GEORGIAN BIOMEDICAL NEWS

VOLUME 4. ISSUE 2. APRIL–JUNE 2026

Vitamin D Supplementation Modulates Early Antioxidant Responses in a Streptozotocin-Induced Diabetes Mellitus Rat Model

Nino Sakhanberidze[1,ID], Manana Namoradze[1,ID], Nino Charkviani[2,ID], Maka Topuridze[1,ID], Nana Momtselidze[3,ID], Maia Mantskava[4,ID]

ABSTRACT

BACKGROUND. Diabetes mellitus represents a systemic metabolic disorder characterized by persistent hyperglycemia, which induces oxidative stress and impairs endogenous antioxidant defense mechanisms, thereby contributing to the pathogenesis and progression of diabetic complications. The streptozotocin (STZ)-induced diabetes mellitus model is widely recognized as a reliable experimental system for investigating oxidative stress–related alterations that underlie disease pathogenesis. Vitamin D is increasingly recognized as a regulator of antioxidant and immunomodulatory pathways; however, the relative efficacy of its preventive and therapeutic administration in diabetes mellitus remains insufficiently investigated, particularly at early stages of disease development.

OBJECTIVES. This study aimed to evaluate the impact of preventive and therapeutic vitamin D supplementation on antioxidant status during the early stage of STZ-induced experimental diabetes mellitus.

METHODS. Forty male rats (10 weeks old, 200±13.4 g) were randomly assigned to four groups: a control (intact) group, an STZ-induced diabetes mellitus group, a preventive vitamin D group (supplemented for 14 days prior to STZ administration and continued throughout the experiment), and a therapeutic vitamin D group (in which supplementation was initiated on day 3 after STZ administration). Experimental diabetes mellitus was induced by intraperitoneal injection of streptozotocin (30 mg/kg), and vitamin D was administered orally at 300 IU/day. Measurements were performed on day 7. Blood samples were analyzed for glucose, catalase (CAT), superoxide dismutase (SOD), vitamin D, and calcium levels.

RESULTS. In the STZ-induced diabetic group, antioxidant enzyme levels were significantly reduced compared with the control group. Preventive vitamin D administration was not associated with meaningful changes in these parameters. In contrast, therapeutic vitamin D treatment significantly improved antioxidant defense, as reflected by increased catalase and superoxide dismutase levels compared with the STZ diabetic group. No significant differences in calcium levels were observed among experimental groups, suggesting that the antioxidant effects of vitamin D are independent of calcium homeostasis.

CONCLUSIONS. STZ-induced diabetes mellitus impairs antioxidant defenses, while short-term therapeutic vitamin D improves antioxidant status, whereas preventive administration does not result in biologically relevant changes at the early stage.

KEYWORDS. Catalase; Early-stage antioxidant defense; Experimental diabetes mellitus; Streptozotocin; Superoxide dismutase; Vitamin D.

DOI. 10.52340/GBMN.2026.01.01.169

BACKGROUND

Diabetes mellitus is a heterogeneous metabolic disease characterized by sustained hyperglycemia resulting from absolute insulin deficiency and/or reduced insulin sensitivity, leading to impaired cellular glucose uptake and disrupted carbohydrate metabolism. [1] Diabetes mellitus continues to expand as a global public health concern, with a progressively increasing prevalence. [2] Despite differences in etiology and clinical presentation—including type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes mellitus, and other specific variants such as latent autoimmune diabetes in adults (LADA), maturity-onset diabetes of the young (MODY), and secondary diabetes mellitus—all forms are characterized by persistent hyperglycemia. This hyperglycemia is a major contributor to tissue damage and the development of chronic complications. Among the underlying mechanisms, oxidative stress plays a central role in mediating hyperglycemia-induced cellular and vascular damage. [3,4] In the literature, it has been demonstrated that hyperglycemia-induced oxidative stress is initiated at

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994.

