and cell death (29). This STZ-induced cytotoxicity can be alleviated by nicotinamide, which inhibits the depletion of pancreatic poly (ADP-ribose) synthetase activity and prevents NAD depletion in beta cells. STZ-NA-induced diabetic rats exhibited moderate hyperglycemia associated with a loss of postprandial early phase insulin secretion, as well as a 50% decrease in pancreatic insulin content (30). In the present study, we evaluated the effect of the oral antidiabetic drugs metformin, acarbose, and a combination of both on fasting blood glucose, HbA1c, lipid profile, serum visfatin, and insulin concentration levels in streptozotocin- nicotinamide-induced mildly diabetic rats.

In T2DM, metformin therapy should be initiated when lifestyle interventions do not achieve or maintain the patient's metabolic goals due to failure to lose weight, regaining weight, progressive disease, or a combination of factors (31). The main effect of metformin is the inhibition of the mitochondrial respiratory-chain complex I and a decrease in hepatic glucose production. The resulting decrease in hepatic energy status activates AMP-activated protein kinase (AMPK), providing a generally accepted mechanism for metformin's action on hepatic gluconeogenesis. Activated AMPK switches cells from an anabolic to a catabolic state, shutting down the AT-consuming synthetic pathways and restoring energy

Archive of SID

www.SID.ir

balance. This regulation involves phosphorylation of key metabolic enzymes and transcription factors/co-activators that modulate gene expression. Therefore, glucose, lipid, and protein synthesis as well as cell growth are inhibited, whereas fatty acid oxidation and glucose uptake are stimulated (32). Metformin is frequently described as an insulin sensitizer, leading to a reduction in insulin resistance and a significant decrease of the plasma fasting insulin level. This improvement in insulin sensitivity could be ascribed to its positive effects on insulin receptor expression and tyrosine kinase activity (33).

If lifestyle interventions and administration of the maximal dose of metformin do not achieve or sustain glycemic goals, another antidiabetic drug should be added any time when the target HbA1c level is not achieved.

Chiasson et al. was the first to examine the use of acarbose as an adjunctive therapy to metformin (34). Acarbose delays glucose absorption and thus attenuates postprandial rises in blood glucose and insulin and reduces HbA1c levels by 0.5 - 0.8 percentage points. However, these antidiabetic drugs induce a significant decrease in the postprandial rise in glucose levels without increasing the circulating insulin levels or causing hypoglycemia.

In this study, fasting blood glucose and glycated hemoglobin decreased significantly in all treated rats, particularly in the group treated with a combination of the two drugs. Our results demonstrated a beneficial effect on overall glycemic control of additional acarbose therapy in T2DM rats that were insufficiently controlled by metformin alone. HbA1c and FBG levels showed a clinically significant decrease in the metformin and acarbose groups compared to the control diabetic group. Therefore, the use of acarbose as an add-on therapy to metformin significantly decreased HbA1c levels and also produced significant reductions in FBG. Our findings were supported by Hoffmann et al. who reported that both active drugs showed the same improvement of efficacy criteria compared with a placebo (35). Fasting blood glucose and HbA1c decreased with acarbose and metformin treatment vs. the placebo. The difference in the effects of acarbose vs. placebo and metformin vs. placebo were statistically significant, but those of acarbose vs. metformin were not (35). On the other hand, our results are in agreement with Van de laar et al. “who reported alpha-glucosidase inhibitors reduce postprandial hyperglycemia, delaying the breakdown of carbohydrates in the gut and, consequently, slowing the absorption of sugars. They are less effective at lowering glycemia values than are metformin and sulfonylureas” (36). Our results regarding the use of acarbose as an add-on therapy to metformin are also supported by Halimi et al. who reported the potential of acarbose add-on therapy for improving the glycemic control of overweight patients with T2DM inadequately controlled with metformin alone; acarbose decreased the HbA1c, FPG, and PPG levels compared with placebo and also improved glycemic control (37).

