Iranian Journal of Basic Medical Sciences

ijbms.mums.ac.ir

IJBMS

Resveratrol attenuates visfatin and vaspin genes expression in adipose tissue of rats with type 2 diabetes

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ARTICLE INFO

Article type:
Original article

Article history:
Received: Oct 27, 2014
Accepted: Feb 13, 2015

Keywords:
Grape
Insulin sensitivity
Resveratrol
Vaspin
Visfatin

ABSTRACT

Objective(s): Visfatin and vaspin are secreted by adipose tissue and play key roles in glucose homeostasis and subsequently are potential targets for diabetes treatment. Resveratrol (RVS) corrects insulin secretion and improves insulin sensitivity. We investigated the RVS effects on serum antioxidants, insulin and glucose levels, also visfatin and vaspin genes expression in adipose tissue of streptozotocin-nicotinamide (STZ-NA) induced type 2 diabetic rats.

Materials and Methods: Diabetes was induced in Wistar rats (n=32) using STZ (60 mg/kg body weight) and NA (120 mg/kg body weight); rats were divided into 4 groups (n=8). Eight untreated normal rats were used as control group; four diabetic rat groups (2–5) were treated with 0, 1, 5 and 10 mg /kg body weight of RVS, respectively for 30 days. After treatment blood and adipose tissue were prepared from all animals. Serum glucose, insulin, HOMA index, total antioxidant capacity (TAC), and malondialdehyde (MDA) were measured. Visfatin and vaspin genes expression in adipose tissue were evaluated using real-time PCR.

Results: RVS reduced blood glucose significantly and increased insulin level, resulting in insulin sensitivity improvement. Furthermore RVS increased weight and TAC, while reducing serum MDA in the diabetic groups. Visfatin gene expression increased in the diabetic group, and RVS treatment reduced it. Vaspin gene expression was reduced in RVS receiving diabetic groups.

Conclusion: The results indicated that RVS has potential hypoglycemic effect, probably by increasing insulin level and changing gene expression of visfatin and vaspin. Moreover RVS showed antioxidant effects through reduction in peroxidiation products and augmented antioxidant capacity.

► *Please cite this paper as:*
Assadi S, Goodarzi MT, Saidijam M, Jamshid Karimi, Yadgar Azari R, Rezaei Farimani A, Salehi I. Resveratrol attenuates visfatin and vaspin genes expression in adipose tissue of rats with type 2 diabetes. Iran J Basic Med Sci 2015; 18:537-543.

Introduction

Diabetes mellitus is a metabolic disorder characterized by elevated blood glucose levels and dysregulation of carbohydrate metabolism due to deficiency in insulin secretion, insulin function or both (1).

Recent studies indicate that adipose tissue is not only responsible for triacylglycerol accumulation, but it also produces and secretes several proteins called adipocytokines such as adiponectin, visfatin and vaspin, which play important roles in the pathogenesis of type 2 diabetes mellitus (T2DM) and insulin resistance (2-4).

Visfatin also known as PBEF (pre-B cell Colony-enhancing Factor) has the highest expression in bone marrow, liver and muscle (5); it is also secreted from adipose tissue and fetal membrane during pregnancy (6). Recently, it was identified as NAMPT (Nicotinamide Phosphoribosyl Transferase) that is involved in NAD^+ biosynthesis (4, 7). Studies revealed visfatin has insulin-mimetic effect and the affinity of visfatin for insulin receptor was found to be similar to that of insulin (4). Studies indicated that visfatin level increased in T2DM (7, 8).

Vaspin is identified as a member of serine protease inhibitor family (9-11). Vaspin expression is associated with insulin resistance and degree of obesity in OLTEF (Otsuka Long- Evans Tokushima Fatty) rats, an animal model for obesity and insulin resistance, and administration of recombinant vaspin to diet-induced obese mice improved glucose tolerance and insulin sensitivity (12, 13).

Some natural compounds derived from plants can affect diabetes. Resveratrol (3, 5, 4`-trihydroxystilbene)

