The Effect of Soybean Oil on Glycaemic Control in Goto-Kakizaki Rats, an Animal Model of Type 2 Diabetes

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Abstract: Several studies in humans and laboratory animals with type 2 diabetes indicate that antioxidant supplements lessen the impact of oxidative damage caused by dysregulation of glucose metabolism. The present study was undertaken to examine the effect of soybean oil on glycaemic control and lipid metabolism in Goto-kakizaki (GK) rats, a model of type 2 diabetes. Rats were divided into three groups, a control group of non-diabetic (Wistar) rats, a group of diabetic GK rats and a group of GK rats treated with soybean oil. Plasma samples from the different groups were analysed for total α-tocopherol, coenzyme Q and glucose levels. Glycated haemoglobin was also compared between the different groups. Fasting and non-fasting blood glucose levels were significantly decreased in soybean oil group compared with GK group. There was also a 14 % reduction in the levels of HbA_{1c} in SO-treated GK when compared with the diabetic control group. Diabetes induced a decrease in coenzyme Q plasma levels that prevailed after treatment with soybean oil. Moreover, the plasma α-tocopherol levels were higher after treatment with soybean oil. Conclusions: Our observations suggest that soybean oil treatment may be beneficial in type 2 diabetes. Since soybean oil has very high amounts of coenzyme Q and other antioxidants one possible mechanism of action could be as an antioxidant.

Key Words: Type 2 diabetes, Goto-Kakizaki rats, soybean oil, antioxidants, CoQ_{10}.

INTRODUCTION

Type 2 diabetes is a disease that is increasing in prevalence worldwide. In genetically predisposed patients, the combination of excess caloric intake and reduced physical activity induces a state of insulin resistance [1, 2]. When β-cells are not able to compensate for insulin resistance by adequately increasing insulin production, impaired glucose tolerance occurs, which is characterized by excessive postprandial hyperglycemia. Impaired glucose tolerance may evolve into overt diabetes. These three conditions (ie, insulin resistance, impaired glucose tolerance, and overt diabetes) are associated with an increased risk of cardiovascular disease. Intervention trials have demonstrated that diabetes can be prevented by means of lifestyle modifications, antidiabetic drugs directed against insulin resistance or simply postprandial hyperglycemia. All of these have intracellular antioxidant effects. Evidence on the efficacy of antioxidant interventions is accumulating, and oxidative stress may be a therapeutic target to prevent diabetes as well as cardiovascular complications [3].

Oxidative stress is a situation in which the amount of reactive oxygen species (ROS) exceeds the levels of neutralizing substances referred to as antioxidants. Numerous studies have shown that oxidative stress is associated with type 2 diabetes, and there is compelling biochemical evidence that suggests that ROS may even play a role, if only secondary, in the pathogenesis of type 2 diabetes [4-9]. In studies of humans and rodents, dietary supplementation with antioxidants is associated with decreased risk of type 2 diabetes and induces changes that could be beneficial in reducing insulin resistance and protecting vascular endothelium, although others studies have reported negative effects [10, 11]. In general, exogenous antioxidants can compensate for the lower plasma antioxidant levels often observed in type 2 diabetic and pre-diabetic patients [9, 12]. However, conflicting results have been reported regarding the beneficial effects of antioxidants in humans [13].

Soybean consumption has been increasing throughout the world, thus reinforcing the need for more research in this area. Soybean oil is one of the few vegetable oils that contain ω-3 and it is a significant source of ω-6 fatty acids [14, 15]. Most studies with soybean oil are related to lipid metabolism [16, 17]. Soybean is used as the single source for lipids in the diet offered to experimental animals and it is also a source of antioxidants.

The spontaneously diabetic Goto-Kakizaki (GK) rat is a genetic type 2 diabetic rat produced by selective breeding of non-diabetic Wistar rats using glucose intolerance on glucose loading as a selection index, repeated over many generations [18]. The GK rats exhibit moderate hyperglycaemia with early mild insulin resistance and a deficient insulin response to glucose *in vivo* and *in vitro* [19-21] with the advantage of avoiding obesity [22]. As the disease progresses, the diabetic GK rats develop characteristic tissue damage as seen in peripheral nerves and kidney, recapitulating systemic complications encountered in human type 2 diabetes [18, 21, 23].

