

# The Effect of CaNa<sub>2</sub>-EDTA on Metabolism of Zinc and Carbohydrate as well as Some Biochemical Factors in Experimental Diabetes

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## Abstract

**Background:** To investigate the effect of CaNa<sub>2</sub>-EDTA and experimental diabetes (IDDM) on zinc and carbohydrate metabolism and the activities of GOT, GPT and alkaline phosphatase. **Methods:** Forty male normal albino (Wistar) rats of 8 weeks of age were fed with a basal diet. Twenty rats were then intraperitoneally injected with alloxan to induce diabetes. Then after one week ten rats from each group (n= 20) were administrated intraperitoneally with CaNa<sub>2</sub>-EDTA for further three weeks. Body weight gain and food intake were recorded regularly. On day 21 animals were killed and blood glucose, serum and tissues zinc concentrations, liver glycogen contents, serum transaminase enzymes (GOT, GPT), and serum alkaline phosphatase activities were determined. **Results:** The administration of CaNa<sub>2</sub>-EDTA significantly altered body weight gain, food intake and serum zinc concentration of either diabetic or non-diabetic animals. Both diabetic and non-diabetic rats given CaNa<sub>2</sub>-EDTA had higher blood glucose than their controls. Liver glycogen was also found to be higher in CaNa<sub>2</sub>-EDTA non-diabetic rats than their controls. CaNa<sub>2</sub>-EDTA also led to increasing of GOT and GPT, and decreasing serum alkaline phosphatase. **Conclusion:** To conclude, the present study demonstrates that CaNa<sub>2</sub>-EDTA had an effect on the previous parameters. Therefore it was appeared that CaNa<sub>2</sub>-EDTA resulted in the development of severe diabetes.

**Keywords:** *Diabetic rats, CaNa<sub>2</sub>-EDTA, GOT, GPT, Alkaline phosphatase*

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## Introduction

EDTA (ethylene diamine tetra acetic acid) was synthesized by Munz in 1935 in an effort to create a substitute for citric acid, and introduced in the United State in 1948. EDTA is a pharmaceutical agent for the treatment of lead and other metal poisoning or exposure (1, 2). It is also used as an anticoagulant in blood used for laboratory studies. The FDA also approved intravenous EDTA treatment as possibly effective in occlusive vascular disorders, it is used for the treatment of pathologic condition to which calcium tissue deposits or hypercalcaemia, EDTA is also utilised for the treatment and prevention of arteriosclerosis and other chronic degenerative disease (3, 4). Moreover it was found bene-

ficial uses of oral EDTA in cardiovascular diseases (5). Early clinical studies with EDTA reported loss of fat in rat, reduction of cholesterol and blood pressure in humans (6). In addition to its remarkable pharmaceutical uses, the FDA has also approved EDTA as a food additive that is generally recognized as safe. EDTA's array of biochemical properties makes it extremely valuable as a food additive. It has the ability to bind with many metals, act synergistically with other antioxidants to stabilize fats, oils and vitamins, prevent discoloration of potato products, fish, shellfish, canned fruit and vegetables, inhibit the thickening of stored condensed milk, prevents oxidation of meat product and flavour changes in milk, colour changes of scrambled

eggs prepared from egg powder and gushing of beer, promote flavour retention and delay loss of carbonation in soft drinks (7, 8). EDTA chelate is a drug beside its important role in the medical and nutrition food. It has also a negative effect. In other words some reports indicated that use of EDTA results in depletion of certain elements such as zinc, which is in general an important element for the metabolism (9). Moreover, tissue zinc concentrations and zinc balances have been both reported to be altered in the diabetic state (10, 11). Therefore, in view of the alteration of zinc status in EDTA chelating therapy, the present study was undertaken to investigate the combined effect of EDTA and experimental diabetes (Insulin Dependent Diabetes) on zinc metabolism, carbohydrate metabolism and some enzymes (GOT, GPT and alkaline phosphatase activities in albino (Wistar) rats.

