The Egyptian Journal of Hospital Medicine (October 2024) Vol. 97, Page 4512-4518

Potential Antidiabetic Effect of Vitamin D and Vitamin K on Streptozotocin- Induced Diabetes Type II in Male Rats

Nermeen A. Zaitoun*[1], Aliaa Talaat[2], Elham Elshawadifi Megahed[3], Amira M. A. Gobran[1]
Departments of [1]Physiology, [2]Medical Biochemistry and [3] Forensic and Clinical Toxicology,
Faculty of Medicine, Zagazig University, Egypt
***Corresponding author:** Nermeen A. Zaitoun, **Mobile:** (+20) 01000089095,
E-mail: nermozaitoun@gmail.com, **ORCHID-ID:** 0000-0001-6593-6596

ABSTRACT

Background: Many variables can lead to one of the most common chronic diseases called type 2 diabetes mellitus. Vitamins K and D are crucial in managing glycemic balance.

Objective: This study aimed to evaluate the vitamin K and D supplementation effects on albino rats with streptozotocin-induced type 2 diabetes mellitus, particularly those on a high-fat diet.

Material and methods: 78 male albino rats, divided into 6 equal groups. Group I: Normal control group. The remaining 65 adult male albino rats were kept on a high-fat diet for 2 weeks, then injected with streptozotocin for induction of T2DM. Then, they were divided into 5 equal groups. Group II: Diabetic control group, group III: Diabetic taking glimepiride. Group IV: Diabetic taking glimepiride and vitamin D. Group V: Diabetic taking glimepiride and vitamin K. Group VI: Diabetic taking glimepiride, vitamin k and D. After that, blood samples had been collected for estimation of blood glucose, insulin, HOMA IR, vitamin D and vitamin K. Also, gene expression of TGF β, SMAD3 and PDX1 were assessed by PCR.

Results: Supplementation with vitamin K or vitamin D3 improved the blood level of glucose, insulin levels, and HOMA-IR compared to the diabetic group. By gene studies the combined-treated group showed a statistically significant decrease in TGF-<0xCE><0xB2> and SMAD3 when compared to other groups, while a statistically significant rise in PDX1 expression was observed.

Conclusion: Vitamin K and D supplementation can lower hyperglycemia and hyperinsulinemia in rats with T2DM.

Keywords: DM, Vitamin D, Vitamin K, SMAD3, PDX1, Metabolic.

INTRODUCTION

Diabetes mellitus (DM) is a life-threatening condition, which is considered to be a significant factor for both morbidity and mortality. The incidence of adult diabetes is estimated by the IDF to be 537 million, and by 2030, it is predicted to reach 783 million by 2045. This multifactorial disease was in 2019 regarded as the 9th death cause [1]. The majority of cases are classified as type 2 diabetes, a complicated metabolic disease marked by chronic hyperglycemia and either decreased insulin production or inability to use insulin [2].

Hereditary factors, environmental factors (as hypercaloric diet and the resulting abdominal obesity) and host-related factors (ageing, oxidative stress imbalance, and inflammatory responses) all contribute to those with decreased glucose tolerance developing type 2 diabetes [3]. Glucose control plays a major role in the development of diabetes and the prevention or delay of microvascular and macrovascular problems associated with it [4]. There is still debate on the exact etiopathogenesis of diabetes, however a lot of data have been acquired regarding its cause. This is due to the fact that the complicated etiology of diabetes mellitus still poses a serious risk to general health [5]. The part that vitamins K and D play in the development and prevention of diabetes has garnered a lot of attention lately [6]. Vitamin D is famous for its essential role beyond bone health, particularly in metabolic processes. This vitamin may not only help regulate blood sugar levels but also enhance overall metabolic health, offering potential therapeutic benefits for those at risk of insulin resistance and related disorders [7]. In addition to this, it is linked to resistance of insulin and considered the main predictor for T2DM development. Moreover, it is linked to a higher chance of complications from diabetes. Thus, maintaining adequate vitamin D levels through nutrition, supplements, and sun exposure can aid in protection from development of type 2 diabetes and its consequences [8]. In recent years, numerous research have illustrated that vitamin K2 may improve glucose metabolism, insulin sensitivity [5] and decrease the risk of T2DM [9], although more research is still needed to detect how much vitamin K intake affects type 2 diabetes. Also, vit K supports a variety of biological functions, such as anti-inflammatory & antioxidant effects, control of calcium metabolism in tissues and control of cell division and development [10].

