Research Article

International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com

UPREGULATION OF PPARγ MEDIATES THE ANTIDIABETIC EFFECTS OF CITRUS FLAVONOIDS IN TYPE 2 DIABETIC RATS

Ayman M Mahmoud*, Osama M Ahmed, Adel Abdel-Moneim and Mohamed B Ashour
Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, Egypt

Received for publication: December 18, 2012; **Revised:** January 12, 2013; **Accepted:** March 20, 2013

Abstract: Type 2 diabetes mellitus is a complicated metabolic disease characterized by impairment of both insulin secretion and insulin sensitivity. The present study was hypothesized to assess the effect of citrus flavonoids on adipose tissue PPARγ, adiponectin and resistin. Diabetes was induced by feeding rats with high fat diet for 2 weeks followed by an intra-peritoneal injection of STZ (35 mg/kg body weight). An oral dose of 50 mg/kg hesperidin or naringin was daily given for 4 weeks after diabetes induction. In the diabetic control group, levels of glucose and glycosylated hemoglobin were significantly increased, while serum insulin was decreased. Both hesperidin and naringin administration significantly reversed these alterations. Moreover, supplementation with either compound significantly ameliorated the declined adipose tissue PPARγ and adiponectin expressions in conjunction with down-regulated resistin expression. These experimental findings demonstrated that hesperidin and naringin exhibit antidiabetic effects by modulating adipose tissue genes.

Keywords: Citrus Flavonoids; Hesperidin; Naringin; GLUT4; Insulin Release; HFD/STZ Diabetes

INTRODUCTION

Type 2 diabetes mellitus (T2DM), a disorder of carbohydrate, fat and protein metabolism is manifested by a number of abnormalities. The myriad of disorders linked with T2DM, include insulin resistance, impaired glucose homeostasis and deficiency of insulin resulting from β-cell dysfunction[1]. Globally, T2DM accounts for a major area of pie in the morbidity and mortality charts and makes it one of the major health concerns accounting for 221 million individuals by this year and 300 million by the year 2025[2].

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily, which also includes the steroid and thyroid hormone receptors [3,4]. PPARs modulate genes that regulate lipid and glucose metabolism and control many cellular and metabolic processes, including fatty acid metabolism, adipocyte differentiation, inflammation, atherosclerosis, and cell cycle[4,5]. There are three isoforms of PPAR: PPARα, PPARδ and PPARγ. Expression of PPARα is greatest in tissues with active metabolism, such as liver, striated muscle and kidney[4,6]. PPARδ has a very broad expression pattern that made identifying its role more difficult. PPARγ is highly expressed in fat, colon, placenta and macrophage[7,8].

The adipokines are signaling proteins involved in the regulation of energy and glucose metabolism[9]. Adipocytes secrete diverse pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α, as well as anti-inflammatory cytokines such as adiponectin[10]. Dysfunction in adipocytes or adipose tissue is associated with insulin resistance and type 2 diabetes[11]. A reduced level of adiponectin and increased levels of IL-6 and TNF-α can induce or exacerbate insulin resistance in adipose tissue[11].

A number of drugs have been used for the treatment of T2DM that act either by enhancing insulin secretion or by improving insulin sensitivity. These drugs when used alone or in combination are found to be effective, but frequently constrained by safety, tolerability, hypoglycemia, lactic acidosis, weight gain and GI disturbances[12]. Hence for better safety and potential therapeutic value, the search for novel molecules has been extended to herbal drugs that offer better protection and a lesser side effect profile[12].

Flavonoids are a group of naturally occurring polyphenolic compounds ubiquitously found in fruits and vegetables[13]. Currently, there is much interest in the usefulness of citrus fruits because of their intake appears to be associated with reduced risk of certain chronic diseases and increased survival as reported by Chen et al[14]. We have reported the antihyperglycemic properties of hesperidin and naringin and their modulatory effects on serum levels of resistin and adiponectin[15]. Moreover, we have demonstrated that the antihyperglycemic effects of citrus flavonoids were due to their antioxidant effects[16]. Thus, the present study was designed to explore whether the improvement in insulin resistance and β-cell dysfunction by hesperidin and naringin in HFD/STZ type 2 diabetic rats was associated with increased adipose tissue PPARγ expression. In addition, the study was extended to evaluate the efect of the tested flavonoids on adipose tissue adiponectin and resistin expression to help tracing out the exact mechanistic pathways.