## Materials and Methods

**Animals and EDTA treatment** Forty male weaning normal albino (Wistar) rats of 8 wk of age with a body weight ranging from 180-250 g. Animals were housed individually in polypropylene cages with stainless-steel gridded tops and bottoms and stainless-steel food hoppers. Trays were placed under each food hopper to collect split food. Humidity was around 70% and temperature was  $22 \pm 2$  °C. Food and distilled water were provided *ad-libitum*. Rats were given a basal diet containing g/kg diet. The dietary carbohydrate source was provided by equal amounts of cornstarch 326 (ONAB EL-Harrouch, Algeria) and sucrose 326, protein 168 (egg white solids), lipids 80 (corn oil), fibre 40 (cellulose), vitamin mix 20 (Sigma), and mineral mix 40. The quantity of zinc in diet was 54 mg/kg quantities, as determined by atomic absorption spectroscopy. Mineral mix supplied (g/kg diet): calcium hydrogen orthophosphate 13; disodium hydrogen orthophosphate 7.4; calcium carbonate 8.2; potassium chloride 7.03; magnesium sulphate 4; ferrous sulphate 0.144;

copper sulphate 0.023; potassium iodate 0.001; manganous sulphate 0.180; zinc carbonate 0.1. The diet was prepared similar to that of the reference (12). Rats were randomly divided into two groups of 20 each. The second group was intraperitoneally injected with freshly prepared alloxan monohydrate solution (Alloxan; Sigma) in a dose of 150 mg/kg of body weight to induce diabetes (13). Then after the stabilization of diabetes for one week, the two groups of animals were divided into two subgroups (10 rats per each). The first subgroup was kept as non-diabetic or diabetic control groups and the second subgroup was administrated intraperitoneally with freshly prepared CaNa<sub>2</sub>-EDTA solution (CaNa<sub>2</sub>-EDTA; Sigma) in a dose 50 mg/kg of body weight, once daily for three weeks (14), and used as a non-diabetic or diabetic test groups. Food intake and body weights were recorded regularly. Rats were fasted over night on day 20, but allowed to feed for two periods of 1 h each, between 11.00- 12.00 am and 17.00-18.00 pm Rats were then killed between 11.00 and 12.30 pm on day 21. Animals were killed by exsanguination from the heart, under diethyl ether anesthesia. The blood was transferred into ice-cold centrifuged tubes and a portion taken for whole-blood glucose analysis, which was performed promptly after exsanguination. The remaining blood was centrifuged for 10 min at 3000 rpm and the serum stored at -20 °C till serum zinc, GOT, GPT, alkaline phosphatase assays. Livers were rapidly excised, weighed, and freeze-clamped at -196 °C, ground under liquid nitrogen and stored at -20 °C for glycogen analysis. The pancreas was washed with isotonic saline (9 g sodium chloride/l distilled water) and blotted to dry. The right femur was taken and the connective tissues and muscle were removed. After that, the pancreases and femurs were weighed, dried at 80 °C for 16 h and zinc concentrations were determined.

**Analytical methods** Blood glucose was measured in 10 µl samples of fresh whole blood by the glucose oxidase (*EC* 1.1. 3. 4) method, using a YSI Model 27 glucose analyzer