Glucotoxicity increases oxidative stress in GK rats as demonstrated by increased 8-hydroxy-2'-deoxyguanosine, a marker of oxidative stress, and 4-hydroxy-2-nonenal-modified proteins in pancreatic β cells. These levels increased proportionally with age and fibrosis within pancreatic islets [24]. It has also been shown that, α-tocopherol supplementation sig-

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nificantly improves glycaemic control, possibly by minimizing free radical damage to the pancreatic β-cells in GK rats [24, 25]. Improvements in glucose metabolism and insulin action in the obese Zucker rats, may also be mediated by a reduction in oxidative stress [26].

We thus hypothesized that GK rat treated with soybean oil, due to its possible antioxidant properties, may ameliorate diabetic profile. We examined the effects of soybean oil in glycaemic control and lipid metabolism in GK rats. We have also evaluated plasma levels of coenzyme Q and α-tocopherol in untreated and treated diabetic rats.

MATERIALS AND METHODS

Animals

Male non-diabetic Wistar and Goto-Kakizaki rats were obtained from our local breeding colonies (Animal Research Center Laboratory, University Hospital, Coimbra, Portugal). Animals were subjected to a constant daily cycle of 12 hours of light and 12 hours darkness and constant temperature (22 – 24 ºC) and humidity (50 – 60%), with free access to water and to a standard commercial pellet chow (Diet AO4-Panlab).

Rats were divided in 3 experimental groups: control non-diabetic Wistar rats (n=12), diabetic GK rats (n=12), and GK rats treated with soybean oil (GK+SO group, n=12). GK+SO rats received, 3 days/week during 8 weeks, an intraperitoneal injection of soybean oil (2 mL/Kg body weight).

The quantity of water and pellets ingested were also evaluated before and at the end of treatment. Blood samples were collected after an overnight fasting at the end of the treatment (12 weeks of age) by cardiac puncture from anaesthetized animals (ketamine chloridrate 75 mg/Kg + chlorpromazine 2.25 mg/Kg, i.m.) for biochemical determinations.

Blood glucose and glycated haemoglobin (HbA_{1c}) levels determination.

Blood glucose levels, fasting and after an intra-peritoneal injection of glucose (1.8 mg/kg of glucose), were determined through the glucose oxidase method using a glucometer (Elite-Bayer, Portugal) [27-29].

Glycated haemoglobin (HbA_{1c}) levels were determined through exchange chromatographic assay (Abbott IMx Glicohemoglobin, Abbott Laboratories, Portugal).

Plasma Lipids Determination

Fasting plasma lipids (total and HDL cholesterol, triglycerides and phospholipids) were measured at the end of treatment (Kits Boehringer Mannheim GmbH, Germany; Olimpus, Tokyo, Japan; Beckmann, Fullerton, CA, USA).

Soybean Oil Composition

Coenzyme Q_{10} 1579.7 μmol/L; coenzyme Q_9 666.8 μmol/L; α-tocopherol 150 μmol/L; palmitate 10.3 %; stearate 3.7%; oleate 22.2 %; linoleate 54.4%, linolenate 8.7 %.

Measurement of α-Tocopherol

The content of α-tocopherol in the plasma and in a sample of soybean oil was determined by high performance liquid chromatography method [30, 31].

Measurement of Coenzyme Q (CoQ9 and CoQ10)

The presence of coenzyme Q in the plasma and in a sample of soybean oil was determined by high performance liquid chromatography method [31, 32].

Ethics

All experiments were performed in accordance to the National Ethical Requirements for Vertebrate Animal Research and in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

Data Analysis and Statistics

Values are expressed as mean ± SEM. Comparisons between 2 parameters were analyzed by Student unpaired t test. Comparisons among 3 or more parameters were analyzed by one-way analysis of variance (ANOVA). P values of less than 0.05 were defined as statistically significant.

Materials

Soybean oil was obtained from Sigma Chemical (ST. Louis). Ketamine chloride was obtained from Merial Portuguesa (Rio de Mouro, Portugal) and chlorpromazine from Laboratórios Vitória (Amadora, Portugal). All others chemicals used were of analytical grade.

