EFFECT OF VITAMIN D SUPPLEMENTATION ON HEPATIC FUNCTION, LIPID PROFILE, AND DIABETIC PROFILE IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is considered one of the leading liver disorders in type 2 diabetes mellitus (T2DM). The progression of NAFLD is faster in T2DM patients than in nondiabetic patients, consequently leading to serious complications such as cirrhosis and hepatocarcinoma. vitamin D3 has been reported to protect the liver from non-alcoholic fatty liver disease (NAFLD) by attenuating liver damage in type 2 diabetes mellitus (T2DM). vitamin D3 also regulates inflammation by reducing the release of pro-inflammatory cytokines and affects insulin action and lipid metabolism. The present study evaluates the role of vitamin D3 in protecting the liver in NAFLD. In the present study, rats were injected intraperitoneally with 30 mg/kg of streptozotocin and fed a high-fat diet to induce diabetes. All rats were administered vehicle or vitamin D3 (300 ng/kg and 600 ng/kg) by oral gavage for 4 weeks. To assess the status of liver Alanine transaminase, Aspartate transaminase was estimated other parameters such as blood glucose, and vitamin D3 lipid profiles were done. Results showed vitamin D3 treatment improved insulin resistance, liver damage and plasma lipid profiles and in diabetic rats. Finally, the present study provides evidence that vitamin D3 could improve dyslipidemia and prevent NAFLD in T2DM.

Keywords: NAFLD, Type 2 Diabetes Mellitus, Vitamin D3, Insulin Resistance.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the chronic metabolic diseases with increasing prevalence worldwide and serious health problems. An estimated 425 million people had DM worldwide in 2017, and the number is expected to rise to 629 million by 2045.1 In T2DM, high blood glucose triggers blood vessel damage and causes micro- and macro-vascular complications, contributing to increasing disability and mortality. Non-alcoholic fatty liver disease (NAFLD) is considered one of the leading liver disorders in T2DM.2 Hepatic lipid dysregulation due to insulin resistance increases fat accumulation in the liver.

The progression of NAFLD is faster in T2DM patients than in nondiabetic patients, 3consequently leading to serious complications such as cirrhosis and hepatocarcinoma. In insulin resistance, insulin signaling to suppress gluconeogenesis is impaired, while signaling to stimulate de novo lipogenesis (DNL) continues to be activated through sterol response element-binding protein 1c (SREBP1c).4

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Meanwhile, impaired insulin signaling suppresses peroxisome proliferator-activated receptor (PPAR)-a and carnitine palmitoyltransferase 1 (CPT1) to decrease b-oxidation. The imbalance between lipid synthesis and breakdown causes excess triglyceride (TG) accumulation in hepatocytes. In addition, hyperglycemia-induced reactive oxygen species (ROS) increase hepatic apoptosis by disturbing the balance between pro- and anti-apoptotic molecules.5

Vitamin D is a well-known hormone precursor that regulates calcium-phosphate homeostasis and bone mineralisation. Vitamin D also regulates inflammation by reducing the release of pro-inflammatory cytokines and affects insulin action and lipid metabolism.6 Recent studies have shown that vitamin D deficiency can cause insulin resistance through inflammation, as vitamin D deficiency is associated with increased inflammation.7 Furthermore, the data collected suggest that 1,25(OH)2D3, a biologically active form of vitamin D, may prevent liver injury by regulating lipogenesis or beta oxidation.8,9

It has been reported that the blood vitamin D levels were generally decreased in NAFLD patients.10, 11 An association between low blood levels of vitamin D3 and the risk of T2DM has also been reported. A previously published study showed that 1,25 (OH)2D3 reduced hepatic triglyceride accumulation and glucose output under insulin-resistant conditions in patients with NAFLD. Furthermore, the benefits of vitamin D3 supplementation for improving insulin resistance depend on the baseline 25 (OH) D3 status.12

Materials and methods

Rats weighing 200-250 gm were used in the study. All rats were housed in three cages in a room maintained at 220C and 50% humidity on a 12-hour light/dark cycle, with ad libitum access to food and distilled water. All rats were assigned to two groups, a control group and a diabetic (DM) group. While the control group was fed a normal chow diet, the DM group was fed a high-fat diet (HFD 40% kcal fat). After four weeks of diet treatment, 12-hour fasting rats were injected with streptozotocin (30 mg/kg body weight, Sigma Aldrich) in citrate buffer (pH 4.5) twice a week intraperitoneally (IP) to induce T2DM.13 Normal saline was administered IP to the normal control rats.13 12-hour fasting blood glucose (FBG) levels were measured once a week from the tail vein using an Accu-Chek Active glucometer (LifeScan Inc., Milpitas, USA) once a week. Rats with FBG above 200mg/dl were considered diabetic and included in further study. All protocols for the animal experiment were approved by the Institutional Animal Ethics Committee of Shri B. M. Patil Medical College, Vijayapur, Karnataka, India.

