**ORIGINAL ARTICLE**

# Effect of Supplementation of Diet with Vitamin C on Induction and Consequences of STZ-Diabetes in Rats

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## ABSTRACT

OBJECTIVE: Streptozotocin (STZ) destroys the beta cells of pancreas by generation of reactive oxygen species and vitamin C has documented antioxidant properties. This study was designed to evaluate the preventive effect of supplementation of diet by vitamin C on induction of STZ-diabetes and its effect on carbohydrate and lipid metabolism of diabetic rats.

MATERIALS AND METHODS: Fifty male Wistar rats were divided randomly into non-diabetic (ND), diabetic (D), $C_1$, $C_2$ and $C_3$ groups. The diet of $C_1$, $C_2$ and $C_3$ groups were supplemented with 2.5, 5 and 10 g/kg of vitamin C, respectively. Four days later all groups exceptND, were made diabetic by IP injection of STZ and blood glucose was measured 72 h later to determine the severity of blood glucose elevation. Weight gain was measured weekly. 21 days after induction of diabetes Glycosylated hemoglobin ($HbA_1C$), triglyceride (TG), total cholesterol (TC), HDL-c and LDL-c were measured or calculated in plasma of 6 diabetic rats in each groups that their glucose was more than 200 mg/dl after STZ injection.

RESULTS: Vitamin C significantly prevented blood glucose elevation after STZ injection in group C2. Weight gain decreased in all diabetic groups. Increase of HbA1c could not be prevented by vitamin C in any groups. TC and LDL-c decreased and HDL% increased in group $C_3$.

CONCLUSION: We suggest that the amount of vitamin C consumption may have an important effect on STZ-diabetes induction and it may be in agreement with opposite effects of free radicals on insulin receptor signaling. Furthermore, vitamin C may have some beneficial effects on lipid metabolism disorders of diabetes.

KEY WORDS: Diabetes, Streptozotocin, Ascorbic Acid, Prevention, Diet, HDL-c, 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 (1,2).

Streptozotocin (STZ) is used to induce experimental diabetes by selectively destroying pancreatic beta cells (3). STZ is taken up by pancreatic beta cells via glucose

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transporter GLUT2 (4). STZ was found to generate reactive oxygen species (ROS), which also contribute to DNA fragmentation and evoke other deleterious changes in the beta cells (5). The inhibition of xanthin oxidase by allopurinol restricts the cytotoxic effect of STZ in vitro. Pre-treatment of beta cells with this inhibitor prevented the STZ-induced decrease of insulin secretion (6). Therefore, intracellular antioxidants attenuate STZ toxicity (3). 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 (7). It has been 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 (8). This idea is supported by observation that antioxidants such as probucol can prevent alloxan-induced diabetes in rats, and superoxide dismutase and catalase protected beta cells of isolated pancreatic islets against alloxan cytotoxicity, as did the hydroxyl radical scavenger dimethyl sulfoxide (DMSO) and butanol (9,10). Furthermore, administration of the antioxidant vitamin E to rats, prior to administration of either STZ or alloxan, provided protection against the diabetogenic effect of both these agents (11).

Diabetes produces myocardial dysfunction that accelerates cardiovascular morbidity and mortality (12). Hyperglycemia and dyslipidemia have been shown to affect physiologic changes in the vasculature leading to atherosclerosis (13). Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, thereby depleting the activity of the antioxidative defense system and promoting the generation of free radicals (14). There is evidence suggesting that antioxidants, especially vitamin E, have potential benefits with respect to cardiovascular disease (15). Vitamin E is a lipid soluble antioxidant and protects LDL particles from oxidative attack. Vitamin C is required for regeneration of α-tocopherol, may thus preventing LDL oxidation (16). Vitamin C, an aqueous phase antioxidant has been reported to improve whole body glucose disposal in healthy subjects and in diabetic patients (17) and animals (18).

However, we do not know if supplementation of diet with vitamins, especially vitamin C, has any effect on diabetes mellitus by STZ. On the other hand, the effect of vitamin C supplement on the carbohydrate and lipid metabolism of diabetics is controversial. We designed this study to evaluate the effects of different amounts of vitamin C on induction of blood glucose elevation after STZ injection and to assess the effect of vitamin C on some lipid parameters important in cardiovascular diseases in STZ-induced diabetic rats.

## MATERIALS AND METHODS

Animals were purchased from the laboratory of Animal House of Veterinary School in Shahid Chamran University. The animal room temperature was 23 ± 1 °C and rats were subjected to 12-h dark/12-h light cycle and had ad libitum access to food and water. All animal manipulations and sample collection were done from 9-12 am.

