http://www.pjbs.org

PJBS

ISSN 1028-8880

# Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (4): 633-637, 2008
ISSN 1028-8880


# The Effects of Oral Vitamin E on Induction and Consequence of Experimental Diabetes Mellitus in Rats

[1]S.R. Fatemi Tabatabaei, [1]A.A. Papahn, [2]M. Razi Jalali and [1]L. Rahimi
[1]Department of Physiology,
[2]Department of Clinical Pathology, Faculty of Veterinary Medicine,
Shahid Chamran University, Ahwaz, Iran

**Abstract:** Streptozotocin destroys the β-cells of pancreas by generation of reactive oxygen species and vitamin E has documented antioxidant properties. To evaluate the preventive effect of vitamin E on induction of diabetes by streptozotocin and effect of oral vitamin E consumption on carbohydrate and lipid metabolism, forty male Wistar rats divided randomly to control, $E_1$, $E_2$ and $E_3$ groups. The diet of $E_1$, $E_2$ and $E_3$ groups were supplemented with 1, 2 and 4 g $kg^{-1}$ of vitamin E, respectively. Four days later all rats were made diabetic by IP injection of 45 mg $kg^{-1}$ streptozotocin and blood glucose was measured 72 h later to determine the severity of blood glucose elevation. Glycosylated hemoglobin, triglyceride, total cholesterol and HDL-c were measured and LDL-c and VlDL-c calculated in plasma of 6 diabetic rats with glucose more than 200 mg $dL^{-1}$ in each groups 21 days after streptozotocin injection. Vitamin E had no effect on diabetes induction by streptozotocin, but elevation of glycosylated Hb and reduction of LDL-c in group $E_3$ were significant. Vitamin E also increased HDL-c although it was not statistically significant. We suggest that oral vitamin E consumption may have some beneficial effect on the correction of lipid metabolism disorders of diabetes, although it may worsen carbohydrate metabolism in mild diabetes.

**Key words:** Glucose, glycosylated Hb, lipid, HDL-c and LDL-c

## INTRODUCTION

Diabetes mellitus is a disease characterized by hyperglycemia and its complications have been attributed to long duration of this condition due to the abnormality of glucose metabolism. On the other hand hyperlipidemia has been frequently observed in diabetes mellitus (Hirano *et al*., 1991; Brownlee *et al*., 1988; Nikkila and Kekki, 1973).

Streptozotocin (STZ) is used to induce experimental diabetes by selectively destroying pancreatic β-cells (Szkudelski, 2001). STZ is taken up by pancreatic β-cells via glucose transporter GLUT2 (Schundle *et al*., 1994). STZ was found to generate Reactive Oxygen Species (ROS), which also contribute to DNA fragmentation and evoke other deleterious changes in the cells (Takasu *et al*., 1991). The inhibition of xanthin oxidase by allopurinol restricts the cytotoxic effect of STZ *in vitro*. Pretreatment of β-cells with this inhibitor prevented the STZ-induced decrease of insulin secretion (Nukatsuka *et al*., 1990). Therefore, intracellular antioxidants attenuate STZ toxicity (Szkudelski, 2001).

All tissues in the body contain adequate amounts of antioxidants to protect them against the toxic actions of free radicals. A deficiency of these antioxidants can result in tissue and organ damage (Krishna and Das, 2001). Roza *et al*. (1995) showed that pancreatic antioxidant enzymes such as SOD, glutathione peroxidase and catalase are low in normoglycemic, diabetic-prone BB rats compared with the low-risk group. This idea is supported by the observation that antioxidants such as vitamin E and probucol can prevent alloxan-induced diabetes in rats, furthermore superoxide dismutase and catalase protected β-cells of isolated pancreatic islets against alloxan cytotoxicity, as did the hydroxyl radical scavengers Dimethyl Sulfoxide (DMSO) and butanol (Jorns *et al*., 1999; Slonim *et al*., 1983; Tibaldi *et al*., 1979).

Diabetes produces myocardial dysfunction that accelerates cardiovascular morbidity and mortality (Wold *et al*., 2001). Hyperglycemia and dyslipidemia have been shown to affect physiologic changes in the vasculature leading to atherosclerosis (Kaur *et al*., 2002). Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic

**Corresponding Author:** Seyed Reza Fatemi Tabatabaei, Department of Physiology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahwaz, Iran Tel: +98 611 3330073 Fax: +98 611 3360807

hyperglycemia, thereby depleting the activity of the antioxidative defence system and promoting the generation of free radicals (Hong *et al*., 2004). There is evidence suggests that antioxidants, especially vitamin E, have potential benefits with respect to cardiovascular disease (Harris *et al*., 2002).