GEORGIAN BIOMEDICAL NEWS

VOLUME 4. ISSUE 2. APRIL-JUNE 2026

the early stages of diabetes mellitus and progressively intensifies as the disease develops, contributing to the onset and progression of diabetic complications. [5-7] Early-stage oxidative stress is therefore considered a critical phase in disease development, as it triggers molecular and cellular alterations that ultimately lead to structural and functional complications in diabetes mellitus. [8,9] In experimental models, including streptozotocin-induced diabetes, alterations in antioxidant enzymes such as superoxide dismutase and catalase have been observed at early stages, reflecting an initial disruption of redox homeostasis. [10,11] Accordingly, assessing these enzymes provides a reliable approach for evaluating early oxidative stress–related changes, as targeted interventions at this stage may influence subsequent disease progression.

Streptozotocin (STZ)-induced experimental diabetes mellitus is a well-established, internationally recognized model widely used to investigate hyperglycemia-induced oxidative stress and the associated redox imbalance. STZ induces hyperglycemia by selectively cytotoxicity to pancreatic β cells, leading to enhanced oxidative stress and impairment of antioxidant defense systems, thereby providing a suitable model for evaluating antioxidant responses and therapeutic strategies. [12,13]

Vitamin D is a fat-soluble secosteroid hormone with diverse systemic effects. While traditionally recognized for its essential role in calcium homeostasis and bone metabolism, an expanding body of evidence highlights its diverse biological functions, including its regulatory influence on antioxidant mechanisms. [14,15] However, evidence comparing preventive and therapeutic vitamin D supplementation, as well as its time-dependent progression, remains limited, particularly regarding early-stage antioxidant responses in diabetes mellitus.

We hypothesized that vitamin D supplementation may affect early antioxidant responses in diabetes mellitus. Therefore, we aimed to determine whether preventive versus therapeutic vitamin D supplementation modulates early-stage antioxidant responses in a Streptozotocin-induced diabetes model.

METHODS

An experiment was conducted on 40 male rats (non-breed-specific), aged 10 weeks and weighing 200±13.4 g at baseline. Only male rats were included because they are generally more sensitive to streptozotocin (STZ) than females, and to avoid potential confounding effects related to hormonal fluctuations.16 Animals were housed under standard laboratory conditions (24±1°C, 55±5% humidity, 12-hour light/dark cycle) and fed a standard laboratory rat diet with free access to water.

Rats were randomly assigned into four experimental groups (n=10 per group): (I) a control (intact) group, (II) a diabetic group comprising rats with STZ-induced diabetes mellitus without vitamin D supplementation, (III) a preventive group in which rats received vitamin D supplementation for 14 days prior to STZ administration and throughout the experimental period, and (IV) a therapeutic group in which vitamin D supplementation was initiated on day 3 post-STZ injection and continued until the end of the study. Outcome assessments were conducted on day 7.

The vitamin D dose was selected based on a preliminary dose–response experiment evaluating daily oral doses of 30, 150, 200, 300, 400, 600, and 800 IU/day to minimize the risk of hypercalcemia while identifying an effective therapeutic range. Higher doses (400–800 IU/day) tended to raise serum calcium levels toward the upper end of the physiological range. The 300 IU/day dose maintained calcium levels within the normal range without approaching the upper threshold and was therefore selected for the present study. Vitamin D was administered orally once daily to mimic the natural route of intake.

Experimental diabetes mellitus was induced by a single intraperitoneal injection of freshly prepared STZ in 50 mM sodium citrate buffer (pH 4.5). Rats in the diabetic, preventive, and therapeutic groups received STZ at 30 mg/kg. To ensure STZ stability, solutions were prepared immediately before injection, and administration was completed within 10 minutes. All animals were fasted prior to STZ injection. Following the injection, rats were provided with 10% sucrose solution. Diabetes induction was confirmed on day 3 post-injection by measuring fasting blood glucose levels using a glucometer. Diabetes mellitus was

Georgian Biomedical News.
ISSN (Online): 2720-8796 ISSN (Print): 2720-7994.

gbmn GEORGIAN BIOMEDICAL NEWS

VOLUME 4. ISSUE 2. APRIL–JUNE 2026

defined as a fasting blood glucose level exceeding 19 mmol/L, accompanied by polyuria.

