

## Dietary Zinc Supplementation in Diabetic Rats: Beneficial Impacts on Glycemic Control and Pancreatic Islet $\beta$ -cells Regeneration

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### Abstract

Implication of Zinc (Zn) deficiency or impairment in the pathogenesis of both type 1 and type 2 diabetes mellitus has long been established. However, only a few studies reported the mechanisms of zinc significance in many biological functions and processes. This experimentally-controlled designed nutritional study, aimed to determine the effects of Zn-supplemented diet on glycemic tolerance and control, body weight and pancreas histomorphometry in diabetic male rats. Twenty-four male Wistar rats each weighing  $\geq 200$ g were randomly categorized into three experimental groups (n = 8, each): Normal control (NC) fed with standard rat feed; Diabetic control (DC) fed with control diet and Diabetic (DZ) on test (zinc-supplemented) diet. Diabetes was induced with freshly prepared alloxan monohydrate solution (150 mg/dL, intraperitoneally). Rats were fed for a period of eight weeks according to the experimental design with water *ad libitum* while their weights were measured twice weekly and recorded. Fasting blood sugar (FBS) levels were measured twice weekly while oral glucose tolerance test (OGTT) was conducted to construct the glycemic response curves. Animals were sacrificed at the end of eight week to extract pancreas for immunocytochemical and histomorphometric analyses. Microsoft Excel and statistical program SPSS version 22.0 were used to analyse data while P values  $< 0.05$  were considered significant. At the end of eight-week study, Zn-supplemented diet caused significant ( $p < 0.05$ ) reductions in mean body weight gain (28.5%) and FBS (31.5%) levels, stimulated islet  $\beta$ -cells regeneration and improved glycemic tolerance and profile in DZ rats compared with the diabetic control rats. In conclusion, zinc-supplemented diet impacts beneficial antidiabetic effect in diabetic rats via stimulation of pancreatic islet  $\beta$ -cells regeneration, reduction of body weight gain and lowering of blood glucose level.

**Keywords:** Body Weight; Diabetic Rats; Glycemic Control; Histomorphometry; Islet B-Cells Regeneration; Zinc-Supplemented Diet

### Introduction

Zinc (Zn) is an essential structural part of important anti-oxidant enzymes such as superoxide dismutase. Its deficiency impairs the synthesis of such enzymes leading to increased oxidative stress [1] that plays a vital role in the pathogenesis of diabetes and its complications. Studies have shown that diabetes is accompanied by hypozincemia [2] and hyperzincuria [3]. Importance of Zinc in insulin action and carbohydrate metabolism has well been documented [4]. The high prevalence of diabetes mellitus in the developing countries may be correlated to zinc deficiency reported to be more common in developing countries [5]. As an important trace element and micronutrient, Zn plays a key role in various biological functions and macronutrient metabolism [6]. Its involvement in synthesis, storage, release and action of insulin [7,8] has proved that its deficiency state is associated with insulin resistance, impaired glucose tolerance and obesity [9,10] which are risk factors for diabetes mellitus. Increased loss of zinc observed in frequent urination in diabetics appears to contribute to the marginal zinc nutritional status which may explain the inadequate efficacy of oral hypoglycaemic agents observed at times as a result of post-receptor events associated with oxidative stress induced by long-term hyperglycaemia. The administration of antioxidants such as

zinc, magnesium, selenium, vitamin A and vitamin E may improve the tissue response to insulin and increase the efficacy of drugs which act through this pathway. Previous studies indicated that marginal zinc deficiency is more prevalent among diabetic adults, compared to the normal adult population [11] while abnormal zinc plasma levels occur more frequently in metabolically uncontrolled diabetic patients [12]. Dietary supplementation of zinc in diabetics has been shown to improve life quality and expectancy [13] as reported by both animal [7] and human [14-17] studies. Hence, it is necessary to carry out more studies in this regard to unravel various mechanisms or pathways through which zinc mediates its antidiabetic activities at cellular and tissue levels. This experimentally-controlled designed nutritional study was therefore carried out to determine the effects of Zn-supplemented diet on glycemic control and tolerance, body weight and pancreas histomorphometry in diabetic male rats with the rationale to ascertain the suitability of zinc supplementation in diabetic diets for euglycemic control. Most studies investigating the efficacy of Zn in glycemic control administered zinc orally in its salt forms without incorporating it in the diet. In this study, the zinc sulphate used was mixed with the diet and rationalized to ensure adequate consumption of recommended dosage necessary for optimal glycemic control and tolerance. The impact of zinc on pancreatic islet  $\beta$ -cells regeneration was also examined in this study.

