Insulin Sensitizing Effects of Hesperidin and Naringin in Experimental Model of Induced Type 2 Diabetes in Rats: Focus on Tumor Necrosis Factor-Alpha and Resistin

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Abstract: Both insulin resistance and decreased insulin secretion are major features of the pathophysiology of type 2 diabetes. Inflammatory pathways are found to be critical in mechanisms underlying insulin resistance, which is a major determinant of increased risk of cardiovascular complications in type 2 diabetes, and so, it is a potential therapeutic target. The present study was designed to study the effects of hesperidin and naringin on serum glucose, insulin, free fatty acids (FFA), tumor necrosis factor alpha (TNF-α) and resistin in a model of type 2 diabetes in rats. In addition, this study was extended to demonstrate the effect of tested compounds on white adipose tissue TNF-α and resistin mRNA expression. Diabetes was induced by feeding rats with a high fat diet (HFD) for 2 weeks followed by an intraperitoneal injection of streptpzotocin (35 mg/kg body weight). An oral dose of 50 mg/kg hesperidin or naringin was daily given for 30 days after diabetes induction. In the diabetic control group, levels of glucose, FFA, TNF-α and resistin were significantly increased, while serum insulin levels were decreased. Both hesperidin and naringin administration significantly reversed these alterations; hesperidin seemed to be more potent. Moreover, supplementation with either compounds significantly ameliorated the up-regulated adipose tissue TNF-α and resistin mRNA expression. These preliminary experimental findings demonstrate that both hesperidin and naringin exhibit antidiabetic effects in a rat model of type 2 diabetes by suppressing serum and adipose TNF-α and resistin.

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1. Introduction

Both insulin resistance and decreased insulin secretion are major features of the pathophysiology of type 2 diabetes (non-insulin-dependent diabetes mellitus) (**Evans *et al.*, 2003**). Insulin resistance is defined as a decreased response of the peripheral tissues to insulin action (**Xu *et al.*, 2003**), and it most often precedes the onset of hyperglycemia and predicts development of type 2 diabetes (**Yki-Jarvinen, 1994**). Adipose tissue represents an active endocrine organ that, in addition to regulating fat mass and nutrient homeostasis, releases a large number of bioactive mediators known as adipokines (**Bastard *et al*., 2006; Gesta *et al*., 2007; Rabe *et al*., 2008**). The release of adipokines by either adipocytes or adipose tissue-infiltrated macrophages leads to a chronic subinflammatory state that could play a central role in the development of insulin resistance and type 2 diabetes (**Aguilera *et al.*, 2008; Antuna-Puente *et al*., 2008**).

Several studies left little doubt that inflammatory pathways are critical in the mechanisms underlying insulin resistance and type 2 diabetes, resulting in deteriorated metabolic homeostasis in general and glucose metabolism in particular (**Wellen and Hotamisligil, 2003**); where even minimal disturbances in glucose tolerance are associated with a chronic, generalized inflammatory reaction that links components of the metabolic syndrome and contributes to the development of diabetic complications (**Lobner and Fuchtenbusch, 2004**). TNF-α can elicit an insulin-resistant state, characterized by an impaired ability of insulin to suppress hepatic glucose production and to stimulate peripheral glucose uptake (**Lang *et al.*, 1992**). Also, TNF-α is implicated to increase the circulating level of FFA and thus indirectly contributes to the pathogenesis of insulin resistance (**Ryden *et al.*, 2002**). Studies utilizing the INS-1 glucose dependent insulin secreting cell line, have shown that TNF-α is capable of inhibiting insulin secretion (**Kim *et al.*, 2008**). Furthermore, TNF-α interferes with insulin signaling in a variety of non-insulin producing cells, essentially inducing a state of insulin resistance (**Del Aguila *et al.*, 1999**). Resistin belongs to a family of cysteine-rich secretory proteins called resistin-like molecules (**Steppan and Lazar, 2004; Hoseen *et al.*, 2010**). In rodents, resistin is derived almost exclusively from adipose tissue, and serum resistin is elevated in animal models of obesity and insulin resistance (**Steppan *et al.*, 2001; Rjala, 2004**). Moreover, the insulin-resistant effects of resistin are

thought to account for the activation of glucose 6-phosphotase, which subsequently prevents glycogen synthesis and increases the rate of glucose production (**Rajala *et al.*, 2003**).

