INTRODUCTION

The significance of zinc (Zn) in human nutrition and public health has recently gained focus due to its proven beneficial impacts and applications. Ensuring adequate levels of zinc intake should be a key concern in efforts to reduce morbidity and mortality. Zinc is a key component of several enzymes and hormones in humans and plants. As an important trace element and micronutrient, Zn plays a key role in various physiological processes.

ABSTRACT

**Background:** Findings on the effects of zinc supplementation on lipid profile (LP) in type 2 diabetic human subjects and animals are conflicting. However, reports of nutritional studies on the relationship between body mass index (BMI), LP, and obesity are well established. This experimentally controlled designed study aimed to determine the metabolic and anthropometric effects of zinc-supplemented diet on LP and BMI in obese diabetic and non-diabetic rats and to correlate the association between BMI and serum triglycerides (TGs). **Materials and Methods:** Twenty-four male Wistar rats weighing 300–350g were categorized into three experimental groups (n = 8, each): Obese diabetic rats on zinc-supplemented diet (diabetic treated [DZSD]); obese diabetic rats on normal diet (diabetic control [DCD]); and obese non-diabetic rats (normal control [NCD]). Obesity and diabetes were inducted, respectively, with hyperlipidemic diet and alloxan monohydrate (150 mg/kg bw. peritonally). Fasting blood sugar (FBS) values >150 mg/dL were considered diabetic. Animals were fed according to the experimental design for 8 weeks. Body weight and nasaonal length used to determine the BMI were measured weekly throughout the study. Blood samples were taken for LP analysis. Data were analyzed using Microsoft Excel and statistical program SPSS version 22 while correlation between BMI and serum TG was determined using Pearson correlation test. *P* < 0.05 was considered significant. **Results:** The mean weight gain (%) and the mean BMI (g/cm²) decreased significantly in DZSD rats (bw – 16.02%, *P* = 0.03; BMI – 0.71 ± 0.01, *P* = 0.02) compared with the DCD rats (bw – 26.28%; BMI – 0.76 ± 0.12). Serum concentrations of TG (10.75 mg/dL), total cholesterol (TC) (16.78 mg/dL), and low-density lipoprotein (LDL) (6.51 mg/dL) decreased while high-density lipoprotein (HDL) concentration (8.12 mg/dL) increased significantly (*P* < 0.05) in treated rats compared with the DCD rats (TG – 12.35 mg/dL; TC – 18.89 mg/dL; LDL – 10.23 mg/dL; and HDL – 6.19 mg/dL). A significant (*P* = 0.023) positive correlation (*r* = 0.069) exists between serum TGs and BMI. **Conclusion:** Dietary zinc supplementation impacts beneficial metabolic and anthropometric effects on BMI and LP in obese diabetic rats.

**Key words:** Body mass index, lipid profile, obesity, rats, type 2 diabetes, zinc-supplemented diet
in several metabolic processes of macronutrients as well as in normal physiological functions of skin, teeth, bones, hair, nails, muscles, nerves, and brain. Its involvement in synthesis, storage, release, and action of insulin has proved that its deficiency state is associated with insulin resistance, impaired glucose tolerance, and obesity which are risk factors for diabetes mellitus. Hyperzincuria observed in frequent urination in diabetics appears to contribute to the marginal zinc nutritional status which may explain the inadequate efficacy of oral hypoglycemic agents observed at times as a result of post-receptor events associated with oxidative stress induced by long-term hyperglycemia. 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. The previous studies indicated that marginal zinc deficiency is more prevalent among diabetic adults, compared to the normal adult population while abnormal zinc plasma levels occur more frequently in metabolically uncontrolled diabetic patients. Dietary supplementation of zinc in diabetes has been shown to improve life quality and expectancy as reported by both animal and human studies. Hence, it is necessary to carry out more interventional studies in this regard to unravel various mechanisms or pathways through which zinc mediates its antilipemic, antidiabetic, and antiobesity activities at cellular and tissue levels. Most studies investigating the efficacy of Zn in glycemic and lipid control administered zinc orally in its salt forms without incorporating it in the diet. In this study, the zinc sulfate used was mixed with the diet and rationalized to ensure adequate consumption of recommended dosage necessary for optimal weight reduction, glycemic, and lipid control. This experimentally controlled design nutritional study aimed to determine the metabolic and anthropometric effects of zinc-supplemented diet on lipid profile (LP) and body mass index (BMI) in obese diabetic and non-diabetic rats and to correlate the association between BMI and serum triglycerides (TGs) with the rationale to ascertain the suitability of such diet in controlling obesity and improving serum LP in diabetic individuals.