MATERIALS AND METHODS

Chemicals:

Hesperidin, naringin and streptozotocin, were purchased from Sigma Chemicals Co., St. Louis, MO, USA, stored at 2-4 °C and protected from sunlight. All other chemicals were of analytical grade and were obtained from standard commercial supplies.

***Corresponding Author:**
Dr. Ayman M Mahmoud,
Physiology Division, Zoology Department, Faculty of Science,
Beni-Suef University, Egypt. Salah Salim St., P.O. 62511, Beni-Suef, Egypt.
Tel.: +2-011-4416-8280.
E-mail: ayman.mahmoud@science.bsu.edu.eg

Experimental animals:

White male albino rats weighting about 190 ± 10 g were used as experimental animals in the present investigation. The animals were housed in standard polypropylene cages and maintained under controlled room temperature (22 ± 2 ◦C) and humidity (55 ± 5%) with 12 h light and 12 h dark cycle and were fed a standard diet of known composition, and water *ad libitum*. The animals used in the present study were maintained in accordance with the principles and guidelines of the Canadian Council on Animal Care as outlined in "Guide for the Care and Use of Laboratory Animals"[17].

Induction of type 2 diabetes:

Type 2 DM was induced by feeding high fat diet (HFD) *ad libitum*, for the initial period of 2 weeks[18]. The composition and preparation of HFD were described elsewhere[19]. After the 2 weeks of dietary manipulation, rats fed by HFD were injected intraperitoneally (i.p.) with STZ (35 mg/kg b.wt.), while the respective control rats were given vehicle citrate buffer (pH 4.5). Seven days after STZ injection, rats were screened for blood glucose levels. Rats having serum glucose ≥ 200 mg/dl, after 2 hours of glucose intake, were considered diabetic and selected for further pharmacological studies. The rats were allowed to continue to feed on their respective diets until the end of the study.

Experimental design:

The experimental animals were divided into four groups, each group comprising six rats as detailed follows.

Group 1: Normal control rats (Fed normal fat diet)

Group 2: Diabetic control rats

Group 3: Diabetic rats administered with hesperidin (50mg/kg b.wt.) orally for 4 weeks

Group 4: Diabetic rats administered with naringin (50mg/kg b.wt.) orally for 4 weeks.

The dosage was adjusted every week according to any change in body weight to maintain similar dose per kg body weight of rat over the entire period of study for each group. By the end of the experiment, animals were sacrificed and blood samples and adipose tissue were obtained.

Biochemical estimations:

Oral glucose tolerance test (OGTT) was performed in normal, diabetic control and diabetic rats treated with hesperidin and naringin. Blood samples were obtained from lateral tail vein of rats deprived of food overnight (10-12 hours). Successive blood samples were then taken at 0, 30, 60, 90 and 120 minutes following the administration of glucose solution (3 g/kg b.w.) through gastric intubation. Serum was obtained for determination of glucose concentration according to the method of Trinder[20], using commercial diagnostic kit (Randox laboratories, UK). Serum insulin level was assayed by Sandwich ELISA using kits purchased from Linco Research (USA), according to the manufacturer instructions. Blood glycated Hb was determined according to the method of Little *et al*[21] using Helena GLYCO-Tek affinity column method (Helena Laboratories, USA). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the following equations[22]:

HOMA-IR = Fasting insulin level (µU/ml) x Fasting blood glucose (mmol/l)/22.5

RNA isolation and real-time quantitative polymerase chain reaction:

Total RNA was isolated from visceral adipose tissue according to the method of Chomzynski and Sacchi[23] with some modifications, using TRIzol reagent (Invitrogen, CA, USA). First strand of cDNA was synthesized from 5 µg of total RNA by using a high-capacity cDNA reverse transcription kit with RNase inhibitor. Quantitative PCR using QuantiTect SYBR Green RT-PCR Kit (QIAGEN) was performed to analyze the mRNA levels of PPARγ, adiponectin and resistin. The following primer sets were used; Up 5′-CCTGAAGCTCCAAGAATACC-3′ and Down 5′-GATGCTTTATCCCCACAGAC-3′ for PPARγ, Up 5′-AATCCTGCCCAGTCATGAAG-3′ and Down 5′-TCTCCAGGAGTGCCATCTCT-3′ for adiponectin, Up 5′-GCTCAGTTCTCAATCAACCGTCC-3′ and Down 5′-CTGAGCTCTCTGCCACGTACT-3′ for resistin and Up 5′-AAGTCCCTCACCCTCCCAAAAG-3′ and Down 5′-AAGCAATGCTGTCACCTTCCC-3′ for β-actin. The PCR cycle was as follows: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s. Fluorescent values were converted into threshold cycle (CT) values using Rotor-Gene Q (Qiagen) and the amount of target genes was analyzed using the $2^{-\Delta\Delta Ct}$ method following the normalization through β-actin. Quantitative amplification of β-actin was used as the house-keeping gene control to normalize the determined mRNA levels.