Pancreatic duodenal homeobox 1, or PDX1, is typically involved in regulating function of β-cell. Pdx1 is an essential insulin gene in the pancreas that also regulates the production and differentiation of pancreatic growth factors [11]. Research conducted on animals has suggested a possible link between the occurrence of type 2 diabetes and β-cell dysfunction and the downregulation of Pdx1 transcription in the β-cell. Consequently, Pdx1 supports the survival of β-cells and the growth of pancreatic islets in mice [12].

TGF-β is a multifunctional cytokine involved in immune regulation, cellular proliferation, and tissue remodeling. Notably, research indicates that patients with both type 1 and type 2 diabetes exhibit considerably higher plasma levels of TGF-β. This elevation may contribute to the complications associated with diabetes, including vascular damage and fibrosis [13]. This is because TGF-β signaling is essential for growth, proliferation, apoptosis, and

Received: 21/07/2024
Accepted: 19/09/2024

function of β cells [14]. Numerous signaling mechanisms, including the TGF β/SMAD signaling system, carefully regulate each of these physiologic outcomes [15].

Therefore, this study aimed to evaluate the effects of vitamin K and D supplementation in albino rats with streptozotocin-induced type 2 diabetes mellitus, particularly those on a high-fat diet. Additionally, the study aimed to assess how these vitamins influence immunochemical and biochemical parameters.

MATERIALS AND METHODS

Animals and the design of the study: Seventy-eight adult male albino rats weighing 250-300 g. 4-5 rats/steel wire cage were kept under hygienic conditions with unrestricted access to standard chow and water and in room temperature with a normal cycle of light/dark in Physiology Department Animal House, Zagazig Faculty of Medicine. **The experiment design was endorsed by Zagazig University Institutional Animal Care and Use Committee (ZU-IACUC/3/F/224/2023).**

Chemicals: Streptozotocin (STZ) [*N*-(Methylnitrosocarbamoyl)-α-glucosamine] (SIGMA-ALDRICH Co.-USA). The best pH for injection is 4.5, which is achieved by dissolving STZ in either saline or citrate buffer. Because the solution is sensitive to light and tubes, it should be made right before injection and utilized within five minutes of dissolution. A single dose of 35 mg/kg BW was administered intraperitoneally [16].

- **Glimepiride (Amaryl 2mg)** (Sanofi, Egypt)**:** It was given orally by oral gavage once/day for eight weeks at a dosage of 100 mcg/kg body weight [17].
- **Vitamin D** (Fermenta Biotech): It was given orally by oral gavage once daily for eight weeks at a dosage of 4000 IU/kg body weight [17].
- **Vitamin K2** (DevartLab / devart Lab Pharmaceutical): It was taken orally by gavage once daily for eight weeks at a dose of 30 mg/kg body weight [18].

Induction of type II diabetes mellitus: Diabetes type II was turned on by a single intraperitoneal injection of 35 mg/kg STZ diluted in citrate buffer solution after two weeks of a high-fat diet consisting of 58% fat, 27% carbs, and 15% protein. After seven days of injection, animals were considered diabetic and were included in the research if their plasma glucose levels were greater than 300 mg/dl [16].

Experimental design: The rats were left to adapt to laboratory conditions for one week then divided equally into 6 groups: **Group (I) control group** rats were treated with normal pellet diet for 2 weeks followed by injection with single dose of citrate buffer (pH 4.4, 0.1 M). Type II diabetes were induced in the remaining animals then they were divided equally into: **Group (II) diabetic control group** left without any treatment for 8 weeks. **Group (III) diabetic** glimepiride-treated group**, Group (IV) diabetic** glimepiride-treated **+ vitamin D supplementation, group (V) diabetic** glimepiride-treated + vitamin K supplementation **and Group (VI) diabetic** glimepiride-treated + vitamin D and K supplementation. Liquid paraffin was used as a vehicle for the administration of Vit D, K and glimepiride.

Blood sampling: Using the Sorg and Buckner technique, blood samples were taken from the retro-orbital plexus after the animals were put to sleep with urethane (1.3 g/kg) at the end of the trial [4]. Before the blood samples were taken, the animals were fasted for 12 hours. The levels of fasting plasma glucose (FPG) were measured using the glucose oxidase technique (Spinreact, Girona, Spain). Fasting serum insulin (FSI) was measured using the high-sensitivity enzyme-linked immunosorbent assay (ELISA) kit from Biosource Europe S.A., Nivelles, Belgium. Insulin resistance was estimated using the homeostasis model evaluation method. HOMA-IR was calculated as FSI (IU/ml) × FPG (mg/dL)/405. Using a commercial ELISA kit and its methodology, **Vit K2** was measured (Glory science Co., Ltd: U.S.A.).