RESULTS

Body Weight

During the observation period, mean body weights were significantly lower in GK rats than those in age-matched non-diabetic Wistar rats (Table **1**). The soybean oil treatment given to diabetic rats for 8 weeks did not affect daily food intake and body-weight gain (Table **1**).

Diabetic Control

Mean fasting blood glucose levels were significantly higher in GK compared to control Wistar rats throughout the experimental period (Table **1**, at 12 weeks of age, 7.3 ± 0.3 and 3.6 ± 0.2 mmol/L, respectively). GK rats treated with soybean oil (GK+SO) presented a significant decrease (~ 20 %) in fasting blood glucose levels when compared to GK control rats (Table **1**, from 7.3 ± 0.3 mmol/L to 5.8 ± 0.2). On intraperitoneal glucose tolerance tests, blood glucose levels after glucose load were markedly elevated in GK rats compared with Wistar rats. With soybean oil supplementation for 8 weeks, there was no change of glucose levels on the intraperitoneal glucose tolerance test (Fig. (**1**)). At 4 and 12 weeks of age, GK rats showed a marked glucose intolerance not affected by the treatment with soybean oil.

Glycated Haemoglobin Evaluation and Lipid Profile

We have also evaluated the effect of soybean oil on HbA_{1c} levels in GK rats after the treatment with soybean oil. The levels of HbA_{1c} in GK rats were significantly higher when compared to non-diabetic Wistar rats (Table **2**, 6.9 ± 0.3 and 5.2 ± 0.1 %, respectively). There was a 14 % reduction in the levels of HbA_{1c} in SO-treated GK when compared with the diabetic control group (Table **2**). Non-fasting blood glucose levels were also significantly higher in GK compared to non-diabetic Wistar rats (Table **2**, at 12 weeks of

Table 1. General Characteristics (Body Weight, Food Intake, and Fasting Glycaemia) of Wistar, GK and GK+SO Rats Before (4 Weeks of Age) and After Treatment with Soybean Oil (12 Weeks of Age)

	Wistar	GK	GK + SO
Body weight – 4W (g)	125.9 ± 1.5	79.51 ± 1.7***	80.6 ± 2.9***
Body weight – 12 W (g)	344 ± 8.9	272.2 ± 6.8***	270.4 ± 6.4***
Body weight gain (g)	218.1 ± 7.2	192.7 ± 6.5*	189.8 ± 6.8*
DFI - 4 W (g/100 g BW)	17.28	17.66	16.9
DFI - 12 W (g/100 g BW)	7.11	8.97	8.14
FBG at 4 weeks of age (mmol/L)	3.66 ± 0.3	5.05 ± 0.44*	5.04 ± 0.27*
FBG at 12 weeks of age (mmol/L)	3.63 ± 0.16	7.27 ± 0.28***	5.77 ± 0.17*** §§§

SO – soybean oil; DFI- daily food intake; FBG- fasting blood glycaemia.

Data are mean ± SEM. * P <0.05, *** P <0.001 *vs* Wistar group; §§§ P <0.001 *vs* GK group.

age, 11.8 ± 1.0 and 5.3 ± 0.3 mmol/L, respectively). GK rats treated with soybean oil (GK+SO) presented a significant decrease (~ 24 %) in non-fasting blood glucose levels when compared to GK control rats (Table **2**, from 11.8 ± 1.0 mmol/L to 8.9 ± 0.7).

The triglycerides, phospholipids and cholesterol levels observed in the different groups (Table **2**) were within their normal physiological range [33].

Plasma Contents of α-Tocopherol and Coenzyme Q

We measured α-tocopherol and coenzyme Q plasma contents of the different groups of animals (Table **3**). The diabetic animals presented an amount of α-tocopherol not significantly different from Wistar rats, but plasma levels of CoQ_9 + CoQ_{10} were significantly lower (99.8 ± 17.9 and 218.5 ± 42.2 nmol/L in GK and control Wistar rats, respectively). Treatment with soybean oil augmented by 50 % plasma α-tocopherol levels (from 5.7 ± 0.8 to 8.6 ± 0.8 μmol/L) and had no effect on the amount of coenzyme Q (Table **3**).