Statistical analysis:

The data were analyzed using the one-way analysis of variance (ANOVA) (PC-STAT, University of Georgia, 1985)[24] followed by LSD test to compare various groups with each other's. Results were expressed as mean ± SE and values of $P>0.05$ were considered non-significantly different, while those of $P<0.05$ and $P<0.01$ were considered significant and highly significant, respectively.

RESULTS

The oral glucose tolerance curve of diabetic rats showed a highly significant elevation at fasting state and 30, 60, 90 and 120 min after oral glucose loading as compared to normal animals. The treatment of diabetic animals with hesperidin and naringin induced a potential improvement of elevated values at all points of oral glucose tolerance curve (Fig. 1).

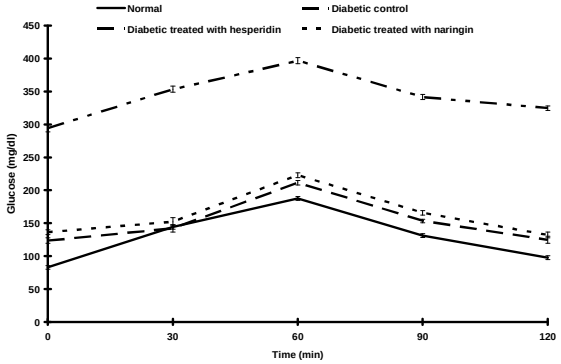

Fig. 1: OGTT of normal, diabetic control and diabetic rats treated with hesperidin and naringin.

Diabetic group of rats have highly significantly ($p<0.01$; LSD) elevated HbA1c% as compared with normal control group of rats. Oral administration of hesperidin as well as

naringin to diabetic rats significantly (p<0.01; LSD) improved the altered level. Serum insulin level exhibited an opposite pattern; it was significantly (p<0.01; LSD) decreased in diabetic rats as compared to normal ones and was significantly increased as a result of treatment with both hesperidin and naringin (Table.1).

Table.1: Blood glycosylaed hemoglobin (HbA1c %), and serum insulin level of normal, diabetic control and diabetic rats treated with hesperidin and naringin.

Parameter / Group	HbA1c%	Insulin (µU/ml)
Normal	4.71 ± 0.18^{d}	26.84 ± 1.40^{a}
Diabetic control	8.96 ± 0.23^{a}	15.50 ± 0.76^{c}
Diabetic treated with hesperidin	5.85 ± 0.18^{c}	21.55 ± 1.13^{b}
Diabetic treated with naringin	6.26 ± 0.17^{b}	20.67 ± 1.08^{b}
F- prob	P< 0.001	P< 0.001
LSD at 5%	0.40	2.32
LSD at 1%	0.54	3.17

Data are expressed as Mean ± SE. Number of animals in each group is six.
Means which share the same superscript symbol (s) are not significantly different.

HOMA-IR of normal, diabetic and diabetic treated with hesperidin and naringin is depicted in figure 2. Diabetic rats showed a significant (p<0.01; LSD) elevation of HOMA-IR that was decreased significantly upon administration of either hesperidin or naringin.

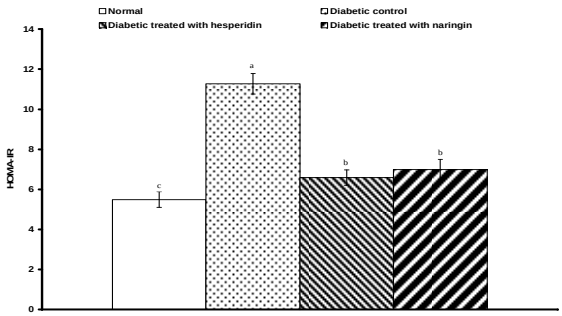

Fig. 2: HOMA-IR index of normal, diabetic control and diabetic rats treated with hesperidin and naringin.

HFD/STZ diabetic rats exhibited a highly significant (LSD; P<0.01) declined adipose tissue PPARγ mRNA expression level when compared to normal control rats. Hesperidin and naringin supplementations potentially increased (LSD; P<0.01) the altered mRNA expression level, with more potent effect for hesperidin (Fig. 3).