Diabetes is commonly associated with abnormalities in plasma lipid and lipoprotein levels. In particular, it usually presents with concomitant elevations in plasma TG and reductions in plasma HDL-C concentrations. Abnormalities in the lipid profile are one of the most common complications in diabetes and are associated with an increased risk of coronary heart disease; therefore, an ideal treatment for diabetes should have a favorable effect on the lipid profile in addition to offering good glycemic control (38). According to our results, the diabetic condition in rats raised serum TG levels significantly and lowered TC, LDL-C, and HDL-C levels compared to control rats. The reduction in LDL-C was significant. The changes in TG and HDL-C that were noted in this study are in agreement with the findings of Pierre et al., while our results for TC and LDL-C were in opposition to their findings (24). Our results showed that the serum TG levels decreased significantly in all three treated groups but were lower in the group that received a combination of metformin and acarbose. Also, in this group, the TC was significantly decreased. Treatment with metformin increased while treatment with acarbose decreased the total cholesterol, but the difference was not significant. However, the serum HDL-C was significantly increased in diabetic rats treated with metformin, while it decreased in the other treated groups. LDL-C significantly decreased in diabetic rats treated with acarbose alone. However, this reduction in the LDL-C in rats treated with a combination of drugs was not significant, and the LDL-C actually increased in the metformin group. Both as a first line and an add-on therapy, treatment with acarbose was more effective than placebo in improving HbA1c, fasting plasma glucose, and postprandial glucose levels as well as the lipid profile. This action on the lipid profile is interesting considering that postprandial hyperglycemia and postprandial hyperlipidemia are strong independent risk factors for CVD.

Serum visfatin levels were found to be significantly elevated in diabetic rats. Several other studies have reported increased blood visfatin levels in patients with T2DM (10). Consistent with most findings, we found significantly higher visfatin levels in diabetic rats compared with the controls. There are limited data about the effect of antidiabetic drugs on adipokines, especially on visfatin. Hsieh reported that metformin had no effect on insulin sensitivity and serum visfatin in patients with T2DM (39). On the contrary, we found that visfatin levels in the serum decreased after treatment with metformin. The mean serum levels of visfatin in all treated groups receiving antidiabetic drugs decreased when compared to untreated diabetic rats, but only metformin decreased the serum visfatin levels significantly. A recent study conducted in patients with polycystic ovarian syndrome revealed that visfatin levels decreased after metformin treatment (40). It was reported that visfatin secretion is regulated by insulin and glucose by means of the phosphatidylinositol 3 kinase and protein kinase B pathways (41). Other studies have shown that metformin has a direct effect on adipocytes and skeletal tissue, inhibiting plasminogen activator inhibitor 1 in the

Archive of SID

www.SID.ir

adipose tissue and stimulating AMP-activated protein kinase in mice (42), which may explain our observations in the present study. Our results showed that visfatin levels decreased after acarbose and a combination of metformin and acarbose treatment, although the differences were not significant. Acarbose treatment may reduce the mRNA levels of inflammatory cytokines in adipose tissues. The mechanism by which acarbose reduces the expression of visfatin is still unknown. Moreover, our study identified fasting plasma glucose as an independent predictor of the serum visfatin level. Consistent with our findings, Haider et al. (41) showed that the release of visfatin in response to hyperglycemia by adipocytes is dependent on the duration and extent of glucose elevation and is also inhibited by the administration of insulin. However, the insulin dose not influence visfatin synthesis in adipocytes, and there is no difference in the serum visfatin level between type 2 diabetic patients treated with insulin infusion or those who are prescribed oral hypoglycemic agents (43). The influence of insulin-sensitizing agents on the serum visfatin level has not yet been confirmed (44). These findings suggest that fasting glucose levels (but not insulin resistance) may play an important role in the elevation of visfatin concentrations in newly diagnosed type 2 diabetics.

Serum insulin levels were decreased in diabetic rats; after treatment, the insulin continued to decrease, but this reduction was only significant in rats treated with acarbose alone and also with the combination therapy. Our results are in agreement with Tahara et al.; they reported the pancreatic insulin content was approximately 1/2 of normal levels in STZ-NA-induced diabetic rats (45). These mildly diabetic rats exhibited moderate hyperglycemia and impaired glucose tolerance due to the loss of early phase insulin secretion. Their study evaluated the effects of antidiabetic drugs voglibose, metformin, glibenclamide, sitagliptin, and insulin on glucose tolerance, as well as insulin and glucagon-like peptide-1 (GLP-1) secretion in STZ-NA diabetic rats. In particular, the insulin secretagogues, glibenclamide and sitagliptin, were found to cause a significant increase in plasma insulin levels; Masiello et al. (46) reported similar results. In contrast, plasma insulin levels were significantly low in the group treated with voglibose or metformin. Plasma GLP-1 levels did not significantly differ between normal and mildly diabetic rats, but they were significantly increased in the sitagliptin-treated group; no significant differences were noted in the other drug-treated groups.

5.1. Conclusions

Compared with acarbose or metformin monotherapy, the addition of acarbose to metformin had superior antihyperglycemia efficacy and provided an officious and safe alternative for treatment of type 2 diabetic rats. Acarbose/metformin reduced the fasting blood glucose and glycated hemoglobin without producing significant changes in the serum visfatin levels.