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is a polyphenol found in high concentration in grapes and red wine (14). Resveratrol (RVS) has anti-inflammatory, anti-diabetic and anti-oxidant properties (15, 16). Studies revealed RVS corrects insulin secretion and improves insulin sensitivity (17, 18) and decreases blood glucose levels and oxidative stress. J. K Bhatt *et al* reported that in patients with T2DM that received RSV for 3 months, glycemic control improved and hemoglobin A1c level decreased (19); also oral supplementation of RSV for short periods, decreased fasting blood glucose and insulin resistance (20). One hypothesis for hypoglycemic effects of RSV and its effect on increasing insulin secretion is that RSV in pancreatic β cell binds to sulfonylurea receptors; the activation of these receptors resulting in inhibition of ATP-sensitive K^+ channels and β cell depolarization and subsequently insulin secretion from β cell (21, 22). There are very limited data about the effect of RSV on visfatin and vaspin gene expression and the details of this effect are still largely unknown. Some studies indicate that RSV modulatory role leads to decrease in visfatin gene expression in SGBS (Simpson-Golabi-Behmel syndrome) adipocytes and zebrafish liver (23, 24). Since the mechanism of RVS on these processes have not been studied, this study was aimed to examine the RVS effect on the vaspin and visfatin genes expression in adipose tissue, insulin secretion, body weight, blood glucose and oxidative status in type 2 diabetes induced rats.

Materials and Methods

Materials

Resveratrol supplementation was from Amazon (USA); streptozotocin (STZ) and nicotinamide (NA) were purchased from Sigma Aldrich (Germany).

Animals

Male Wistar rats (6–8 weeks old, weighing 150–200 g) were purchased from the animal house of Razi Institute, Iran, and maintained in the Central Animal House, Hamadan University of Medical Sciences (Hamadan, Iran). All animals were fed standard pellet and fresh water, and were housed under standard conditions with 12 hr light/dark cycles. The research protocol was approved by the ethical committee of the university.

Induction of type2 diabetes, treatment, and sample collection

Forty rats were divided into five groups as follows: group 1 (n=8): healthy rats fed normal standard diet (Cont); groups 2–5 (n=8 in each group)were diabetic; group 2: diabetic rats (diabetic control); groups 3, 4 and 5 received 1, 5, and 10 mg RVS/kg body weight (bwt), respectively for 30 days.

The diabetes induction and RVS treatment protocol were briefly as follow: overnight fasted rats were injected intraperitoneally 60 mg /kg bwt STZ (in 0.1 M sodium citrate pH 4.5) followed by 120 mg/kg bwt NA after 15 min (25). STZ enters β cell selectively and alkylates DNA (26). In β cell, STZ results in increased activity of PARP-1 (Poly ADP ribose Polymerase-1) and subsequently NAD and ATP levels decrease in the nucleus and type 1 diabetes mellitus is induced (27); but T2DM induction is achieved by administration of NA with STZ, NA prevents excess damage to β cell and type 2 diabetes is induced (28). To confirm T2DM in the rats, 72 hr after injection of STZ/NA fasting blood glucose was measured using a glucometer. Rats with fasting blood glucose levels higher than 150 mg/dl were considered diabetic (29).

One week after induction of T2DM, three diabetic groups (groups 2, 3 and 4) were treated with different doses of resveratrol (1, 5, 10 mg/kg bwt, administration performed orally using gavage syringe) for a month. Assessment of blood glucose levels was performed weekly and the animals were fasted before sampling. After completion of treatment period, the rats were anesthetized using ketamine: xylenes (100 mg/kg bwt: 5–10 mg/kg bwt, IP) (30), and subsequently the animals were sacrificed. Visceral adipose tissue was separated from each rat and immediately frozen in liquid nitrogen and stored at -80 ˚C until analysis. To measure biochemical parameters, a blood sample was collected from the cardiac puncture; serum was separated and stored at -20 ˚C.

RNA Extraction and Quantitative Real Time PCR

RNA extraction was performed manually using TRIzol (Invitrogen) (31). cDNA synthesis was carried out by reverse transcription of 1 µg of RNA using RevertAid First Strand cDNA Synthesis Kit (Fermentas, Burlington, ON, Canada), following the manufacturer's protocol. Vaspin and visfatin genes expression were measured by real-time polymerase chain reaction (qPCR) in a fluorescent temperature cycler using Takara kit (No. RR820L), and fluorescence was detected on CFX96 Real-Time PCR Detection System (BioRad, USA). qPCR reaction mixture was prepared

Table 1. The primers sequences used in PCR

Genes	Primers (5/ - 3/)	GC %	Tm (˚C)	Product length (base pair)
visfatin	F: CCTTACCTTAGAGTCATTCA	40	57.6	99
	R: GACATTCTCAATACTCCAC	42.1	56.5	
vaspin	F: CTGAAACTGGCTAAGAACT	42.1	58.6	191
	R: CACCTGCCTTGAAAGTAAAT	40	60.1	
18s rRNA	F: GTAACGCGTTGAACCCCATT	54.8	64.5	151
	R: CCATCCAATCGGTAGTAGCG	54.4	64.2	

