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Alterations in Somatostatin Cells and Biochemical Parameters Following Zinc Supplementation in Gastrointestinal Tissue of Streptozotocin-Induced Diabetic Rats

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Chronic hyperglycemia in diabetes is a major causative factor of free radical generation which further leads to many secondary diabetic complications via the damage to cellular proteins, membrane lipids, and nucleic acids. Zinc is an essential trace element in all living systems and plays a structural role in many proteins and enzymes. Somatostatin is known to have inhibitory effects on various gastrointestinal functions. Therefore, we determined somatostatin protein production and secretion levels, and biochemical and light microscopical changes following zinc supplementation in the gastrointestinal tract of streptozotocin (STZ)-diabetic rats. The animals were divided into four groups: Group I: control (untreated) animals; Group II: control animals given zinc sulfate; Group III: diabetic animals; and Group IV: diabetic animals given zinc sulfate. Zinc sulfate was given to the animals by gavage at a daily dose of 100 mg/kg body weight for 60 days. Diabetes was induced by intraperitoneal (i.p.) injection of STZ in a single dose of 65 mg/kg. For histological studies, stomach and duodenum tissues were fixed in Bouin solution and sections stained with Masson's trichrome and Periodic-Acid-Schiff. Tissue homogenates were used for protein, lipid peroxidation (LPO), glutathione (GSH), and nonenzymatic glycosylation (NEG) analyses. Zinc supplementation to the STZ-diabetic rats revealed the protective effect of zinc on these parameters. Zinc supplementation may contribute to prevent at least some complications of diabetes mellitus.

Key words: somatostatin, diabetes, stomach, small intestine, rat

I. Introduction

Various substances have recently been utilized to prevent the complications of diabetes mellitus in patients or animals. Zinc complexes have recently been shown to exhibit insulinomimetic activities and an antioxidant role [1, 6, 8, 12, 26, 32]. Zinc homeostasis is maintained via the gastrointestinal tract by the processes of absorption of exogenous zinc and excretion of endogenous zinc [19]. It is reported that supplementation of zinc to STZ diabetic animals reduces the blood glucose levels [18, 26, 31]. However, the action mechanism of zinc has yet to be clarified. Zinc sulfate affects a glucose transporter 4, which is involved in the glucose uptake [32]. Zinc deficiency in diabetics could result from the hyperglycemia or the impaired intestinal zinc absorption or increased oxidative stress [9, 14, 15, 17, 25, 28]. Zinc supplementation studies in diabetes are very few and their results are contradictory. Somatostatin is produced in D cells and intrinsic neurons of the stomach and intestines. It could directly regulate the secretion of gut hormones and enzymes [22]. Somatostatin has been shown to be an effective inhibitor of insulin [24, 29]. In the present study, we aimed to investigate somatostatin production and secretion levels, and biochemical and light microscopical changes following zinc supplementation in the gastrointestinal tract of STZ-diabetic rats.

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II. Materials and Methods

Animals and tissue preparation

Diabetes was induced by intraperitoneal injection of STZ in a single dose of 65 mg/kg body weight. STZ was dis-

solved in a freshly prepared 0.01 M citrate buffer (pH 4.5). Six–6.5 months old female Swiss albino rats weighing 150–200 g were used. The experiments were reviewed and approved by Institute's Animal Care and Use Committee of the University of Istanbul. The animals were fed laboratory pellet chow and given water *ad libitum*. All rats were clinically healthy. The animals were divided into four groups: Group I: control (untreated) animals (n=5); Group II: Control animals given zinc sulfate (n=6); Group III: Diabetic animals (n=6); and Group IV: Diabetic animals given zinc sulfate (n=9). Zinc sulfate was given to the animals by gavage at a daily dose of 100 mg/kg body weight for 60 days. At the end of experimental period, stomach (fundus region) and duodenum tissues were obtained from animals sacrificed under ether anesthesia after an overnight fast. Tissue samples were immediately washed with saline and frozen until needed for study. For histological studies, stomach and duodenum tissues were fixed in Bouin solution and sections stained with Masson's trichrome and Periodic-Acid-Schiff (PAS).