Acknowledgments

We are grateful to the Exir Pharmaceutical Company in Tehran, Iran.

Footnotes

Authors' Contribution: Proposal design: Zahra Salemi, Mohamad Taghi Goodarzi; Material preparation; Elham Rafie, Mohamad ali Ghaffari; Carrying out the experiment: Elham Rafie; Data analysis: All authors; Manuscript preparation: Zahra Salemi.

Financial Disclosure: The authors declare that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Funding/Support: Arak University Department of Medical Sciences

References

1. Alberti KG, Zimmet P, Shaw J, I. D. F. Epidemiology Task Force Consensus Group The metabolic syndrome--a new worldwide definition. *Lancet.* 2005;**366**(9491):1059–62. doi: 10.1016/S0140-6736(05)67402-8. [PubMed: 16182882]
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care.* 2004;**27**(5):1047–53. [PubMed: 15111519]
3. Caro JJ, Ward AJ, O'Brien JA. Lifetime costs of complications resulting from type 2 diabetes in the U.S. *Diabetes Care.* 2002;**25**(3):476–81. [PubMed: 11874933]
4. Toh SA, Rader DJ. Dyslipidemia in insulin resistance: clinical challenges and adipocentric therapeutic frontiers. *Expert Rev Cardiovasc Ther.* 2008;**6**(7):1007–22. doi: 10.1586/14779072.6.7.1007. [PubMed: 18666851]
5. Bloomgarden ZT. Adiposity and diabetes. *Diabetes Care.* 2002;**25**(12):2342–9. [PubMed: 12453983]
6. Papanas N, Maltezos E. Oral antidiabetic agents: anti-atherosclerotic properties beyond glucose lowering? *Curr Pharm Des.* 2009;**15**(27):3179–92. [PubMed: 19754390]
7. Bulcao C, Ferreira SR, Giuffrida FM, Ribeiro-Filho FF. The new adipose tissue and adipocytokines. *Curr Diabetes Rev.* 2006;**2**(1):19–28. [PubMed: 18220614]
8. Haider DG, Schaller G, Kapiotis S, Maier C, Luger A, Wolzt M. The release of the adipocytokine visfatin is regulated by glucose and insulin. *Diabetologia.* 2006;**49**(8):1909–14. doi: 10.1007/s00125-006-0303-7. [PubMed: 16736128]
9. Hug C, Lodish HF. Medicine. Visfatin: a new adipokine. *Science.* 2005;**307**(5708):366–7. doi: 10.1126/science.1106933. [PubMed: 15604359]
10. Chen MP, Chung FM, Chang DM, Tsai JC, Huang HF, Shin SJ, et al. Elevated plasma level of visfatin/pre-B cell colony-enhancing factor in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab.* 2006;**91**(1):295–9. doi: 10.1210/jc.2005-1475. [PubMed: 16234302]
11. Berndt J, Kloting N, Kralisch S, Kovacs P, Fasshauer M, Schon MR, et al. Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. *Diabetes.* 2005;**54**(10):2911–6. [PubMed: 16186392]
12. Gligori R, Zdremti D, Pilati L, Matei I. Correlation of visfatin with the lipidic metabolism in diabetic and obese patients. *RJME.* 2012;**1**(1):37–43.
13. Pagano C, Pilon C, Olivieri M, Mason P, Fabris R, Serra R, et al. Reduced plasma visfatin/pre-B cell colony-enhancing factor in obesity is not related to insulin resistance in humans. *J Clin Endocrinol Metab.* 2006;**91**(8):3165–70. doi: 10.1210/jc.2006-0361. [PubMed: 16720654]
14. Sun G, Bishop J, Khalili S, Vasdev S, Gill V, Pace D, et al. Serum visfatin concentrations are positively correlated with serum triacylglycerols and down-regulated by overfeeding in healthy young men. *Am J Clin Nutr.* 2007;**85**(2):399–404. [PubMed: 17284735]