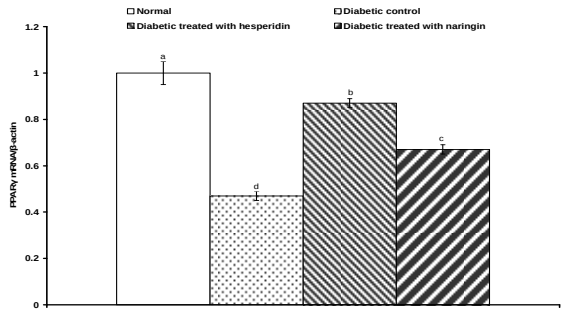

Fig. 3: Adipose tissue PPARγ mRNA expression levels in normal, diabetic control and diabetic rats treated with hesperidin and naringin.

In view of the results of adipose tissue adiponectin mRNA expression, it was observed that HFD/STZ diabetic rats exhibited a notable (LSD; P<0.01) down-regulation of adiponectin gene expression levels, as indicated in figure 4. Treatment with either hesperidin or naringin produced a highly significant (LSD; P<0.01) up-regulation of adiponectin mRNA levels.

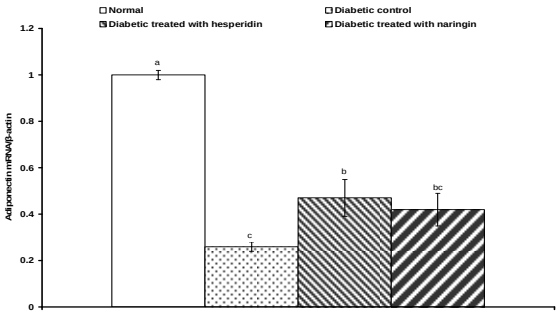

Fig. 4: Adipose tissue adiponectin mRNA expression levels in normal, diabetic control and diabetic rats treated with hesperidin and naringin.

Resistin exhibited an opposite behavioral pattern; its expression was up-regulated highly significantly (LSD; P<0.01) in diabetic control rats as compared to normal ones. Treatment of the HFD/STZ diabetic rats with hesperidin and naringin induced a highly significant amelioration (P<0.01; LSD) of the elevated resistin expression level; hesperidin seemed to be more potent than naringin (Fig. 5).

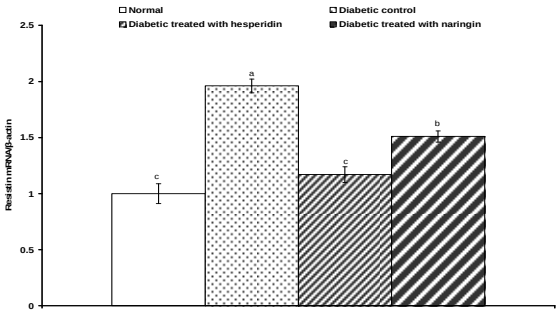

Fig. 5: Adipose tissue resistin RNA expression levels in normal, diabetic control and diabetic rats treated with hesperidin and naringin.

DISCUSSION

Type 2 diabetes mellitus is a complicated metabolic disease characterized by impairment of both insulin secretion and insulin sensitivity[25]. Kraegen et al established that rats fed with HFD have significantly higher fasting plasma insulin and glucose levels[26]. The HFD induces the intracellular accumulation of lipid metabolites in the liver and skeletal muscle that leads to insulin resistance via decreased tyrosine phosphorylation of insulin receptor substrate (IRS), a key mediator in insulin action[27]. STZ, on the other hand, at a low dose resulted in decreased insulin biosynthesis, the generation of reactive oxygen species, DNA fragmentation and β-cell damage which ultimately leads to hyperglycemia[28]. Altogether, the rats fed the HFD and given a low dose STZ resulted in an array of pathophysiological changes such as hyperglycemia and hyperlipidemia[19,29]. Similarly in our study, the HFD/STZ treated rats developed T2DM with significant increases in serum glucose, HbA1c% and HOMA-IR. In addition, the HFD/STZ treated rats exhibited diminished serum insulin levels as well as declined adipose tissue PPARγ

and adiponectin mRNA levels, while that of resistin was significantly increased.