Flavonoids are naturally occurring polyphenolic phytoconstituents. They are present in fruits, vegetables, tea, wine grains (**Hertog *et al.*, 1993; Fernandes *et al.*, 2009**). Flavonoids exhibit a multitude of biological activities such as antioxidant, antibacterial, antiinflammatory, antiallergic, vasodilatory, anticarcinogenic, immune-stimulating, antiviral, and estrogenic effects, as well as being inhibitors of several enzymes such as phospholipase A2, cyclooxygenase, lipoxygenase, and xanthine oxidase (**Rice-Evans *et al.*, 1996**). 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.* (2002)**. Naringin is responsible for the characteristic sour flavor of the fruits (**Kim *et al.*, 1998**) and has been empirically proven to have no side effects, as humans have been ingesting grapes and citrus fruits for a long time (**Choe *et al.*, 2001**). Hesperidin is an abundant and inexpensive byproduct of citrus cultivation and isolated from the ordinary orange *Citrus aurantium* and other species of the genus *Citrus* (**Kakadiya *et al.*, 2010**). **Choi (2008)** has demonstrated antioxidant effects of hesperetin, the aglycone form of hesperidin, against 7,12-dimethylbenz(a)anthracene-induced oxidative stress in mice. Hence, this study is aimed to investigate the ameliorative potential of those two flavonoid compounds on the proinflammatory cytokine TNF-α and resistin in HFD/STZ-induced type 2 diabetic rats.

2.Material and Methods

Chemicals

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

Experimental animals

White male albino rats (*Rattus norvegicus*) 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" (Canadian Council on Animal Care, 1993).

Development of HFD-fed low dose STZ-treated type 2 diabetic rats

The rats were allocated into two dietary regimens by feeding either normal or high fat diet (HFD) *ad libitum*, for the initial period of 2 weeks (Srinivasan *et al.,* 2005). After the 2 weeks of dietary manipulation, the group of 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 ≥ 300 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 served as control rats; Group 2 served as diabetic control rats; Group 3 served as diabetic rats administered with hesperidin (50 mg/kg b.wt.) orally for 30 days, and Group 4 served as diabetic rats administered with naringin (50 mg/kg b.wt.) orally for 30 days. 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 study

Serum glucose concentration was determined according to the method of Trinder (1969), using commercial diagnostic kit (Randox laboratories, UK). Serum insulin level was assayed by Sandwich ELISA using kits purchased from Linco Research, USA. Serum FFA was determined according to the method of Harris (1974) using diagnostic kit purchased from Roche Diagnostics (Germany).

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the following equation (Haffner *et al.,* 2002):

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

The levels of TNF-α and resistin in serum of control and experimental groups of rats were determined by specific ELISA kits purchased from R&D Systems (USA) and Biovendor (USA), respectively. The concentration of either TNF-α or resistin was determined spectrophotometrically at 450 nm. Standard plots were constructed by using

standards and the concentrations for unknown samples were calculated from the standard plot.

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 (1987) 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 (Applied Biosystems, CA, USA). Quantitative PCR using QuantiTect SYBR Green RT-PCR Kit (QIAGEN) was performed to analyze the mRNA levels of TNF-α and resistin. The following primer sets were used: Up 5′-AAATGGGCTCCCTCTCATCAGTTC-3′ and Down 5′-TCTGCTTGGTGGTTTGCTACGAC-3′ for TNF-α; Up 5′-GCTCAGTTCTCAATCAACCGTCC-3′ and Down 5′-CTGAGCTCTCTGCCACGTACT-3′ for resistin; 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 ABI PRISM® 7000 Sequence Detector Program 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) followed by LSD test to compare various groups with each others. 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.