Body weight and fasting blood glucose levels were recorded at baseline (day 1, prior to STZ injection) and on day 7. Blood glucose levels were monitored throughout the study using a Contour Plus glucometer (Ascensia Diabetes Care, Basel, Switzerland), with blood samples obtained via tail vein puncture to confirm the stability of the diabetic state.

Blood samples were collected after an 18-hour fast, and vitamin D administration was discontinued 1 day prior to blood collection. Serum glucose concentration was determined using the glucose oxidase–peroxidase (GOD–POD) enzymatic colorimetric method (BIO-LABO, France) measured on a semi-automated biochemical analyzer (URIT-880, China). Serum antioxidant enzyme levels were evaluated by measuring catalase (CAT) and superoxide dismutase (SOD) using FineTest ELISA kits (Fine Biotech Co., Ltd., China) according to the manufacturer's instructions. Catalase results were expressed in mIU/mL, and superoxide dismutase results in ng/mL.

Statistical analyses were performed using the licensed software OriginPro 8.1. Data are presented as mean±standard deviation (SD). The normality of data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. Differences among groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons when overall statistical significance was detected. A p-value < 0.05 was considered statistically significant. Effect size was estimated using eta squared (η^2), calculated as the ratio of the between-group sum of squares (SS_between) to the total sum of squares (SS_total): η^2=SS_between/SS_total. Effect sizes were interpreted according to conventional thresholds: 0.01 (small), 0.06 (moderate), and ≥0.14 (large). Percentage changes in experimental groups were calculated relative to the control group.

All animal experiments were conducted in accordance with institutional regulations and internationally accepted guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Ethics Committee of Tbilisi State Medical University (Protocol No. 60-1002, approved 10 February 2023). All procedures were performed to minimize animal pain and distress and in accordance with the principles of the 3Rs (Replacement, Reduction, and Refinement).

RESULTS

The antioxidant effects of Vitamin D supplementation in a streptozotocin-induced diabetes mellitus rat model are represented in **TABLE 1**.

TABLE 1. Effects of preventive and therapeutic vitamin D administration on antioxidant parameters in streptozotocin (STZ)-induced diabetes mellitus on experimental day 7

	CAT (mIU/mL)		SOD (ng/mL)		Ca (µmol/L)	
Groups	Mean±SD	vs. Control (%)	Mean±SD	vs. Control (%)	Mean±SD	vs. Control (%)
Control	302.2±3.8	0	7.4±0.65	0	2.40±0.20	0
STZ Control	277.8±2.1	−8.1	5.57±0.49	−24.8	2.40±0.02	0
Preventive STZ+Vit D	281.6±2.7	−6.8	5.37±0.47	-27.4	2.44±0.04	+1.7
Therapeutic STZ+Vit D	406.5±5.0	+34.5	8.30±0.30	+12.2	2.50±0.03	+4.2
ANOVA p-value	$p=2.26\times10^{-10}$		$p=2.11\times10^{-4}$		p=0.59	
Effect size	η^2=0.997		η^2=0.90		η^2=0.20	

Abbreviations: Ca, calcium; CAT, catalase; SD, standard deviation; SOD, superoxide dismutase; STZ, streptozotocin; Vit D, vitamin D

Catalase and superoxide dismutase (SOD) were markedly altered in the STZ-induced diabetic group compared with the control group, with catalase decreasing by approximately 8.1% and SOD decreasing by 24.8%, reflecting an oxidative imbalance. Preventive vitamin D administration did not result in meaningful changes in antioxidant enzyme activity, with only a marginal increase in catalase (~1.4%) compared with the STZ-induced diabetic group. In contrast, therapeutic vitamin D treatment resulted in a pronounced restoration of antioxidant defense, with catalase activity increasing by approximately 46% and SOD levels by approximately 49% relative to the STZ-induced diabetic group, indicating a marked improvement.