**MATERIALS AND METHODS**

**Experimental animals and design**

Twenty-four adult male Wistar rats (*Rattus norvegicus*) weighing ≥150g were purchased from the disease-free stock of Olu Animal Research Farm in Iwo, Osun State, Nigeria. They were fed initially with hyperlipidemic diet (60% fat) to induce obesity until their weight reaches the range of 300–350 g necessary for this experimental study. Thereafter, the animals were allowed to acclimatized for 2 weeks before exposure to experimental diets in raised stainless steel cages with 6 mm mesh floor (to maintain same physical activity) kept in a well-ventilated animal house maintained under standard conditions (12:12 h light:dark cycle; 25 ± 2°C, relative humidity). After acclimatization, rats were randomly divided into three groups of eight rats each: Obese diabetic rats fed with zinc-supplemented diet (diabetic treated [DZSD]); obese diabetic rats fed with normal diet as diabetic control (DCD); and obese non-diabetic rats (normal control [NCD]) fed with normal diet as NCD. The experimental diets were introduced after 2 weeks acclimatization according to the experimental design: DCD and NCD rats were fed with normal diet while DZSD rats were fed with test diet, as shown in Table 1. 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. Each group had a close entry value of mean body weight and coefficient of variation. All animal weights were measured weekly and recorded. This study using experimental animals was conducted in accordance with the internationally accepted principles for laboratory animal use and care 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 on 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 (expressed in percentage per 100 g feed) were prepared from ingredients purchased from a commercial market in Ibadan Metropolis, Oyo State, Nigeria, and composed as shown in Table 1. Both control and test diets contain same calories while zinc sulfate heptahydrate (ZnSO₄·7H₂O) from Koch-Light Laboratories Ltd., Colnbrook, 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 ZnSO₄·7H₂O 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.

**Induction of diabetes and obesity**

Obesity was induced with hyperlipidemic diet (60% fat) until weight range of 300–350 g was achieved. After 15 h overnight fast, rats in DCD and DZSD 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. Since the level of serum glucose considered being normal in *R. norvegicus*, ranges from 50 to 135 mg/dL, rats with fasting blood sugar (FBS) level >150 mg/dL were considered diabetic to be used in this study. Diabetes was allowed to stabilize for 5 days before exposure to test diets.

**Measurement of anthropometric parameters**

Anthropometric parameters (body weight, length, and waist circumference) of all the rats were measured twice a week according to standard methods over a period of 8 weeks. Weight was measured to the nearest gram using automated weighing balance while the length and waist circumference expressed in centimeters were measured using a measuring tape beginning from the nose to the anus and around the waist at hip region above the iliac crest, respectively. Values obtained were used to calculate the BMI expressed as weight (g)/square of length (cm²).

**Blood collections and biochemical assays**

**Glucometric assay**

With the use of glucometer (On Call Plus Blood Glucose Monitoring System, ACON Laboratories Inc., San Diego, USA) and compatible strips, blood samples collected from the cordal veins of the rats were used to determine the FBS concentrations of each rat on weekly basis throughout the study period.

**LP assay**

The LP was conducted at the beginning and at the end of the study. Blood samples obtained by cardiac puncture under light anesthesia were collected and transferred into the k₃ EDTA (ethylenediaminetetraacetic acid) sample bottles. Samples were centrifuged at 3000 revolutions to obtain the plasma fractions which was kept in a refrigerator (at −70°C) until used and the sera obtained were used for the biochemical assay of the LP. Plasma concentration of total cholesterol (TC), high-density lipoprotein (HDL), and triacylglycerol (TAG) was measured by the enzymatic colorimetric method after centrifugation using a dry chemical automatic analyzer AU-5200 OLYMPUS (Randox Laboratories, San Francisco, USA). Low-density lipoprotein (LDL) level was determined by the Friedewald formula as follows:

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VLDL (mg/dL) = \frac{TAG}{5}.
\]

\[
LDL (mg/dL) = TC - VLDL - HDL.
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**Statistical Analysis**

Data were analyzed using appropriate statistical methods and programs of Microsoft Excel and SPSS v. 22. Results are expressed as group mean ± SEM. Comparison between control and treated groups was analyzed using one-way analysis of variance (ANOVA) while the correlation between BMI and serum TGs was determined by Pearson correlation test. *P* < 0.05 was considered statistically significant.

**RESULTS**

**Effect of zinc-supplemented diet on body anthropometry**

**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* = 0.03) reduced in DZSD rats compared with DCD and NCD rats as suggested by standard ANOVA. No significant (*P* > 0.05) difference observed in total food intake and food conversion ratio between experimental groups.
Length and waist circumference
Effect of test diet on length and waist circumference is shown in Table 3. A significant ($P < 0.05$) decrease in waist circumference without alteration in body length was observed in zinc-treated rats compared with the diabetic and healthy controls.