Elevation of blood glucose may be attributed to reduced entry of glucose to peripheral tissues, muscle and adipose tissue[30], increased glycogen breakdown[31] and increased gluconeogenesis and hepatic glucose production[32]. In addition, the hyperglycemia observed in our study could be explained through glucose–fatty acid cycle[33], where the high free fatty acids (FFAs) reduce the glucose uptake and utilization, through the increased endogenous glucose production[34]. The excess of glucose present in blood reacts with hemoglobin to form glycosylated hemoglobin[35]. In our study, oral administration of hesperidin and naringin significantly decreased the levels of fasting blood glucose and HbA1c%. Recently, we have demonstrated decreased serum resistin levels of diabetic rats administered with hesperidin and naringin[36]. These results indicated the beneficial effects of both hesperidin and naringin in preventing the pathogenesis of diabetic complications caused by impaired glucose metabolism.

Our study revealed a significant increase in resistin mRNA expression level in HFD/STZ diabetic group in comparison with that of controls, which runs parallel to serum glucose and HOMA-IR index. The findings of this study are in line with that of Kushiyama *et al* who found that transgenic mice with hepatic resistin overexpression exhibit significant hyperglycemia, hyperlipidemia, fatty liver, and pancreatic islet enlargement, when fed a HFD[37]. These effects may be due to resistin-induced impairment of glucose homeostasis and insulin action, thus modulating one or more steps in the insulin signaling pathway and possibly playing a role in the pathogenesis of insulin resistance[38]. The mechanism whereby resistin decreases insulin sensitivity involves several impacts. First, resistin reduces adenosine 5′-monophosphate activated protein kinase activity in skeletal muscle, adipose tissue, and liver. These alterations decrease tissue insulin sensitivity that results in glucose intolerance, elevated FFA levels, and hypertriglyceridemia[39]. Secondly, the resistin-induced reduction in IRS-1 and IRS-2 elevates mRNA levels of gluconeogenetic enzymes, such as glucose-6-phosphatase and phosphoenol pyruvate carboxykinase, thus suggesting a direct resistin induction of insulin resistance in the liver[40]. Thirdly, resistin decreased glycogen synthase (GS) activity both in the presence or absence of insulin; this suggests that resistin directly down-regulates GS activity[41]. Furthermore, it has been reported that resistin promotes lipid accumulation in human macrophages by up-regulating CD36 cell surface expression, which is one of the scavenger receptors in macrophages involved in the uptake of modified LDL[42]. Based on the current data, the resistin lowering effect of hesperidin and naringin may directly participate to their hypoglycemic, hypolipidemic and insulin sensitizing effects.

In contrast to resistin, HFD/STZ diabetic rats exhibited diminished adiponectin mRNA level and treatment with either hesperidin or naringin significantly alleviated adiponectin expression. Adiponectin has been reported to sensitize the body tissues toward actions of insulin. The proposed mechanism of action for adiponectin include its insulin sensitizing effect which in turn regulates glucose metabolism through stimulation of AMPK[43], enhanced oxidation of muscle fat and glucose transport mediated through AMPK activation and acetyl-CoA carboxylase inhibition[44], inhibition of hepatic gluconeogenesis through decrease in the expression of phosphoenolpyruvate carboxylase and glucose-6-phosphatase[43], and increased fatty acid combustion and energy consumption, partly through PPARα activation, leading to decreased triglyceride content in skeletal muscles and liver[45]. Moreover, it has been shown that mice lacking adiponectin expression have reduced insulin sensitivity or are more likely to suffer from insulin resistance[46]. Though, the insulin sensitizing effects of the tested flavonoids may be attributed to increasing adipose tissue adiponectin expression.

PPARγ is a key regulator of glucose and lipid metabolism by controlling energy homeostasis in adipose tissue, liver and skeletal muscle[47]. The effect of PPARγ on lipid and glucose control may be explained according to Feige *et al*[48] and Lefebvre *et al*[49] who stated that PPARγ promotes pre-adipocyte differentiation, stimulates the storage of fatty acids in adipocytes and enhance insulin sensitivity. The action of PPARγ on insulin sensitivity results from its ability to channel FFAs into adipose tissue, thus decreasing plasma FFAs concentration and alleviating lipotoxicity in skeletal muscle, liver and pancreas. Also, PPARγ activation was reported to improve insulin resistance by lowering the hepatic triglyceride content[50], activating hepatic glucokinase expression[51] and exhibited an antiatherogenic effect synergistic with a hydroxymethylglutaryl CoA reductase inhibitor in rabbits[52]. In addition, PPARγ can affect insulin sensitivity by regulating adipocyte hormones, cytokines and proteins that are involved in insulin resistance. Indeed, PPARγ downregulates the expression of genes encoding resistin and tumor necrosis factor (TNFα), whereas it induces adiponectin expression, which increases fatty acid oxidation by activation of the AMP-activated protein kinase pathway[48,49]. Moreover, Willson *et al* demonstrated that activation of PPARγ improves insulin sensitivity and lowers circulating levels of glucose, triglycerides and FFAs without stimulating insulin secretion in rodent models of T2DM[53]. Also, Hevener *et al* stated that PPARγ agonists increase glucose uptake in adipose tissue and skeletal muscle[54].