### Materials and Methods

#### Experimental Animals and Design

Twenty-four adult male Wistar rats (*Rattus norvegicus*) weighing  $\geq 200$ g were purchased from the disease-free stock of Olu Animal Research Farm Sango, Oyo State, Nigeria. They were fed initially with standard rat chow and water *ad libitum* for the 2 weeks acclimatization in raised stainless steel cages with 6 mm<sup>2</sup> mesh floor (to maintain same physical activity) kept in a well-ventilated animal house (at 23°C and a 12h light and dark cycle). Replaceable numbered blotters papers were placed under each cage to catch the spilled diet that was measured to make up for the daily serving ration. After acclimatization, the rats were randomly divided into four groups of 8 rats each: Normal control (NC) fed with standard rat feed; Diabetic control (DC) fed with control diet and Diabetic fed on Zn-supplemented diet (DZ). Each group had a close entry value of mean body weight (Table 2) and coefficient of variation. All animal weights were measured twice weekly and recorded. This study using experimental animals was conducted in accordance with the internationally accepted principles for laboratory animal use and care [18] with the approval of the Animal Care and Use Review Committee of the Institution.

#### Diets, Composition and Feeding

The composition of the diets in this study was based upon the standard diet formulas used to assess weight gain in rodents during commercial feeding studies. The control (normal ration) and the test (Zn-supplemented ration) diets were prepared from ingredients purchased from a commercial market in Ibadan metropolis, Oyo State, Nigeria according to the compositions (expressed in percentage per 100g feed) shown in Table 1. Both control and test diets contain same calories while zinc sulphate heptahydrate ( $ZnSO_4 \cdot 7H_2O$ ) from Koch-light laboratories Ltd Colinbrook Berks England was used as the source of the zinc which was mixed with the control diet to form the test diet. The amount of zinc used in the test diets for daily serving size was based on the total weight of the number of rats per group equivalent to 50 mg  $ZnSO_4 \cdot 7H_2O$  per kg diet. The animals were fed according to the experimental design for 8 weeks with water *ad libitum*. Body weight and total food intake of each group of rats were measured and recorded weekly while the food conversion ratio (food intake/weight gain) was calculated.

Nutrient Components	Ingredients	Control Diet (%)	Test Diet (%)
Carbohydrate	Maize	40	40
Fibre	Wheat offal	15	15
Protein	Soya bean meal	10.5	10.5
Fats and Oils	Palm kernel cake	20	20
	Groundnut cake	10	10
	Vitamins B, C, D	0.25	0.25
Minerals	Oyster shell	1.0	1.0
	Bone meal	3.0	3.0
Amino Acids	Methionine, Lysine	0.2	0.2
Supplement (trace element)	Zinc Sulphate ( $ZnSO_4$ )	-	0.005
Metabolizable Energy kcal/kg)		2337.45	2337.45
Crude protein (%)		18.58	18.58

**Table 1:** Composition of Control and Test Diets (%/100g feed).

### Induction of Diabetes

After 15 hour overnight fast following acclimatization, rats in DC and DHP groups were injected by single intraperitoneal injection of 150 mg/kg body weight of freshly prepared 2% Alloxan monohydrate (Sigma chemicals, USA) dissolved in sterile 0.9% normal saline in a standard volumetric flask strapped with foil to prevent alloxan instability. Diabetes was confirmed 4 - 7 days later by use of glucometer (On Call Plus Blood Glucose Monitoring System, ACON Laboratories, Inc. San Diego, USA.) and compatible strips. Rats with Fasting Blood Glucose (FBG) level > 150 mg/dl were considered diabetic and used for this study since the level of serum glucose considered to be normal in *Rattus norvegicus* ranges from 50 - 135 mg/dL [19]. Diabetes was allowed to stabilize for 5 days before animal grouping and exposure to experimental diets. Fasting blood glucose level of all rats in each experimental group was measured on weekly basis for the eight-week study period.