It is well known that vitamin E, a lipid soluble and antioxidant vitamin protect unsaturated fatty acid, a main component of cell membrane, from the attack of oxygen-derived free radicals. However, we do not know that consumption of antioxidant vitamins have any effect on induction of diabetes mellitus by STZ. On the other hand, the effect of vitamin E consumption on the carbohydrate and lipid metabolism of diabetics is controversial. We designed this study to evaluate the effects of different doses of vitamin E on the induction of diabetes mellitus in STZ-rats and to assess the effect of vitamin E on some lipid parameters important in cardiovascular disease.

## MATERIALS AND METHODS

In the laboratory animal research center of veterinary school in Shahid Chamran University forty male Wistar rats (approximately 195 g) were divided randomly to four groups, control, $E_1$, $E_2$ and $E_3$. The control group was fed with commercial rat diet and the diet of group $E_1$, $E_2$ and $E_3$ were supplemented with 1, 2 and 4 g $kg^{-1}$ of vitamin E, respectively. The animal room temperature was 23±1°C and rats were subjected to 12 h dark/12 h light cycle. The study was done at last May and all of animal manipulation and sample collection were done from 8-10 am.

Rats were made diabetic by IP injection of 45 mg $kg^{-1}$ body weight STZ, 4 days after the beginning of vitamin consumption. To compare the amount of blood glucose elevation in different groups, the blood glucose was measured by glucometer (Glucomen, Italy) in tail vein blood 72 h after the injection of STZ (n = 10 in each group). Six rats with blood glucose greater than 200 mg $dL^{-1}$ were maintained in each group and others omitted from the remaining parts of the study. Blood samples were collected by heart puncture, 21 days after STZ injection under deep inhalation anaesthesia by chloroform. Blood were mixed with EDTA, as an anticoagulant and centrifuged at 3000 rpm for 10 min, to separate blood cells and plasma. Plasma was freeze at -20°C until biochemical assay. RBCs were washed immediately three times with normal saline and glycosylated hemoglobin was measured by a commercial kit (Mahsayaran) by cyanomethhemoglobin method one day later.

The Total Cholesterol (TC), HDL-cholesterol and triglycerides (TG), were measured by an enzymatic assay commercial kits (Zyst-chimi). LDL-c and VLDL-c were calculated by the Friedewald formula (Friedewald *et al*., 1972):

$$VLDL\text{-}c = TG.5^{-1}$$
$$LDL\text{-}c = TC - HDL\text{-}c - VLDL\text{-}c$$

Results obtained were expressed as the means±SEM. Data were analyzed by one way ANOVA with Tukey post hoc test. The analyses were performed using the Sigmastat software.

## RESULTS AND DISCUSSION

Blood glucose of rats 72 h after STZ injection was not statistically different (Table 1). Glycosylated hemoglobin increased by vitamin E consumption and it was statistically greater in group $E_3$ in comparison with control group (Table 1).

TC and LDL-c decreased and HDL-c increased in diabetic rats by vitamin E consumption, but only the reduction of LDL-c was statistically significant in group $E_3$ vs. control group (Table 2).

Table 1: Blood glucose level 72 h after streptozotocin injection and glycosylated haemoglobin 3 week after diabetes induction in control, $E_1$, $E_2$ and $E_3$ groups

| Groups | Glucose (mg $dL^{-1}$) (n = 10) | Glycosylated Hb (%) (n = 6) |
|---|---|---|
| Control | 240.7±35.66 | 7.87±0.54* |
| $E_1$ | 224.9±35.77 | 8.05±0.40 |
| $E_2$ | 239.3±45.63 | 8.62±0.11 |
| $E_3$ | 258.5±44.30 | 9.88±0.66* |

*: $p<0.05$

Table 2: Triglyceride, total cholesterol, HDL-c, LDL-c and VLDL-c in control, $E_1$, $E_2$ and $E_3$ groups (n = 6 in each groups)

| Groups | Triglyceride | Total cholesterol | HDL-c | LDL-c | VLDL-c |
|---|---|---|---|---|---|
| | (mg $dL^{-1}$) | | | | |
| Control | 94.33±14.07 | 266.35±13.5 | 8.83±30.0 | 238.65±12.47* | 18.87±2.81 |
| $E_1$ | 100.00±18.30 | 248.33±10.60 | 8.13±0.46 | 220.20±7.75 | 20.00±3.60 |
| $E_2$ | 63.67±11.69 | 227.00±6.81 | 10.25±0.99 | 204.05±7.08 | 12.70±2.33 |
| $E_3$ | 108.83±22.00 | 227.00±13.43 | 9.85±0.38 | 195.45±12.03* | 21.70±4.371 |