Fifty male Wistar rats (approximately 195 g) were randomly divided into five groups, non-diabetic (ND), diabetic (D), $C_1$, $C_2$ and $C_3$. The ND and D groups were fed with commercial rat diet without any supplementation, and the diet of group $C_1$, $C_2$ and $C_3$ were supplemented with 2.5, 5, and 10 g/Kg of vitamin C, respectively. Groups D, $C_1$, $C_2$ and $C_3$ were made diabetic by IP injection of 45 mg/kg STZ, four days after vitamin supplementation in the diet of groups $C_1$, $C_2$ and $C_3$. Similar volumes of saline were injected to ND group.

All rats in each group were weighed at STZ injection and 1, 2, and 3 weeks after STZ injection. To compare the amount of blood glucose elevation in each group, the blood glucose was measured by glucometer (Glucomen, Italy) in tail vein blood 72 h after injection of STZ (n = 10 in each group). Six rats with blood glucose greater than 200 mg/dl were maintained in each group and others

omitted from the remaining parts of the study.Three weeks after STZ injection, the rats were euthanized by anesthesia using chloroform vapor. Blood was collected from heart by cardiac puncture, and transferred into EDTA treated tubes immediately. Blood was then centrifuged at 3000 rpm for 10 minutes, to separate blood cells and plasma. The plasma tubes were frozen at -20°C until biochemical analysis. RBCs were washed immediately three times with normal saline and glycosylated hemoglobin (HbA1c) measured by a commercial kit (Mahsayaran) by cyanomethhemoglobin method one day later.

The total cholesterol (TC), HDL-cholesterol (HDL-c) and triglycerides (TG) were measured by enzymatic assay commercial kits (Zyst-chimi). LDL-c was calculated by the Friedewald formula (19):

LDL-c = TC - HDL-c – TG/5

Results obtained were expressed as the means ± SEM. Data were analyzed by one-way ANOVA using the Sigmastat software followed by Tukey post hoc test. Differences between groups were considered significant at $P < 0.05$.

## RESULTS

Blood glucose of group $C_2$ did not increased significantly in comparison with group ND (Figure 1). However, the glucose level of all other groups were significantly greater than group ND. Between diabetic groups, blood glucose of group $C_2$ was also lower than group $C_1$ ($P < 0.05$). As there were not enough diabetic rats with blood glucose greater than 200 mg/dl in group $C_2$, this group was completely discarded from the remaining parts of the experiment.

As illustrated in Figure 2, weight gain in diabetic groups was stopped after induction of diabetes, and supplementation of their diets with vitamin C did not have any effect on it.

HbA1c has been increased by diabetes induction in all diabetic groups in comparison with group ND ($P < O.05$) (Table 1).

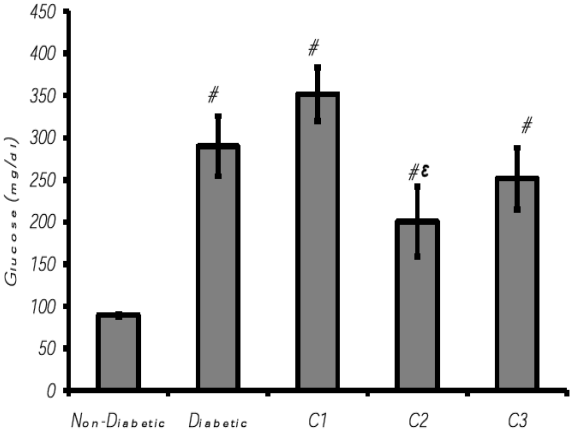


**Figure 1- Blood Glucose 72h after STZ injection**
#: Significant differences with nondiabetic group.
ε: Significant differences with C1