### Blood Collections and Biochemical Assays

#### Weekly Fasting Blood Glucose Concentrations Measurement

Fasting blood glucose level of all rats in each experimental group was measured twice on weekly basis for the eight-week study period by use of glucometer (On Call Plus Blood Glucose Monitoring System, ACON Laboratories, Inc. San Diego, USA.) and compatible strips. Blood samples were collected from the cordal veins of the rats.

#### Oral Glucose Tolerance Test (OGTT) Analysis

Animals in all groups were fasted overnight with free access to water before the last day of eight-week and were administered oral D-glucose load of 2g kg<sup>-1</sup> (dissolved in distilled water) by means of cannula after taking the initial fasting blood glucose (FBG) concentration. Thereafter, blood samples were withdrawn from the tail vein of each animal (tail snipping) to determine the fasting blood sugar concentration at intervals of 30, 60, 90, 120 and 150 minutes using glucose analyzer (On Call Plus Blood Glucose Monitoring System, ACON Laboratories, Inc. San Diego, USA).

#### Pancreas Extraction and Histological Analysis

At the end of the study, animals in all groups were anesthetized using Ethyl Ether in a glass dome and then dissected to extract the pancreas which were rinsed and weighed. Immunocytochemistry and histomorphometry of the pancreatic tissues were carried out using standard laboratory techniques. Extracted pancreatic tissues were placed in 10% formalin solution for a day. All samples were then de-

hydrated in graded ethanol series, cleared in toluene and embedded in paraffin wax; 5 - 6  $\mu$ m sections were routinely stained with Harris hematoxylin and eosins stains (Sigma-Aldrich) and were assessed under light microscope (Nikon Eclipse E400).

**Statistical Analysis**

Data was analyzed using appropriate statistical methods and program of Microsoft Excel and SPSS v. 22.0 (SPSS Inc., Chicago, USA). Results are expressed as group mean  $\pm$  SEM (Standard Error of Mean). Comparisons between groups and the significant difference between the control and the treated groups were analyzed using one way analysis of variance (ANOVA) and students' t - test. P values of < 0.05 were considered statistically significant.

**Results**

**Effect of Zinc-supplemented Diet on Body Weight Gain**

**Body Weight and Weight Gain**

The effect of Zn-supplemented diet on mean body weights is presented in Table 2. Overall percentage weight gain after 8 weeks was significantly (P = 0.020) reduced in DZ rats compared with DC and NC rats as suggested by standard ANOVA. Paired T-test analysis showed that there is a significant reduction in anthropometric parameter in the DZ rats. No significant (P > 0.05) difference observed in total food intake between experimental groups while a significant (p < 0.05) difference was observed in the food conversion ratio (food intake/weight gain) between DZ and DC rats.

Parameters	Experimental Animal Categories		
	Normal Control NC	Diabetic Control DC	Diabetic Zn-Treated DZ
Initial Weight (g)	200.2 $\pm$ 1.78	202.14 $\pm$ 1.82	205.12 $\pm$ 2.10
Final Weight (g)	226.12 $\pm$ 2.20	228.15 $\pm$ 1.60	175.30 $\pm$ 0.18
Weight change (%)	13.09	12.86	-14.71*

**Table 2:** Effect of Zinc-supplemented Diet on Body Weights (n = 8).

Values are expressed in mean  $\pm$  SEM, \*Significant (p < 0.05) when compared with diabetic control - DC.

**Effect of Zinc-supplemented Diet on Serum Fasting Blood Sugar (FBS) Concentrations**

Table 3 depicts the hypoglycemic effect of Zn-supplemented diet on serum FBS (mg/dL) in diabetic rats. A significant (P = 0.04) reduction (31.52%) in serum FBS concentrations was observed in DZ rats as revealed by the paired T-test analysis while the diabetic control (DC) rats showed no significant difference in their serum FBS concentrations. Difference between the mean percentage change in serum FBS values of the DZ and DC rats was comparably significant (p < 0.05).