Gene expression analysis: The method used in rats scar faction was decapitation. After removal of pancreas, it was kept at -80 °C for RNA extraction. Qiagen RNeasy mini kit was used for total RNA extraction from pancreatic tissue homogenate according to the instructions of the manufacturer. The quality of the RNA was assessed using the A260/A280 ratio. For each preparation, the range was between 1.8 and 2.0. The QuantiTect Reverse Transcription Kit was used to reverse transcribe cDNA in accordance with the manufacturer's instructions.

By qRT-PCR using 5 uL of the cDNA, 10 pmol/uL of each primer (1 uL each), and 10 uL of SYBR Green 2x Master Mix Green (QuantiTect SYBR Green PCR Kits, Qiagen) the gene expression study was evaluated.

Mx3005P Real-Time PCR system (Agilent Stratagene, USA) was used. The PCR cycling parameters were denaturation at 95°C for 10 s, enzyme activation at 95 °C for 8 min as a single cycle and annealing and extension at 60 °C for 60 s for 40 cycles, the real-time RT-PCR was carried out. After normalizing the data to GAPDH, the 2-ΔΔCt approach was used to compute the relative expression [19]. The primer sequences are presented in table 1.

Table (1): Primer sequences of TGF β, SMAD3, PDX1and GAPDH

Gene	Forward primer	Reverse primer
TGF β	**AGGGCTACCATGCCAACTTC**	**CCACGTAGTAGACGATGGGC**
SMAD3	**GGCTTTGAGGCTGTCTACCA**	**GGTGCTGGTCACTGTCTGTC**
PDX1	**GGATGAAATCCACCAAAGCTC**	**TTCCACTTCATGCGACGGT**
GAPDH	**GCATCTTCTTGTGCAGTGCC**	**GGTAACCAGGCGTCCGATAC**

Statistical analysis

SPSS software for statistical analysis version 26.0 for Windows was used. The quantitative data were expressed as mean ± SD. The normality was tested using the Kolmogorov-Smirnov test. When comparing more than two groups statistically, one-way ANOVA was used. P-value for statistical significance was ≤ 0.05 (S).

RESULTS

As shown in table (2), group II (diabetic control group) had a statistically significant rise in **glucose levels** when compared to each of the control groups, III, IV, V, and VI. The control group had the lowest mean glucose level, followed by IV, while VI and V did not significantly differ from one another. Group II (diabetic control group) had the lowest mean insulin level compared to the other groups, while group VI (diabetic glimepiride-treated + vitamin D and K supplementation) was next in line.

The comparison of the groups showed a statistically significant increase in insulin levels in the control group relative to all other groups, while groups VI and V did not significantly differ from one another. Regarding HOMA IR, there was a statistically significant difference among the different studied groups (P value < 0.001**).

Group II (diabetic control group) had a statistically significant increase in HOMA IR when compared to the control group, IV, V, and IV groups. The control group had the lowest mean HOMA IR, followed by group VI. Group III did not significantly differ from either groups II or IV or from groups IV or V.

Table (2): Diabetic investigations among the studied groups

Variable	Group I (n=6)	Group II (n=13)	Group III (n=13)	Group IV (n=13)	Group V (n=13)	Group VI (n=13)	Tests	
							F	*P* value
Glucose (gm/dl) Mean ± SD	76.5±3.22	515.3±36.91 *	298.34±24.83 *♯	209.84±15.77 *♯@	206.75±14.28 *♯@	164.99±8.02 *♯@$¥	543.9	<0.001*
Insulin (µg/ l) Mean ± SD	7.33±0.47	2.81±0.3*	4.5±0.37 *♯	5.93±0.37 *♯@	5.94±0.33 *♯@	6.42±0.43 *♯@$¥	201.9	<0.001*
HOMA IR Mean ± SD	1.39±0.13	3.55±0.31 *	3.32±0.44 *	3.09±0.42 *♯	3.02±0.07 *♯@	2.61±0.18 *♯@$¥	48.6	<0.001*

(f)= ANOVA test, (*) significant with control group, (♯) significant with group II, (@) significant with group III, ($) significant with group VI, (¥) significant with group V.

