RESEARCH

Effect of Selenium and Zinc Supplementation on Reproductive Organs Following Postnatal Protein Malnutrition

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Abstract

Protein diets are required for the normal development of the reproductive system and their inadequacy or deficiency might have hazardous functional complications during maturational and developmental stages. The study was carried out to evaluate the effect of selenium (Se) and zinc (Zn) supplementation on the male and female reproductive organs of rats with postnatal protein malnutrition. Male and female weanling rats were randomly assigned to six groups respectively. The adequate protein diet rats were fed with 16% casein diet while the protein malnourished diet (PMD) rats were fed with 5% casein diet. After the 8th week of feeding, Se (sodium selenite; Na_2SeO_3) and Zn (zinc sulfate; $ZnSO_4 \cdot 7H_2O$) were supplemented for 3 weeks. The growth curve of body weights, lipid profile, testosterone and progesterone level, Na^+-K^+-ATPase activity, oxidative stress, and antioxidant status were evaluated. The results showed that PMD reduced the body weights of male and female rats. It also reduced the activities of catalase and glutathione peroxidase in the testes, but reductions in superoxide dismutase and glutathione-S-transferase activities, glutathione, vitamins C and E, testosterone, and progesterone levels were observed in both the testes and ovaries. Furthermore, PMD increased the nitric oxide level in both organs and altered the plasma lipid profiles in both sexes. Se and Zn supplementation, however, restored almost all the alterations observed in all the parameters analyzed. In conclusion, Se and Zn supplementation protects the male and female reproductive organs of rats against postnatal protein malnutrition.

Keywords Antioxidant · Ovary · Progesterone · Lipid profile · Testis · Testosterone

Introduction

Protein malnutrition (PM) is a nutritional disorder, which poses a global concern, especially among young infants in Africa, Latin America, Asia, and many underdeveloped or developing countries [1]. Proper nutrition is essential for the normal growth and development of the reproductive system and its associated processes. PM has been reported to cause hazardous functional complications during maturational and developmental stages to both male and female reproductive organs. It has been demonstrated that PM changes the testicular structure and causes a permanent effect on their capacity to produce spermatozoa and/or decreases daily sperm production [2]. Moreover, evidence shows that PM reduces the weights of the testis, seminal vesicle, and epididymis [3, 4]. Also, reduction in the level of serum testosterone and impairment in sperm production have been reported in protein-malnourished rats [5]. Furthermore, PM reduces the thickness of the uterine endometrium, and the level of androgen receptors, and increased the estrogen receptor β [6].

It has been reported that oxidative damage from free radicals is accentuated in protein-malnourished animals leading to loss of protein function and altered cellular redox balance

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resulting in impairment in tissue function [7]. In addition, reactive oxygen species (ROS) has been implicated in male infertility [8] as well as female infertility and impaired ovarian functions [9]. Antioxidants are essential for normal function and the maintenance of redox balance in both testes and ovaries. For example, testes possess high concentrations of antioxidants that protect against oxidative stress and thus play critical roles in the prevention of testicular atrophy and maintenance of spermatogenesis [10]. However, it has been reported that deficiency of Se, Zn, and other micronutrients usually occurs in plasma and cells after exposure to a low-protein diet [11, 12].

Selenium (Se), with atomic number 34, is a trace element required for cellular functions in animals and other organisms. It is covalently incorporated into peptide chains as a component of selenoproteins occurring as selenocysteine [13]. Se, via the functions of seleno-dependent enzymes and selenoproteins, plays an essential role in redox reactions and male reproduction as it is highly expressed in the testes [14]. Se is an essential component of glutathione peroxidase (GPx) selenoprotein P (Sel P), thioredoxin reductase (TXNRD), and selenoprotein V (Sel V) which are highly expressed in the testes [15, 16]. The GPx has been documented as an important antioxidant for spermatogenesis [15]. Moreover, Abedelahi et al in an in vitro study showed that Se reduced ROS levels and increased the total antioxidant capacity and GPx activity resulting in improved follicular development [17]. Furthermore, Se confers protection on ischemia/reperfusion injury in a rat ovary model [18]. Li et al. showed that dietary selenium-deprived chicks exhibited poor development of testis and impaired sex hormone synthesis. It also reduces antioxidant enzyme activity and increased the expressions of mRNA and protein autophagy-related factors [19]. Se enhanced reproductive performance, progesterone levels, and animal health [20]. It improves semen quality and reproductive hormone concentration of Saanen goat kids [21].

Zn, another trace element with atomic number 30, is essential as a cofactor in stabilizing proteins structurally and it is useful in enzymatic catalysis. The antioxidant capacity of Zn has been associated with its ability to induce metallothioneins, confer protection on protein sulfhydryls, and also reduce the formation of hydroxyl radicals through the antagonism of redox-sensitive transition metals [7, 22]. Deficiency of Zn has been linked to insufficient dietary intake, malabsorption, chronic liver and renal diseases, and other sicknesses [23]. Deficiency in Zn in the male reproductive system has been associated with impotence, hypogonadism, or delayed sexual development [22, 24] whereas several pathological conditions like abnormal ovarian development, prolonged gestation period, impaired synthesis and/or secretion of follicle-stimulating hormone and luteinizing hormone (LH), and disruption of the menstrual cycle have been reported for female [25, 26]. Zn supplements have been used for cancer prevention and it regulates primary ovarian tumor growth and metastasis [27] and also for a reduction of prostate size in benign prostate hyperplasia [28]. Zn supplementation can improve testosterone levels and sexual function in postmenopausal women [29].

Although several studies have been reported on male and female reproductive organs, and the effects of Se and Zn supplementation on them, yet there is a paucity of information on the role of Se and Zn on the reproductive organs of rats subjected to postnatal protein malnutrition. We hypothesize that Se and Zn supplementation reverses biochemical alterations arising from postnatal protein malnutrition in male and female reproductive organs. Therefore, the study was carried out to investigate biochemical parameters and the effects of Se and Zn supplementation on the male and female reproductive organs of rats exposed to postnatal protein malnutrition.