Biochemical assays

At the end of experimental period, stomach and duodenum tissues were homogenized in cold 0.9% serum physiologic by means of a glass homogenizer to make up a 10% (w/v) homogenate, centrifuged, and the clear supernatants used for GSH, LPO, NEG, and protein analysis. Stomach and duodenum LPO and GSH were determined by the methods of Ledwozyw *et al.* [20] and Beutler using Ellman's reagent, respectively [3]. Nonenzymatic glycosylation (NEG) levels were assessed by 2-thiobarbituric acid [23]. Total protein levels measured by the method of Lowry using bovine serum albumin as a standard [21].

Immunohistochemistry

Sections were dewaxed and rehydrated. After washing in phosphate-buffered saline (PBS), sections were immersed in a solution of 3% H_2O_2 for 10 min. The sections were then pre-incubated with non-immune serum for 10 min. After overnight incubation of the sections in a dilution of the rabbit anti-somatostatin antibody (Zymed, San Francisco, CA, USA) at 4°C, specific reactivity was detected with biotinylated second antibody and streptavidin-biotin-peroxidase complex. The localization of the antigen was indicated by a red color obtained with 3-amino-9-ethyl-carbazole (AEC) as chromogen substrate for peroxidase activity. The Histostain SP kit (Zymed) was used for immunohistochemistry. Detection procedures were carried out as described by the manufacturer. Slides were counterstained with hematoxylin. The specificity of the immunohistochemical staining was checked by omission of the primary antibody or by using an inappropriate antibody. All these controls gave negative results. Control pancreas sections were used as a positive control.

Statistical analysis

The biochemical results were statistically evaluated with SPSS/10 software. Variance analysis was used for comparison of the group. One way ANOVA was applied to find the difference between the groups. Using a *post hoc* multiple comparison test, Duncan's and Scheffe's multiple range tests were used to find the significant difference among means. The mean count of somatostatin-immunoreactive cells per field of vision by a light microscope was determined for each animal by averaging the number of cells in 10 randomly selected fields of vision in which the entire thickness of the mucosa was visible in a single section that was immunohistochemically stained. The immunoreactivity was evaluated by two different blinded observers. The significance of changes in somatostatin-immunoreactive cells was evaluated statistically using the Kruskal-Wallis One-way ANOVA test. Analysis of differences between the control and experimental groups was performed by using the Dunn multiple comparison test. Results are expressed as the mean±SD and considered significantly different at the level of $p<0.05$.

III. Results

Biochemical results

Table 1 shows the content of LPO, GSH, and NEG in the stomach of normal and experimental groups. In the diabetic rats, an increase in stomach LPO levels was observed. Administration of zinc sulfate was found to reduce stomach LPO levels in diabetic rats, but in the nondiabetic control groups, it significantly increased the stomach LPO levels ($^{a}p<0.0001$). In diabetic rats, a significant decrease in stomach GSH levels was observed when compared with control group ($^{b}p<0.0001$). In the diabetics rats treated with zinc sulfate, the stomach GSH levels increased significantly compared with the diabetic group ($^{c}p<0.009$). In the diabetic rats, NEG levels were increased significantly when compared with the control group ($^{d}p<0.0001$). In the diabetic rats treated with zinc sulfate, the stomach NEG levels decreased significantly when compared with the diabetic group ($^{e}p<0.0001$).

Table 2 shows the content of LPO and GSH in the duodenum of normal and experimental groups. In the diabetic rats, LPO levels were significantly different between groups ($P_{ANOVA}=0.0001$). In diabetic rats treated with zinc sulfate, the duodenum LPO levels decreased significantly when compared with the diabetic group ($^{b}p<0.0001$). The duodenum GSH levels were significantly reduced in the diabetic rats as compared with the control group ($p<0.0001$) and diabetic+zinc sulfate group. In diabetic rats a significant decrease in duodenum GSH levels was observed ($^{c}p<0.0001$). Treatment with zinc sulfate for 60 days was found to increase in duodenum GSH levels in diabetic rats ($^{d}p<0.010$). The NEG levels in duodenum tissue were very low (data not shown).

