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Effect of vitamin D on blood glucose and lipid profile in streptozotocin-induced diabetic rats

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***Abstract*---**Introduction and Objectives: One of the vital nutrients, vitamin D, has a role in fat metabolism and other metabolic processes in addition to its direct impact on calcium and bone metabolism. This study intends to look at how vitamin D intake affects the levels of

lipids, glucose, and insulin in rats with experimentally induced diabetes mellitus. Materials and Methods: 24 male albino Wistar rats, weighing between 250 to 300 g, were placed into four groups at random (n=6). Group 1 served as the control group, while the other three groups were given an i.p. injection of 35 mg/kg streptozotocin (STZ) to induce diabetes and a high-fat diet for three weeks. Then, for the following three weeks, groups III and IV received treatment with vitamin D (400 IU/kg) and Vitamin D + Glimepiride (50 mcg/kg). Results: Vitamin D supplementation was observed to dramatically lower lipid profile, raise insulin, and decrease HbA1c concentration. Additionally, when Vitamin D and Glimepiride were administered together, a substantial decrease in the serum concentrations of blood glucose, HbA1c, and lipid profile was seen (p 0.05). Conclusion: According to the findings of this study, treatment with glimepiride and vitamin D could dramatically lower the fasting plasma glucose, insulin, and lipid profiles in an experimental type 2 diabetic animal.

***Keywords*---**vitamin D, diabetes mellitus, streptozotocin, HbA1c, rats.

Introduction

Diabetes mellitus type 2 is a non-communicable disease that is spreading over the world at an alarming rate [1]. According to the WHO, the prevalence of diabetes will increase from 17.1 crores people in 2000 to 36.6 people in 2030 [2]. Type 2 DM is linked to hypertension[3] cardiovascular [4,5] disease, blindness, nephropathy, and neuropathic consequences [6,7]. The main role of metabolism and bone health is played by vitamin D, a fat-soluble vitamin. Vitamin D insufficiency has been linked to a variety of non-skeletal ailments [8], including osteoporosis, cancer [9], and immunological disorders; type 1 and type 2 diabetes; hypertension; cardiovascular disease; and others. The vitamin D receptor (VDR) and the 1-hydroxy enzyme (25-hydroxyvitamin D is converted to 25-hydroxyvitamin D) are both found in numerous organs [10].

Vitamin D insufficiency has been linked to reduced glucose tolerance and diabetes mellitus in numerous cross-sectional and interventional investigations [11,12,13] . As a result, blood hydroxyl cholecacitriol concentrations in T2DM patients are lower than in healthy individuals. The adverse association between vitamin D and type 2 diabetes has been explained by many processes. Through genetic and non-genomic processes, On insulin synthesis, beta-cell function, and insulin resistance, vitamin D has both direct and indirect effects [14,15]. Contrarily, the major cause of death in those with type 2 diabetes is cardiovascular disease. Vitamin D is necessary for endothelial function, blood pressure regulation, calcification of the coronary vasculature, raising vascular resistance, and avoiding CVD, claims a study [16]. One of the hypothesised mechanisms for the association between vitamin D levels and lipid profile is vitamin D's action on lipid profile modulation. The goal of our research is to see if there's a link between vitamin D levels in the blood and lipid profiles such as cholesterol, TG, HDL, and LDL in type 2 diabetic rats.

Materials and Methods

24 male Wistar rats weighing 200–250 g were used in the experiment. The investigation was conducted at BLDE University in Vijayapur's main animal house. Prior to and after the experiment, rats were housed with free access to food and water, a 12:12 h light/dark cycle, and stable environmental conditions. The handling of the animals during the experiment, sampling, and sacrifice all adhered to CPCSEA regulations. The IAEC of BLDE University, Vijayapur, authorised the study. Four groups of rats were randomly assigned; group 1 served as the control group, while groups 2, 3, and 4 were fed a high-fat diet [17]. for three weeks and received a single intraperitoneal (i.p.) 35 mg/kg injection of freshly prepared STZ, dissolved in 0.1 M sodium citrate buffer with pH 4.5, within a few minutes of preparation. Three days following STZ injection, diabetes was assessed by checking the blood glucose level using glucometer (Aqua Chek Active) strips. Diabetic rats were defined as those with blood glucose levels greater than 250 mg/dL and were included in the study. Following 72 hours of diabetes induction, the treatments started and continued each day for 21 (three weeks) straight days.

Animals were grouped as follows

Group 1: oral distilled water was administered.
Group 2: diabetic untreated wistar rats were given vehicle daily orally.
Group 3: diabetic rats received 400 iu/kg body weight of vitamin d daily orally.
Group 4: diabetic treated 400 iu/kg body weight of vitamin d and glimepiride (glim) 50 mcg/kg body weight daily orally.