No significant differences in calcium levels were observed among experimental groups, suggesting that vitamin D did not exert a major effect on calcium homeostasis under the present conditions.

Georgian Biomedical News.
ISSN (Online): 2720-8796 ISSN (Print): 2720-7994.

GEORGIAN BIOMEDICAL NEWS

VOLUME 4. ISSUE 2. APRIL-JUNE 2026

DISCUSSION

In the present study, we found that vitamin D differentially modulates early-stage antioxidant responses in STZ-induced experimental diabetes mellitus. In particular, therapeutic vitamin D improved antioxidant defense, whereas preventive administration did not result in meaningful changes at this stage.

Hyperglycemia-induced oxidative stress plays a key role in the pathogenesis of diabetes mellitus. Streptozotocin (STZ) is a well-established and experimentally validated model for investigating the effects of interventions on redox imbalance. The induction of an experimental hyperglycemic state with STZ is widely used because of its selective toxicity toward pancreatic β-cells. The observed alterations in antioxidant parameters, including catalase activity and superoxide dismutase levels, following STZ administration enabled evaluation of vitamin D's short-term effects on oxidative damage.

Catalase is a key antioxidant enzyme that catalyzes the breakdown of hydrogen peroxide, thereby protecting cells against oxidative damage. STZ administration reduced catalase activity, consistent with previous basic research and the authors' earlier studies. [17,18] This reduction is associated with the chronic hyperglycemia induced in this study, which promotes oxidative stress, overwhelms antioxidant defense systems, facilitates protein glycation, and may impair antioxidant enzymes, including catalase. [19,20] Consequently, hydrogen peroxide and other reactive oxygen species accumulate, leading to tissue damage characteristic of diabetic neuropathy and other complications. [21]

Elevated oxidative stress, depletion of endogenous antioxidant enzymes, and reduced catalase activity disrupt the body's antioxidant defense system. Our study demonstrated that prophylactic vitamin D administration resulted in only a slight increase in catalase activity, indicating a limited protective effect. In contrast, therapeutic vitamin D administration increased catalase activity above control levels. These results suggest that vitamin D may enhance antioxidant defense mechanisms following the development of oxidative stress. Several studies have reported that vitamin D increases the expression of antioxidant enzymes and reduces oxidative damage by modulating cellular signaling pathways and anti-inflammatory mechanisms. [22,23]

The decrease in catalase activity observed in our experiments was paralleled by similar changes in superoxide dismutase (SOD) activity, a key antioxidant enzyme that dismutates superoxide radicals to hydrogen peroxide. In the STZ-induced diabetes group, SOD levels were significantly lower than in the control group, confirming the presence of oxidative stress. Treatment with vitamin D significantly increased SOD levels compared with the STZ diabetic group. This improvement suggests that vitamin D may help restore antioxidant balance. Vitamin D has been shown to regulate oxidative stress by modulating mitochondrial function, reducing inflammation, and activating antioxidant pathways. The observed increase in SOD levels in the treatment group supports the hypothesis that vitamin D plays an important role in enhancing antioxidant defenses under conditions associated with increased reactive oxygen species (ROS).

Unlike catalase and superoxide dismutase (SOD), calcium levels did not differ significantly between the experimental groups. STZ administration did not induce notable alterations in this parameter, and vitamin D treatment resulted in only a slight, statistically nonsignificant increase. This relative stability may suggest that calcium homeostasis is preserved during the early stages of STZ-induced metabolic disturbances. Accordingly, the observed improvements in antioxidant defense are likely attributable to vitamin D's regulatory effects on oxidative stress pathways.