DISCUSSION

In the present study we show that after 8 weeks of treatment with soybean oil GK rats had a significant decrease in glycated haemoglobin accompanied by a significantly decrement in fasting blood glucose levels, while no changes were observed in glucose tolerance test and lipid metabolism. The improved diabetic profile is probably due to the soybean oil antioxidant composition.

There is growing evidence that a general increase in antioxidant status achieved by dietary supplementation can help to diminish oxidative stress associated with type 2 diabetes [9, 11, 12, 29]. Although some contradictory studies have been reported regarding antioxidants beneficial effects in control of diabetic profile, particularly in humans [13].

Soybean oil is a triglyceride derived from soybean rich in C-18 unsaturated components namely linoleic and oleic acids. It contains high amounts of tocopherols, coenzyme Q (see Materials and Methods section) and other antioxidants

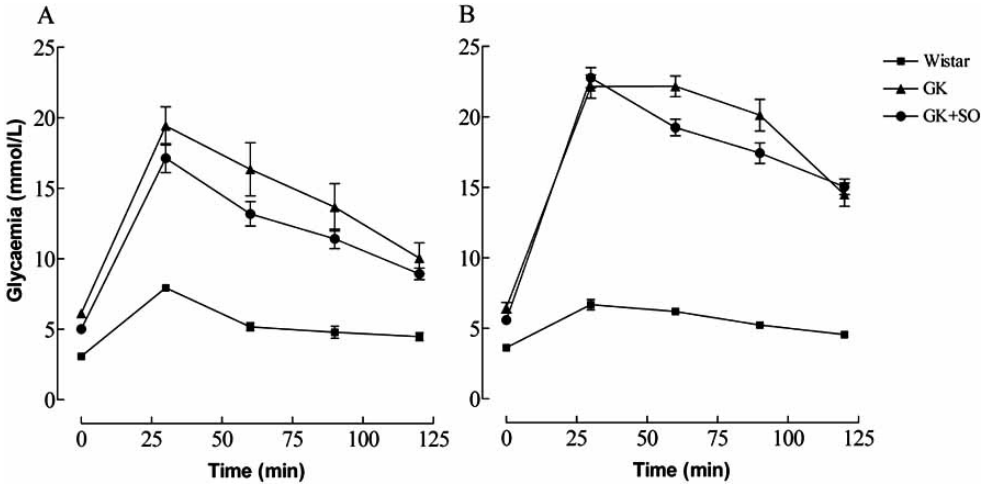

Fig. (1). Glucose tolerance in Wistar and GK rats before (A, 4 weeks of age) and after (B, 12 weeks of age) supplementation with soybean oil. After an overnight fast, glucose was injected intraperitoneally and the glucose tolerance tests were performed as described in Materials and Methods.

Table 2. Lipid Characterization, Glycated Haemoglobin (HbA_{1c}) and Non-Fasting Glycaemia of Wistar, GK and GK Rats Treated with Soybean Oil at 12 Weeks of Age

	Wistar	GK	GK + SO
Non-FBG (mmol/L)	5.37 ± 0.3	11.78 ± 1.03***	8.91 ± 0.7** §
HbA1c (%)	5.24 ± 0.09	6.86 ± 0.28***	5.93 ± 0.07* §§
Cholesterol (mmol/L)	2.5 ± 0.08	2.86 ± 0.17	2.62 ± 0.09
Cholesterol HDL (mmol/L)	1.22 ± 0.05	1.58 ± 0.09	1.30 ± 0.03
Triglycerides (mmol/L)	0.87 ± 0.19	1.14 ± 0.18	1.19 ± 0.11

Non-FBG- non-fasting blood glycaemia; SO – soybean oil.