Experimental procedures and laboratory measurements

At the end of the study period, anaesthetic was administered to the animals, and blood samples were taken through the retro-orbital plexus for evaluation of the following parameters: HbA1c, Triglycerides (TG), Total cholesterol (TC), High-Density Lipoprotein-Cholesterol (HDL-C), and Low-Density Lipoprotein-Cholesterol (LDL-C). A 50mcg/kg body weight oral dosage of glimepiride was given. Carboxymethylcellulose was used as a vehicle for the administration of glimepiride and vitamin D. Throughout the study, all efforts were taken to reduce rats' suffering. Statistical analysis was done by using SPSS version-17 and methods used were oneway ANOVA and Student’s t-test.

Results

Table 1. Body weight at the beginning and after the end of every week in gm (Mean±SD)

Groups	Week 0	Week 1	Week 2	Week 3
Control	212±8.2	228±7.6	241±8.1	253±5.7
Diabetic Control (DC)	345±10.1	380±11.5	398±8.4	402±9.2
DC + Vit D	314±6.1	341±4.4	345±6.6	342±9.5
Glim + Vit D	315±4.4	280±4.7	260±6.4	235.5±5

Animals in group 1-3 gained body weight consistently. However, rats in group 2

(Diabetic control) gained significant ($p<0.05$) body weight compared to normal control rats. Whereas, body weights in group 4 (Diabetic + Glimiperide and Vitmain D) rats were decreased signicantly compared to the group 1 (normal control) and group 2 (diabetic control).

Table 2. Blood glucose(FBS) before 1 hour of stz administration and on subsequently at the end of every week (mg/dL) (Mean±SD)

Groups	Week 0	Week 1	Week 2	Week 3
Control	80±4.3#	80±4.1#	78±3.4#	73±4.7#
Diabetic Control (DC)	125±6.1*	404±25.7*	518±34.1*	526±37.7*
DC + Vit D	118±6.2*	368±16.5*	459±12.1*	471±9.4*#
Glim + Vit D	120±8.1*	130±8.3*#	123±10.3*#	90±5.2*#

*p<0.05 compared with the control group. #p<0.05 compared with the diabetic group

The blood glucose levels of diabetic rats and diabetes treatment groups (groups 3 and 4) were significantly ($p<0.05$) elevated compared to the normal control group. Group 3 rats treated with Vitmain D alone demonstrated a modest reduction in blood glucose levels. Vitmain D in conjunction with Glimepiride significantly decreased blood glucose levels compared to Group 2 (Diabetic control).

Table 3. Lipid profile of diabetic male rats treated with glimepiride, vitamin D, and their mixtures (MeanSD) and control rats

Groups	TG (mg/dL)	TC (mg/dL)	LDL(mg/dL)	VLDL (mg/dL)	HDL (mg/dL)	CAL (mg/dL)
Control	85±10.7	156±13.9	65±6.3	20±5.2	27±5.1	10.78±0.5
Diabetic Control (DC)	149±15.1#	324±23.1#	244±22.2#	28±2.3#	13±1.9#	7.3±0.6
DC + Vit D	135±12.2*	298±11.1*	222±5.4*	24±2*	15±1.7*	9.53±1*
Glim + Vit D	111±8*	220±6.9*	156±11.7*	20±2.7*	20±2.4*	10±0.9*

#p < 0.05 compared with the control group. *p < 0.05 compared with the diabetic group.

After Vitmain D administration, lipid profile (TG, TC, LDL-c, and VLDL-c) levels reduced in comparison to the diabetic group., by 9.39%, 8.0%, 9%, and 14.2%, and Vitmain D + Glimepiride by 25.50%, 32%, 36%, 28.57%, respectively. Whereas, there is increase in HDLc and Calcium levels in group 3 and 4 by 15.38%, 30.54%, and 53.86%, 36.98%, respectively. Similarly the values of calcium in group 3 and 4 are comparable with group 1 ($p < 0.05$).

Table 4. Insulin, HbA1c, and Vitamin D levels in control and diabetic male rats treated with vitamin D, glimepiride and their combinations (Mean±SD)

Groups	Insulin (mU/L)	HbA1c (mmol/mol	Vit D (µg/L)

		%)	
Control	7.7+0.3	7+1	272.5±21
Diabetic Control (DC)	2.7 +0.5	14.26.5+2	236.7±22.7
DC + Vit D	3.1+0.4*	12.19.33+1.32*	415.2±35.6*#
Glim + Vit D	6.6+0.6#	8.6+1.2#	387.2±35.6*#

#p value <0.05 Compared to diabetic group. *p value <0.05 Compare to control group.