3. Results

Table 1 shows the effect of hesperidin and naringin on the levels of fasting serum glucose, FFA and insulin in the control and experimental groups of rats. Diabetic group of rats have highly significantly ($p<0.01$; LSD) elevated glucose and FFA 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 levels. 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.

HOMA-IR of normal, diabetic and diabetic treated with hesperidin and naringin is depicted in figure 1. Diabetic rats showed a significant ($p<0.01$; LSD) elevation of HOMA-IR that was decreased significantly upon administration of either hesperidin or naringin. However, while both hesperidin and naringin have more or less similar effects, hesperidin seemed to be more effective on serum glucose and HOMA-IR.

Regarding serum TNF-α and resistin levels, administration of HFD and STZ produced a highly significant elevation ($P<0.01$; LSD) of both TNF-α and resistin as compared with normal rats. The treatment of HFD/STZ diabetic rats with hesperidin and naringin induced a highly significant amelioration ($P<0.01$; LSD) of the elevated serum resistin and TNF-α (Table 2).

Concerning the adipose tissue TNF-α mRNA expression, it was highly significantly (LSD; $P<0.01$) increased in diabetic control rats as compared to normal ones and treatment with hesperidin as well as naringin produced a potential decline in TNF-α mRNA expression as illustrated in figure 2. Resistin exhibited the same 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. 3).

4. Discussion

The rats fed with HFD can result in insulin-resistance mainly through Randle or glucose–fatty acid cycle (**Zhang *et al.*, 2003**). It was also reported that in addition to a direct effect on glucose, which is characteristic of this model, other pathophysiological changes are seen, such as insulin resistance in adipose tissue (**Reed *et al.*, 2000**) and diabetic kidney lesions such as glomerulosclerosis and proteinuria (**Danda *et al.*, 2005**). Also, high fat diet feeding to rodents was shown to affect the respiratory capacity, reactive oxygen species generation, fatty acid beta oxidation, mitochondrial ADP/ATP translocator inhibition and regulation of kinases involved in carbohydrate and lipid metabolism (**Ciapaite *et al.*, 2007; Raffaella *et al.*, 2008**). Since the combination of HFD-fed and low-dose STZ-treated rat which serves as an alternative animal model for diabetes has been proved to be suitable for testing antidiabetic agents (**Srinivasan *et al.*, 2005**), we chose this model to carry out our evaluations.

Table 1: Serum glucose, FFA and insulin levels of normal, diabetic control and diabetic rats treated with hesperidin and naringin.

Parameter / Group	Glucose (mg/dl)	Insulin (µU/ml)	Free fatty acids (mmol/L)
Normal	82.83 ± 2.11[d]	26.84 ± 1.40[a]	0.57 ± 0.07[c]
Diabetic control	294.87 ± 4.19[a]	15.50 ± 0.76[c]	1.68 ± 0.06[a]
Diabetic treated with hesperidin	124.03 ± 3.90[c]	21.55 ± 1.13[b]	0.92 ± 0.10[b]
Diabetic treated with naringin	136.73 ± 3.19[b]	20.67 ± 1.08[b]	1.04 ± 0.12[b]
F- prob	P< 0.001	P< 0.001	P< 0.001
LSD at 5%	6.96	2.32	0.18
LSD at 1%	9.49	3.17	0.25

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.

Table 2: Serum TNF-α, and resistin of normal, diabetic control and diabetic rats treated with hesperidin and naringin.

Parameter / Group	Resistin (ng/ml)	TNF-α (pg/ml)
Normal	6.52 ± 0.54[c]	14.66 ± 0.52[d]
Diabetic control	14.52 ± 0.40[a]	48.24 ± 1.15[a]
Diabetic treated with hesperidin	8.86 ± 0.51[b]	23.86 ± 1.31[c]
Diabetic treated with naringin	7.87 ± 0.27[bc]	29.74 ± 0.65[b]
F- prob	P< 0.001	P< 0.001
LSD at 5%	1.44	2.02
LSD at 1%	2.09	2.75

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.