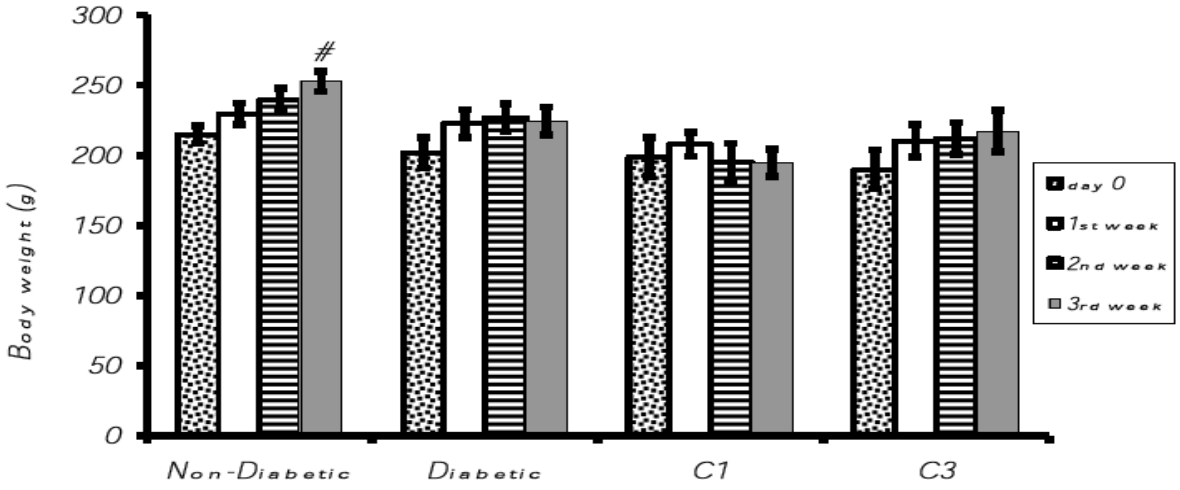


**Figure 2- Weight Gain After STZ Injection.**
#: Significant difference with weight at STZ injection

**Table 1- HbA1c, Triglyceride, Total Cholesterol, HDL-C, LDL-Cand VLDL-C in Non-Diabetic, Diabetic, C1 and C3 Groups (N=6 in Each Group)**

| Group | HbA1C (mg/dl) | TG (mg/dl) | TC (mg/dl) | HDL-c (mg/dl) | HDL-c% | LDL-c (mg/dl) | LDL-c% |
|---|---|---|---|---|---|---|---|
| Non-Diabetic | 6±0.35 | 69.33±16.91 | 213.5±22.49 | 7.28±0.45 | 3.41±0.24 | 192.35±21.73 | 90.1±1.9 |
| Diabetic | 7.95±0.45# | 94.33±14.07 | 256±12.45 | 8.83±0.36# | 3.45±0.29 | 228.3±12.49 | 89.18±0.92 |
| $C_1$ | 8.28±0.41# | 62.33±14.04 | 212.67±4.34 | 8.4±0.28 | 3.95±0.15 | 191.8±6.56 | 90.19±1.45 |
| $C_3$ | 9.02±0.6# | 52.33±5.32 | 155.33±16.16* | 7.73±0.36 | 4.98±0.42* | 137.13±16.23* | 88.28±1.7 |

**#**: significant difference with non-diabetic group.
*****: significant difference with diabetic group

TC, HDL-c and LDL-c increased by diabetes induction, but only HDL-c was significantly greater in group D ($P < 0.05$). However, the percentage of HDL-c did not change by diabetes in group D, whereas it was increased by high level of vitamin C in the diet. The minimum level of TG, TC and LDL-c were seen in group $C_3$, although only TC and LDL-c of this group was significantly lower than group D ($P < 0.05$).

## DISCUSSION

Diabetes mellitus is a metabolic disease associated with impaired glucose metabolism which in effect adversely alters intermediary metabolism of lipids and proteins. Formation of protein glycation products releases free radicals; subsequently causing oxidative stresses (20). Most of the complications of the diabetic state are initiated by the generation of free radicals, for instance LDL oxidative modification, leading to atherosclerosis (21).

In the recent study, supplementation of diet with 5 g/kg vitamin C reduced the severity of glucose elevation after STZ injection. Tsujinaka et al. (22) showed that diet high in lipid hydroperoxide by vitamin E deficiency accelerates glucose intolerance through impairments of both sensitivity and secretion of insulin as a result of increased lipid peroxidation. On the other hand, pre-treatment of pancreatic beta cells with allopurinol (xanthin oxidase inhibitor) prevented the STZ-induced decrease of insulin secretion in vitro (6). Prior administration of SOD, catalase, monomethyl, dimethyl, or monoethyl urea could block alloxan-induced cytotoxic action on pancreatic beta cells (9,10). Pre-treatment with tert-butylhydroquinone, a synthetic antioxidant and vitamin E reduced the severity of STZ-diabetes in rats (11). All these agents can quench either a superoxide anion or a hydroxyl radical, thus may be able to protect against alloxan-induced damage to the pancreatic beta cells both in vitro and in vivo. Therefore, inhibition of diabetes induction by supplementation of diet with 5 g/kg vitamin C may be related to the antioxidative properties of the vitamin.