The significant increase in CAT and SOD observed in the group receiving therapeutic vitamin D underscores vitamin D's potential to modulate oxidative stress and enhance antioxidant defense mechanisms.

However, several limitations of this study should be considered. First of all, only male rats were included, in accordance with recommendations outlined in STZ guidelines. Secondly, although the selected dose was based on prior dose-control experiments to avoid the risk of hypercalcemia, using a single dose is a limitation, as dose-dependent effects were not assessed. These aspects should be taken into consideration in future investigations.

Georgian Biomedical News.
ISSN (Online): 2720-8796 ISSN (Print): 2720-7994.

GEORGIAN BIOMEDICAL NEWS

VOLUME 4. ISSUE 2. APRIL–JUNE 2026

CONCLUSIONS

To conclude, the present study demonstrated that STZ-induced hyperglycemia significantly impairs antioxidant defense systems. Therapeutic vitamin D administration, even for a short duration, led to a more pronounced improvement in antioxidant parameters. In contrast, preventive administration did not produce biologically relevant changes during the early stage of STZ-induced diabetes mellitus.

AUTHOR AFFILIATION

1. Department of Pathophysiology, Tbilisi State Medical University, Tbilisi, Georgia
2. Department of Endocrinology, Tbilisi State Medical University, Tbilisi, Georgia
3. Ivane Beritashvili Experimental Center of Biomedicine, Tbilisi, Georgia
4. Department of Physics, Biophysics, Biomechanics and Informational Technologies, Tbilisi State Medical University, Tbilisi, Georgia