Data are mean ± SEM. *P<0.05, **P<0.01, ***P<0.001 *vs* Wistar group; §P<0.05, §§P<0.01 *vs* GK control group.

such as bioflavonoids and aromatic compounds [34, 35]. α-Tocopherol and flavonoids are capable of reduce protein glycation processes and consequently reduce glycated haemoglobin [36] although we cannot rule out a link between the diminished HbA_{1c} and glycaemic reduction. α-Tocopherol exerts moderate beneficial effects on β-cell functions during short to intermediate length of high glucose exposure [37, 38] and it has also been reported to affect glucose transport [11]. Soybean oil supplementation was able to increase plasma α-tocopherol levels and thus could be the main explanation for the decrement in glycated haemoglobin and blood glucose levels.

It has recently been shown by Zhang and co-workers [39] that a post-weaning isocaloric hyper-soybean oil versus a hypercarbohydrate diet reduced body weight, body fat content, as well as blood lipid, glucose, insulin and leptin levels in male Wistar rats. Furthermore, other study [40] showed that the excretion of triacylglycerol and cholesterol in faeces were higher in soybean-oil treated Sprague-Dawley rats. There are also descriptions of anti-inflammatory effects for soybean oil [41, 42]. In 90% pancreatectomized diabetic rats (Px rats) fermented, unsalted soybeans seem to improve the diabetic profile of these rats by enhancing insulin secretion through the enhancement of insulin/IGF-1 signaling in the pancreatic islets, when compared to cooked soybeans [43].

Another antioxidant reported to have beneficial effects for diabetes is coenzyme Q_{10}, a lipophilic antioxidant and mitochondrial respiratory chain redox coupler, with the potential to improve energy production in mitochondria by bypassing defective components in the respiratory chain as well as by reducing the effects of oxidative stress [44]. Coenzyme Q can ameliorate β cell function [45] particularly if the endogenous levels are low. In this study systemic levels of CoQ are low and supplementation with soybean oil did not further increased those levels. We cannot rule out the possibility that CoQ was taken up by other tissue and/or was regenerating other antioxidants. It has been shown that CoQ_{10} is capable of improvements in glycaemic control, blood pressure and endothelial dysfunction [46].

Previous studies have concluded that soybean oil enriched-diets increase the response of insulin secretion to glucose stimulus in pancreatic islets [47], although we could not observed this effects in GK rats with a short term treatment with soybean oil [27]. In the face of the improvements in fasting blood glucose and glycated haemoglobin, we cannot exclude a long-term effect of the oil treatment on peripheral insulin action [47-49].

Our results suggest that soybean oil treatment can exert beneficial effects in type 2 diabetes. The precise mechanism for the beneficial effects of soybean oil observed is unknown. Since soybean oil has very high amounts of coenzyme Q compounds and other antioxidants one possible mechanism of action could be as an antioxidant improving blood glucose levels and reducing glycated haemoglobin in GK rats.

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Table 3. Plasma Levels of α-Tocopherol and Coenzyme Q of Wistar, GK and GK Rats Treated with Soybean Oil at 12 Weeks of Age

	Wistar	GK	GK + SO
α-Tocopherol (mmol/L)	6.15 ± 0.6	5.7 ± 0.8	8.6 ± 0.8 §*
CoQ (mmol/L)	218.5 ± 42.2	99.8 ± 17.9*	110.1 ± 19.2*

Data are mean ± SEM. * P< 0.05 *vs* Wistar group; § P< 0.05 *vs* GK group.