Materials and Methods

Animal and Diets

Seventy-eight rats, 39 male and 39 female weanlings Wistar rats (3–4 weeks old), were procured from Animal House, University of Lagos Teaching Hospital (LUTH), Lagos, Nigeria. The rats were housed in polypropylene cages and were allowed to acclimatize for 2 weeks. The rats were fed with commercial rat chow and water during the period and maintained at 12 h of light-dark cycles at room temperature throughout the experiment. The procedures on animal handling were approved by the Animal Ethical Committee of Olabisi Onabanjo University, Ogun State, Nigeria, and were following the principle of *NIH Guidelines for Humane Use and Care of Laboratory Animals.* The protein malnourished diet (PMD) contained 5% casein while the adequate protein diet (APD) contained 16% casein. The other components of the PMD diets include carbohydrates, vitamins, fats, and minerals mixture; all these are in sufficient amounts with an adequate amount of iodine (32.5 mg per 100 g) as previously reported by Adebayo et al. [7, 30].

Experimental Protocols

Male and female weanling rats were randomly assigned to six groups respectively after acclimatization. The APD rats which serve as the control group contained five rats per group and were fed with 16% casein diet, while the PMD rats contained eight rats per group and were fed with 5% casein diet. After the 8th week of feeding, Se (sodium selenite; Na_2SeO_3) and Zn (zinc sulfate; $ZnSO_4 \cdot 7H_2O$) were supplemented at a concentration of 0.15 mg^{-1}L and 227 mg^{-1}L

respectively in drinking water (for both male and female groups) for 3 weeks as previously reported by Adebayo et al. [7, 31]. Both the APD and PMD rats continue with their respective diets until the 11th week when the experiment was terminated and rats were sacrificed.

The grouping and treatments are as follows:

Group A: APD containing 16% casein diet.

Group B: PMD containing 5% casein diet.

Group C: APD containing 16% casein diet was supplemented with Se (0.15 mg^{-1}L).

Group D: PMD containing 5% casein diet was supplemented with Se (0.15 mg^{-1}L)

Group E: APD containing 16% casein diet was supplemented with Zn (227 mg^{-1}L)

Group F: PMD containing 5% casein diet was supplemented with Zn (227 mg^{-1}L).

The rats were weighed every week to determine the growth curve of body weights and to evaluate the effect of Se and Zn supplementation on the weight by sex.

At the end of the treatments, blood samples from male and female rats were collected via ocular sinus using micro-hematocrit capillary tubes. The blood samples were carefully kept in sterilized anticoagulant-free bottles and centrifuged at 5000 rpm for 5 min using Eppendorf Centrifuge 5415R. The plasma collected was refrigerated and stored at −20 °C to be used later for lipid profile and hormonal assays.

Testes and Ovaries Collection

Male and female rats were sacrificed after diethyl ether anesthesia by decapitation and the testes were excised from males and ovaries were removed from female rats in each of the groups. The testes and ovaries were kept in the freezer at −20 °C until used.

Sample Preparation for Biochemical Estimations

Ten percent (w/v) testis and ovary homogenates were prepared in 0.1 M phosphate buffer, pH 7.4, by using a Potter-Elvehjem-type glass homogenizer. The homogenates were centrifuged at 5000 rpm in a cold centrifuge for 5 min. The supernatants were kept at −20 °C and used for various biochemical assays.

Biochemical Assays

The oxidative markers were assessed to determine the level of oxidative stress in the testes and ovaries of PMD-fed rats and the effects of Se and Zn supplementation on them by analyzing the following parameters.

Estimation of Lipid Peroxidation Level

Malondialdehyde (MDA), a by-product of lipid peroxidation (LPO), was quantified by measuring the amount of MDA formed as described by Varshney and Kale [32]. The method is based on the formation of pink chromophores when malondialdehyde is treated with 2-thiobarbituric acid. Briefly, 0.5 mL of 30% trichloroacetic acid (TCA) was added to a mixture of homogenate (0.4 mL from the supernatant of tissue samples) and 1.6 mL Tris-KCl buffer. This was followed by the addition of 0.5 mL of 0.75% thiobarbituric acid and then incubated in a water bath for 45 min at 80 °C. The mixture, after it was cooled down in ice, was centrifuged at 3000 ×g. The clear supernatant was collected and absorbance was measured against a reference blank of distilled water at 532 nm. The results were expressed as micromoles/milligram of protein using a molar extinction coefficient chromophore of 1.56×10^{-5} M^{-1} cm^{-1}.

Estimation of Nitric Oxide Level

Nitric oxide (NO) in conjunction with other reactive oxygen species contributes to oxidative stress. NO level was determined as described by Tracey et al. [33]. Fifty microliters of the sample supernatants was mixed with 1.5 mL of 0.1 M phosphate-buffered saline and 0.5 mL of Griess' reagents and the reaction mixtures were incubated at 25 °C for 15 min. The pink chromophore formed was measured at 540 nm and the results were expressed as micromoles per milligram of protein.

Estimation of Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity in the supernatants of both the testis and ovary was determined as described by Mistra and Fridovich [34] based on the inhibition of autoxidation of epinephrine (pH 10.2) at 30 °C. The assay mixture contained 20 µL of sample and 2.5 mL of 0.05 M carbonate buffer (pH 10.2). After equilibration in the spectrophotometer, 300 µL of freshly prepared solution of 0.3 mM epinephrine was added and mixed by inversion. The increase in absorbance at 480 nm was monitored in a spectrophotometer for 150 s at 30-s intervals. The activity of SOD was expressed as units/milligram of protein.

Estimatimation of Catalase Activity

Catalase (CAT) activity in the tissue supernatants was determined following the procedure of Sinha [35]. The diluted sample was added to the mixture of hydrogen peroxide-phosphate. The principle is based on the formation of chromic acetate when hydrogen peroxide reacts with a dichromate-glacial acetic mixture at 100 °C. Briefly, 1 mL

of 0.01 M phosphate buffer (pH 7.0), 0.1 mL of sample, and 0.4 mL of 0.2 M H_2O_2 were mixed. This was followed by the addition of 2 mL of dichromate-glacial acetic acid reagent (5% potassium dichromate and glacial acetic acid in a ratio of 1:3). The absorbance of the decomposition of hydrogen peroxide when acted upon by CAT and the reduction in the green coloration was measured at 570 nm. The results were expressed as micromoles/minute/milligram of protein using an extinction coefficient of 40 M^{-1} cm^{-1}.