Normal insulin levels were observed in the normal control group (group 1). These levels were decreased significantly ($p<0.05$) in the diabetic control group compared to normal rats. Vitamin D treatment increased insulin levels marginally in diabetic rats but failed to achieve normal levels. HbA1c levels were significantly ($p<0.05$) increased in the diabetic control rats compared to the normal control. Treatment with Vitamin D did not reduce HbA1c levels significantly. However, Vitamin D in combination with Glimepiride reduced the HbA1c levels significantly ($p<0.05$) compared to diabetic untreated rats. Vitamin D levels were reduced slightly in the diabetic rats compared to normal rats. Whereas Vitamin D levels were significantly high in Vitamin D alone and in combination groups (groups 3 and 4).

Discussion

There is accumulating proof that from both animal and human studies through both direct and indirect methods, vitamin D may potentially lower the risk of acquiring both type 1 and type 2 diabetes mellitus [18]. According to several report [19,20] , low vitamin D levels have been associated with an elevated risk of type-I diabetes mellitus incidence and type-II diabetes mellitus development in general population. The evidence from experimental studies and clinical trials, however, is scant and ambiguous, making it insufficient to establish a link between vitamin D intake and glycemic control. In our study, vitamin D intake led to a slight drop in FPG. Additionally, there was a slight improvement in the insulin level. The significance of vitamin D in bringing the glucose level back to normal is explained by a number of different mechanisms, according to earlier research. Vitamin D insufficiency has been demonstrated to impair glucose metabolism by increasing insulin resistance, which is brought on by a drop in adipose PPAR- expression and a decline in beta-cell mass and function [21]. The restoration of calcium-mediated insulin secretion was facilitated by the reversal of the altered IP3 and AMPA expression level in the pancreatic islets, this demonstrated the vitamin's therapeutic impact on glutamatergic function in diabetes patients [22]. In our investigation, it was shown that vitamin D administration not only brought blood sugar levels close to normal but also raised insulin levels (p 0.05). Additionally, it has been noted that 1, 25$(OH)_2$ D_3 therapy restored the pathogenic alterations in the mTOR signaling pathway brought on by high hyperglycemia, successfully preventing apoptosis in beta-cells [23]. Additionally, vitamin D appears to improve the IRS-1-mediated intracellular mechanisms of insulin action and increase the expression of the total protein GLUT4 [24,25] . Numerous studies show that oral administration of dietary vitamin D to older mice enhances glucose metabolism by enhancing GLP-1 [26]. According to some reports, cholecalciferol supplementation

in skeletal muscle can reverse drug-induced reductions in Pik3r1 expression, a crucial gene acting downstream of the insulin receptor, and concurrent calcitriol can improve impaired insulin-stimulated glucose uptake into the myotube in a PI3K-dependent manner [27]. The significant dyslipidemia in untreated diabetic rats was indicated by an increase in plasma triacylglycerol, total cholesterol, very-low-density lipoprotein (VLDL) cholesterol, and low-density lipoprotein (LDL), and low-density lipoprotein cholesterol, and a decrease in high-density lipoprotein (HDL). Similar findings were found in a number of investigations using animal or experimental models of diabetes[28,29,30,31]. The hypertriglyceridemia associated with diabetes is thought to be caused by a number of metabolic processes, one of these is a rise in hormone-sensitive lipase activity, which catalyses the mobilisation of fatty acids from triacylglycerols stored in adipocytes [29, 32]. The volume of fatty acids that reach the liver increases as a result, and it subsequently reassembles those fatty acids into triacylglycerols that are then secreted in VLDL. The activity of the enzyme lipoprotein lipase, which catalyses the hydrolysis of triacylglycerols in VLDL and chylomicrons, has also been shown to be decreased by diabetes [32,33]. This encourages hypertriglyceridemia in diabetics. In the current investigation, vitamin D treatment in STZ-induced diabetic rats, there was a reduction in VLDL, triacylglycerols, and LDL and an increase in HDL. This may be related to better glycemic management through a process involving more effective insulin due to vitamin D [34,35].

Overall, this study demonstrated that supplementing with vitamin D might considerably lower blood glucose levels, increase insulin sensitivity, and alters lipid profiles in an experimental diabetic model. The combination of vitamin D and glimepiride had the greatest positive impact. To include vitamin D in the treatment of diabetes mellitus, further research including the general population, interventional studies, and clinical trials are required.

Conclusion

In the above study we found that Vitamin D is one of the important vitamins which not only has its direct action on calcium and bone metabolism, it is also involved in other metabolic functions including glucose homeostasis and fat metabolism, in the above study it was found to be effective in combination with glimepiride in controlling blood glucose levels and also in reducing all the components of lipid profile and increasing HDLc levels.

Conflict of interests: None Declared
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