REFERENCES

[1] Efendic S.; Luft R.; Wajngot A. *Endocr. Rev.*, **1984**, *5*, 395.
[2] DeFronzo, R.A.; Bonadonna, R.C.; Ferrannini, E. *Diabetes Care*, **1992**, *15*, 318.
[3] Robertson, R.P.; Olson, L.K.; Zhang, H.J. *Diabetes*, **1994**, *43*, 1085.
[4] Giugliano, D.; Ceriello, A.; Paolisso, G. *Diabetes Care*, **1996**, *19*, 257.
[5] Du, X.L.; Edelstein, D.; Rossetti, L.; Fantus, I.G.; Goldberg, H.; Ziyadeh, F.; Wu, J.; Brownlee, M. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 12222.
[6] Nishikawa, T.; Edelstein, D.; Du, X.L.; Yamagishi, S.; Matsumura, T.; Kaneda, Y.; Yorek, M.A.; Beebe, D.; Oates, P.J.; Hammes, H.P.; Giardino, I.; Brownlee, M. *Nature*, **2000**, *404*, 787.
[7] Kaneto, H.; Suzuma, K.; Sharma, A.; Bonner-Weir, S.; King, G.L.; Weir, G.C. *J. Biol. Chem.* , **2002,** *277*, 3680.
[8] Wiernsperger, N.F. *Biofactors*, **2003**, *19*, 11.
[9] Opara, E.C. *J. Investig. Med.*, **2004**, *52*, 19.
[10] Strain, J.J. *Proc. Nutr. Soc.*, **1991**, *50*, 591.
[11] Ceriello, A. *Metabolism*, **2000**, *49*, 27.
[12] Gokkusu, C.; Palanduz, S.; Ademoglu, E.; Tamer, S. *Endocr. Rev*., **2001**, *27*, 377.
[13] Evans, J.L., Goldfine, I.D., Maddux, B.A., Grodsky, G.M. *Endocr. Rev.*, **2002**, *23*, 599.
[14] Yaqoob, P.; Sherrington, E.J.; Jeffery, N.M.; Sanderson, P.; Harvey, D.J.; Newsholme, EA; Calder, P.C. *Int. J. Biochem. Cell Biol.,* **1995**, *27*, 297.
[15] Gilani, G.S.; Ratnayake, W.M.N.; Brooks, S.P.J.; Botting, H.G.; Plouffe, L.J; Lampi, B.J. *Nutr. Res.*, **2002**, *22*, 297.
[16] Lichtenstein, A.H.; Matthan, N.R.; Jalbert, S.M.; Resteghini, N.A.; Schaefer, E.; Ausman, L.M. Am. *J. Clin. Nutr.*, **2006**, *84*, 497.
[17] Vega-Lopez, S.; Ausman, L.M.; Jalbert, S.M.; Erkkila, A.T.; Lichtenstein, A.H. *Am. J. Clin. Nutr*., **2006**, *84*, 54.
[18] Goto, Y.; Suzuki, K.; Sasaki, M.; Ono, T.; Abe, S. In Frontiers in Diabetes Research: Lessons from Animal Diabetes II, Shafrir A.R., Ed.; John Libbey: London, **1988**; pp. 301–303.
[19] Goto, Y.; Kakizaki, M. *Proc. Jpn. Acad*., **1981**, *57*, 381.
[20] Seiça, R.M.; Suzuki, K.I.; Santos, R.M.; Rosário, L.M. *Acta Med Port.*, **2004,**17,42.
[21] Östenson, C.G.; Khan, A.; Abdel-Halim, S.M.; Guenifi, A.; Suzuki, K.; Goto, Y.; Efendic, S. *Diabetologia*, **1993**, *36*, 3.
[22] Bisbis, S.; Bailbe, D.; Tormo, M.A.; Picarel-Blanchot, F.; Derouet, M.; Simon, J.; Portha, B. *Am. J. Physiol.*, **1993**, *265*, E807.
[23] Seiça, R.; Santos, D.L.; Palmeira, C.M.; Moreno, A.J.; Sena, C.M.; Xavier da Cunha, F.; Santos, M.S. *Toxicol. Mechan. Meth.,***2005**, *15*, 253.
[24] Ihara, Y.; Toyokuni, S.; Uchida, K.; Odaka, H.; Tanaka, T.; Ikeda, H.; Hiai, H.; Seino, Y.; Yamada, Y. *Diabetes*, **1999**, *48*, 927.
[25] Ihara, Y.; Yamada, Y.; Toyokuni, S.; Miyawaki, K.; Ban, N.; Adachi, T.; Kuroe, A.; Iwakura, T.; Kubota, A.; Hiai, H.; Seino, Y. *FEBS Lett.*, **2000**, *473*, 24.