and the kit constitute of phosphate buffer containing the enzymes (GOD, POD) and D-glucose (Sigma). The determination of total liver glycogen was as that of glucose following an enzymatic hydrolysis with amyloglucosidase (*EC* 3. 2. 1. 3) obtained from *Asperigillus niger*; Sigma) (15). Both dried pancreas and femur were heated in silica crucibles at 480 °C for 48 h and the ash taken up in hot hydrochloric acid (11.7 M) for Zn analysis by atomic absorption spectrophotometer (Pye Unicam SP 9000) (12). Using standard reference materials: bovine liver and wheat flour checked the accuracy of zinc recovery using this method. These standards were prepared and analyzed identically to assess recovery. The recovery of zinc in the standard reference material exceeded 96%. Zinc in serum was also measured in duplicate, after a twenty-fold dilution of serum in double distilled water by flame Atomic Absorption Spectrophotometer (Pye Unicam SP 9000). Zinc standards were prepared from a 1mg/ml zinc nitrate standard solution (BDH) using 5% glycerol to approximate the viscosity characteristics, and to avoid zinc contamination from exogenous sources. All tubes were soaked in HCl (10% v/v) for 16 h and rinsed with double distilled water. GOT, GPT and alkaline phosphatase activities were determined using commercial test kits following the enzyme listing GOT, GPT (16) and alkaline phosphatase (17). The results were compared using one-way analysis of variance (ANOVA). A  $p < 0.05$  was considered the limit for the statistical significance.

## Results

Induction of a type I diabetic state caused a decrease ( $P < 0.05$ ) in body weight gain and higher food intake in the diabetic rats compared to the non-diabetic rats. CaNa<sub>2</sub>-EDTA was also significantly ( $P < 0.05$ ) altering both body weight gain and food intake of both diabetic and non-diabetic (Table 1). Femur and pancreatic zinc concentrations taken as an index of zinc status indicated that both diabetic and non-diabetic rats given CaNa<sub>2</sub>-EDTA were not able to maintain a similar status to their control groups (Table 2). Pancreatic Zn concentration was significantly lower in diabetic individual compared to the non-diabetic animals. In this experiment, mean femur zinc concentration for diabetic was relatively lower than those for non-diabetic rats, but the difference was not always significant. Analysis of blood glucose, serum zinc concentrations, GOT, GPT and alkaline phosphates activities and liver glycogen content indicated that both CaNa<sub>2</sub>-EDTA-diabetic and CaNa<sub>2</sub>-EDTA-non diabetic rats had low serum zinc concentration, alkaline phosphatase activity, but a high fasting blood glucose level, GOT and GPT activities compared to the control animals. Liver glycogen contents were similar in both diabetic groups, but the CaNa<sub>2</sub>-EDTA-non-diabetic rats had higher liver glycogen contents than that of controls. Both diabetic animals had a high ( $P < 0.05$ ) blood glucose concentration, serum GOT and serum GPT activities, decreased serum zinc concentrations, alkaline phosphatase activity and liver glycogen contents compared with the non-diabetic rats (Table 3).

**Table 1:** Mean body weight gain (g/day), food intake (g/day), feed efficiency (Body weight gain/food intake×100) of diabetic and non-diabetic rats administrated with CaNa<sub>2</sub>-EDTA or not

Diet	Diabetic rats				Non-diabetic rats			
	Control (n = 10)		With EDTA (n = 10)		Control (n = 10)		With EDTA (n = 10)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body wt gain	2.4 <sup>a</sup>	0.09	1.2 <sup>b</sup>	0.05	5.2 <sup>c</sup>	0.04	3.2 <sup>d</sup>	0.06
Food intake	17.1 <sup>a</sup>	0.04	17.3 <sup>b</sup>	0.04	12.4 <sup>c</sup>	0.04	10.4 <sup>d</sup>	0.07
Feed efficiency	14.3 <sup>a</sup>	0.6	7.4 <sup>b</sup>	0.3	41.9 <sup>c</sup>	0.3	30.7 <sup>d</sup>	0.7

a, b, c, d: values within a horizontal line with different superscript letters were significantly different ( $P < 0.05$ ).

**Table 2:** Pancreas dry wt (g), pancreatic zinc content ( $\mu\text{g}$ ), pancreatic zinc concentration ( $\mu\text{g/g}$  dry weight), femur zinc concentration ( $\mu\text{g/dry weight}$ ), serum zinc ( $\mu\text{g/100 ml}$ ) of diabetic and non-diabetic rats administrated with CaNa2-EDTA or not.