Estimation of Glutathione Peroxidase Activity

GPx activity was determined as described by Ellman [36]. The reaction mixture contained 200 µL of 0.4 M phosphate buffer (pH 7.0), 100 µL of 10 mM sodium azide, 200 µL of 4 mM reduced glutathione, 100 µL of 0.2 mM hydrogen peroxide, and 200 µL of sample. The mixture was incubated at 37 °C for 10 min and the reaction stopped by adding 400 µL of 10% TCA and was later centrifuged at 5000 rpm. One milliliter of the supernatant was added to 0.5 mL of Ellman's reagent and 3 mL of 0.2 M phosphate buffer (pH 8.0). The absorbance was read at 412 nm. The results were expressed as µmol/min/mg protein.

Estimation of Glutathione-S-Transferase Activity

Glutathione-S-transferase (GST) activity was determined according to Habig et al [37]. Briefly, the assay mixture containing 0.03 mL of tissue supernatant, 2.79 mL of 0.1 M sodium phosphate buffer (pH 7.4), and 0.15 mL of 20 mM 1-chloro-2, 4,-dinitrobenzene in 95% alcohol was incubated at 37 °C for 5 min and then 0.03 mL of 20 mM glutathione (GSH) was added and mixed by inversion. The mixture was immediately read at 340 nm against a blank containing all the components except the sample for 180 s at 60-s intervals in a spectrophotometer. The results were expressed as micromoles/minute/milligram of protein using a molar extinction coefficient of 9.6×10^3 M^{-1} cm^{-1}.

Estimation of Reduced Glutathione Level

The level of glutathione (GSH) in the testis and ovary was estimated by the method of Sedlak and Lindsay [38]. The method is based on the development of a relatively stable yellow complex formed from the reaction between Ellman's reagent and free sulphydryl groups that absorbs maximally at 412 nm. The absorbance at 412 nm is proportional to the level of GSH in the sample. Briefly, 2 mL of 10% TCA was added to 1 mL of the sample and centrifuged at 5000 rpm for 10 min. One milliliter of the supernatant was added to the mixture of 0.5 mL of 0.01 M Ellman's reagent (5,5′-dithio-bis-2-nitrobenzoic acid) and 3 mL of 0.2 M phosphate buffer (pH 8.0). The absorbance was read at 412 nm and the results

were expressed as nanomoles/milligram of protein using 1.34×10^4 M^{-1} cm^{-1} as the molar extinction coefficient.

Estimation of Vitamin C Level

Vit C level was determined as described by Roe and Kuether [39]. To 0.1 mL of tissue supernatant, 0.3 mL of 6% TCA was added, stirred, allowed to stand for 5 min, and then centrifuged. To the supernatant was added 0.15 g of acid-washed norit, vigorously stirred, and filtered. To the filtrate, 0.1 mL of 0.1 M 2,4-dinitrophenylhydrazine was added and incubated at 37 °C for 3 h. The color was produced by adding 0.4 mL of 85% H_2SO_4 and then incubated for 30 min. The color developed was read at 540 nm and results were expressed as milligrams per 100 g.

Estimation of Vitamin E Level

The level of Vit E in the tissue sample was estimated as described by Baker et al. [40]. Lipid was extracted from the tissues according to Folch et al. [41]. 0.5 mL of lipid extract from the tissue was added to 1.5 mL of absolute ethanol and 2 mL of petroleum ether. The mixture was centrifuged at 3500 rpm for 5 min and the supernatant was evaporated to dryness at 80 °C. To this was added 0.5 mL of 0.2% 2–2′dipyridyl solution and 0.1 mL of 0.5% ferric chloride and kept in the dark for 5 min before 4 mL of butanol was added. The developed color was read at a wavelength of 520 nm and the result was expressed as milligrams per 100 g.

Determination of Na^+-K^+-ATPase Activity

The activity of Na^+-K^+-ATPase was determined according to the method of Lardy and Wellman [42] with slight modification. Briefly, 0.5 mL of sample was added to 0.4 mL of reaction medium (65 mM Tri-HCl buffer (pH 7.4), 0.5 mM KCl, 25 mM sucrose), and 0.1 mL of 1 mM ATP. The reaction was incubated in a shaker at 25 °C for 30 min and later stopped by the addition of 1 mL of 10% TCA. The mixture was centrifuged at 3000 rpm for 5 min. Zero-time tubes were deproteinized by the addition of chilled 1 mL of 10% TCA before the addition of ATP. All tubes were chilled after deproteinization and analyses for inorganic phosphate were made by the procedure of Stewart [43]. The supernatant was measured at a wavelength of 660 nm and the results were expressed as nanomoles P_i/minute/milligram of protein.

Hormonal Level Determination

Estimations of Testosterone and Progesterone

Considering the importance of sex hormones, testosterone (the primary male hormone that is required for
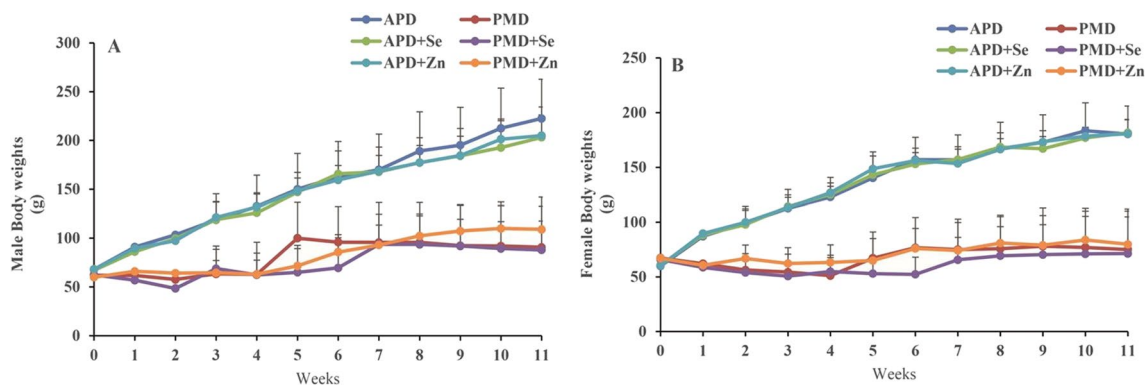
spermatogenesis and the activities of Sertoli cells) and progesterone (a progestogen sex hormone involved in the menstrual cycle, pregnancy, and embryogenesis) levels were estimated in the plasma using the ELISA Assay kits (DiaMetra, Spello (PG), Italy; Cat. No: DCM002-10 and DCM006-8; for testosterone and progesterone respectively). Competitive immunoenzymatic colorimetric procedures for the quantitative determination of total testosterone and progesterone concentrations in the plasma were carried out according to the manufacturer's manuals. The absorbance was read at 450 nm against the respective blank within 5 min.