Furthermore, PPARγ is involved in governing the inflammatory response, especially in macrophages. Currently, two different molecular mechanisms have been proposed by which anti-inflammatory actions of PPARγ are effectuated: (1) *via* interference with pro-inflammatory transcription factors including STAT, NF-κB, and AP-1[55], and (2) by preventing removal of co-repressor complexes from gene promoter regions resulting in suppression of inflammatory gene transcription[56]. This mechanism involves ligand dependent SUMOylation of PPARγ followed by binding of PPARγ to nuclear receptor co-repressor (NCoR)-histone deacetylase-3 (HDAC3) complexes localized on inflammatory gene promoters. The binding of PPARγ prevents the removal of co-repressor complexes, thus retaining inflammatory genes in a suppressed state[57]. Hence, hesperidin and naringin may exert their anti-inflammatory action via PPARγ up-regulation and this contributes to the insulin sensitizing effects of both compounds. In this context, our lab had provided evidence that the protective effects are, possibly, due to decline in oxidants and pro-inflammatory cytokines production[16].

In the present study, amelioration of the glycemic state of the HFD/STZ diabetic rats in response to treatment with hesperidin and naringin may also be attributed to the increased expression of PPARγ. Hesperidin showed much potent effect on the expression of PPARγ than naringin and this may explains why hesperidin has more effective hypoglycemic and hypolipidemic effects than naringin.

CONCLUSION

This study revealed that the anti-hyperglycemic efficacy of the citrus flavonoids, hesperidin and naringin, may be mediated via up-regulating adipose tissue PPARγ and adiponectin in conjunction with down-regulating adipose tissue resistin.