Diet	Diabetic rats				Non-diabetic rats			
	Control (n = 10)		With EDTA (n = 10)		Control (n = 10)		With EDTA (n = 10)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Pancreas dry wt	0.88 <sup>a</sup>	0.01	0.92 <sup>a</sup>	0.01	0.90 <sup>a</sup>	0.01	0.88 <sup>a</sup>	0.02
Pancreatic Zn content	44.1 <sup>a</sup>	0.4	45 <sup>a</sup>	0.07	85.4 <sup>b</sup>	0.5	86.9 <sup>b</sup>	0.1
Pancreatic Zn Concentration	52.7 <sup>a</sup>	0.5	48.9 <sup>b</sup>	0.5	105.1 <sup>c</sup>	1.2	94.8 <sup>d</sup>	1.1
Femur Zn Concentration	117 <sup>a</sup>	0.4	117.7 <sup>a</sup>	0.1	150.4 <sup>b</sup>	0.3	117.9 <sup>a</sup>	0.1
Serum zinc	80.6 <sup>a</sup>	2.7	72.2 <sup>b</sup>	3.9	114.8 <sup>c</sup>	1.5	105.3 <sup>d</sup>	2.7

a, b, c, d: values within a horizontal line with different superscript letters were significantly different ( $P < 0.05$ ).

**Table 3:** Mean blood glucose (m mole/l), liver wet weight (g), liver glycogen content (mg), liver glycogen concentration (mg/g fresh weight), serum GOT, GPT and alkaline phosphatase activities (UI/l) of diabetic and non-diabetic rats administrated with CaNa2-EDTA or not.

Diet	Diabetic rats				Non-diabetic rats			
	Control (n = 10)		With EDTA (n = 10)		Control (n = 10)		With EDTA (n = 10)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Blood glucose	15.1 <sup>a</sup>	0.08	19.3 <sup>b</sup>	0.14	5.0 <sup>c</sup>	0.07	7.2 <sup>d</sup>	0.07
Liver wet wt	12.4 <sup>a</sup>	0.8	13.5 <sup>a</sup>	0.9	12.9 <sup>a</sup>	0.9	13.2 <sup>a</sup>	0.6
Glycogen Concentration	5.2 <sup>a</sup>	0.9	4.0 <sup>a</sup>	1.0	11.7 <sup>b</sup>	3.1	24.3 <sup>c</sup>	2.5
Serum GOT	45.8 <sup>a</sup>	3.4	65 <sup>b</sup>	4.3		2.7	160.9 <sup>d</sup>	3.6
Serum GPT	86 <sup>a</sup>	9.3	114 <sup>b</sup>	7.3	19 <sup>c</sup>	0.5	20 <sup>c</sup>	1.0
Alkaline phosphatase	239.3 <sup>a</sup>	0.5	179.3 <sup>b</sup>	0.7	346.2 <sup>c</sup>	0.5	272.2	0.4

a, b, c, d values within a horizontal line with different superscript letters were significantly different ( $P < 0.05$ ).

## Discussion

In this experiment, diabetic administrated with CaNa2-EDTA or not weighed less than the non-diabetic rats. Body weight gain in diabetic and non-diabetic rats was also affected by CaNa2-EDTA chelate. [This is in a good agreement and similar with some previously published reports when zinc reduced in diet] (18, 19). It is suggested, thus that CaNa2-EDTA was bounded to zinc and it was resulted in depletion of zinc free level and confirmed by the result of low

zinc concentration, which was found in serum. The diabetic rats given either CaNa2-EDTA or not had a lower feed efficiency than the non-diabetic rats although the food intake of these animals was higher (hyperphagia) than that of the non-diabetic rats. The daily mean of consumed diet by rat is 14 g (20). This rises the possibility of the metabolic state disturbance of animal, suggesting that the diabetic condition had exacerbated reduced the ability of the diabetic rats to utilize food intake as normal subjects.