Group I: control group, Group II: diabetic group (high fat diet for 2 w then streptozotocin intraperitoneal)
Group III: diabetic as above taking treatment, Group IV: diabetic taking treatment and vit D for 6 weeks
Group V: diabetic taking treatment and Vit K, Group VI: diabetic taking treatment and vit D and K

Group VI had a statistically significantly higher **vitamin D3** level than the other groups, followed by groups IV and V, and finally groups III and II. While, there was no discernible difference between group II, control group, and group III. The control group had the lowest mean vitamin D3 levels. There was statistically insignificant difference between studied groups concerning vitamin K (Table 3).

Table (3): Vitamin D and Vitamin K supplementation of the studied groups:

Variable	Group I (n=6)	Group II (n=13)	Group III (n=13)	Group VI (n=13)	Group V (n=13)	Group IV (n=13)	Tests	
							F	*P* value
Vit D (µg/ l) Mean ± SD	248.63± 19.53	250.13±18.23	269.27± 19.18 *♯	339.35±31.7 *♯@	305.05±12.72 *♯@$	380.8±14.85 *♯@$¥	79.4	<0.001*
Vit K (nmol/l) Mean ± SD	1.4±0.13	1.39±0.2	1.54±0.3	1.61±0.2	1.92±0.27	1.71±0.23	0.92	0.472

(f)= ANOVA test, (*) significant with control group, (♯) significant with group II, (@) significant with group III, ($) significant with group VI, (¥) significant with group V
Group I: control group, Group II: diabetic group (high fat diet for 2 w then streptozotocin intraperitoneal)
Group III: diabetic as above taking treatment, Group IV: diabetic taking treatment and vit D for 6 weeks
Group V: diabetic taking treatment and Vit K, Group VI: diabetic taking treatment and vit D and K.

TGF β levels were statistically significantly higher in group II (diabetic control group) than in any other group, whereas the control group had the lowest TGF β mean values, followed by group VI, while VI and V did not significantly differ from one another. Group VI (diabetic receiving therapy and vitamin D and K) had the lowest **SMAD** mean values, while group II had a statistically significant increase in SMAD when compared to all other groups, meanwhile groups VI and V did not significantly differ from one another. Group VI (diabetic receiving therapy + vitamin D + K) had a statistically significant rise in **PDX1** when compared to all other groups, whereas group II had the lowest PDX1 mean values, followed by group IV (diabetic receiving treatment + vitamin D for six weeks). Group III did not differ significantly from either groups IV or V (Table 4).

Table (4): Gene expressions between the studied groups

Variable	Group I (n=6)	Group II (n=13)	Group III (n=13)	Group IV (n=13)	Group V (n=13)	Group VI (n=13)	Tests	
							F	*P* value
TGF Mean ± SD	1.12±0.12	3.37±0.22 *	2.17±0.19 *♯	2.33±0.2 *♯@	2.43±0.18 *♯@	1.4±0.17 *♯@$¥		<0.001
SMAD Mean ± SD	1.14±0.11	2.17±0.19 *	1.49±0.15 *♯	1.68±0.17 *♯@	1.71±0.13 *♯@	1.36±0.14 *♯@$¥		<0.001
PDX1 Mean ± SD	1.08±0.07	0.6±0.1 *	0.74±0.11 *♯	0.69±0.11 *♯	0.72±0.1 *♯	1.28±0.09 *♯@$¥		<0.001

(f)= ANOVA test, (*) significant with control group, (♯) significant with group II, (@) significant with group III, ($) significant with group VI, (¥) significant with group V

Group I: control group

Group II: diabetic group (high fat diet for 2 w then streptozotocin intraperitoneal)

Group III: diabetic as above taking treatment

Group IV: diabetic taking treatment and vit D for 6 weeks

Group V: diabetic taking treatment and Vit K

Group VI: diabetic taking treatment and vit D and K.

DISCUSSION

A complicated interaction between environmental and genetic factors causes varying degrees of insulin resistance and β-cell malfunction in type 2 diabetes, a non-communicable disease. Age, inactivity, and weight gain are its defining characteristics. It is characterized by persistently high blood sugar levels brought on by an insufficient pancreatic β-cell response to the developing insulin resistance [20].

Despite ongoing studies, there is still no effective cure for diabetes mellitus. Additionally, nearly all currently available medications have serious side effects, ranging from obesity, pulmonary edema, and hypoglycemic coma to simple diarrhea, stomach discomfort, and anemia. In an effort to find new novel methods for diabetes prevention and treatment, vitamin D supplementation has drawn more and more attention. Because vitamin D reduces peripheral insulin resistance through the liver and muscle vitamin D receptors and stimulates insulin secretion through the pancreatic beta cell vitamin D receptor [21].