Estimation of Lipid Profile

Cholesterol (CHOL), high-density lipoprotein (HDL), triglycerides (TG), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) were estimated in the plasma using Randox kits. Assays were carried out as described in the kits' manuals. VLDL and LDL were obtained through calculation as described by Njike et al. [44].

Statistical Analyses

The significant difference between the evaluations of the rats subjected to protein restriction compared to the controls with adequate diet was evaluated. All data are expressed as the mean ± standard deviation and were analyzed using one-way analysis of variance (ANOVA). Post hoc multiple comparisons to assess the significant differences between the means across the groups were determined using Duncan multiple range test, and values with $p < 0.05$ were considered statistically significant. IBM SPSS (Statistical Package for the Social Sciences) software version 23 (Armonk, NY) was used for the analysis.

Results

Effect of Protein Malnourished Diet, Se, and Zn Supplementation on the Growth Curve of Body Weights of Male and Female Rats

The effect of PMD on the *growth curve of body weights* of male and female rats is presented in Fig. 1 A and B. At the end of the 11th week of feeding, male and female PMD-fed rats showed significant reductions in their body weights as compared to APD-fed rats. Se and Zn supplementation for 3 weeks did not have significant effects on both PMD- and APD-fed rats.
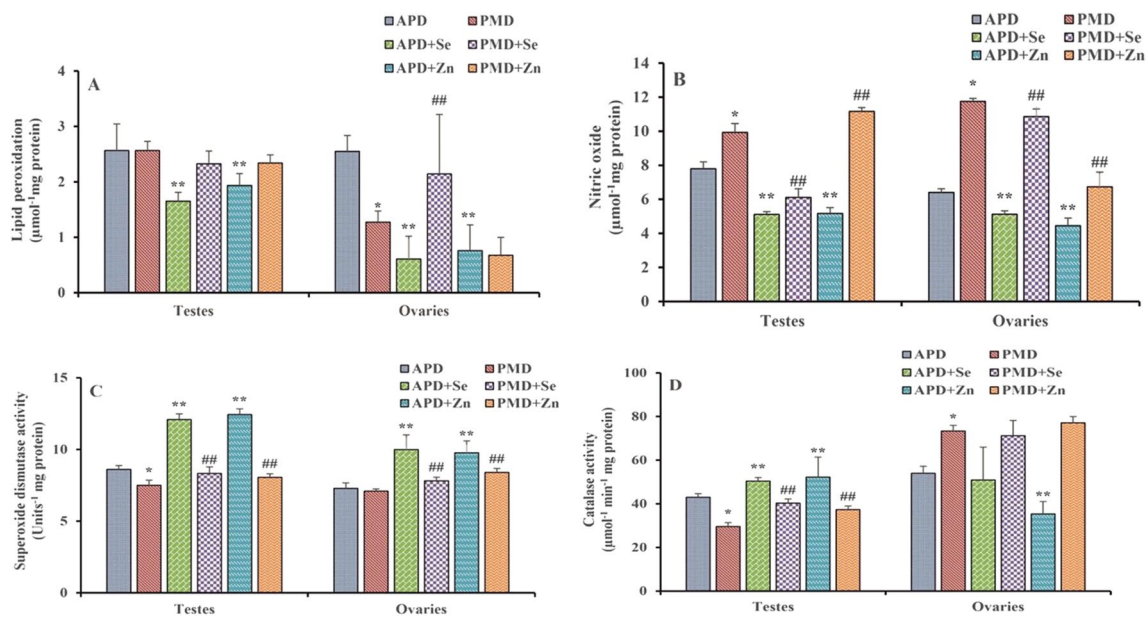
Effect of Se and Zn Supplementation on the Oxidative Markers in the Testes and Ovaries of Protein Malnourished and Adequate Protein Diet–Fed Rats

Oxidant Parameters

Lipid Peroxidation As presented in Fig. 2 A, the level of MDA in the testes of PMD-fed rats did not show any significant effect compared to APD-fed rats whereas a significant reduction was observed in the ovaries when compared to APD-fed rats. Se and Zn supplementation, in APD-fed rats compared to non-supplemented APD-fed rats, significantly reduced the level of MDA in the testes and in the ovaries. Se and Zn supplementation did not affect MDA level in PMD-fed rats when compared to the non-supplemented PMD-fed rats in the testes but a significant increase was observed after Se supplementation in the ovaries.

Fig. 1 The growth curve of body weights of protein malnourished diet (PMD)- and adequate protein diet (APD)-fed rats. The body weights of male PMD rats fed with 5% casein diet versus the body weights of male APD rats fed with 16% casein diet (**A**). The body weights of female PMD rats fed with 5% casein diet versus the body weights of female APD rats fed with 16% casein diet (**B**). Both males and females were supplemented with Se (sodium selenite; Na_2SeO_3) and Zn (zinc sulfate; $ZnSO_4·7H_2O$) at a concentration of 0.15 mg^{-1}L and 227 mg^{-1}L respectively in drinking water. APD $n = 5$, PMD $n = 8$. The level of significance was assessed at $p < 0.05$

Fig. 2 Oxidative markers and the effects of Se and Zn supplementation in the testes and ovaries of PMD-fed and APD-fed rats. The level of MDA, a by-product of lipid peroxidation (**A**), assessment of NO level (**B**), the activity of superoxide dismutase (SOD) (**C**), and the activity of catalase (CAT) (**D**) and supplementation with Se and Zn were assessed in the testes and ovaries of PMD- and APD-fed rats. APD $n = 5$, PM $n = 8$. *PMD significantly different from APD group; **APD treated significantly different from untreated group; ##PMD treated significantly different from untreated group ($p < 0.05$)