Diabetic and non-diabetic rats injected with CaNa<sub>2</sub>-EDTA had differences in pancreatic and femur zinc concentrations. This finding again indicated the effect of CaNa<sub>2</sub>-EDTA on body zinc status and it is an agreement with some previous investigations (21, 22). The reduced total pancreatic and serum zinc in diabetic rats either given CaNa<sub>2</sub>-EDTA or not compared with the non-diabetic rats, is probably due to the degranulation, cytolysis and to other pathological changes in the pancreatic tissue, associated with progression of the condition (23), or to the high excretion of zinc in the urine (24-26). However, the marked no reduction in femur zinc level of diabetic rats compared with the non-diabetic rats, supports the work of Levine et al. (13). In the current study, when the time of feeding was strictly controlled and the amount of food eaten by each animal before an over night fast was known to be similar, the mean fasting blood glucose concentration in the CaNa<sub>2</sub>-EDTA- diabetic or CaNa<sub>2</sub>-EDTA- non diabetic rats were found to be higher than that of controls. This suggests that CaNa<sub>2</sub>-EDTA was reduced zinc free level (12). Thus it had exacerbated the ability of diabetic and non-diabetic rats to utilize glucose. Results from previous studies have also showed an increased blood glucose concentration, after intra-venous injection with glucose in rats fed on a zinc deficiency diet (27) suggesting a relation between carbohydrate utilization and the zinc status. The fasting glycogen content of both CaNa<sub>2</sub>-EDTA diabetic and control diabetic rats was lower compared to that of non-diabetic groups, although liver weight was similar in different groups. This might be related to liver dysfunction (28). Liver glycogen concentration in CaNa<sub>2</sub>-EDTA-non-diabetic rats was higher than that of control non-diabetic rats. These observations indicate that the carbohydrate metabolism of these animals is sensitive to variation in zinc status and it is interesting to note that Reeves and O'Dell (29) also have observed an increased glycogen synthesis in low zinc rats. In this experiment it was also found a significant rise in

serum GOT and GPT levels in diabetic rats, which could relate to excessive accumulation of amino acids (glutamic and alanine) in serum of diabetic animals as a result of amino acids mobilization from protein stores (30). These excessive amino acids are then converted to ketonic bodies ( $\alpha$  keto-glutaric and pyruvate) for which the enzyme GOT and GPT are needed, leading to increased enzyme activity. The higher levels of GOT and GPT in the CaNa<sub>2</sub>-EDTA-animals, confirm the result of high concentration of their blood glucose. In other words, the gluconeogenic action of GOT and GPT plays the role to provide new supply of glucose from other sources such as amino acids. It is interesting to note that Grefley and Sandstead (31) found evidence of decreased oxidation of the carbon chain of alanine when zinc was restricted and led to alanine accumulation in blood. The decrease of serum alkaline phosphatase activity in rats administrated with CaNa<sub>2</sub>-EDTA probably related to the decreased serum zinc concentration. Another possible explanation for the observed variation in alkaline phosphatase could result from the increased call of energy through glycolytic and oxidative pathway of glucose 6 phosphate, rather than alkaline phosphatase activity. Since these animals had higher blood glucose than their controls (32), serum alkaline phosphatase activity was also found low in the diabetic compared to non-diabetic rats, which could be attributed also to the decrease of serum zinc. Prasad et al. (33) showed that zinc was present in several metalloenzymes such as alkaline phosphatase, and hence it is needed for their activities.

In conclusion, the combination of CaNa<sub>2</sub>-EDTA and diabetes had affected growth rate, blood glucose concentration, liver glycogen content, body zinc status, GOT, GPT and alkaline phosphatase activities. Therefore CaNa<sub>2</sub>-EDTA chelate has beside its important role in the pharmaceutical and nutrition field it has also a negative effect on the metabolism and appeared to result in the development of severe diabetes.

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