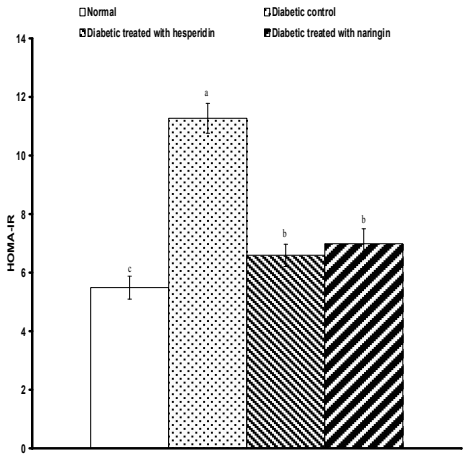

Fig. 1: HOMA-IR index of normal, diabetic control and diabetic rats treated with hesperidin and naringin.

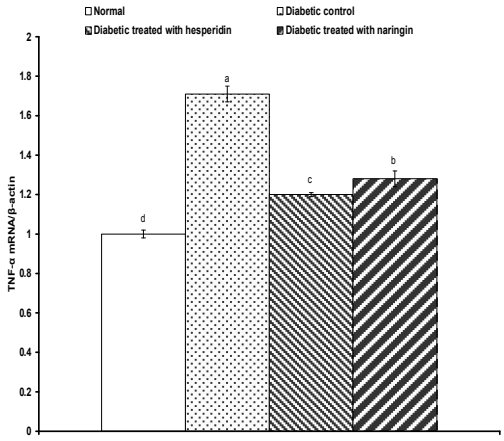

Fig. 2: Adipose tissue TNF-α mRNA expression levels in normal, diabetic control and diabetic rats treated with hesperidin and naringin.

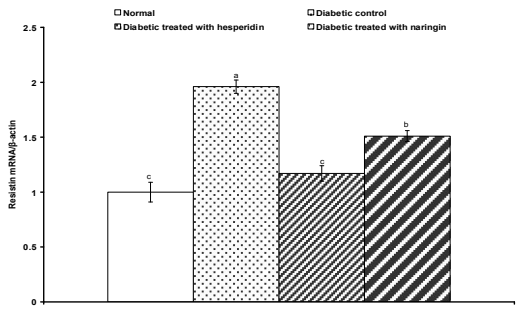

Fig. 3: Adipose tissue resistin mRNA expression levels in normal, diabetic control and diabetic rats treated with hesperidin and naringin.

In our study, diabetic control rats exhibited significantly elevated fasting blood glucose and HOMA-IR, accompanied with diminished serum insulin levels. Hence, it is suggested that insulin resistance has been developed in these animals. Therefore, this rat model exhibits hyperglycemia and insulin resistance that would closely reflect the natural history and metabolic characteristics of humans, and it is further sensitive to pharmacological testing.

Several mechanisms of how elevated FFA levels decrease insulin sensitivity have been proposed, including the Randle hypothesis concerning inhibition of insulin-stimulated glucose transport. It also should be noted that FFAs regulate gene expression, especially those involved in lipid and carbohydrate metabolism (**Evans *et al*., 2003**). Chronically elevated FFAs may also impair insulin secretory function through toxic effects on pancreatic β-cells as predicted by the "lipotoxicity hypothesis" (**Unger, 1995**). Finally, increased flux of FFAs from adipose tissue due to lipolysis of visceral adipose depots (triglycerides) to the nonadipose tissue (e.g., liver, skeletal muscle) may lead to excessive endogenous glucose production and progression to frank type 2 diabetes (**Lewis *et al*., 2002**). Therefore, decreasing plasma FFA levels is proposed as a strategy for prevention and treatment of insulin resistance as stated by **Na *et al*. (2010)**. Upon treatment of the diabetic animals with hesperidin and naringin there was a decreased level of serum FFA which may participate in the insulin sensitizing effects of both tested compounds.