Nitric Oxide As shown in Fig. 2 B, there was a significant increase in the level of NO in the testes of PMD-fed rats compared to APD-fed rats. Supplementation with Se and Zn significantly reduced the level of NO in the testes of APD-fed rats but only Se reduces the level of NO in the testes of PMD-fed rats while Zn supplementation increases it. In the ovaries, there was a significant increase in the level of NO in PMD-fed rats compared to the APD-fed rats. Supplementation with Se and Zn significantly reduced the level of NO in both the ovaries of PMD- and APD-fed rats.
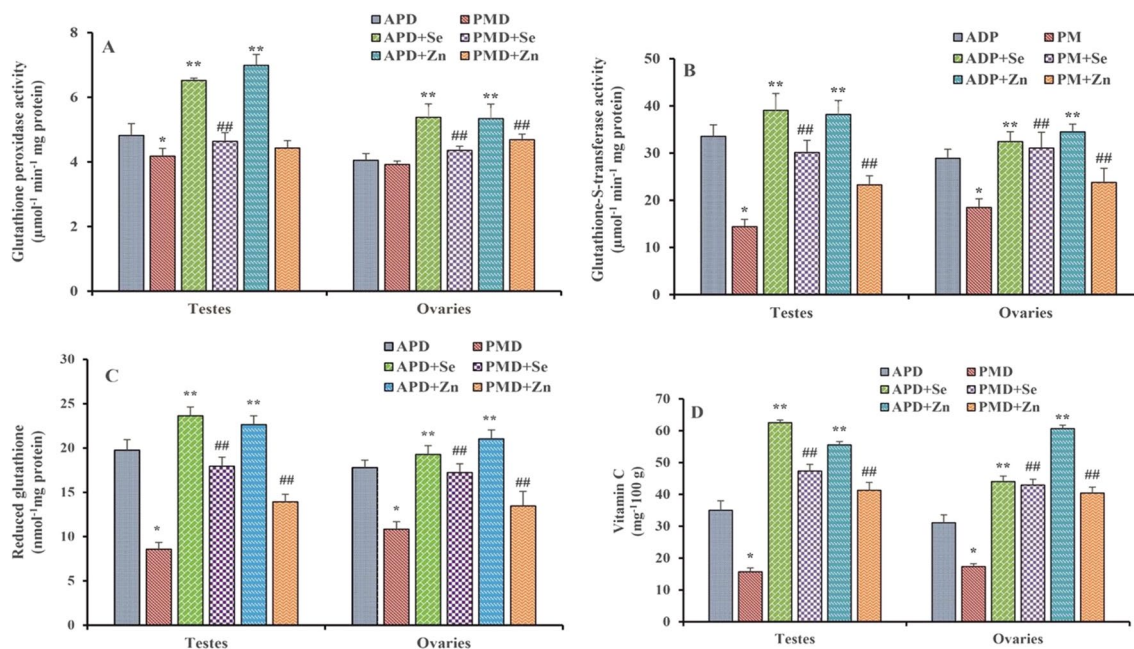
Antioxidants Parameters

As shown in Fig. 2 C and D, there were significant reductions in the activities of SOD and CAT in the testes of PMD-fed rats compared to APD-fed rats. Se and Zn supplementation increased the activities of SOD and CAT in the testes of both PMD- and APD-fed rats. Also, the activity of CAT was significantly increased in the ovaries of PMD-fed rats compared to APD-fed rats. Supplementation with Se and Zn significantly increased the ovarian SOD activity in both PMD- and APD-fed rats.

The activities of GPx and GST, as presented in Fig. 3 A and B, were significantly reduced in the testes of PMD-fed rats compared to the APD-fed rats. Se and Zn supplementation significantly increased the activity of GST in the testes of PMD- and APD-fed rats. Se increased the activity of GPx in the testes of PMD- and APD-fed rats and Zn supplementation increased GPx activity only in the testes of APD-fed rats. On the other hand, PMD did not affect GPx activity in the ovaries when compared to the control rats but there was a significant reduction in the activity of GST in the ovaries of PMD-fed rats when compared to APD-fed rats. However, supplementation with Se and Zn significantly increased the activities of both GPx and GST in the ovaries of both PMD- and APD-fed rats. Moreover, there were significant reductions in the level of GSH (Fig. 3C) in both the testes and ovaries of PMD-fed rats when compared to the APD-fed rats. Supplementation with Se and Zn to PMD- and APD-fed rats significantly increased the GSH levels in both tissues.

Assessment of vitamins C (Fig. 3D) and E (Fig. 4A) in PMD-fed rats showed significant reductions when compared to the APD-fed rats in both the testes and ovaries. Zn supplementation significantly increased the levels of both vitamins in the testes and ovaries of rats fed PMD and APD. Se increased vitamin C levels in the testes and ovaries of PMD- and APD-fed rats. Se also increased vitamin E levels in the testes of PMD- and APD-fed rats whereas Se only increase vitamin E levels in the ovaries of PMD-fed rats.

Fig. 3 Antioxidant parameters and the effect of Se and Zn supplementation in the testes and ovaries of PMD and APD rats. The activity of glutathione peroxidase (**A**), the activity of glutathione-S-transferase (**B**), the level of reduced glutathione (**C**), and the level of vitamin C (**D**), and supplementation with Se and Zn were assessed in the testes and ovaries of PMD and APD rats. APD $n = 5$, PM $n = 8$. *PMD significantly different from APD group; **APD treated significantly different from untreated group; ##PMD treated significantly different from untreated group ($p < 0.05$)
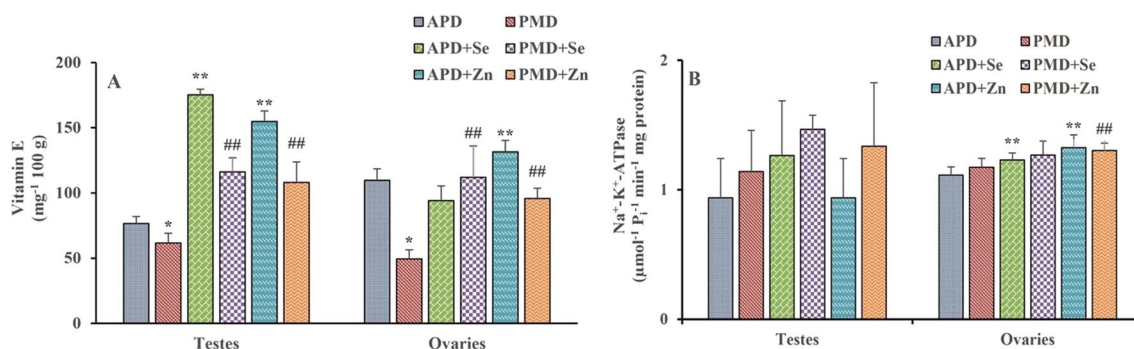
Fig. 4 Assessment of vitamin E (**A**), the activity of Na$^+$-K$^+$-ATPase (**B**) supplemented with Se and Zn in the testes and ovaries of PMD- and APD-fed rats. APD $n = 5$, PM $n = 8$. *PMD significantly different from APD group; **APD treated significantly different from untreated group; ##PMD treated significantly different from untreated group ($p < 0.05$)