Parameters	Experimental Animal Categories		
	Normal Control NC	Diabetic Control DC	Diabetic Zn-Treated DZ
Baseline FBS (mg/dL)	55.40 $\pm$ 4.74	169.48 $\pm$ 4.20	256.00 $\pm$ 10.21
Final FBS (mg/dL)	55.20 $\pm$ 4.99	170.33 $\pm$ 7.01	175.30 $\pm$ 19.19
% change (8 weeks)	0.36	0.50	31.52*

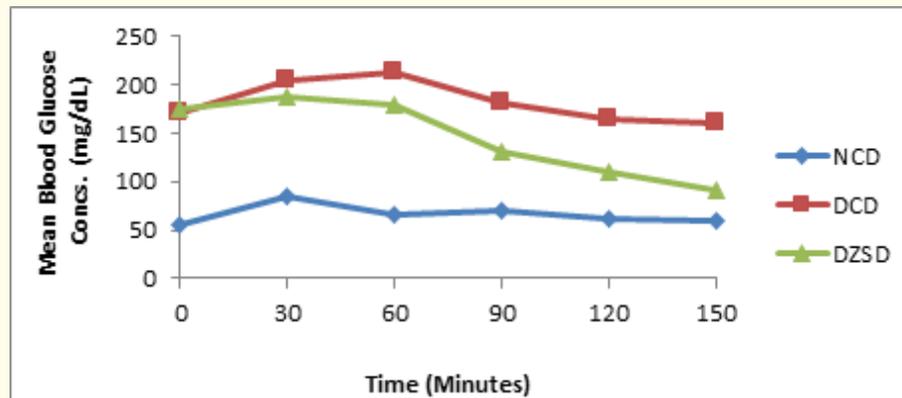
**Table 3:** Effect of Zinc-supplemented Diet on Serum Fasting Blood Sugar Concentrations (n = 8).

Values are expressed in mean  $\pm$  SEM, \*Significant (p < 0.05) when compared with diabetic control - DC.

**Effect of Zinc-supplemented Diet on Glycemic Tolerance**

Effect of Zn-supplemented diet on glycemic tolerance is depicted by the glycemic response curves shown in Figure 1 below. Zn-supple-

mented diet caused improved glycemic tolerance in DZ rats over that of the diabetic control as assessed by the incremental areas under the glycemic response curves.



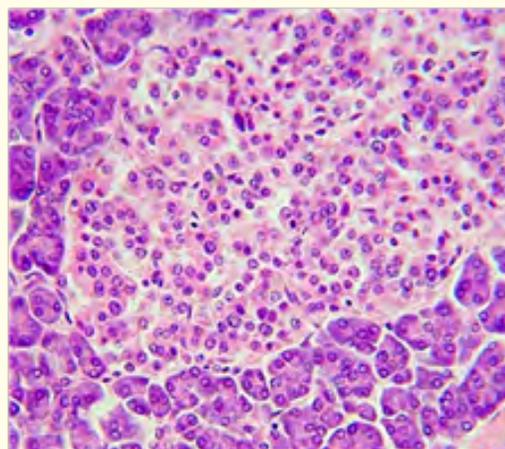
**Figure 1:** Glycemic response curves of experimental rats after 8 weeks ( $n = 8$  each/group).

*NCD: Normal control group; DCD: Diabetic control group; DZSD: Diabetic group fed on Zn-supplemented diet*