Light microscopical results

A moderate degree of mucosal hyperemia and cystic dilatation were observed in fundus of diabetic rats. It was shown that zinc sulfate treatment moderately improves

Table 1. *Mean levels of stomach GSH, LPO, and NEG for all groups*

Groups	n	LPO (nmol MDA/mg protein)*	GSH (nmol GSH/mg protein)*	NEG (nmol fructose/mg protein)*
Control	5	0.38±0.06	22.32±1.06	14.80±0.87
Control+Zinc Sulfate	6	1.44±0.14[a]	18.68±2.62	25.84±1.37
Diabetic	6	0.56±0.04	14.56±0.9[b]	23.63±1.05[d]
Diabetic+Zinc Sulfate	9	0.53±0.05	18.67±2.00[c]	17.37±0.88[e]
P_{ANOVA}		0.0001	0.0001	0.0001

* Mean±SD, n=Number of animals.
[a, b, d] p<0.0001 versus control group, [c] p<0.009 versus diabetic group, [e] p<0.0001 versus diabetic group.

Table 2. *Mean levels of duodenum LPO and GSH for all groups*

Groups	n	LPO (nmol MDA/mg protein)*	GSH (nmol GSH/mg protein)*
Control	5	0.42±0.01	20.72±1.84
Control+Zinc Sulfate	6	0.48±0.05	18.63±1.34
Diabetic	6	0.73±0.14[a]	13.39±1.42[c]
Diabetic+Zinc Sulfate	9	0.43±0.06[b]	17.79±2.75[d]
P_{ANOVA}		0.0001	0.0001

* Mean±SD, n=Number of animals.
[a, c] p<0.0001 versus control group, [b] p<0.0001 versus diabetic group, [d] p<0.010 versus diabetic group.

Table 3. *Densities of somatostatin-producing cells for all groups*

Groups	n	Stomach Cell Density*	Duodenum Cell Density*
Control	4	116.75±45.84	79.00±10.89
Control+Zinc sulfate	5	156.00±96.71	117.20±85.03
Diabetic	4	266.75±15.94[a]	59.25±9.35
Diabetic+Zinc sulfate	5	96.40±25.42[b]	24.00±18.17
P_{ANOVA}		0.027	0.031

* Mean±SD, n=Number of animals.
[a] p<0.05 versus control group, [b] p<0.05 versus diabetic group.

gastrointestinal damage in the diabetic+zinc sulfate group. Stomach surface and mucous neck cells had dense PAS-positive material in four groups. Somatostatin-immunoreactive cells in the four groups were generally found to be scattered in the neck region of fundic glands (Fig. 1). There was a significant difference in somatostatin-immunoreactive cells between the four groups (P_{ANOVA}=0.027) (Table 3). Control group given zinc sulfate was not different from untreated control group considering somatostatin immunoreactivity. A significant increase in fundic somatostatin-immunoreactive cells was observed in diabetic group as compared to controls ([a]p<0.05). There was no statistically significant change in fundic somatostatin-immunoreactive cells in the diabetic +zinc sulfate group as compared to the controls. A significant decrease was observed in fundic somatostatin immunoreactive cells of the diabetic animals given zinc sulfate as compared to the diabetic animals ([b]p<0.05) (Fig. 1).

Dense positive PAS reaction observed in the duodenal mucosa was more common in the diabetic+zinc sulfate group as compared to the diabetics. Irregular villi and edema in lamina propria were observed in significant numbers in the diabetic rats. But, we observed that zinc sulfate treatment does not improve duodenal damage in the diabetic+zinc sulfate group (Fig. 2). A small but scattered number of somatostatin-immunoreactive cells in the four groups was observed mainly at the base of the crypts in the duodenum. There was a significant difference in somatostatin-immunoreactive cells between the four groups (P_{ANOVA}=0.031) (Table 3). There was no statistically significant difference in duodenal somatostatin-immunoreactive cells in the control+zinc sulfate group as compared to the untreated control. On the other hand, a notable decrease was observed in duodenal somatostatin-immunoreactive cells of diabetic group as compared to controls but this was not statistically significant. There was an insignificant difference in duodenal somatostatin-immunoreactive cells in the diabetic+zinc sulfate group as compared to diabetics (p=0.81).