Beside the results obtained from this study, supplementation of diet with 5 g/kg vitamin C could decrease the severity of STZ diabetes but lower and greater doses did not have any effects on the induction of STZ diabetes. It is possible that lower dose of vitamin C (2.5 g/kg diet) have not been sufficient to protect beta cells from oxidative damage. If vitamin C has reduced the diabetogenic effects of streptozotocin by reduction of oxidative stress, why similar effect did not occur by supplementation of diet with 10 g/kg vitamin C?

Signaling by insulin requires autophosphorylation of the insulin receptor (23). In intact cells, high concentrations of hydrogen peroxide and thiol-reactive agents induce insulin-like effects in the absence of insulin (24). 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 (25), indicating that the redox signal has a coregulatory function in insulin receptor activation under physiologically relevant conditions (26). Hydrogen peroxide directly interacts with insulin receptor kinase domain and enhances its autophosphorylation and kinase activity in the presence of ADP (27). On the other hand, autophosphorylation of insulin receptor can be down-regulated by a redox-sensitive protein-tyrosine phosphatase (28). Thus it is possible that high dose of vitamin C in the present study have reduced hydrogen peroxide and thol-reactive agent by increasing the anti oxidative potency of the organism. Therefore, the ability of hydrogen peroxide to trigger the autophosphorylation of the insulin receptor in the presence of low level of insulin (25) may be reduced by 10 g/kg of vitamin C in group $C_3$.

At the present study vitamin C not only could not inhibit decrease of weight gain, but also increased glycosylated hemoglobin in diabetic rats. Sridulyakul et al. (29) showed that weight and plasma vitamin C reduction and glycosylated hemoglobin elevation in STZ-diabetic rats were prevented by supplementation of 1 g/l vitamin C in drinking water but the same dose and route of vitamin C administration could not prevent weight loss in STZ diabetic rats in Amatyakul et al. study (30). The effect of vitamin C on glycozylated hemoglobin is controversial. Some investigator reported that vitamin C decreases HbA1c in diabetic patients (31,32) and animal models (18,29), but in some other studies it did not have beneficial effect on HbA1c (33,34). Glucose and vitamin C might occupy the same membrane transport system (35). Taking of the ascorbic acid in the dosage of 1000 mg/per day for seven days (36), and 2000 mg/day for 14 days intensifies the level of glycemia during OGGT in normoglycemic individual, perhaps by competitively inhibiting glucose uptake by pancreatic beta cells (37). The severity and duration of diabetes, dose of vitamin, period and route of vitamin consumption, and different methodologies may be an explanation for these controversies.

During the course of type 1 diabetes, there was a serum lipid profile disturbance characterized by an increase of blood cholesterol (15). This is one of the significant factors in development of cardiovascular diseases (18). Also oxidative modification of LDL is an important step in the development of atherosclerosis (21). This oxidation is initiated and propagated by free radicals where antioxidants become depleted (38). There are reports that show the ability of vitamin C to scavenge superoxide, hydrogen peroxide, hydroxyl radicals etc, in DM (39). In this study vitamin C decreased TC and LDL-c of diabetic rats, dose dependently. Many reports accentuate that vitamin C improves lipid profile in diabetes (18,31,32,33 and 40) but some did not show any benefit (34,41 and 42). As shown in the recent study, it seems that higher amounts of vitamin C may be more effective in lipid profile correction of diabetes (31).

The possible explanation for the hypocholesterolaemic effect of vitamin C is that vitamin C prevents LDL-cholesterol from oxidative damage and aids in degradation of cholesterol (18). Furthermore, this vitamin is needed by the enzyme in the first step of bile acid synthesis (cholesterol 7α-hydroxylase) thus directing cholesterol towards bile acid synthesis and reduces its level in serum (43). Kaviarasan et al. (35) reported that the level of total cholesterol, triglyceride, lipid peroxidation and glucose increased in hyperlipidemic patients with or without diabetes mellitus, whereas there was decreased plasma concentration of vitamin C and other antioxidants. Taking the above evidence together suggest that vitamin C supplementation dose dependently improves the lipid profile in diabetic rats by acting through cholesterol 7α-hydroxylase to direct cholesterol into bile synthesis. Furthermore, by scavenging free radicals, it decreases oxidative damage to oxidized LDL-cholesterol.

## CONCLUSION

We suggest that supplementation of diet with different amounts of vitamin C have different

effects on glucose elevation in STZ diabetic rats, and this is in agreement with opposite effects of oxidative stress on insulin receptor signaling. Furthermore, administration of vitamin C in diabetics may be beneficial for the improvement of lipid profile. This may, at least in part, reduce the risk of cardiovascular events seen in diabetes mellitus.

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