Deficiency in vitamin K has been associated with poor glucose metabolism and insulin resistance. The participation of vitamin K-dependent protein osteocalcin, as well as the anti-inflammatory and lipid-lowering effects of vitamin K2, were shown to improve insulin sensitivity. When it came to treating type 2 diabetes, vitamin K2 was more beneficial than vitamin K1 [22].

The current study illustrated that the diabetic group's glucose level increased statistically significantly when compared to each of control group, group III, group IV, group V, and group IV. Group III, V had the lowest mean glucose levels, but group VI and group V did not significantly vary from the control group. In agreement with our findings, **Moharir *et al.*** [17] stated that rats with experimentally-induced T2DM have higher fasting blood glucose levels. All group's blood glucose levels were similar. Blood glucose levels increased in the diabetic control group and stayed high for the duration of the trial. In contrast, blood glucose levels dramatically decreased in the diabetic with therapy group and the diabetic with treatment + vitamin D group. In a study by **Calle *et al.*** [23], vitamin D therapy increased the reduced basal glucose transfer in streptozotocin-induced diabetic rats by 107%, which is correlated with lower glucose levels in our investigation. Vitamin D treatment significantly increased insulin concentration, which may help diabetic rats with their hyperglycemia. Rats given vitamin D had considerably lower serum HbA1c.

The present study revealed that the control group had a statistically significant rise in insulin compared to all other groups, followed by the diabetes group receiving therapy and vitamin D and K group compared to groups II, III, IV, V, and II. Among the groups, the diabetic group had the lowest mean insulin level. Group VI and group V did not differ significantly from one another. These results are compatible with

Moharir *et al.* [17] who reported that serum insulin levels were lower in rats with experimentally-created type 2 diabetes during fasting. In diabetic control rats, serum insulin levels were much higher and HbA1c levels were significantly lower. **Zoair** [24] showed that it has been shown that in response to glucose stimulation, vitamin D enhances insulin secretion rather than having an effect on baseline insulinemia.

Our current findings regarding HOMA IR clearly revealed that the diabetic group's HOMA IR increased statistically significantly when compared to the control group, group IV, group V, and group IV. Between groups II and IV as well as between groups IV and V, there was no discernible difference. The diabetic group getting treatment together with vitamin D and K had the second lowest mean HOMA IR, after the control group. In line with our results, **Zoair** [24] found that HOMA-IR values of diabetic rats returned to a reasonably normal value after they received vitamin D-treatment and pre-treatment. The diabetic rats' HOMA-IR readings, however, were significantly greater than the corresponding controls. **Kavadar *et al.*** [25] showed that in reaction to the elevated blood sugar brought on by insulin resistance, vitamin D lowers insulin resistance, which lowers the overproduction of insulin. It raises insulin sensitivity as a result. Vitamin D increases the development of β-cells and speeds up the conversion of proinsulin. Vitamin D acts as a supportive factor in managing blood sugar levels through insulin sensitivity enhancement and regulation of inflammation. It enhances the function of insulin receptors on cells, allowing for more effective glucose uptake and utilization. This can lead to better control of blood sugar levels, particularly in individuals with insulin resistance. Vitamin D plays a role in modulating the immune system and reducing inflammation. Chronic inflammation is often associated with insulin resistance and poor glycaemic control. By mitigating inflammatory responses, vitamin D may help improve insulin action and overall glucose metabolism, thereby contributing to better glycemic control [26].

Concerning antidiabetic effect of vitamin D on streptozotocin-induced diabetes type II in male albino rats. Analysis of groups IV, V, III, and II revealed that in comparison with the other groups, the diabetic group undergoing treatment showed a statistically significant increase in their vitamin D levels. Despite the fact that the control group's mean level of vitamin D was the lowest, the control group, groups II, and III did not vary in any noticeable ways. This was in accordance with **Moharir *et al.*** [17] who stated the infusion of vitamin D significantly improved insulin sensitivity, hyperinsulinemia, and hyperglycemia in diabetic rats as compared to those animals without treatment. The combination of glimepiride and vitamin D did not cause the same drop in blood sugar levels as glimepiride monotherapy. The improvement obtained with vitamin D supplementation is in agreement with the study of **Zeitz *et al.*** [27].