Our study revealed a significant increase in both serum resistin levels and adipose tissue resistin mRNA expression in HFD/STZ diabetic group in comparison with that of controls, which runs parallel to serum glucose levels, insulin levels and HOMA-IR index. The findings of this study are in line with that of **Kushiyama *et al*. (2005)**, who found that transgenic mice with hepatic resistin overexpression exhibit significant hyperglycemia, hyperlipidemia, fatty liver, and pancreatic islet enlargement, when fed a HFD. 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 (**Muse *et al*., 2004**). The majority of *in vivo* studies showed that resistin has a negative effect on insulin signaling in the liver (**Qi *et al*., 2006**). In contrast with our results, no significant correlation was found between resistin levels and glucose levels in high fat-fed rats (**Li *et al*., 2007**), and also in patients with type 2 diabetes mellitus (**Mojiminiyi and Abdella, 2007**). Also, some studies have observed significant low resistin mRNA levels in adipose tissue in different obese mouse models, such as db/db, or high-fat-diet-induced obesity, and in rat models characterized by IR (**Way *et al*., 2001**).

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. In addition, insulin receptor substrate (IRS-1 and IRS-2) protein levels and phosphorylation states, as well as protein kinase B activity, were decreased in hyperresistinemic animal tissues. These alterations decrease tissue insulin sensitivity that results in glucose intolerance, elevated free fatty acid levels, and hypertriglyceridemia (**Rajala *et al*., 2003**). Secondly, the resistin-induced reduction in IRS-1 and IRS-2 elevates mRNA levels of gluconeogenetic enzymes, such as glucose-6-phosphatase (G-6-Pase) and phosphoenolpyruvate carboxykinase, thus suggesting a direct resistin induction of insulin resistance in the liver (**Moon *et al*., 2003**). Thirdly, it was found that resistin decreased glycogen synthase (GS) activity both in the presence or absence of insulin, this suggests that resistin directly down-regulates GS activity (**Ferrer *et al*., 2003**). In addition, **Samuel *et al*. (2004)** reported that resistin caused the transcriptional levels of key enzymes for lipid metabolism to be modestly altered; a lipogenic enzyme, fatty acid synthase was increased, and lipolytic enzyme, carnitine palmitoyl transferase, and PPAR-γ were decreased. The altered hepatic lipolytic and lipogenic enzymes promoted lipid accumulation in the liver. Moreover, insulin signaling in pancreatic islets plays an important role in the maintenance of β-cell functions and glucose-induced insulin secretion in islets of pancreas (**Otani *et al*., 2004**). Therefore, the inhibition of insulin signaling could underlie the impairment of glucose-induced insulin secretion by resisitn (**Nakata *et al*., 2007**).

Concerning the insulin signaling pathway, binding of insulin to its receptor inducing

autophosphorylation at multiple tyrosine sites is a key element in insulin signaling pathway. Activated receptor further phosphorylates IRS. Finally, insulin signals are transduced from IRS to major pathway of intracellular serine-threonine kinase namely phosphatidyl inositol (PI) 3-kinase. PI-3 kinase stimulates the translocation of glucose transporters GLUT4 from intracellular pool to cell membrane for uptake of glucose by the cell (**Virkamaki *et al.*, 1999**). The cytokine TNF-α has direct inhibitory effect on tyrosine kinase and phosphorylation cascade of insulin signaling pathway (**Gao *et al.*, 2003; Pickup, 2004**). TNF-α mediates insulin resistance also through indirect effects including increasing FFA in circulation, stimulation of insulin counter-regulatory hormones, impairment of endothelial function, or inhibiting the glucose-stimulated insulin release by pancreatic β-cells (**Tataranni and Ortega, 2005**). The above interference of TNF-α in various pathways is justified by its elevated levels in type 2 diabetic rats. Elevated TNF-α indicates the activated innate immune system followed by chronic systemic inflammation associated with type 2 diabetes. Decrease in elevated TNF-α by hesperidin and naringin along with their blood glucose lowering effect suggests that the immunomodulatory properties of both flavonoids could be related with their potential anti-diabetic activity.

In conclusion, the insulin sensitizing effects of both hesperidin and naringin may be attributed to their modulation of adipose tissue TNF-α and resistin gene expression.

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References

Aguilera, C. M.; Gil, C. M.; Canete, R. and Gil, A. (2008): Alterations in plasma and tissue lipids associated with obesity and metabolic syndrome. Clin. Sci., 114: 183–193.