*: $p<0.05$

Tsujinaka *et al.* (2005) showed that diet high in lipid hydroperoxide by vitamin E deficiency accelerate glucose intolerance through impairments of both sensitivity and secretion of insulin. Srinivasan *et al.* (2005) reported that combination of high fat diet-fed and low dose STZ-treated rats serves as an alternative animal model for type 2 diabetes. Pretreatment of pancreatic β-cells with allopurinol (xanthine oxidase inhibitor) prevented the STZ-induced decrease of insulin secretion *in vitro* (Nukatsuka *et al.*, 1990). In present study, vitamin E had no protective effects against STZ-diabetes induction.

Prior administration of SOD, catalase, monomethyl, dimethyl, or monoethyl urea can block alloxan-induced cytotoxic action on pancreatic β-cells (Jorns *et al.*, 1999; Gandy *et al.*, 1982; Grankvist *et al.*, 1979; Tibaldi *et al.*, 1979). According to Nishizono *et al.* (2000) pre-treatment with tert-butylhydroquinone, a synthetic antioxidant reduced the severity of STZ-diabetes in rats and Slonim *et al.* (1983) demonstrated that vitamin E prevent alloxan-induced diabetes in rats. All these agents can quench either a superoxide amion or a hydroxyl radical and thus are probably able to protect against alloxan-induced damage to the pancreatic β-cells both *in vitro* and *in vivo*. This suggests that alloxan-induced damage to the pancreatic B cells is a free-radical-dependent process. Oils rich in ω-3 and ω-6 long-chain fatty acids also can significantly attenuate alloxan-induced diabetes in these experimental animals (Krishna and Das, 2001).

Beside the results obtained from this study, vitamin E with current doses used in this study is not effectively potent to inhibit the toxicity of STZ in β-cells of pancreas.

In present study carbohydrate metabolism has been worsen by vitamin E consumption in diabetic rats. Studies on the effects of vitamin E on carbohydrate metabolism are controversial. Skrha *et al.* (1997) reported an increase in glycosylated hemoglobin after daily administration of 600 mg of vitamin E during 3 months in obese type 2 diabetes. However, in most of the studies vitamin E has no effect (Garg *et al.*, 2005; Craven *et al.*, 1997) or has beneficial effect (Maning *et al.*, 2004; Al Shamsi *et al.*, 2004) on carbohydrate metabolism. The severity of diabetes, dose, period and route of vitamin E consumption and different methodologies may be an explanation for these controversies. Signaling by insulin requires autophosphorylation of the insulin receptor kinase (Hubbard, 1997). In intact cells high concentrations of hydrogen peroxide on the order of 1 mM pervanadate and thiol-reactive agents induce insulin-like effects in the absence of insulin (Heffetz *et al.*, 1990). Lower and physiologically relevant concentrations (<0.1 mM) of hydrogen peroxide are not sufficient to trigger the autophosphorylation of the insulin receptor in the absence of insulin, but do enhance the response to 100 nM insulin (Schmid *et al.*, 1999), indicating that the redox signal has a coregulatory function in insulin receptor activation under physiologically relevant conditions (Droge, 2002). However, low dose of STZ injection produced a mild hyperglycemia in our study and may be vitamin E consumption (as an antioxidant) counteracted with low level of insulin in receptor autophsphorylation.

During the course of type 1 diabetes, there was a serum lipid profile disturbance characterized by an increase of blood cholesterol (Harris *et al.*, 2002). According to Gokkusu and Mostafazadeh (2003), plasma and tissue cholesterol concentrations in hypercholesterolemic animals did not appear to be affected by vitamin E treatment. However, Komaratat *et al.* (1985) observed decreased total cholesterol, LDL-c and VLDL-c and an increase in HDL-c, with high doses of vitamin E in rabbits. Jeon *et al.* (2005) suppressed an increase in plasma total cholesterol and LDL-c in high cholesterol diet rabbits by vitamin E. Vieira da Costa and Vianna (2005) showed an increase of HDL-c and a decrease of LDL-c, but the concentrations of triglycerides and total cholesterol did not changed.