Table 2. Effect of different doses of resveratrol on rat body weights (g) in different groups

Period	Cont	Dia	Dia+RSV1	Dia+RSV5	Dia+RSV10
Pre-treatment	218.13±17.10	220.00±14.14	231.25±18.08	228.00±15.04	213.67±18.00
7 days after diabetes induction	229.37±16.78	205.75±14.89[a]	208.87±10.85	208.37±7.61	206.33±17.00
One month after RVS treatment	260.62±14.78	191.25±14.48[a*]	222.62±17.67[b]	221.50±14.97[b]	211.00± 18.01[b]

Cont: normal control rats, Dia: diabetic control rats, Dia+RSV1, Dia+RSV5 and Dia+RSV10 are diabetic rats receiving 1, 5, and 10 mg/kg bwt of resveratrol, respectively
[a] *P*-value<0.05 comparing with normal Cont. [a*] *P*-value<0.001 comparing with normal Cont
[b] *P*-value<0.01 comparing with diabetic control

according to the kit manufacturer's instructions. 18srRNA level was measured and served as the reference gene. Gene-specific primers were designed using the AlleleID7 software (Premier Biosoft Corporation, USA). The characteristics of the primers are shown in Table 1. Delta Ct analysis was applied to detect the rate of gene expression in each group.

Analyzing the biochemical parameters

The serum glucose was measured using glucose oxidase method in Pars Azmun kit (Iran). Insulin was determined using rat Insulin (INS) ELISA Kit. HOMA (insulin resistance index) was calculated using the formula [insulin (μU/ml)×glucose (mmol/l)/22.5] (32). Total antioxidant capacity (TAC) in serum samples was measured using ferric reducing antioxidant power assay (FRAP) (33); serum maolndialdehyde (MDA) level as a marker for lipid peroxidation was determined using a fluorometric method (34).

Statistical analysis

One-way analysis of variance followed by Tukey test was used for statistical analysis of the obtained data using the SPSS (ver. 16) software. *P*-values <0.05 were considered significant.

Results

The effect of RVS on body weight of rats

Body weight was measured at three different periods (Table 2): pre-diabetes, 7 days after induction of T2DM, and one month after treatment with RVS. Seven days after diabetes induction body weight significantly decreased in diabetic groups. After one month of treatment with different doses of RVS, body weight in treated groups significantly increased compared to the diabetic control group.

The Effect of RVS on fasting blood glucose and insulin levels

Blood glucose was measured at three different times: pre-diabetes, seven days after diabetes induction and one month after RVS treatment. Table 3 shows the effect of different doses of RVS on reduction of fasting blood glucose compared to the control groups. Seven days after diabetes induction, blood glucose in diabetic groups significantly increased compared to the control (*P*-value <0.001).

Administration of 3 different doses of RVS led to decrease in blood glucose levels in treated groups compared to the untreated diabetic group. Doses of 5 and 10 mg/kg bwt RSV were more effective (*P*-value <0.001) in reducing blood glucose levels than 1mg/kg bwt. After induction of diabetes, insulin levels significantly decreased (*P*-value<0.001) in diabetic rats compared to the control group. Doses of 5 and 10 mg/kg bwt RVS led to increase (*P*-value <0.001) in insulin levels to near normal.

Table 3. Effect of different doses of resveratrol on rats' fasting blood glucose (FBS), serum insulin and HOMA index in different studied groups

Factor	Cont	Dia	Dia+RSV1	Dia+RSV5	Dia+RSV10
FBS (mg/dl), pretreatment	87.3±10.6	95.62 ± 11.34	93.2±14.8	84.1±11.0	90.5±7.23
FBS (mg/dl), 7 days after diabetes induction	89.12±10.7	279.2±95.3[a]	289.75±79.30	219.37±120.00[b]	241.22 ± 99.80[b]
FBS (mg/dl), one month after RVS treatment	92.0±10.8	303.3±92.1[a]	271.37±77.55	192.50±84.80[b]	190.33±68.63[b]
Insulin (μU/ml)	11.17±1.06	7.23±1.15[a]	8.13±0.97	9.52±1.05[b*]	9.83±0.86[b*]
HOMA	2.51±0.37	5.46±2.09[a]	5.52±1.92	3.73±2.01[b*]	4.77±1.84[b*]

Cont: normal control rats, Dia: diabetic control rats, Dia+RSV1, Dia+RSV5 and Dia+RSV10 are diabetic rats receiving 1, 5, and 10 mg/kg bwt of resveratrol, respectively
[a] *P*-value <0.001comparing with normal Cont. [b] *P*-value <0.01 comparing with diabetic control
[b*] *P*-value <0.001 comparing with diabetic control