Effect of Se and Zn Supplementation on Na$^+$-K$^+$-ATPase Activity in the Testes and Ovaries of Protein Malnourished and Adequate Protein Diet–Fed Rats

As shown in Fig. 4 B, the activity of Na$^+$-K$^+$-ATPase in the testes and ovaries of PMD-fed rats was not statistically different from APD-fed rats. While there was a significant increase in the ovarian Na$^+$-K$^+$-ATPase activity in Se- or Zn-supplemented APD-fed rats as well as a significant increase in Zn-supplemented PMD-fed rats, the activity of testicular

Na$^+$-K$^+$-ATPase in Se- or Zn-supplemented rats does not affect PMD- and APD-fed rats.

Effect of Se and Zn Supplementation on the Hormonal Level of Protein Malnourished and Adequate Protein Diet–Fed Rats

Table 1 shows that testosterone and progesterone levels were significantly reduced in PMD-fed rats when compared to APD-fed rats. Supplementation with Se significantly increased testosterone levels in both APD- and PMD-fed

Table 1 Effect of selenium and zinc supplementation on testosterone and progesterone levels in the plasma of PMD- and APD-fed rats

Treatments	Testosterone (ng/mL)	Progesterone (ng/mL)
APD	1.60 ± 0.10	0.30 ± 0.05
APD + Se	2.20 ± 0.10**	0.36 ± 0.05
APD + Zn	0.36 ± 0.02**	0.46 ± 0.05**
PMD	0.30 ± 0.10*	0.20 ± 0.01*
PMD + Se	1.20 ± 0.10##	0.33 ± 0.05##
PMD + Zn	0.67 ± 0.21##	0.37 ± 0.02##

APD $n = 5$, PMD $n = 8$. *PMD significantly different from APD group; **APD treated significantly different from untreated group; ##PMD treated significantly different from untreated group ($p < 0.05$)

rats. Also, Zn supplementation increased the level of testosterone in PMD-fed rats. Furthermore, progesterone level was significantly increased in PMD- and APD-fed rats after Zn supplementation. In the Se-supplemented rats, the progesterone level was increased in the PMD-fed rats.

Effect of Se and Zn Supplementation on Lipid Profile in the Testes and Ovaries of Protein Malnourished and Adequate Protein Diet–Fed Rats

Lipid profiles from the plasma of male and female rats, as presented in Table 2 shows significant reductions in CHOL, HDL, and LDL in the plasma of male PMD-fed rats and significant reductions in HDL, VLDL, and TG in the plasma of female PMD-fed rats when compared to the APD-fed rats. Se supplementation significantly decreased plasma levels of HDL, and LDL in both males and females and CHOL in females but a significant increase in TG was observed in both males and females and VLDL only in male APD-fed rats. Moreover, Se supplementation to PMD-fed rats significantly reduced plasma CHOL, LDL, VLDL, and TG in females and significantly increased plasma HDL levels in females. Also, Zn supplementation in male APD-fed rats significantly decreases CHOL, HDL, VLDL, LDL, and TG while it increases all the parameters in the plasma of female APD-fed rats. Zn supplementation to the PMD-fed rats significantly increased CHOL and HDL in the plasma of both males and females rats and the increase in the levels of VLDL, LDL, and TG was only observed in the female while the LDL and TG levels were reduced in the plasma of male PMD-fed rats.

Discussion

The present study was carried out to evaluate the effect of Se and Zn supplementation on the male and female reproductive organs of rats subjected to postnatal protein malnutrition.

Table 2 Effect of Se and Zn supplementation on lipid profile in the plasma of male and female PMD- and APD-fed rats

Treatments	Cholesterol (mg⁻¹dL)		High-density lipoprotein (HDL) (mg⁻¹dL)		Very low-density lipoprotein (VLDL) (mg⁻¹dL)		Low-density lipoprotein (LDL) (mg⁻¹dL)		Triglycerides (TG) (mg⁻¹dL)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
APD	144.2 ± 1.3	94.9 ± 1.1	43.6 ± 1.3	43.0 ± 1.2	33.1 ± 0.1	34.1 ± 6.5	66.2 ± 0.8	22.9 ± 0.9	174.7 ± 5.1	169.1 ± 0.9
APD + Se	142.0 ± 1.1	73.0 ± 0.4**	38.2 ± 0.8**	21.8 ± 0.5**	45.1 ± 1.3**	38.9 ± 1.2	61.1 ± 1.59**	12.7 ± 0.5**	232.7 ± 2.7**	205.8 ± 1.1**
APD + Zn	82.0 ± 1.5**	124.6 ± 0.6**	26.5 ± 0.1**	52.8 ± 0.5**	17.5 ± 1.0**	39.9 ± 1.5**	36.2 ± 2.1**	32.9 ± 0.6**	102.3 ± 3.6**	206.6 ± 2.8**
PMD	87.4 ± 1.1*	95.4 ± 0.9	30.7 ± 0.9*	16.2 ± 0.7*	43.1 ± 1.5	27.3 ± 0.7*	18.8 ± 1.5*	51.1 ± 0.3*	219.7 ± 1.7*	146.4 ± 0.9*
PMD + Se	89.3 ± 1.3	80.1 ± 0.6##	28.4 ± 0.8	24.2 ± 1.3##	42.2 ± 0.4	24.5 ± 0.6##	20.9 ± 1.1	32.1 ± 0.6##	273.0 ± 1.0##	133.8 ± 1.0##
PMD + Zn	96.7 ± 1.1##	115.8 ± 0.9##	45.8 ± 0.2##	19.6 ± 0.6##	38.9 ± 1.7	35.8 ± 1.1##	14.9 ± 0.2##	60.9 ± 0.9##	196.8 ± 2.3##	196.9 ± 0.4##

APD $n = 5$, PMD $n = 8$. *PMD significantly different from APD group; **APD treated significantly different from untreated group; ##PMD treated significantly different from untreated group ($p < 0.05$)

Protein is an important nutrient containing essential amino acids that are required for biological functions. Evidence has shown that reproductive maturation and function are influenced by protein malnutrition which is accompanied by changes in testicular structure resulting in a reduction in daily sperm production and/or has a permanent effect on their capacity to produce spermatozoa [2, 45].