Archive of SID

www.SID.ir

15. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science.* 2005;**307**(5708):426–30. doi: 10.1126/science.1097243. [PubMed: 15604363]
16. Kloting N, Kloting I. Visfatin: gene expression in isolated adipocytes and sequence analysis in obese WOKW rats compared with lean control rats. *Biochem Biophys Res Commun.* 2005;**332**(4):1070–2. doi: 10.1016/j.bbrc.2005.05.058. [PubMed: 15922301]
17. Mercader J, Granados N, Caimari A, Oliver P, Bonet ML, Palou A. Retinol-binding protein 4 and nicotinamide phosphoribosyltransferase/visfatin in rat obesity models. *Horm Metab Res.* 2008;**40**(7):467–72. doi: 10.1055/s-2008-1065324. [PubMed: 18401839]
18. International Diabetes Federation. *2012 Clinical Guidelines Task Force.* Available from: www.idf.org/sites/default/files/IDF-Guideline-for-Type2-Diabetes.
19. Standl E, Schnell O. Alpha-glucosidase inhibitors 2012 - cardiovascular considerations and trial evaluation. *Diab Vasc Dis Res.* 2012;**9**(3):163–9. doi: 10.1177/1479164112441524. [PubMed: 22508699]
20. Hanefeld M, Fischer S, Schulze J, Spengler M, Wargenau M, Schollberg K, et al. Therapeutic potentials of acarbose as first-line drug in NIDDM insufficiently treated with diet alone. *Diabetes Care.* 1991;**14**(8):732–7. [PubMed: 1954810]
21. Pan CY, Gao Y, Chen JW, Luo BY, Fu ZZ, Lu JM, et al. Efficacy of acarbose in Chinese subjects with impaired glucose tolerance. *Diabetes Res Clin Pract.* 2003;**61**(3):183–90. [PubMed: 12965108]
22. Fischer S, Patzak A, Rietzsch H, Schwanebeck U, Kohler C, Wildbrett J, et al. Influence of treatment with acarbose or glibenclamide on insulin sensitivity in type 2 diabetic patients. *Diabetes Obes Metab.* 2003;**5**(1):38–44. [PubMed: 12542723]
23. Delgado H, Lehmann T, Bobbioni-Harsch E, Ybarra J, Golay A. Acarbose improves indirectly both insulin resistance and secretion in obese type 2 diabetic patients. *Diabetes Metab.* 2002;**28**(3):195–200. [PubMed: 12149599]
24. Pierre W, Gildas AJ, Ulrich MC, Modeste WN, Benoit NT, Albert K. Hypoglycemic and hypolipidemic effects of Bersama engleriana leaves in nicotinamide/streptozotocin-induced type 2 diabetic rats. *BMC Complement Altern Med.* 2012;**12**:264. doi: 10.1186/1472-6882-12-264. [PubMed: 23267560]
25. Shirwaikar A, Rajendran K, Barik R. Effect of aqueous bark extract of Garuga pinnata Roxb. in streptozotocin-nicotinamide induced type-II diabetes mellitus. *J Ethnopharmacol.* 2006;**107**(2):285–90. doi: 10.1016/j.jep.2006.03.012. [PubMed: 16644162]
26. Matsuyama-Yokono A, Tahara A, Nakano R, Someya Y, Hayakawa M, Shibasaki M. Chronic inhibition of dipeptidyl peptidase-IV with ASP8497 improved the HbA(1c) level, glucose intolerance, and lipid parameter level in streptozotocin-nicotinamide-induced diabetic mice. *Naunyn Schmiedebergs Arch Pharmacol.* 2009;**379**(2):191–9. doi: 10.1007/s00210-008-0348-x. [PubMed: 18762913]
27. Hypponen E. Micronutrients and the risk of type 1 diabetes: vitamin D, vitamin E, and nicotinamide. *Nutr Rev.* 2004;**62**(9):340–7. [PubMed: 15497767]
28. Chen H, Carlson EC, Pellet L, Moritz JT, Epstein PN. Overexpression of metallothionein in pancreatic beta-cells reduces streptozotocin-induced DNA damage and diabetes. *Diabetes.* 2001;**50**(9):2040–6. [PubMed: 11522669]
29. Schnedl WJ, Ferber S, Johnson JH, Newgard CB. STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. *Diabetes.* 1994;**43**(11):1326–33. [PubMed: 7926307]
30. Oguri S, Motegi K, Endo Y. Augmented lipopolysaccharide-induction of the histamine-forming enzyme in streptozotocin-induced diabetic mice. *Biochim Biophys Acta.* 2003;**1637**(1):83–90. [PubMed: 12527411]
31. Derosa G, Maffioli P. Effects of thiazolidinediones and sulfonylureas in patients with diabetes. *Diabetes Technol Ther.* 2010;**12**(6):491–501. doi: 10.1089/dia.2009.0172. [PubMed: 20470234]