Antuna-Puente, B.; Feve, B.; Fellahi, S. and Bastard, J. P. (2008): Adipokines: the missing link between insulin resistance and obesity. Diabetes and Matabol., 34: 2-11.

Bastard, J. P.; Maachi, M.; Lagathu, C.; Kim, M. J.; Caron, M.; Vidal, H.; Capeau, J. and Feve, B. (2006): Recent advances in the relationship between obesity, inflammation and insulin resistance. Eur. Cytokine Netw., 17: 4–12.

Canadian Council on Animal Care (1993): Guide to the care and use of experimental animals. Vol. 2, CCAC, Ottawa, Ontario, Canada, 1993.

Chen, H.; Ward, M. H. and Graubard, B. I. (2002): Dietary patterns and adenocarcinoma of the esophagus and distal stomach. Am. J. Clin. Nutr., 75: 137-144.

Choe, S. C.; Kim, H. S.; Jeong, T. S.; Bok, S. H. and Park, T. B. (2001): Naringin as an anti atherogenic effect with the inhibition of intracellular adhesion molecule-1 in hypercholesterolemic rabbits. J. Cardiovasc. Pharmacol., 38: 947-955.

Choi, E. J. (2008): Antioxidative effects of hesperetin against 7,12-dimethylbenz(a)anthracene-induced oxidative stress in mice. Life Sci., 82: 1059–1064.

Chomzynski, P. and Sacchi, N. (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Annual review of Biochemistry, 162: 156–159.

Ciapaite, J.; Bakker, S. J.; Van Eikenhorst, G.; Wagner, M. J.; Teerlink, T.; Schalkwijk, C. G.; Fodor, M.; Ouwens, D. M.; Diamant, M.; Heine, R. J.; Westerhoff, H. V. and Krab, K. (2007): Functioning of oxidative phosphorylation in liver mitochondria of high-fat diet fed rats. Biochimica. Biophysica. Acta, 1772 (3): 307–316.

Danda, R. S.; Habiba, N. M.; Rincon-Choles, H.; Bhadaria, B. K.; Barnes, J. L.; Abboud, H. E. and Pergola, P. E. (2005): Kidney involvement in a nongenetic rat model of type 2 diabetes. Kidney Int., 68: 256–71.

Del Aguila, L. F.; Claffey, K. P. and Kirwan, J. P. (1999): TNF-alpha impairs insulin signaling and insulin stimulation of glucose uptake in C2C12 muscle cells. Am. J. Physiol., 276:E849-E855.

Evans, J. L., Goldfine, I. D., Maddux, B. A., & Grodsky, G. M. (2003). Are oxidative stress-activated signaling pathways mediators of insulin resistance and β-cell dysfunction? Diabetes, 52, 1−8.

Fernandes, A. A. H.; Novelli, E. L. B.; Junior, A. F. and Galhardi, C. M. (2009): Effect of naringerin on biochemical parameters in the streptozotocin-induced diabetic rats. Braz. Arch. Biol. Technol., 52 (1): 51-59.

Ferrer, J. C.; Favre, C.; Gomis, R. R.; Fernaóndez-Novell, J. M.; Garcióa-Rocha, M. and de la Iglesia, N. (2003): Control of glycogen deposition. FEBS. Lett., 546:127-32.

Gesta, S.; Tseng, Y. H. and Kahn, C. R. (2007): Developmental origin of fat: tracking obesity to its source. Cell, 131: 242-256.

Haffner, S. M.; Greenberg, A.S.; Weston, W. M.; Chen, H.; Williams, K. and Freed, M. I. (2002): Effect of rosiglitazone treatment on nontraditional markers of cardiovascular disease in patients with type 2 diabetes mellitus. Circulation, 106:679−684.

Harris, R. J. (1974): Plasma nonesterified fatty acid

and blood glucose levels in healthy and hypoxemic newborn infants. Pediatrics, 84:578-584.

Hertog, M.G.L.; Hollman, P.C.H. and van de Putte, B. (1993): Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juices, Journal of Agricultural and Food Chemistry, 41: 1242–1246.