Protein Malnourished Diet, Se, and Zn Supplementation on the Body Weights of Male and Female Rats

Body weight has been associated with reproductive potential in animals [46]. The finding from this study showed a significant decrease in the body weights of male and female PMD-fed rats as compared to the APD-fed rats' counterparts. Reduction in the body weights of PMD-fed rats has previously been reported as a demonstration of the importance of protein for normal development [47]. Moreover, reduction of body weight due to PM has been linked with appetite failure and metabolic disturbances, consequently leading to increased transamination or deamination of amino acids arising from increased endogenous protein degradation [48]. Supplementation with Se and Zn did not have any significant effect on the body weights of both male and female rats fed with either low-protein or adequate-protein diets. There are conflicting reports about the effect of Se and Zn on body weight. Hasani et al. [49] reported that Se improves weight control in obese rats and this was corroborated by another report showing that sodium selenite attenuated increased body weight in high-fat-diet-fed mice [50]. Some have shown that Se in high doses has been associated with weight gain [51]. Similarly, some have reported an increase in body weight and some reduction after Zn supplementation [49]. However, the present study corroborated the earlier report that Se and Zn supplementation does not affect body weight [7]. The reason for these conflicting results may be attributed to dosage, route of administration, duration of exposure, the type and species of animals used, and probably some other mechanisms which unfortunately are not the focus of this present study but may serve as proposed future studies.

Se and Zn Supplementation on Oxidative Markers in the Testes and Ovaries of Protein Malnourished and Adequate-Protein-Diet-Fed Rats

Oxidative stress has been implicated with compromised reproduction and fertility and this includes impaired ovarian functions, deteriorated oocyte quantity, and gynecological disease [52]. In this study, PMD did not influence the testicular level of LPO products whereas a reduction in the ovarian level of LPO products was observed. This result contradicts the normal trends that are usually observed in PMD-fed rats [7, 53, 54] and the reason for this discrepancy could not yet be ascertained. However, NO levels increased in both tissues. NO, produced from L-arginine by NO synthase, is a signaling molecule that is essential for normal cellular function. In this case, NO is acting as a potent oxidant species because it contains an unpaired electron in its highest orbital making it a highly reactive molecule. In the presence of superoxide anion ($O_2^{\cdot-}$), NO is converted to peroxynitrite ($ONOO^-$) which exerts pro-oxidant actions more than NO itself and this can modify lipids, proteins, and DNA [7, 55]. The report has shown that increased NO production has been linked to PM resulting in a higher rate of apoptosis after cytokines or oxidative stress aggregation [56]. Se reduced NO level in the testes and ovaries of both PMD- and APD-fed rats.

Se prevents peroxynitrite formation from NO and controls the level of cellular free radicals via its various selenoproteins. Reduction in NO level by Se has been reported in rat models of ischemia/reperfusion injury in various tissues and a rat ovary model [18, 57]. Se was also reported to protect against NO production and gene expression of inflammatory cytokines in chicken splenic lymphocytes induced by cadmium [58] The antioxidant property of Zn was demonstrated by reducing the level of NO in the ovaries of PMD-fed rats. Zn exerts its protective role through the formation of metallothionein complex thereby protecting against free radical attack. The exact mechanism by which Se and Zn lower NO is not yet fully understood but a study reported that Se attenuates an increase in cytokines expression by inhibiting the binding of nuclear factor kappa B (NF-κB) and thus decrease NO production [59]. Likewise, Zn has been shown to downregulate mRNA and protein expressions of inducible nitric oxide synthase (iNOS) and decreased cytokine-mediated activation of the iNOS promoter which has been attributed to inhibit NF-κB transactivation activity [60].

Additionally, data from the present study show reductions in the activities of SOD, CAT, GPx, and GST in the testicular tissue of PMD-fed rats. A similar observation had earlier been reported for the cortex and cerebellum of PMD-fed rats [7]. Studies have shown that the balance between ROS and antioxidants greatly influences reproductive activities in male and female animals [61, 62]. Supplementation with Se and Zn increased the activities of SOD, GPx, and GST in both tissues of APD- and PMD-fed rats. Zn is a cofactor for SOD activity and it might exert its antioxidant property through the formation of metallothionein which protects against free radical attack. Moreover, Se is incorporated into selenocysteine (SeCys) and then forms part of selenoenzymes which includes glutathione peroxidase (GPx). The protection of GPx against free radical attack is due to the induction of its selenoproteins property and thereby increasing its total catalytic activity [63, 64]. Se and

Zn supplementation increased the level of CAT in the testes in both diets but maintained its high activity in the ovaries only in the PMD-fed rats, as observed in the study, protecting the ovaries against free radical attack. PMD reduced the level of GSH in both the testes and the ovaries. The level of cellular GSH is a function of several factors including consumption through the formation of conjugates via glutathione-S-transferase, which may be contributing to the reduction in GST level as observed in this study: de novo synthesis, the extent of oxidation, and reduction of oxidized GSH by glutathione reductase [65]. Se and Zn increased the level of GSH and GST in both the testes and ovaries of APD- and PMD-fed rats. The antioxidant role of Zn has been associated with its competitive effect on iron and copper thereby preventing them from producing toxic hydroxyl radicals. Zn also prevents protein oxidation by binding to the thiol (–SH) group of some proteins [66].

Vitamin C is an essential nutrient with antioxidant properties to protect tissues from oxidative damage [67]. Also, vitamin E acts as an antioxidant and a free radical scavenger by protecting fatty acids mainly phospholipids in the plasma membrane from oxidation by ROS and is considered the core of the antioxidant system [68]. The level of vitamins C and E was reduced in the testes and ovaries of PMD-fed rats. Similar reduction and or deficiency in the level of vitamin C has been reported in critically ill patients despite recommended enteral and parenteral intakes [69]. Supplementation with Se and Zn increased the level of vitamins C and E in both testes and ovaries of APD- and PMD-fed rats. The efficacy of vitamin E, for example, has been reported to be strongly dependent on the action of ascorbic acid and other dietary components such as Se and carotenoids [68, 70, 71]. We may say that a positive correlation exists between Se and Zn supplementation and enhancement of vitamins C and E levels which might be contributing to improving reproductive organ functions by protecting them against free radical attacks.