32. Viollet B, Guigas B, Leclerc J, Hebrard S, Lantier L, Mounier R, et al. AMP-activated protein kinase in the regulation of hepatic energy metabolism: from physiology to therapeutic perspectives. *Acta Physiol (Oxf).* 2009;**196**(1):81–98. doi: 10.1111/j.1748-1716.2009.01970.x. [PubMed: 19245656]
33. Gunton JE, Delhanty PJ, Takahashi S, Baxter RC. Metformin rapidly increases insulin receptor activation in human liver and signals preferentially through insulin-receptor substrate-2. *J Clin Endocrinol Metab.* 2003;**88**(3):1323–32. doi: 10.1210/jc.2002-021394. [PubMed: 12629126]
34. Chiasson JL, Josse RG, Hunt JA, Palmason C, Rodger NW, Ross SA, et al. The efficacy of acarbose in the treatment of patients with non-insulin-dependent diabetes mellitus. A multicenter controlled clinical trial. *Ann Intern Med.* 1994;**121**(12):928–35. [PubMed: 7734015]
35. Hoffmann J, Spengler M. Efficacy of 24-week monotherapy with acarbose, metformin, or placebo in dietary-treated NIDDM patients: the Essen-II Study. *Am J Med.* 1997;**103**(6):483–90. [PubMed: 9428831]
36. Van de Laar FA, Lucassen PL, Akkermans RP, Van de Lisdonk EH, Rutten GE, Van Weel C. Alpha-glucosidase inhibitors for type 2 diabetes mellitus. *Cochrane Database Syst Rev.* 2005; (2):CD003639. doi: 10.1002/14651858.CD003639.pub2. [PubMed: 15846673]
37. Halimi S, Le Berre MA, Grange V. Efficacy and safety of acarbose add-on therapy in the treatment of overweight patients with Type 2 diabetes inadequately controlled with metformin: a double-blind, placebo-controlled study. *Diabetes Res Clin Pract.* 2000;**50**(1):49–56. [PubMed: 10936668]
38. Azizi R, Salemi Z, Goodarzi MT. Effect of Biochanin-A on serum visfatin levels in STZ-induced diabetic rats. *Iran Red Crescent Med J.* 2014;**16**(9):1–7.
39. Hsieh CH, He CT, Lee CH, Wu LY, Hung YJ. Both slow-release and regular-form metformin improve glycemic control without altering plasma visfatin level in patients with type 2 diabetes mellitus. *Metabolism.* 2007;**56**(8):1087–92. doi: 10.1016/j.metabol.2007.03.018. [PubMed: 17618954]
40. Ozkaya M, Cakal E, Ustun Y, Engin-Ustun Y. Effect of metformin on serum visfatin levels in patients with polycystic ovary syndrome. *Fertil Steril.* 2010;**93**(3):880–4. doi: 10.1016/j.fertnstert.2008.10.058. [PubMed: 19111298]
41. Haider DG, Holzer G, Schaller G, Weghuber D, Widhalm K, Wagner O, et al. The adipokine visfatin is markedly elevated in obese children. *J Pediatr Gastroenterol Nutr.* 2006;**43**(4):548–9. doi: 10.1097/01.mpg.0000235749.50820.b3. [PubMed: 17033537]
42. Erdem G, Dogru T, Tasci I, Bozoglu E, Muhsiroglu O, Tapan S, et al. The effects of pioglitazone and metformin on plasma visfatin levels in patients with treatment naive type 2 diabetes mellitus. *Diabetes Res Clin Pract.* 2008;**82**(2):214–8. doi: 10.1016/j.diabres.2008.07.021. [PubMed: 18778865]
43. Kralisch S, Klein J, Lossner U, Bluher M, Paschke R, Stumvoll M, et al. Hormonal regulation of the novel adipocytokine visfatin in 3T3-L1 adipocytes. *J Endocrinol.* 2005;**185**(3):R1–8. doi: 10.1677/joe.1.06211. [PubMed: 15930160]
44. Kadoglou NP, Tsanikidis H, Kapelouzou A, Vrabas I, Vitta I, Karayannacos PE, et al. Effects of rosiglitazone and metformin treatment on apelin, visfatin, and ghrelin levels in patients with type 2 diabetes mellitus. *Metabolism.* 2010;**59**(3):373–9. doi: 10.1016/j.metabol.2009.08.005. [PubMed: 19815243]
45. Tahara A, Matsuyama-Yokono A, Nakano R, Someya Y, Shibasaki M. Hypoglycaemic effects of antidiabetic drugs in streptozotocin-nicotinamide-induced mildly diabetic and streptozotocin-induced severely diabetic rats. *Basic Clin Pharmacol Toxicol.* 2008;**103**(6):560–8. doi: 10.1111/j.1742-7843.2008.00321.x. [PubMed: 18793271]
46. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, et al. Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes.* 1998;**47**(2):224–9. [PubMed: 9519717]

Archive of SID

www.SID.ir