REFERENCES

1. Koning EJ, Weir S B, Rabelink TJ, Preservation of betacell function by targeting beta-cell mass, Trends Pharmacol Sci, 2008, 29, 218–227.
2. Shaw JE, Sicree RA, Zimmet PZ, Global estimates of the prevalence of diabetes for 2010 and 2030, Diabetes research and clinical practice, 2010, 87(1), 4-14.
3. Decker M, Hofflich H, Elias AN, Thiazolidinediones and the preservation of β-cell function, cellular proliferation and apoptosis, Diabetes Obes Metab, 2008, 10(8), 617-625.
4. Soldo-Jureša D, Metelko Ž, PPARγ-A new concept of treatment? Diabetologia Croatica, 2009, (38)2, 23-29.
5. Rosenson RS, Effects of peroxisome proliferators activated receptors on lipoprotein metabolism and glucose control in type 2 diabetes mellitus, Am J Cardiol, 2007, 99, 96B-104B.
6. Bookout AL, Jeong Y, Downes M, Yu RT, Evans RM, Mangelsdorf DJ, Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network, Cell, 2007, 126 (4), 789–799.
7. Rosen ED, Spiegelman BM, Molecular regulation of adipogenesis, Annu Rev Cell Dev Biol, 2000, 16, 145–171.
8. Barish GD, Downes M, Alaynick WA, Yu RT, Ocampo CB, Bookout AL, Mangelsdorf DJ, Evans RM, A nuclear receptor atlas: macrophage activation, Mol Endocrinol, 2005, 19 (10), 2466– 2477.
9. Kershaw EE, Flier JS, Adipose tissue as an endocrine organ, J Clin Endocrinol Metab, 2004, 89, 2548-56.
10. Sowers J, Endocrine functions of adipose tissue: focus on adiponectin, Clin Cornerstone, 2008, 9, 32-38.
11. Blüher M, Adipose tissue dysfunction in obesity, Exp Clin Endocrinol Diabetes, 2009, 117, 241-250.
12. Goldberg RB, Holman R, Drucker DJ, Management of type 2 diabetes, N Engl J Med, 2008, 358, 293–297.
13. Mahmoud AM, Influence of rutin on biochemical alterations in hyperammonemia in rats, Exp Toxicol Path, 2012, 64, 783-789.
14. Chen H, Ward MH, Graubard BI, Dietary patterns and adenocarcinoma of the esophagus and distal stomach, Am J Clin Nutr, 2002, 75, 137-144.
15. Abdel-Moneim A, Ashour MB, Mahmoud AM, Ahmed OM, Insulin sensitizing effects of hesperidin and naringin in experimental model of induced type 2 diabetes: focus on tumor necrosis factor-alpha and resistin, Nature and Science, 2011, 9(10), 134-141.
16. Mahmoud AM, Ahmed OM, Ashour MB, Abdel-Moneim A, Hesperidin and naringin attenuate hyperglycemia-mediated oxidative stress and proinflammatory cytokine production in high fat fed/strptozotocin-induced type 2 diabetic rats, Diabetes and its Complications, 2012, 26, 483–490.
17. Canadian Council on Animal Care, Guide to the care and use of experimental animals. Vol. 2, CCAC, 1993, Ottawa, Ontario, Canada.
18. Srinivasan K, Viswanand B, Asrat L, Kaul CL, Ramarao P, Combination of high-fat diet-fed and low dose of streptozotocin- treated rat: a model for type 2 diabetes and pharmacological screening, Pharmacol Res, 2005, 52, 313–320.
19. Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM *et al*, A new rat model of type 2 diabetes, the fat-fed, streptozotocin- treated rat, Metabol, 2000, 49, 1390–1394.
20. Trinder P, Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor, Ann Clin Biochem, 1969, 6, 24–27.
21. Little RR, Wiedmeyer HM, England JD, Wilke AL, Rohlfing CL, Wians FH *et al*, Interlaboratory standardization of measurements of glycohemoglobins, Clin Chem, 1992, 38, 2472-2478.
22. Haffner SM, Greenberg AS, Weston WM, Chen H, Williams K, Freed MI, Effect of rosiglitazone treatment on nontraditional markers of cardiovascular disease in patients with type 2 diabetes mellitus, Circulation, 2002, 106, 679–684.
23. Chomzynski P, Sacchi N, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Annual review of Biochemistry, 1987, 162, 156–159.
24. PC-STAT, One way analysis of variance. Version IA (C), Program coded by Roa M, Blane K, Zonneberg M, 1985, University of Georgia, USA.
25. Yi P, Lu FE, Xu LJ, Chen G, Dong H, Wang KF, Berberine reverses free-fatty-acid–induced insulin resistance in 3T3-L1 adipocytes through targeting IKKbeta, World J Gastroenterol, 2008, 14, 876-83.
26. Kraegen EW, Clark PW, Jenkins AB, Daley EA, Chisholm DJ, Storlien LH, Development of muscle insulin resistance after liver insulin resistance in high fat fed rats, Diabetes, 1991, 40, 1397–1403.
27. Petersen KF, Shulman GI, Etiology of insulin resistance, Am J Med, 2006, 119, 10S–16S.
28. Mythili MD, Vyas R, Akila G, Gunasekaran S, Effect of streptozotocin on the ultrastructure of rat pancreatic islets. Microsc Res Tech, 2004, 63, 274–281.
29. Parveen K, Khan MR, Mujeeb M, Siddiqui WA, Protective effects of pycnogenol on hyperglycemia-induced oxidative damage in the liver of type 2 diabetic rats, Chem Biol Interact, 2010, 186, 219–227.
30. Beck-Nielsen H, Insulin resistance: organ manifestations and cellular mechanisms, UgeskrLaeger, 2002, 164, 2130-2135.
31. Gold AH, The effect of diabetes and insulin on liver glycogen synthetase activation, J Biol Chem, 1970, 245, 903-905.

32. Raju J, Gupta D, Rao AR, Yadava PK, Baquer NZ, *Trigonella foenum graecum* (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reserving the altered glycolytic, gluconeogenic and lipogenic enzymes, Mol Cell Biochem, 2001, 224, 45-51.

33. Randle PJ, Garland PB, Hales CN, Newsholme EA, The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus, Lancet, 1963, 1, 785–789.

34. Song H, Shojima N, Sakoda H, Ogiharaa T, Fujishiroa M, Katagiric H et al, Resistin is regulated by C/EBPs, PPARs, and signal-transducing molecules, Biochem Biophys Res Commun, 2002, 29, 291–298.