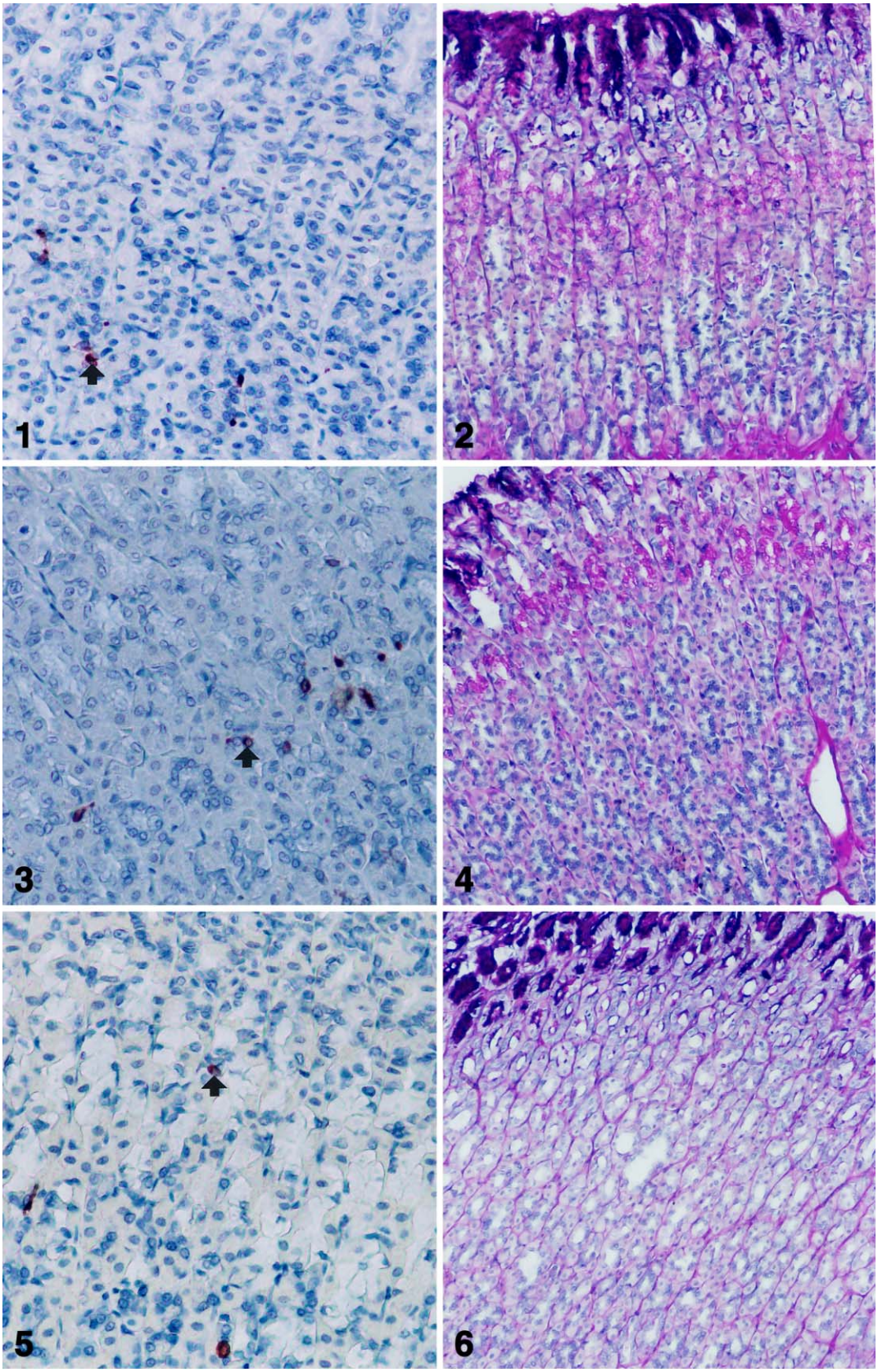

Fig. 1. Somatostatin-immunoreactive cells (arrow) are generally seen in the neck region of fundic glands of stomach. A control rat (**1, 2**), STZ-diabetic rat (**3, 4**), and diabetic rat given zinc sulfate (**5, 6**). Streptavidin-biotin-peroxidase technique (**1, 3, 5**), magnifications ×200, PAS (**2, 4, 6**) magnifications ×100.