Hoseen, M. I.; Hassan, M. M.; Abd-Alaleem, D. I. and Faragallah, E. M. (2010): Serum resistin levels and haemostatic changes in experimentally induced diabetic and high fat fed rats. J Am Science, 6(11):217-227.

Kakadiya, J.: Shah, M. and Shah, N. J. (2010). Effect of nobivolol on serum diabetic marker and lipid profile in normal and streptozotocin-nicotinamide induced diabetic rats. RJPBCS, 1, 329-334.

Kim, D. H.; Jung, E. A.; Song, I. S.; Han, J. A.; Kim, T. H. and Han, M. J. (1998): Intestinal bacterial metabolism of flavonoids and its relation to some biological activities. Arch. Pharmacol. Res., 21: 17-23.

Kim, H. E.; Choi, S. E.; Lee, S. J.; Lee, J. H.; Lee, Y. J.; Kang, S. S.; Chun, J. and Kang, Y. (2008): Tumour necrosis factor-alpha-induced glucose-stimulated insulin secretion inhibition in INS-1 cells is ascribed to a reduction of the glucose-stimulated Ca^{2+} influx. J. Endocrinol., 198: 549-560.

Kushiyama, A.; Shojima, N.; Ogihara, T.; Inukai, K.; Sakoda, H. and Fujishiro, M. (2005): 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., 280: 42016−42025.

Lang, C. H.; Dobrescu, C. and Bagby, G. J. (1992): Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. Endocrinol., 130: 43– 52.

Lewis, G. F.; Carpentier, A.; Adeli, K. and Giacca, A. (2002): Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocrine Reviews, 23 (2): 201–229.

Li, Y.; Wang, Y.; Li, Q.; Chen, Y.; Sun, S. Z.; Zhang, W. D. and Jia, Q. (2007): Effect of resistin on vascular endothelium secretion dysfunction in rats. Endothelium, 14: 207-14.

Lobner K., and Fuchtenbusch M., (2004): Inflammation and diabetes. MMW Fortschr Med. 146(35-36):32–3, 35–6.

Mojiminiyi, O. A. and Abdella, N. A. (2007): Associations of resistin with inflammation and insulin resistance in patients with type 2 diabetes mellitus. Scand. J. Clin. Lab. Invest., 67: 215-225.

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

Muse, E. D.; Obici, S.; Bhanot, S.; Monia, B. P.; McKay, R. A. and Rajala, M. W. (2004): Role of resistin in diet-induced hepatic insulin resistance. J. Clin. Invest., 114: 232– 239.

Na, L-X.; Zhang, Y-L.; Li, Y.; Liu, L-Y.; Li, R.; Kong, T. and Sun, C-H. (2010): Curcumin improves insulin resistance in skeletal muscle of rats. Nutrition, Metabolism & Cardiovascular Diseases, 1-8.

Nakata, M.; Okada, T.; Ozawa, K. and Yada, T. (2007): Resistin induces insulin resistance in pancreatic islets to impair glucose-induced insulin release. Biochem. Biophys. Reas. Commun., 353: 1046-51.

Otani, K.; Kulkarni, R. N.; Baldwin, A. C.; Krutzfeldt, J.; Ueki, K.; Stoffel, M.; Kahn, C. R. and Polonsky K. S. (2004): Reduced beta-cell mass and altered glucose sensing impair insulin-secretory function in beta IRKO mice. Am. J. Physiol. Endocrinol. Metab., 286: E41–E49.

PC-STAT (1985): One way analysis of variance. Version IA (C). Program coded by Roa, M.; Blane, K. and Zonneberg, M. University of Georgia, USA.

Pickup, J.C. (2004): Inflammation and activated immunity in the pathogenesis of type 2 diabetes. Diabetes Care, 27: 813-823.

Qi, Y.; Nie, Z.; Lee, Y. S.; Singhal, N. S.; Scherer, P. E.; Lazar, M. A. and Ahima, R. S. (2006): Loss of resistin improves glucose homeostasis in leptin deficiency. Diabetes, 55: 3083–3090.