Scragg *et al.* [28] showed that Low 25(OH)D levels were strongly inversely correlated with diabetes, and bringing blood vitamin D levels back to normal reducing the incidence of type 2 diabetes by 55%. This might be explained by the finding that 1 alpha-hydroxylase and vitamin D receptors are present in pancreatic insulin secretions. Additionally, vitamin D has been demonstrated to have an indirect influence on insulin secretion, possibly through a calcium action. By helping extracellular calcium to return to normal, vitamin D helps to maintain the proper flow of calcium across cell membranes. Other possible routes that link vitamin D and diabetes involve improving responsiveness of insulin for glucose transport, convalescing insulin action by boosting insulin receptor expression, and perhaps influencing insulin function indirectly through calcium effect on insulin secretion.

Vitamin D may influence glucose metabolism in two ways: Either by a delayed genomic effect that upregulates VDR expression to promote insulin release, or through a quick non-genomic action. Another possible strategy is to inhibit the synthesis of proinflammatory cytokines, which are believed to be the root cause of insulin resistance. The latter theory is supported by studies showing a link between low blood 25(OH)D and high C-reactive protein levels. Furthermore, vitamin D may have an indirect effect on the intracellular and extracellular regulation of calcium in target tissues. The control of glucose transport depends on this modulation [29].

The change in IP3 and AMPA receptor, a non-NMDA-type ionotropic transmembrane receptor for glutamate expression in the pancreatic islets, was shown to be corrected by cholecalciferol supplementation. By assisting in the restoration of calcium-mediated insulin production, this showed the vitamin's potential therapeutic role in controlling glutamatergic activity in diabetic rats [30].

In terms of effect of vitamin K on streptozotocin-induced diabetes type II in male albino rats, the comparison between groups revealed statistically non-significant difference in vitamin K mean value in all groups compared to each other. Unlikely, **Helmy *et al.*** [31] illustrated that patients with uncontrolled T2DM were shown to have lower vitamin K2 levels than those with managed diabetes. The relationship between vitamin K2 level and insulin resistance was demonstrated by the finding of a negative association between vitamin K2 level and fasting insulin and HOMA IR in patients with uncontrolled type 2 diabetes. Additionally, vitamin K2 was found to be negatively correlated with fasting insulin, FBG, 2-hour PPBG, HbA1c, and HOMA-IR in diabetics. The findings further confirmed that blood vitamin K2 is a useful diagnostic tool for those with type 2 diabetes who have uncontrolled hyperglycemia. It was established

that the only reliable independent predictor of blood vitamin K2 levels was FBG. They discovered that whereas vitamin K2 supplementation was linked to an increased insulin sensitivity index, placebo treatment had no effect on the index. **Manna *et al.*** [32] demonstrated that vitamin K2 was more advantageous than vitamin K1 in terms of insulin sensitivity and glucose metabolism, and that vitamin K1 and K2 intake was required to lower the risk of type 2 diabetes.

Blood vitamin K status and plasma insulin level are positively correlated, while Vit K intake had no discernible effect on fasting plasma glucose status. **Yoshida *et al.*** [33] stated that in the 2-hour oral glucose tolerance test, increased vitamin K1 ingestion corresponded with higher insulin sensitivity and glycemic status. They investigated how vitamin K1 consumption and insulin sensitivity in older persons are related to each other. Conversely, during the oral glucose tolerance test, men who ingested less vitamin K1 had greater glucose and lower insulin levels than men who consumed more vitamin K1. This can be explained by how vitamin K affects OC and adiponectin levels and how they relate to glycemic management and insulin sensitivity. Furthermore, vitamin K has anti-inflammatory properties because it can reduce lipid levels and deactivate the tumor necrosis factor κB (NF κB) signaling pathway [9].

Our current findings regarding gene expressions clearly revealed that TGF and SMAD 3 levels were statistically significantly higher in the diabetes group than in any other group. This is in concordance with **Wang *et al.*** [14] who found that the growth, function, proliferation, apoptosis, and dedifferentiation of β cells are all influenced by TGF-β_signaling. According to **Wang *et al.*** [13]. TGF-β _/SMAD 3 signaling is a crucial route in the pathophysiology of diabetes and diabetic nephropathy, which helps to explain this. The MPK/TGF-β1/Smad pathway promotes the development of fibrosis and metabolic abnormalities in type 2 diabetes and insulin pathway.

Our results showed that, in comparison with the other groups, the diabetic group receiving treatment with vitamin D and K had a statistically significant decrease in SMAD. SAMD 3 deficiency causes the regression of overt diabetes by promoting β cell proliferation and function produced by insulin demand, inhibiting cell apoptosis and dedifferentiation induced by stress, and eliminating peripheral insulin resistance. In SMAD 3 deletion mouse models of type 2 diabetes, SAMD 3 loss results in the regression of overt diabetes by promoting peripheral insulin resistance, inhibiting stress-induced cell death and dedifferentiation, and promoting insulin demand-induced β cell proliferation and function [14].