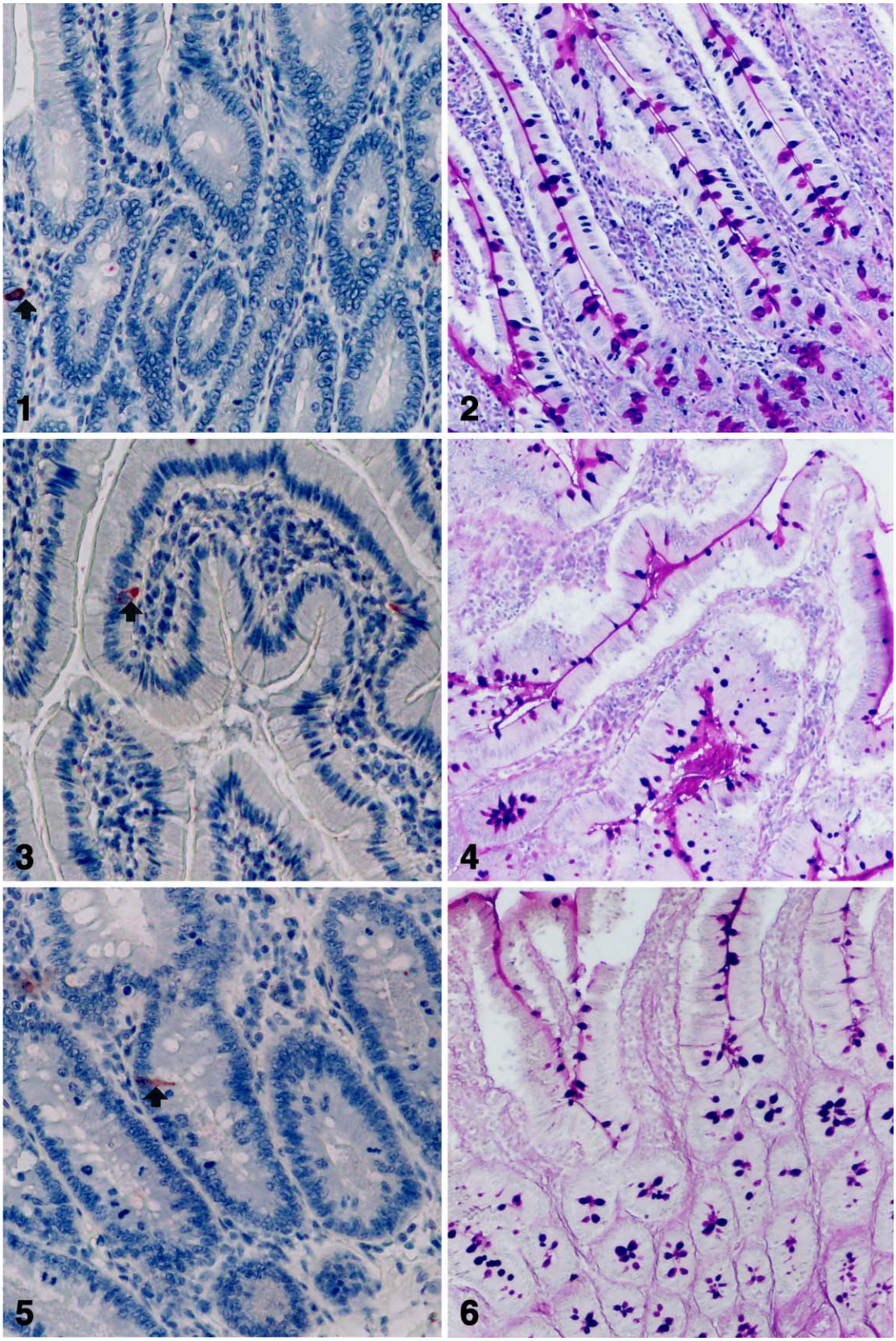

Fig. 2. Somatostatin-immunoreactive cells (arrow) localized immunohistochemically in duodenum. A control rat (**1, 2**), STZ-diabetic rat (**3, 4**), and diabetic rat given zinc sulfate (**5, 6**). Streptavidin-biotin-peroxidase technique (**1, 3, 5**), magnifications ×200, PAS (**2, 4, 6**), magnifications ×100.