Rabe, K.; lehrke, M.; parhofer, K. G. and Broedl, U. (2008): Adipokines and insulin resistance. Mol. Med., 14: 741-751.

Raffaella, C.; Francesca, B.; Italia, F.; Marina, P.; Giovanna, L. and Susanna, I. (2008): Alterations in hepatic mitochondrial compartment in a model of obesity and insulin resistance. Obesity (Silver Spring), 16 (5): 958–964.

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.

Rajala MW. Role of resistin in diet-induced hepatic insulin resistance. J Clin Invest 2004; 114:232– 239.

Reed, M. J.; Meszaros, K.; Entes, L. J.; Claypool, M. D.; Pinkett, J. G.; Gadbois, T. M. and Reaven, G. M. (2000): A new rat model of type 2 diabetes, the fat-fed, streptozotocin- treated rat. Metabolism, 49: 1390–1394.

Rice-Evans CA, Miller NJ, Paganga G, Structure–antioxidant activity relationships of flavonoids and phenolic acids, Free Radical Biology and Medicine, 20, 1996, 933–956.

Ryden, M.; Dicker, A.; van Harmelen, V.; Hauner, H.; Brunnberg, M. and Perbeck, L. (2002): Mapping of

early signaling events in tumor necrosis factor-alpha-mediated lipolysis in human fat cells. J. Biol. Chem., 277: 1085– 1091.

Samuel, V. T.; Liu, Z. X. and Qu, X. et al. (2004): Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. Journal of Biological Chemistry, 279: 32345–32353.

Srinivasan, K.; Viswanand, B.; Asrat, L.; Kaul, C. L. and Ramarao, P. (2005): Combination of high-fat diet-fed and low dose of streptozotocin- treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res., 52:313–320.

Steppan, C. M.; Bailey, S. T.; Bhat, S.; Brown, E. J.; Banerjee, R. R.; Wright, C. M.; Patel, H. R.; Ahima, R. S. and Lazar, M. A. (2001): The hormone resistin links obesity to diabetes. Nature, 409(6818): 307-312.

Steppan, C. M. and Lazar, M. A. (2004): The current biology of resistin. J. Intern. Med., 255(4): 439-447.

Tataranni, P.A. and Ortega, E. (2005): Does an adipokine induced activation of the immune system mediate the effect of over nutrition on type 2 diabetes? Diebetes, 54: 917-927.

Trinder, P. (1969): Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor, Ann. Clin. Biochem., 6: 24–27.

Unger, R. H. (1995): Lipotoxicity in the pathogenesis of obesity-dependent NIDDM: genetic and clinical implications. Diabetes, 44: 863–870.

Virkamaki, A.; Ueki, K. and Kahn, C.R. (1999): Protein-protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. J Clin Invest., 103: 931-943.

Way J.M., Gorgun C.Z., Tong Q., Uysal K.T., Brown K.K., Harrington W.R., Oliver Jr., T.M., Willson S.A. Kliewer G.S., Hotamisligi G.S. (2001): Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor gamma agonists. J. Biol. Chem., 276: 25651–25653.

Wellen, K. E., and Hotamisligil, G. S. (2003). Obesity-induced inflammatory changes in adipose tissue. Journal of Clinical Investigation, 112(12), 1785−1788.

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., Sole, J., Nichols, A., Ross, J. S., Tartaglia, L. A., & Chen, H. (2003). Chronic inflammation in fat plays a crucial role in the development of obesityrelated insulin resistance. Journal of Clinical Investigation, 112(12), 1821−1830.

Yki-Jarvinen, H. (1994). Pathogenesis of non-insulin-dependent diabetes mellitus. Lancet, 343, 91−95.

Zhang, F.; Ye, C.; Li, G.; Ding, W.; Zhou, W.; Zhu, H.; Chen, G.; Luo, T.; Guang, M.; Liu, Y.; Zhang, D.; Zheng, S.; Yang, J.; Gu, Y.; Xie, X. and Luo, M. (2003). The rat model of type 2 diabetes mellitus and its glycometabolism characters. Experiment Animals, 52: 401–407.

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