35. Sheela CG, Augusti KT, Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic, Allium sativum Linn, Ind J Exp Biol, 1992, 30, 523-526.

36. Ahmed OM, Mahmoud AM, Abdel-Moneim A, Ashour MB, Antidiabetic effects of hesperidin and naringin in type 2 diabetic rats, Diabetologia Croatica, 2012, 41(2), 53-67.

37. Kushiyama A, Shojima N, Ogihara T, Inukai K, Sakoda H, Fujishiro M, Resistin-like molecule beta activates MAPKs, suppresses insulin signaling in hepatocytes, and induces diabetes, hyperlipidemia, and fatty liver in transgenic mice on a high fat diet, J Biol Chem, 2005, 280, 42016–42025.

38. Muse ED, Obici S, Bhanot S, Monia BP, McKay RA, Rajala MW, Role of resistin in diet-induced hepatic insulin resistance, J Clin Invest, 2004, 114, 232– 239.

39. Rajala MW, Obici S, Scherer PE, Rossetti L, Adipose-derived resistin and gut-derived resistin-like molecule-beta selectively impair insulin action on glucose production, J Clin Invest, 2003, 111, 225–230.

40. Moon B, Kwan JJ, Duddy N, Sweeney G, Begum N, Resistin inhibits glucose uptake in L6 cells independently of changes in insulin signaling and GLUT4 translocation, Am J Physiol Endocrinol Metab, 2003, 285, E106-15.

41. Ferrer JC, Favre C, Gomis RR, Fernaóndez- Novell JM, Garcıóa-Rocha M, de la Iglesia N, Control of glycogen deposition, FEBS Lett, 2003, 546, 127-32.

42. Xu W, Yu L, Zhou W, Luo M, Resistin increases lipid accumulation and CD36 expression in human macrophages, Biochem Biophys Res Commun, 2006, 351, 376–382.

43. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S *et al*, Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase, Nat Med, 2002, 8, 1288-95.

44. Tomas E, Tsao TS, Saha AK, Murrey HE, Zhang CC, Itani SI *et al*, Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation, Proc Natl Acad Sci USA, 2002, 99, 16309–16313.

45. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S *et al*, Cloning of adiponectin receptors that mediate antidiabetic metabolic effects, Nature, 2003, 423, 762–769.

46. Nawrocki AR, Rajala MW, Tomas E, Pajvani UB, Saha AK, Trumbauer ME, Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor gama agonists, J Biol Chem, 2006, 281, 2654-2660.

47. Semple RK, Chatterjee, VK, O'Rahilly S, PPAR gamma and human metabolic disease, J Clin Invest, 2006, 116(3), 581-589.

48. Feige JN, Gelman L, Michalik L, Desvergne B, Wahli W, From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions, Prog Lipid Res, 2006, 45, 120-159.

49. Lefebvre P, Chinetti G, Fruchart JC, Staels B, Sorting out the roles of PPAR alpha in energy metabolism and vascular homeostasis, J Clin Invest, 2006, 116, 571-580.

50. Yamauci T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, The fat derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity, Nat Med, 2001, 7, 941-946.

51. Kim SY, Kim HI, Park SK, Im SS, Li T, Cheon HG, Liver glucokinase can be activated by peroxisome proliferators-activated receptor-γ, Diabetes, 2004, 53, S66–S70.

52. Shiomi M, Ito T, Tsukada T, Tsujita Y, Horikoshi H, Combination treatment with troglitazone, an insulin action enhancer, and pravastatin an inhibitor of HMG-CoA reductase, shows a synergistic effect on atherosclerosis on WHHL rabbits, Atherosclerosis, 1999, 142, 345–353.

53. Willson TM, Brown PJ, Sternbach DD, Henke BR, The PPARs: From orphan receptors to drug discovery, J Medicinal Chem, 2000, 43, 527-550.

54. Hevener AL, He W, Barak Y, Le J, Bandyopadhyay G, Olson P, Wilkes J, Evans RM, Olefsky J, Muscle- specific PPARγ deletion causes insulin resistance, Nat Med, 2003, 9, 1491-1497.

55. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK, The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation, *Nature*, 1998, 391(6662), 79–82.

56. Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, Rose DW, Willson TM, Rosenfeld MG, Glass CK, A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. Nature, 2005, 437, 759–763.

57. Stienstra R, Duval C, Müller M, Kersten S, PPARs, Obesity, and Inflammation, PPAR Res, 2007, 2007, 2-10.

Source of support: Nil
Conflict of interest: None Declared