Se and Zn Supplementation on Na^+-K^+-ATPase Activity in the Testes and Ovaries of Protein Malnourished and Adequate-Protein-Diet-Fed Rats

Na^+/K^+-ATPase is an enzyme that regulates ROS and intracellular calcium signaling among several other functions. The present study showed that PMD does not have any significant effect on the activity of Na^+/K^+-ATPase either in the testes or in the ovaries. A previous report by Calderon Guzman et al. [72] showed that PMD causes alteration in the activity of Na^+/K^+-ATPase in the brain of rats. The discrepancy might be due to differences in the tissues, animal species, and duration of administration. However, Se and Zn supplementation increases ovarian Na^+/K^+-ATPase activity.

The reason why Se and Zn are not influencing testicular Na^+/K^+-ATPase activity awaits further investigation.

Se and Zn Supplementation on the Hormonal Level of Protein Malnourished and Adequate-Protein-Diet-Fed Rats

The finding from this study shows that PMD lowers the levels of testosterone and progesterone. Testosterone is an integral component of the hypothalami-pituitary gonadal axis which functions to modulate the release of gonadotropins by the anterior pituitary gland. The reduction in testosterone level observed in this study correlates with the finding of Oliveira and co-workers. They observed that testosterone production was lower in rats subjected to a low-protein diet. Similarly, maternal protein malnutrition has been implicated in lower progesterone levels in rats [73]. Supplementation with Se and Zn increased testosterone and progesterone levels in PMD-fed rats. Several reports have shown the influence of Se on testosterone and progesterone levels. For example, Se supplementation to goat kids and rams showed a marked increase in the level of testosterone and this was attributed to increasing responsiveness of Leydig cells to gonadotropins which are stimulated by luteinizing hormone (LH) receptor mechanisms controlling the storage and release of testosterone [21, 74]. Moreover, Dkhil et al. [75] corroborated that Se nanoparticles elevated testosterone levels in streptozotocin-diabetic rats which have been associated with stimulation of LH and thus positively affects the biosynthesis of testosterone. Furthermore, reports have shown that Se supplementation increased plasma progesterone in pregnant heifers [76] as well as in postpartum dairy cows [77]. In addition, *Saccharomyces cerevisiae* enriched with Se enhanced progesterone levels in local Iraqi female goats [20]. Zn supplementation modulated and increased the level of testosterone in the blood serum [20, 29]. As reported for Se, *Saccharomyces cerevisiae* enriched with Zn also enhanced progesterone levels in local Iraqi female goats [20]. Increased progesterone level was also shown in ovariectomized rats supplemented with Zn as compared to its ovariectomized control rats [78].

Se and Zn Supplementation on Lipid Profile in the Testes and Ovaries of Protein Malnourished and Adequate-Protein-Diet-Fed Rats

Malnutrition causes lipid metabolism disorder [79] in both males and females. The increase in TG, as observed in male PMD-fed rats, might be associated with the increase in VLDL and reduction in HDL. Also, the reductions in TG and HDL in female PMD rats were accompanied by increased LDL. The major carrier of triglycerides in the plasma is VLDL. The increase in VLDL may be a major factor in increasing the TG level observed. In addition, the increase

in triglyceride levels may also be due to the decrease in the activity of lipoprotein lipase; an enzyme that hydrolyzes triglycerides in chylomicrons. Xue et al. [79] have shown that undernutrition triggered severe lipid metabolism disorder and causes enhanced fatty acid oxidation, ketogenesis, and TG synthesis. Supplementation with Se reduced the levels of CHOL, VLDL, LDL, and TG with an increase in HDL levels in the plasma of female PMD-fed rats. Reductions in these parameters have been reported by Hasani et al. [80] and they concluded that Se supplementation could decrease TG and VLDL levels. A reduction in LDL, as well as an increase in the level of HDL after Se supplementation, was also reported [80, 81]. Conversely, supplementation with Zn increases CHOL in the plasma of male and female PMD-fed rats as well as an increase in VLDL, LDL, and TG in the plasma of female rats only. The reason for the increase cannot be presently explained. However, Zn increased HDL levels in both male and female PMD-fed rats and reduced TG, LDL, and VLDL levels in male PMD-fed rats. An increase in HDL and reduction in TG levels were reported in type 2 diabetes mellitus after Zn supplementation [82]. Moreover, dietary supplementation of Zn oxide and Zn methionine was reported to reduce triglyceride and LDL cholesterol levels in the blood of laying hens [83].

One of the main limitations of the study is that we did not study the combined effects of Se and Zn. It can be speculated that there could be synergy between the action of Se and Zn and the mechanism could involve the antioxidant action of both trace elements. Studies have reported the possibility of potential interaction and mutual influence between Se and Zn and that Zn intake alone may increase Se levels [49, 84]. This study provides further insight into the role of Se and Zn supplementation in the reproductive organs of postnatal protein-malnourished rats. However, an explanation of the synergistic effects of Se and Zn on the male and female reproductive organs of rats exposed to protein-depleted diets awaits further study. Given the antioxidant capacity of Se and Zn, and given that most of the problems derived from protein malnutrition are associated with oxidative stress, it is suggested to promote the use of these trace elements in protein-malnourished populations; since they mediate vital biochemical reactions, acting as cofactors of many enzymes, in addition to acting as centers to stabilize enzymatic and protein structures, related not only to their nutritional status but also to their reproductive potential.

Conclusion

The results of this present study indicate that postnatal protein malnutrition alters the lipid profile, increases the level of oxidants, and reduces the antioxidant status, as well as hormonal levels in the testicles and ovaries. Se and Zn

supplementation, however, reversed almost all the alterations observed in the parameters analyzed suggesting that Se and Zn supplementation might protect the reproductive organs of rats against postnatal protein malnutrition.

Declarations

Ethics Approval This study was performed in line with the NIH Guidelines for Humane Use and Care of Laboratory Animals. Approval was granted by the Ethics Committee of Olabisi Onabanjo University, Ogun State, Nigeria.

Consent to Participate Not applicable

Consent to Publish Not applicable

Competing Interests The authors declare no competing interests.

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