Table 4. The effect of resveratrol on the serum TAC and MDA in studied rat groups

Factor	Cont	Dia	Dia+RSV1	Dia+RSV5	Dia+RSV10
MDA(µmol/ml)	0.48±0.12	1.24±0.19[a]	1.21±0.18	0.85±0.1[b]	0.69±0.12[b]
TAC(mmol/ml)	0.22±0.02	0.12±0.01[a]	0.15±0.01[b*]	0.18±0.01[b*]	0.19±0.04[b]

Cont: normal control rats, Dia: diabetic control rats, Dia+RSV1, Dia+RSV5 and Dia+RSV10 are diabetic rats receiving 1, 5, and 10 mg/kg bwt of resveratrol, respectively
[a] *P*-value <0.001comparing with normal Cont. [b] *P*-value <0.001 comparing with diabetic control
[b*] *P*-value <0.01 comparing with diabetic control

The effect of RVS on the TAC and MDA

Table 4 shows the effects of 3 different doses of RVS on serum TAC and MDA levels. MDA was significantly increased in the diabetic group (*P*-value <0.001), whereas treatment with three different doses of RVS resulted in decreased levels of MDA compared to diabetic control group (*P*-value <0.001). The TAC levels decreased in diabetic group (*P*-value <0.001) and treatment with three different doses of RVS resulted in significant increase (*P*-value<0.01) in the treated diabetic groups.

Gene expression of visfatin and vaspin in rat's adipose tissue

Expression of visfatin and vaspin was assessed by real-time PCR. Table 5 illustrates the mean delta Ct comparisons in studied groups. It is important to notice that higher delta Ct values mean lower gene expression.

Results indicated that expression of the visfatin gene was significantly increased in the diabetic group (*P*-value<0.05) compared to the healthy control group and treatment with various doses of RVS led to decrease in its gene expression compared to untreated diabetic rats, however the difference was not statistically significant.

Vaspin gene expression in the diabetic group decreased compared to the healthy control group (*P*-value<0.05). While RVS treatment in 1 and 5 mg/kg bwt doses did not change the vaspin gene expression significantly; in 10 mg RVS/kg bwt, it significantly reduced this gene expression compared to the untreated diabetic group (*P*-value<0.001).

Discussion

Adipocytokines secreted from adipose tissue might be important in the pathogenesis of T2DM and insulin resistance (35, 36). Natural compounds derived from plants can affect diabetes, for example Mohammadi *et al* indicated that *Zataria multiflora* improved insulin sensitivity and reduced glucose levels in fructose fed insulin resistant rats (37). In this research we studied the effect of RSV on vaspin and visfatin genes expression in adipose tissue, blood glucose, serum insulin, and body weight in animal model of type 2 diabetes. Moreover we examined any possible relation between vaspin and visfatin genes expression in adipose tissue and insulin resistance and also the effect of RSV on this relation. Furthermore we studied antioxidant properties of RSV. Based on the recent studies, RSV can affect diabetes via several mechanisms. RSV can lead to weight gain, decreased blood glucose and increased insulin secretion (38). The results of our study indicate that treatment with three different doses of RVS, induced weight gain in diabetic rats compared to the diabetic control.

RSV is a potent activator of SIRT-1(silent information regulator 2) and activation of SIRT-1 results in decreased hyperglycemia and improved insulin sensitivity (39). Palsamy *et al* demonstrated that treatment with RVS in STZ/NA induced diabetic rats, results in decreased blood glucose levels and increased insulin sensitivity and indicated that RSV has anti-hyperglycemic activity (40). Rivera *et al* found that RVS at dose of 10 mg/kg improved insulin sensitivity and decreased hyperglycemia in Zucker fat rats (41), and administration of RVS to STZ/NA-induced diabetic rats significantly decreased insulin resistance (42). Rezaei *et al* in a recent study showed that SNARE (soluble N-ethylmaleimide-sensitive factor activating protein receptors) gene expression, which is involved in uptake of glucose, was significantly decreased in adipose tissue of STZ/NA-induced diabetic rats and oral treatment with resveratrol significantly increased the expression of this gene in adipose tissue of the diabetic model (43). Based on this study RSV has hyperglycemic effects and improves insulin sensitivity and increases glucose uptake; parallel with these studies our

Table 5. Visfatin and vaspin gene expression in visceral tissue (ΔCt Value)

Factor	Cont	Dia	Dia+RSV1	Dia+RSV5	Dia+RSV10
Visfatin	11.37±1.03	9.32±1.08[a]	9.87±1.05	10.22±0.97	10.77±1.26
Vaspin	4.72±0.95	6.24±1.12	5.39±2.63	7.05±1.48	9.7±1.50 [b]