According to **Shehata *et al.*** [34], pancreatic expression of the PDX1 and Ins1 genes, insulin level, total lipid profile results, and total antioxidant capacity (TAC) all demonstrated significant improvements, which is consistent with our findings. Also, **El-Gohary *et al.*** [35] reported that Pdx1 significantly increases the proliferation of β cells in mice that had a partial pancreatectomy. Vitamin D has anti-diabetic properties via increasing the expression of the PDX1 and Ins1 genes in mice with STZ diabetes.

CONCLUSION

Vitamin K and D injections can lower hyperglycemia and hyperinsulinemia in rats with type 2 diabetes mellitus induced by streptozotocin. Hence, they may be used as an adjuvant treatment in addition to other antidiabetic medications. Before their using to treat type 2 diabetes, additional randomized clinical control studies must confirm the dosage and duration.

Conflict of interest: None.
Financial disclosures: None.

REFERENCES

1. **International Diabetes Federation (2021):** IDF Diabetes Atlas Tenth Edition. Pp: 101. https://idf.org/aboutdiabetes/what-is- diabetes/facts-figures.html.
2. **Keane K, Calton E, Carlessi R *et al.* (2017):** The bioenergetics of inflammation: insights into obesity and type 2 diabetes. Eur J Clin Nutr., 71 (7): 904-912.
3. **Harinarayan C (2014):** Vitamin D and diabetes mellitus. Hormones, 13: 163-181.
4. **Chagas C, Borges M, Martini L *et al.* (2012):** Focus on vitamin D, inflammation, and type 2 diabetes. Nutrients, 4: 52-67.
5. **Sun H, Saeedi P, Karuranga S *et al.* (2022):** IDF Diabetes Atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract., 183: 109119. doi: 10.1016/j.diabres.2021.109119.
6. **Raghuvanshi D, Chakole S, Kumar M (2023):** Relationship Between Vitamins and Diabetes. Cureus, 15 (3): e36815. doi: 10.7759/cureus.36815.
7. **Miller E, Pastor-Barriuso R, Dalal D *et al.* (2005):** Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Ann Intern Med., 142: 37-46.
8. **Juanola-Falgarona M, Salas-Salvadó J, Estruch R *et al.* (2013):** Association between dietary phylloquinone intake and peripheral metabolic risk markers related to insulin resistance and diabetes in elderly subjects at high cardiovascular risk. Cardiovasc Diabetol., 12: 7. doi: 10.1186/1475-2840-12-7
9. **Ho H, Komai M, Shirakawa H (2020):** Beneficial Effects of Vitamin K Status on Glycemic Regulation and Diabetes Mellitus: A Mini-Review. Nutrients, 12: 2485. doi: 10.3390/nu 12082485.
10. **Akbari S, Rasouli-Ghahroudi A (2018):** Vitamin K and Bone Metabolism: A Review of the Latest Evidence in Preclinical Studies. BioMed Res Int., 18: 4629383. doi: 10.1155/2018/ 4629383.
11. **Siwan D, Nandave M, Gilhotra R *et al.* (2024):** Unlocking β-cell restoration: The crucial role of PDX1

in diabetes therapy. Pathol Res Pract., 254: 155131. doi: 10.1016/j.prp.2024.155131.