BMI
Table 4 depicts the impact of zinc-supplemented diet on BMI (g/cm$^2$). A significant ($P = 0.02$) reduction in mean BMI was observed in diabetic rats fed with zinc-supplemented diet compared with the diabetic and NCD.

Effect of zinc-supplemented diet on LP
Effect of Zn-supplemented diet on the LP of grouped rats is illustrated in Figure 1. Values of the lipid parameters at the onset were similar across groups. At the end of the 8th week, a significant ($P < 0.05$) decrease in serum concentrations of TG (41.02 mg/dL), TC (80.68 mg/dL), and LDL (14.22 mg/dL) with concomitant increase in HDL concentration (58.26 mg/dL) was observed in treated (DZSD) rats compared with the DCD rats (TG – 55.93 mg/dL; TC – 102.12 mg/dL; LDL – 51.67 mg/dL; and HDL – 39.26 mg/dL). Pearson correlation test revealed a positive correlation between serum TGs and BMI [Table 5].

DISCUSSION
The anthropometric and metabolic effects of zinc-supplemented diet on BMI and LP were assessed in this study while the correlation between the BMI and the serum TGs was determined. Findings revealed that Zn-supplemented diet significantly reduced mean body weight gain, weight circumference, and BMI with improved LP in experimental diabetic male rats. These antiobesity and antilipemic effects of Zn-supplemented diet displayed its beneficial potentials in dietary control of obesity-related type 2 diabetes mellitus more especially in uncontrolled diabetics in developing countries where the prevalence of zinc deficiency is relatively high. Diabetic rats treated with Zn-supplemented diet also showed improved glycemic control as reported in our previous studies [12,13].
The composition of the diets used in this study was based on the standard diet formulas used to assess weight gain in rodents during commercial feeding studies. The effects of zinc-supplemented diet on body weight gain and BMI were assessed in this study. A significant reduction in mean body weight gain and BMI was observed in diabetic rats fed on Zn-supplemented diet compared with their DCD. This observation agrees with the findings of other previous studies using animal\(^\text{[12,21]}\) and human subjects\(^\text{[22,23]}\) which reported reduction in the observed values of anthropometric parameters such as weight, waist circumference, and BMI.

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.\(^\text{[24]}\) Possible mechanisms of weight reduction by zinc could arise either from the role of zinc on appetite regulation through leptin system and its receptor through changes in hypothalamic neurotransmitter metabolism,\(^\text{[25]}\) predictive role of zinc in the gene mutation which can increase the risk of obesity,\(^\text{[26]}\) or similarity of zinc to insulin action in terms of insulin sensitivity and resistance.\(^\text{[27]}\)

Figure 1 illustrates the effects of Zn-supplemented diet on LP. Values of the lipid parameters at the onset were similar across groups. At the end of the 8th week, a significant decrease in serum concentrations of TG, TC, and LDL concentrations with concomitant increase in HDL concentration occurred in Zn-treated rats compared with the control groups. An observed positive correlation exists between serum TGs and BMI in this study indicating the possibility of identifying individuals with tendency to develop obesity and its related cardiometabolic complications. Thus, consecutive values of serum TG concentrations can be used to monitor progress during management of hyperlipidemia and obesity-related disorders.

Hyperlipidemia resulting from consumption of high-fat diet has been demonstrated to affect the blood lipid chemistry.\(^\text{[28,29]}\) Thus, it is essentially advisable to have routine check of serum zinc level in individuals for possible detection of deviations from normal (dyslipidemia) which may affect body physiology and overall health. Moderate consumptions of fatty foods according to some epidemiological studies\(^\text{[30-32]}\) have revealed reasonable reductions in metabolic and cardiovascular risks associated with obesity.

While findings from some studies on the effects of zinc supplementation on LP in type 2 diabetic human subjects and animals are conflicting, results obtained from this study, revealed the beneficial potentials and impacts of zinc on LP and BMI. These observed effects in animal study, tally with the findings of other studies using human subjects which reported beneficial effects of Zn supplementation on serum concentrations of TC, TGs, LDL cholesterol, and HDL cholesterol in type 2 diabetic patients at the dosage of <100 mg/day.\(^\text{[33,34]}\) To avoid this conflicting variability, more interventional studies in this regard should be carried out for elucidation while standardization of the mode of administration, type, dose, and time of zinc supplementation is also necessary.

**CONCLUSION**

This study provides clear evidence of beneficial anthropometric and metabolic impacts of zinc-supplemented diets on LP and BMI in diabetic rats which may be attributed to its antiobesity, antilipemic, and antioxidant properties. It also depicts the positive correlation between BMI and serum TGs. For optimal control of dyslipidemia in obese-related diabetes and minimization of the associated risks and complications, consumption of zinc-rich diets or otherwise, zinc supplements, under nutritionist and physician guide, should be encouraged and recommended with moderation.

**REFERENCES**

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