Cont: normal control rats, Dia: diabetic control rats, Dia+RSV1, Dia+Rsv5 and Dia+RSV10 are diabetic rats receiving 1, 5, and 10 mg/kg bwt of resveratrol, respectively
[a] *P*-value<0.05 comparing with normal Cont. [b] *P*-value<0.001 comparing with diabetic control

findings indicate that treatment with various doses of RVS decreased blood glucose levels , increased insulin levels, improved insulin sensitivity and decreased insulin resistance index (HOMA) in diabetic rats treated with different doses of RSV.

Effect of RVS on MDA level as a lipid peroxidation product was also examined in this study. Prasad demonstrated that serum and pancreatic MDA levels in diabetic rats were significantly higher than those of control groups (44). Another study on the effect of RVS on rats receiving ethanol indicated that RVS significantly decreased liver, heart, brain, and testis MDA levels in these animals (45). Opara found that plasma TAC levels in patients with type 2 DM was significantly reduced compared with control group (46). Experiments on rats fed with low-protein and high-carbohydrate (LPHC) diet showed, while treatment with RSV in control group increased levels of plasma TAC, in rats fed with LPHC, it did not show any significant effect on plasma TAC levels (47). In our study treatment with RVS reduced MDA levels and increased serum levels of TAC in treated groups compared with the untreated diabetic group, hence our findings indicate that RSV, by increasing levels of TAC and decreasing level of MDA, ameliorated oxidative status in diabetes.

Li *et al* indicated visfatin levels increased in obesity and T2DM (48). Some studies demonstrated visfatin serum levels are associated with insulin resistance and T2DM but are not correlated with body fat or body mass index (49-51).

Derdemezis *et al* demonstrated that visfatin levels increased in obesity and diabetes mellitus, whereas RVS decreased visfatin levels (23). In another study conducted by Eseberri *et al* it was found that RVS increased visfatin gene expression in adipocytes (52). In our study, we found that visfatin gene expression significantly increased in diabetic control group compared with the healthy group and treatment with different doses of resveratrol decreased visfatin gene expression. We can suggest that in the diabetic control group, due to increased blood glucose and decreased insulin levels, expression of visfatin gene was increased compensatory, whereas treatment with RVS led to decreased hyperglycemia and improved insulin sensitivity and hence the expression of visfatin gene in the treated groups decreased.

Kloting *et al* indicated that vaspin expression increases in obesity and diabetes and it is correlated with insulin resistance and amount of visceral adipose tissue (53). It is proved that vaspin has an insulin-sensitizing effect in obese mice (12) and administration of recombinant vaspin to obese mice improved glucose tolerance and insulin sensitivity (54). Li *et al* indicated that vaspin might correlate with insulin sensitivity and have compensatory roles in insulin resistance and T2DM (55). Another study conducted by Youn *et al* revealed significant association between serum vaspin, insulin sensitivity and body mass index but this correlation was not statistically significant in patients with T2DM (56). In our study, the highest expression of vaspin was observed in the healthy control group and RVS at dose of 10 mg/kg led to reduction in vaspin gene expression. Vaspin gene expression depends on diabetes, insulin sensitivity and amount of visceral adipose tissue. Since vaspin gene expression was slightly higher (however non-significant), in healthy rats compared to diabetic rats, it can be the result of higher body weight in healthy the control group compared to diabetic rats. Vaspin gene expression decreased in diabetic rats which received RSV (10 mg/kg/day) and we suppose that insulin resistance might stimulate vaspin production via a compensatory mechanism in diabetic rats as a counter action to insulin resistance, and when insulin sensitivity and hyperglycemia improved, vaspin gene expression decreased and we can conclude presence of a positive association between insulin resistance and vaspin gene expression.

Conclusion

As a final conclusion, we showed RVS significantly decreased blood glucose level and increased insulin level; thereby it can improve insulin resistance. Furthermore, RVS increased serum TAC levels while it decreased MDA level which shows antioxidant effect of RVS. Also we demonstrated that RVS decreased visfatin and vaspin genes expression in diabetic rats' adipose tissue, which can indicate a mechanism for the RVS anti-diabetic effects. Further study in human subjects can complete this conclusion.

Acknowledgment

The authors would like to thank the Deputy for Research of Hamadan University of Medical Sciences (Hamadan, Iran) for financial support of this project. Also we appreciate Mrs N Shabab for her technical assistance. The results reported in this paper were part of S Asadi's MSc thesis.

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