12. **Kaneto H, Kimura T, Shimoda M *et al.* (2022):** Molecular mechanism of pancreatic β-cell failure in type 2 diabetes mellitus. Biomedicines, 10 (4): 818. doi: 10.3390/biomedicines 10040818.
13. **Wang L, Wang H, Liu T *et al.* (2021):** TGF-Beta as a Master Regulator of Diabetic Nephropathy. Int J Mol Sci., 22 (15): 7881. doi: 10.3390/ijms22157881.
14. **Wang H, Wang L, Zhao C *et al.* (2022):** Role of TGF-Beta Signaling in Beta Cell Proliferation and Function in Diabetes. Biomolecules, 12 (3): 373. doi: 10.3390/biom12030373.
15. **Weiss A, Attisano L (2013):** The TGFbeta Superfamily Signaling Pathway. Wiley Interdiscip Rev Dev Biol., 2: 47–63.
16. **Srinivasan K, Viswanad B, Kaul C *et al.* (2005):** Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacological Research, 52: 313-20.
17. **Moharir G, Naikawadi A, Patil J *et al.* (2020):** Effect of Vitamin Don Blood Sugar, HbA1c and Serum Insulin Levels in Streptozotocin-Induced Diabetic Rats. Maedica (Bucur), 15 (3): 327-331.
18. **Hussein A, Mohamed R, Shalaby S *et al.* (2018):** Vitamin K 2 alleviates type 2 diabetes in rats by induction of osteocalcin gene expression. Nutrition, 47: 33–38.
19. **Livak K, Schmittgen T (2001):** Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Δ Δ C (T)) Method. Methods (San Diego, Calif.), 25 (4): 402–408.
20. **Padhi S, Nayak A, Behera A (2020):** Type II diabetes mellitus: a review on recent drug based therapeutics. Biomedicine & Pharmacotherapy, 131: 110708. doi: 10.1016/j.biopha.2020. 110708.
21. **Dominguez E, Peñafiel A, Pedraza A *et al.* (2021):** Molecular mechanisms from insulin-mimetic effect of vitamin D: treatment alternative in Type 2 diabetes mellitus. Food & Function, 12 (15): 6682-6690.
22. **Rahimi Sakak F, Moslehi N, Niroomand M *et al.* (2021):** Glycemic control improvement in individuals with type 2 diabetes with vitamin K 2 supplementation: A randomized controlled trial. European Journal of Nutrition, 60: 2495-2506.
23. **Calle C, Maestro B, García-Arencibia M (2008):** Genomic actions of 1, 25-dihydroxyvitamin D3 on insulin receptor gene expression, insulin receptor number and insulin activity in the kidney, liver and adipose tissue of streptozotocin-induced diabetic rats. BMC Molecular Biology, 9 (1): 1-12.
24. **Zoair M (2021):** Effect of pre-treated and treated vitamin D on insulin resistance, lipid profile and platelet indices in adult diabetic male albino rats. Al-Azhar Medical Journal, 50 (1): 809-820.
25. **Kavadar G, Demircioğlu D, Özgönenel L *et al.* (2015):** The relationship between vitamin D status, physical activity and insulin resistance in overweight and obese subjects. Bosnian Journal of Basic Medical Sciences, 15 (2): 62-66.
26. **Szymczak -Pajor I, Śliwińska A (2019):** Analysis of Association between Vitamin D Deficiency and Insulin Resistance. Nutrients, 11 (4): 794. doi: 10.3390/nu11040794.
27. **Zeitz U, Weber K, Soegiarto D *et al.* (2003):** Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. The FASEB Journal, 17 (3): 1-14.
28. **Scragg R, Sowers M, Bell C (2004):** Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care, 27 (12): 2813-2818.
29. **Helmy M, Elsaid N, Gwad M (2022):** The association of vitamin K2 level with the glycaemic status in type 2 diabetic patients: a case-control study. Indian Journal of Endocrinology and Metabolism, 26 (1): 87-92.
30. **Wu J, Atkins A, Downes M *et al.* (2023):** Vitamin D in Diabetes: Uncovering the Sunshine Hormone's Role in Glucose Metabolism and Beyond. Nutrients, 15 (8): 1997. doi: 10.3390/nu15081997.
31. **Jayanarayanan S, Anju T, Smijin S *et al.* (2015):** Vitamin D3 supplementation increases insulin level by regulating altered IP3 and AMPA receptor expression in the pancreatic islets of streptozotocin-induced diabetic rat. J Nutr Biochem., 26 (10): 1041-49.
32. **Manna P, Kalita J (2016):** Beneficial role of vitamin K supplementation on insulin sensitivity, glucose metabolism, and the reduced risk of type 2 diabetes: A review. Nutrition, 32 (7-8): 732-739.
33. **Yoshida M, Booth S, Meigs J *et al.* (2008):** Phylloquinone intake, insulin sensitivity, and glycemic status in men and women. The American Journal of Clinical Nutrition, 88 (1): 210-215.
34. **Shehata N, Abo zeid S, Abd El Aziz S *et al.* (2022):** Mitigation of streptozotocin-induced alterations by natural agents via upregulation of PDX1 and Ins1 genes in male rats. Journal of Food Biochemistry, 46 (5): e14086. doi: 10.1111/jfbc.14086.
35. **El-Gohary Y, Tulachan S, Wiersch J *et al.* (2014):** A Smad Signaling Network Regulates Islet Cell Proliferation. Diabetes, 63: 224–236.