IV. Discussion

The zinc requirement of gastrointestinal cells is high, since undifferentiated cells require zinc for mucosal cell function, differentiation, growth, and repair. It is suggested that zinc is protective and enhances epithelial repair in gastrointestinal tract [19]. In the diabetic+zinc sulfate group, a certain degree of repair of mucosa was observed in stomach. However, we observed that zinc sulfate treatment does not improve duodenal damage in the diabetic+zinc sulfate group. Zinc supplementation to STZ-diabetic rats reduced blood glucose concentration (data not shown). This observation indicates that zinc can improve some of the degenerative changes induced in the gastrointestinal tract. The number of fundic somatostatin-immunoreactive cells significantly increased in diabetic group when compared to the other groups. A marked decrease in duodenal somatostatin-immunoreactive cells was also found in diabetic group. Therefore, we concluded that somatostatin-immunoreactive cells are reciprocally related to insulin in diabetic stomach and duodenum. On the other hand, a notable decrease was observed in fundic and duodenal somatostatin immunoreactive cells of the diabetic animals given zinc sulfate as compared to the diabetic animals. It is suggested that the expression of somatostatin-immunoreactive cells is reversed by treatment with zinc sulfate for 60 days. To our knowledge, this is the first reported investigation on the relation of zinc supplementation and somatostatin in the diabetic stomach and duodenum. Therefore, more research is needed in order to clarify its useful role in diabetics.

Alterations in the antioxidant enzymes and increased oxidative damages have been demonstrated in different tissues of diabetic animals. It is suggested that zinc is protective against oxidative stress and free radical injury [5, 11]. Some investigators [2, 7, 33] reported that zinc deficiency increased lipid peroxidation. Faure *et al.* [10] reported that zinc supplementation decreases in lipid peroxidation and improves glutathione peroxidase activity in diabetic patients. Oxidative stress is characterized by increased lipid peroxidation. In the diabetic rats, stomach and duodenum LPO levels were significantly higher than those of the other groups. Significant increases in LPO during diabetes show the occurrence of oxidative damage in diabetic rats. Increased oxidative stress may cause some of the morphological alterations occurring during diabetes. In the stomach of the diabetic +zinc sulfate group, LPO and NEG levels decreased as compared to the diabetic group. In the duodenum of the diabetic+zinc sulfate group, LPO levels also decreased as compared to the diabetic group. We can say that zinc has a role in controlling LPO. In this study, zinc supplementation did not cause important changes in lipid peroxidation in stomach but decreased its levels in intestine, because intestinal absorption and excretion of zinc in STZ-diabetic rats may be affected more than stomach.

GSH is one of the most important nonprotein sulfhydryls present in animal cells. Glutathione plays a major role as a reductant in the oxidation-reduction process [27]. In the diabetic+zinc sulfate group, GSH levels significantly increased as compared to the diabetic group in the stomach and duodenum. However, the observed increase in glutathione concentration induced by zinc sulfate may not be enough to complete the protective mechanism in stomach and duodenum cells. This study showed that zinc improves the antioxidant defense system, in particular tissue-reduced glutathione levels. It has been shown that zinc sulfate plays an important role in the maintenance of GSH. Previous studies reported that some free radical scavengers, such as glutathione, can prevent the development of diabetes induced by STZ in rats [4, 13]. Johnson and Canfield [16] reported that the amount of zinc excreted by the intestines was less in the diabetic rats. Abnormalities in excretion or absorption may be due to the impairment of the oxidative balance. It is suggested that the long term exposure of tissues to high glucose concentration may be responsible for accumulation of nonenzymatically glycosylated products [30]. Increased reactive oxygen species in diabetes may result from increased generation of nonenzymatically glycated proteins.

In conclusion, zinc supplementation to STZ-induced diabetic rats revealed the protective effect of zinc on oxidative stress and free radical metabolism. Our results demonstrate that zinc supplementation on somatostatin-immunoreactive cells in the stomach and duodenum has different effects. All of these data suggest a role for zinc in the protection of the somatostatin cell against oxidative stress. Zinc supplementation may be useful in the future care of patients with diabetes through its antioxidant effect against oxidant stress.

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VI. References

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