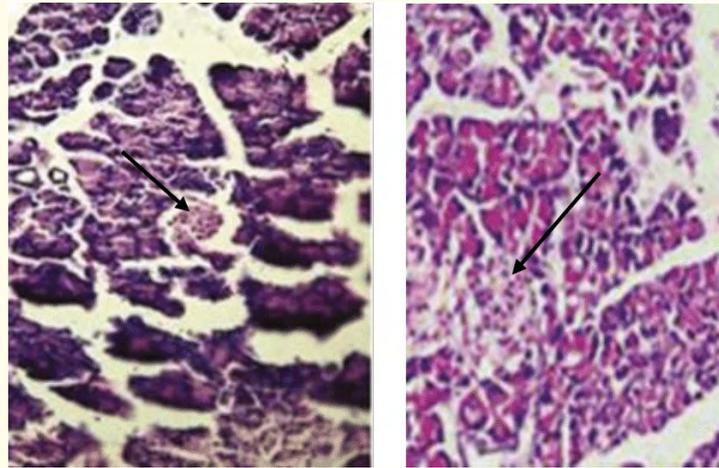
### Pancreas Histological Analysis

#### Effect of Zinc-supplemented Diet on Pancreas Histomorphometry

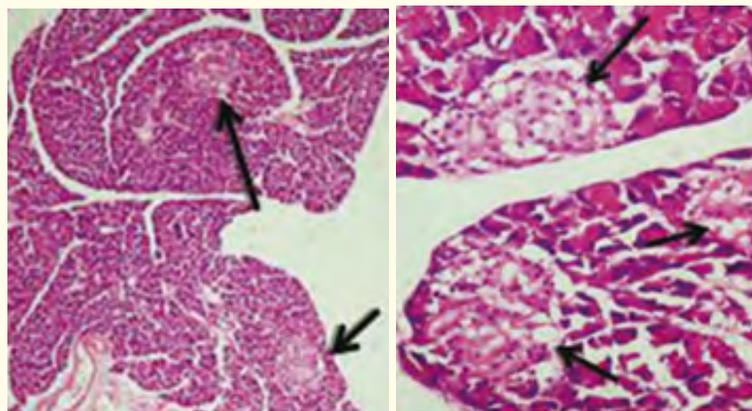
Under high power magnification ( $\times 400$ ) light microscopic examination, the photomicrographs (H and E stained) of the pancreas sections were closely examined. Photomicrograph of the pancreas from NC rats (Figure 2) demonstrates normal histoarchitecture of islet cells without visible regeneration or proliferation while that of the diabetic control (DC) rats (Figure 3) showed indefinite margin of the islet of Langerhans with some necrotic parenchyma and mild adipose tissue and lymphocytic infiltration. Photomicrographs of the DZ rats (Figure 4) revealed pronounced changes in pancreas histomorphometry with numerous and clustered islet cells showing some visible regeneration of some islet  $\beta$ -cells interspersed with scanty necrotic areas of parenchyma.



**Figure 2:** Photomicrograph of pancreas from normal control (NC) rats after 8 weeks demonstrating normal histoarchitecture of islet cells without visible islets  $\beta$  cells regeneration or proliferation.



**Figure 3:** Photomicrograph of pancreas from diabetic control (DC) rats after 8 weeks showing indefinite margin of the islet of Langerhans (arrows) with some necrotic parenchyma, mild adipose tissue infiltration and lymphocytic cells aggregation.



**Figure 4:** Photomicrographs of pancreas from Zn-supplemented diet-fed diabetic (DZ) rats after 8 weeks depicting variable small sized and shaped regenerating islets of Langerhans (left arrows) with some visible proliferating islet  $\beta$ -cells (right arrows).

## Discussion

The effects of zinc-supplemented diet on body weight gain, pancreas histomorphometry, islet  $\beta$ -cells regeneration, glycemic tolerance and control in diabetic rats was assessed in this experimentally-controlled nutritional study which lasted for eight weeks. Findings obtained revealed that Zn-supplemented diet significantly reduced mean body weight gain and fasting blood glucose concentrations, stimulated islet  $\beta$ -cells regeneration and improved glycemic tolerance and control in diabetic rats. These antidiabetic, antiobesity and islet  $\beta$ -cells regenerative activities of Zn-supplemented diet displayed its beneficial potentials in dietary control of diabetes mellitus more especially in developing countries where the prevalence of zinc deficiency is relatively high.