Present data suggests that oral vitamin E consumption may have some beneficial effect on the correction of lipid metabolism disorders of diabetes although further studies should be taken on vitamin E supplementation as an alternative therapy to treat lipid disorder for diabetic patients and more attention on the effect of vitamin E on carbohydrate metabolism in mild diabetes.

## REFERENCES

Al-Shamsi, M.S., A. Amin and E. Adeghate, 2004. Beneficial effect of vitamin E on metabolic parameters of diabetic rats. Mol. Cell Biochem., 261 (1-2): 35-42.

Brownlee, M., A. Cerami and H. Vlassara, 1988. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N. Engl. J. Med., 318 (20): 1315-1321.

Craven, P.A., F.R. DeRubertis, V.E. Kagan, M. Melhem and R.K. Studer, 1997. Effect of supplementation with vitamin C or E on albuminuria, glomerular TGF-β and glomerular size in diabetes. J. Am. Soc. Nephrol., 8 (9): 1405-1414.

Droge, W., 2002. Free radicals in the physiological control of cell function. Phsyol. Rev., 82 (1): 47-95.

Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18 (6): 499-502.

Gandy, S.E., N.G. Buse and R.K. Crouch, 1982. Protective role of superoxide dismutase against diabetogenic drugs. J. Clin. Invest., 70 (3): 650-658.

Garg, M.C., D.P. Chaudhary and D.D. Bensal, 2005. Effect of vitamin E supplementation on diabetes induced oxidative stress in experimental diabetes in rats. Indian J. Exp. Biol., 43 (5): 177-180.

Gokkusu, C. and T. Mostafazadeh, 2003. Changes of oxidative stress in various tissues by long-term administration of vitamin E in hypercholesterolemic rats. Clin. Chem. Acta, 328 (1-2): 155-161.

Grankvist, K., S. Marklund, J. Sehlin and I.B. Taljedal, 1979. Superoxide dismutase, catalase and scavengers of hydroxyl radicals protect against toxic action of alloxan on pancreatic islet cells *in vitro*. Biochem. J., 182 (1): 17-25.

Harris, A., S. Devaraj and I. Jialal, 2002. Oxidative stress, alpha-tocopherol therapy and atherosclerosis. Curr. Atheroscler. Rep., 4 (5): 373-380.

Heffetz, D., I. Bushkin, R. Dror and Y. Zick, 1990. The insulinomimetic agents $H_2O_2$ and vanadate stimulate protein tyrosine phosphorylation in intact cells. J. Biol. Chem., 265 (5): 2896-2902.

Hirano, T., J.C. Mamo, H. Takeuchi, S. Nagano and T. Takahashi, 1991. Correlation of insulin deficiency and hypertriglyceridemia in diabetic rats. Diabetes Res. Clin. Pract., 12 (3): 173-180.

Hong, J.H., M.J. Kim, M.R. Park, O.G. Kwag, I.S. Lee, B.H. Byun, S.C. Lee, K.B. Lee and S.J. Rhee, 2004. Effects of vitamin E on oxidative stress and membrane fluidity in brain of streptozotocin-induced diabetic rats. Clin. Chem. Acta, 340 (1-2): 107-115.

Hubbard, S.R., 1997. Crystal structure of the activated insulin receptor tyrosine kinase in complex with peptide substrate and ATP analog. EMBO. J., 16 (18): 5572-5581.

Jeon, S.M., Y.B. Park, O.S. Kwon, T.L. Huh, W.H. Lee, KM. Do, T. Park and M.S. Choi, 2005. Vitamin E supplementation alters HDL-cholesterol concentration and paraoxonase activity in rabbits fed high-cholesterol diet: Comparison with probucol. J. Biochem. Mol. Toxicol., 19 (5): 336-346.

Jorns, A., M. Tiedge, S. Lenzen and R. Munday, 1999. Effect of superoxide dismutase, catalase, chelating agents and free radical scavengers on the toxicity of alloxan to isolated pancreatic islets *in vitro*. Free Radic. Biol. Med., 26 (9-10): 1300-1304.

Kaur, J., P. Singh and J.R. Sowers, 2002. Diabetes and cardiovascular diseases. Am. J. Ther., 9 (6): 510-515.