SUPPLEMENTARY MATERIALS

N/A

ACKNOWLEDGEMENTS

N/A

REFERENCES

1. American Diabetes Association. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes—2025. Diabetes Care. 2025;48(suppl 1):S17-S42.
2. International Diabetes Federation. Diabetes facts and figures. Accessed April 4, 2026. https://idf.org/about-diabetes/diabetes-facts-figures/
3. Srikanth KK, Orrick JA. Biochemistry, polyol or sorbitol pathways. In: StatPearls. StatPearls Publishing; 2022. Updated November 14, 2022. Accessed April 4, 2026. https://www.ncbi.nlm.nih.gov/books/NBK576381/
4. Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: sparking the development of diabetic vascular injury. Circulation. 2006;114(6):597-605. doi:10.1161/CIRCULATIONAHA.106.621854.
5. Rajlic S, Treede H, Münzel T, Daiber A, Duerr GD. Early detection is the best prevention—characterization of oxidative stress in diabetes mellitus and its consequences on the cardiovascular system. Cells. 2023;12(4):583. doi:10.3390/cells12040583.
6. Raza H, Prabu SK, John A, Avadhani NG. Impaired mitochondrial respiratory functions and oxidative stress in streptozotocin-induced diabetic rats. Int J Mol Sci. 2011;12(5):3133-3147. doi:10.3390/ijms12053133.
7. Chen X, Xie N, Feng L, et al. Oxidative stress in diabetes mellitus and its complications: from pathophysiology to therapeutic strategies. Chin Med J (Engl). 2025;138(1):15-27. doi:10.1097/CM9.0000000000003230.
8. González P, Lozano P, Ros G, Solano F. Hyperglycemia and oxidative stress: an integral, updated and critical overview of their metabolic interconnections. Int J Mol Sci. 2023;24(11):9352. doi:10.3390/ijms24119352.
9. Abdel-Raheem A, Ibrahim H, Fahim ES, Saber AM. Oxidative stress markers as early predictors of diabetes complications in type 2 diabetic patients. Indian J Physiol Pharmacol. 2022;66. doi:10.25259/IJPP_120_2022.
10. Kisacam MA, Kocamuftuoglu GO, Ufat H, Ozan S. The evaluation of early stage oxidative status in streptozotocin-induced diabetes in rats. Arch Physiol Biochem. Published online June 10, 2020. doi:10.1080/13813455.2020.1776736.
11. Lee JY, Kim M, Oh SB, et al. Superoxide dismutase 3 prevents early-stage diabetic retinopathy in streptozotocin-induced diabetic rat model. PLoS One. 2022;17(1):e0262396. doi:10.1371/journal.pone.0262396.
12. Ghasemi A, Jeddi S. Streptozotocin as a tool for induction of rat models of diabetes: a practical guide. EXCLI J. 2023;22:274-294. doi:10.17179/excli2022-5720.
13. Damasceno DC, Netto AO, Iessi IL, et al. Streptozotocin-induced diabetes models: pathophysiological mechanisms and fetal outcomes. Biomed Res Int. 2014;2014:819065. doi:10.1155/2014/819065.
14. Gu JC, Wu YG, Huang WG, et al. Effect of vitamin D on oxidative stress and serum inflammatory factors in patients with type 2 diabetes. J Clin Lab Anal. 2022;36:e24430. doi:10.1002/jcla.24430.
15. Sepidarkish M, Farsi F, Akbari-Fakhrabadi M, et al. The effect of vitamin D supplementation on oxidative stress parameters: a systematic review and meta-analysis of clinical trials. Pharmacol Res. 2019;139:141-152. doi:10.1016/j.phrs.2018.11.011.
16. Furman BL. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc Pharmacol. 2015;70:5.47.1-5.47.20. doi:10.1002/0471141755.ph0547s70.
17. Yazdi HB, Hojati V, Shiravi A, Hosseinian S, Vaezi G, Hadjzadeh MA. Liver dysfunction and oxidative stress in streptozotocin-induced diabetic rats: protective role of Artemisia turanica. J Pharmacopuncture. 2019;22(2):109-114. doi:10.3831/KPI.2019.22.014.
18. Erejuwa OO, Sulaiman SA, Wahab MSA, Sirajudeen KNS, Salleh MSM, Gurtu S. Differential responses to blood pressure and oxidative stress in streptozotocin-induced diabetic Wistar-Kyoto rats and spontaneously hypertensive rats: effects of antioxidant (honey) treatment. Int J Mol Sci. 2011;12(3):1888-1907. doi:10.3390/ijms12031888.
19. Alhumaydhi FA, Younus H, Khan MA. Catalase functions and glycation: their central roles in oxidative stress, metabolic disorders, and neurodegeneration. Catalysts. 2025;15(9):817. doi:10.3390/catal15090817.
20. Nakamura A, Kawaharada R. Advanced glycation end products and oxidative stress in a hyperglycaemic environment. In: Fundamentals of Glycosylation. IntechOpen; 2022. doi:10.5772/intechopen.97234.

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994.

21. Pavlova ON, Tulaeva ON, Gulenko ON, Gromova DS, Maslyakov VV. Superoxide dismutase and catalase activity in the blood and liver of rats of different age groups with experimental type 2 diabetes mellitus and skeletal muscle injury. Bull Med Inst Reaviz. 2024;13(6):26-33. doi:10.20340/vmi-rvz.2023.6.PHYS.2.
22. Tohari AM, Alhasani RH, Biswas L, et al. Vitamin D attenuates oxidative damage and inflammation in retinal pigment epithelial cells. Antioxidants. 2019;8(9):341. doi:10.3390/antiox8090341.
23. Vázquez-Lorente H, Herrera-Quintana L, Jiménez-Sánchez L, et al. Antioxidant functions of vitamin D and CYP11A1-derived vitamin D, tachysterol, and lumisterol metabolites: mechanisms, clinical implications, and future directions. Antioxidants (Basel). 2024;13(8):996. doi:10.3390/antiox13080996.

Georgian Biomedical News.
ISSN (Online): 2720-8796 ISSN (Print): 2720-7994.