[26] Laight, D.W.; Desai, K.M.; Gopaul, N.K.; Anggard, E.E.; Carrier, M.J. *Eur. J. Pharmacol*., **1999,** *377*, 89.
[27] Nunes, E., Peixoto, F., Louro, T., Sena, C.M., Santos, M.S., Matafome, P., Moreira, P.I., Seica, R. *Acta Diabetol*., **2007**, *44*, 121.
[28] Sena, C.M., Barosa, C., Nunes, E., Seica, R., Jones, J.G. *Diabetes Metab*., **2007**, *33*, 296.
[29] Sena, C.M., Nunes, E., Louro, T., Proença, T., Fernandes, R., Boarder, M.R., Seiça, R. *Br. J. Pharmacol.,* **2008**, *153*, 894.
[30] Vatassery, G.T.; Morley, J.E.; Kuskowski, M.A. *Am. J. Clin. Nutr.*, **1983**, *37*, 641.
[31] Santos, M.S.; Santos, D.L.; Palmeira, C.M.; Seiça, R.; Moreno, A.J.; Oliveira, C.R. *Diabetes Metab. Res. Rev.*, **2001**, *17*, 223.
[32] Chung, A.P.; Rainey, F.; Nobre, M.F.; Burghardt, J.; Costa, M.S. *Int. J. Syst. Bact.*, **1997**, *47*, 1225.
[33] Harkness, J.E.; Wagner, J.E. In The biology and medicine of rabbits and rodents, Lea; Febiger, Ed.; Philadelphia, **1989**; pp. 47-54.
[34] Lee, K.G.; Mitchell, A.; Shibamoto, T. *Biofactors*, **2000**, *13*, 173.
[35] Cabrini, L.; Barzanti, V.; Cipollone, M.; Fiorentini, D.; Grossi, G.; Tolomelli, B.; Zambonin, L.; Landi, L. *J. Agric. Food Chem.*, **2001**, *49*, 6026.
[36] Manuel y Keenoy, B.; Vertommen, J.; De Leeuw, I. *Diabetes Nutr. Metab*., **1999**, *12*, 256.
[37] Kaneto, H.; Kajimoto, Y.; Miyagawa, J.; Matsuoka, T.; Fujitani, Y.; Umayahara, Y.; Hanafusa, T.; Matsuzawa, Y.; Yamasaki, Y.; Hori, M. *Diabetes*, **1999**, *48*, 2398.
[38] Tajiri, Y.; Grill, V.E. *Pancreas*, **1999**, *18*, 274.
[39] Zhang, X.H., Hua, J.Z., Wang, S.R, Sun, C.H. *Asia Pac. J. Clin. Nutr.,* **2007**, *16*, 368.
[40] Naziroglu, M., Brandsch, C. *J. Nutr. Sci. Vitaminol. (Tokyo),* **2006**, *52*, 83.
[41] Wohlers, M., Nascimento, C.M., Xavier R.A., Ribeiro, Silveira, V.L. *Inflammation*, **2003**, *27*, 1.
[42] Wohlers, M., Xavier R.A., Oyama, L.M., Ribeiro, E.B., do Nascimento, C.M., Casarini, D.E, Silveira, V.L. *Inflammation*, **2005**, *29*, 81.
[43] Kwon, D.Y., Jang, J.S., Hong, S.M., Lee, J.E., Sung, S.R., Park, H.R., Park, S. *Eur. J. Nutr.*, **2007**, *46*, 44.
[44] Crane, F.L. *J. Am. Coll. Nutr.*, **2001**, *20*, 591.
[45] McCarty, M.F. *Med. Hypotheses*, **1999**, *52*, 397.
[46] Watts, G.F., Playford, D.A., Croft, K.D., Ward, N.C., Mori, T.A., Burke, V. *Diabetologia*, **2002**, *45*, 420.
[47] Picinato, M.C.; Curi, R.; Machado, U.F.; Carpinelli, A.R. *Physiol. Behav.*, **1998**, *65*, 289.
[48] Steerenberg, P.A.; Beekhof, P.K.; Feskens, E.J.; Lips, C.J.; Hoppener, J.W.; Beems, R.B. *Diabetes Nutr. Metab*., **2002**, *15*, 205.
[49] van Dam, R.M.; Willett, W.C.; Rimm, E.B.; Stampfer, M.J.; Hu, F.B. *Diabetes Care*, **2002**, *25*, 417.

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