Komaratat, P., N. Chupukcharoen and P. Wilairat, 1985. Effect of vitamin E on cholesterol plasma lipoprotein distribution and metabolism in rabbit. Int. J. Vitamin Nutr. Res., 55 (2): 167-171.

Krishna, M.I. and U.N. Das, 2001. Prevention of chemically induced diabetes mellitus in experimental animals by polyunsaturated fatty acids. Nutrition, 17 (2): 126-151.

Maning, P.J., W.H. Suterland, R.J. Walker, S.M. Williams, Sa. De Jong, A.R. Ryalls and E.A. Berry, 2004. Effect of high-dose vitamin E on insulin resistance and associated parameters in overweight subjects. Diabetes Care, 27 (9): 2166-2171.

Nikkila, E.A. and M. Kekki, 1973. Plasma triglyceride transport kinetics in diabetes mellitus. Metabolism, 22 (1): 1-22.

Nishizono, S., T. Hayami, I. Ikeda and K. Imaizumi, 2000. Protection against diabetogenic effect of feeding tert-butylhydroquinone to rats prior to administration of streptozotocin. Biosci. Biotechnol. Biochem., 64 (6): 1153-1158.

Nukatsuka, M., Y. Yoshimura, M. Nishiada and J. Kawada, 1990. Allopurinol protect pancreatic beta cells from the cytotoxic effects of streptozotocin: *In vitro* study. J. Pharmacobiodyn, 13 (4): 259-262.

Roza, A.M., G.M. Pieper, C.P. Johnson and M.B. Adams, 1995. Pancreatic antioxidant enzyme activity in normoglycemic diabetic prone BB rats. Pancreas, 10: 53-58.

Schmid, E., A. Hotz-Wagenblatt, V. Hack and W. Dröge, 1999. Phosphorylation of the insulin receptor kinase by phosphocreatine in combination with hydrogen peroxide. The structural basis of redox priming. FASEB. J., 13 (12): 1491-1500,

Schundle, W.J., S. Ferber, J.H. Johnson and C.B. Newgard, 1994. STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. Diabetes, 43 (11): 1326-1333.

Skrha, J., G. Sindelka and J. Hilgertova, 1997. The effect of fasting and vitamin E on insulin action in obese type 2 diabetes mellitus. Ann. Acad. Sci. N.Y., 827 (1): 556-560.

Slonim, A.E., M.L. Surber, D.L. Page, R.A. Sharp and I.M. Burr, 1983. Modification of chemically-induced diabetes in rats by vitamin E supplementation minimizes and depletion enhances development of diabetes. J. Clin. Invest., 71 (5): 1282-1288.

Srinivasan, K., B. Viswanad, L. Asrat, C.L. Kaul and P. Ramarao, 2005. Combination of high-fat diet-fed and low dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol. Res., 52 (4): 313-320.

Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in β-cells of the rat pancreas. Physiol. Res., 50 (6): 536-546.

Takasu, N., I. Komiyada, T. Asawa, Y. Nagasawa and T. Yamada, 1991. Streptozotocin and alloxan induced $H_2O_2$ generation and DNA fragmentation in pancreatic islets. $H_2O_2$ as mediator for DNA fragmentation. Diabetes, 40 (9): 1141-1145.

Tibaldi, J., J. Benjamin, F.S. Cabbat and R.E. Heikkila, 1979. Protection against alloxan-induced diabetes by various derivatives: Relationship between protective effects and reactivity with the hydroxyl radical. J. Pharmacol. Exp. Ther., 211 (2): 415-418.

Tsujinaka, K., T. Nakamura, H. Meagawa, M. Fujimiya, Y. Nishio, M. Kudo and A. Kashiwagi, 2005. Diet high in lipid hydroperoxide by vitamin E deficiency induces insulin resistance and impaired insulin secretion in normal rats. Diabetes Res. Clin. Pract., 67 (2): 99-109.

Vieira da Costa, V.A. and L.M. Vianna, 2005. Effect of a-tocopherol supplementation on blood pressure and lipidic profile in streptozotocin-induced diabetes mellitus in spontaneously hypertensive rats. Clin. Chim. Acta, 351 (1-2): 101-104.

Wold, L.E., D.P. Relling, P.B. Colligan, G.I. Scott, K.K. Hintz, B.H. Ren, P.N. Epstein and J. Ren, 2001. Characterization of contractile function in diabetic hypertensive ardiomyopathy in adult rat ventricular myocytes. J. Mol. Cell. Cardiol., 33 (9): 1719-1726.