The composition of the diets used in this study was based upon the standard diet formulas used to assess weight gain in rodents during commercial feeding studies. The effect of zinc-supplemented diet on body weight and weight gain was assessed in diabetic rats. In this study, a significant reduction in mean body weight gain was observed in diabetic rats fed on Zn-supplemented diet compared with their

diabetic control. This observation, tallies with the findings of other previous studies using animal [20] and human subjects [21] which reported reduction in the observed values of anthropometric parameters such as weight, waist circumference and body mass index. Weight loss is an effective approach in controlling obesity and it has been demonstrated that weight loss improves plasma concentration of glucose, insulin and lipids. Moreover, weight loss has a positive effect on increasing plasma zinc concentration [22]. Possible mechanisms of weight reduction by zinc could arise either from the role of zinc on appetite regulation via leptin system and its receptor through changes in hypothalamic neurotransmitter metabolism [23], preventive role of zinc in the gene mutation which can increase the risk of obesity [24] or similarity of zinc to insulin action in terms of insulin sensitivity and resistance [25].

Zn-supplemented diet in diabetic rats demonstrated beneficial blood glucose lowering effect in this study as it resulted in significant reduction in fasting blood glucose level (31.52%) with improved glycemic tolerance and response as shown by the timed glycemic response profile (Figure 1). This significant change observed in the supplemented groups reflects the effective improvement in their glycemic control by the zinc-supplementation diet. This observation corresponds with the results of other studies [17,26] that examined the effect of zinc supplementation on patients with type 2 diabetes. Zinc is readily available in animal foods especially lean red meat, beef, liver, fish, and eggs. Based on these findings, diabetics should be encouraged to consume Zn-rich diets (if no contraindication exists) in moderation in order to achieve optimal glycaemic control. Where this is not feasible, then, the prescriptive use of a multivitamin and minerals supplement may be a suitable alternative.

Effect of zinc on pancreas histoarchitecture and morphometry was remarkably examined in this study. In the supplemented-grouped pancreas micrographs, zinc stimulated and enhanced islets cells proliferation and  $\beta$ -cells regeneration as evidenced by variable clusters of small sized and shaped regenerating islets of Langerhans with some visible proliferating islet  $\beta$ -cells. This observation differs from that of the diabetic control where islets with indefinite margin, infiltrated with lymphocytic cells and mild adipose tissue were observed. The mechanism of this observed  $\beta$ -cells hyperplasia may be as a result of recruitment from proliferating pancreatic stem cells induced by zinc or as a result of pancreatic duct cells stimulation by zinc as reported by other research studies [27,28]. The observed decrease in fasting blood glucose levels and the improved glycemic tolerance in DZ rats may also be contributed by the expansion of beta cell mass stimulated and enhanced by zinc supplementation. This beneficial effect may compensate for increased insulin demands in diabetics necessary for the control of blood glucose homeostasis. The pancreas has been reported as the organ with the lowest levels of antioxidant enzymes; consequently, pancreatic  $\beta$ -cells are exceptionally vulnerable to detrimental actions of oxidative stress [29]. However, recent studies have shown that pancreas has the propensity to undergo repair following damage by a regeneration process needed for maintenance of critical cell mass necessary for organ homeostasis. Considering the above facts, researchers have directed their focus on factors and stimuli that can induce pancreatic tissue regeneration as a means of abating problems associated with diabetes. Accordingly, this study, which was carried out to examine the regenerative ability of zinc on pancreatic islet beta cells, evidently proved that supplementing zinc in diabetic diets beneficially improved good glycemic control in diabetics. While further studies are needed to establish the mechanisms of zinc actions in diabetes control, necessary surveillance should be put in place to avoid abuse use of zinc supplements that might culminate in zinc toxicity which eventually promotes obesity and related diseases in adolescents and makes diabetic patients more susceptible to complications due to elevated HbA1c level associated with zinc toxicity as reported by Singh., *et al* [30].

## Conclusion

Zinc is one of the most important essential trace metals in human nutrition and lifestyle. Its deficiency may severely affect the homeostasis of a biological system. This experimentally-controlled nutritional study provides clear evidence of beneficial impacts of zinc-supplemented diets on glycemic control and pancreatic islets regeneration in diabetic rats which could be attributed to its anti-obesity, antidiabetic and antioxidant properties. Therefore, consumption of zinc-rich diets or otherwise, zinc supplements, should be encouraged with moderation in diabetics for optimal glycemic control.

## Conflicts of Interest

No conflict of interest exists.

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