The diabetic rats were divided into four groups (each n=8) [see table 1] and treated with different levels of vitamin D3 supplement. The normal control rats (NC) fed with a normal chow diet were supplemented with a vehicle (olive oil). The diabetic control rats (DC) fed HFD were supplemented with the vehicle. Diabetic rats treated with vitamin D3 were fed HFD and supplemented with 300 ng/kg (low dose vitamin D, DLD300) and 600ng/kg (high dose vitamin D3 DHD) (Sigma Aldrich, USA) dissolved in olive oil, respectively. The vehicle and vitamin D3 were administered daily for 4 weeks by oral gavage.

Table 1. Grouping of Rats

Groups	**Drugs Intervention**
Normal Control (NC)	Olive oil
Diabetic Control (DC)	STZ + Olive oil
Vit D Low Dose (DLD)	HFD + 300 ng/kg DLD orally in olive oil
Vit D High Dose (DHD)	HFD + 600 ng/kg DHD orally in olive oil

Food intake, body weight and 12-hour FBG of the rats were measured every week. After 4 weeks of treatment, rats fasted for 12 hours and blood was collected from the retro-orbital plexus of the rats using heparinised capillary tubes. Rats were sacrificed with a high dose of diethyl ether. (The collected blood was centrifuged to obtain plasma and used for biochemical estimation).

Oral glucose tolerance test

One week before sacrifice, rats fasted for 12 hours and were treated with 2 g/kg glucose by oral gavage to perform OGTT. After glucose administration, the blood glucose level was determined after 0, 15, 30, 60, 90 and 120 minutes using the Accu-Chek Active blood glucose meter. Liver function test Plasma aspartate transaminase (AST) and alanine transaminase (ALT) were measured using commercially available kits.

Plasma and hepatic lipid profiles and plasma vitamin D concentration

For plasma lipid profiles, triglyceride (TG), total cholesterol (TC), and high-density lipoprotein-cholesterol (HDL-C) were measured by using commercial kits. Analysis of plasma vitamin D3 (EAGLE BIOSCIENCES, INC., NH, USA) was performed according to the manufacturer's protocols.

Statistical analysis

Comparison of significant differences between the groups was analysed by one-way analysis of variance (ANOVA) with post hoc Duncan's multiple range test using SPSS (version 22 for Windows, SPSS Inc., IL, USA). Statistical significance was determined at a P-value <0.05. The results were expressed as the mean ±standard error of the mean (SEM).

Results

Effect of vitamin D3 supplementation on food intake, body weight, and liver weight in T2DM rats Food intake was not

significantly different between all groups. Body weight gain was similar in the diabetic groups. However, a low-dose vitamin D3 supplementation significantly reduced liver weight (% BW) compared to the DC group. On the other hand, the HC group showed no significant difference in liver weight (Table 2).

Table 2. Effect of vitamin D3 supplementation on food intake, body weight, and liver weight in type 2 diabetic rats.

	Groups			
	NC	**DC**	**DLD**	**DHD**
Body weight (g)				
Before treatment	28.32 ± 0.69[a]	32.42 ±0.91[b]	33.31 ± 1.70[b]	34.76 ± 1.05[b]
After treatment	31.41 ± 0.86[a]	41.29 ± 1.23[b]	44.30 ± 2.90[b]	41.29 ± 1.60[b]
Weight gain	3.11 ± 0.33[a]	8.88 ± 0.94[b]	10.99 ± 2.01[b]	8.53 ± 1.14[b]
Liver weight (%BW)	3.23 ± 0.28[a]	5.77 ± 0.30[c]	4.52 ± 0.35[b]	4.93 ± 0.28[bc]

Note: Data were presented as mean±SEM (n8). Values with the different superscript letters were significantly different (P<0.05; ANOVA with post hoc Duncan's multiple range test).

There was no statistical difference in plasma vitamin D3 concentration between the control and the DC groups. However, high-dose vitamin D3 supplementation in diabetic rats significantly increased the plasma vitamin D3 levels compared to the DC group (Table 2).

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Table 3. Effect of vitamin D3 supplementation on plasma vitamin D3 concentration, blood glucose, hepatic function, and lipid profiles in type 2 diabetic rat.

	Groups			
	NC	**DC**	**DLD**	**DHD**
Plasma vitamin D_3 (ng/mL)	78.00 ± 5.61	83.32 ± 3.74	89.54 ± 10.28[ab]	106.96 ± 4.16[b]
FBG (mg/dL)				
Week 0	111.00 ± 8.35[a]	155.20 ±6.65[b]	169.40 ± 6.69[b]	175.80 ± 7.26[b]
Week 2	119.00 ± 4.95[a]	160.80 ± 5.38[b]	153.40 ± 13.67[b]	176.20 ± 9.23[b]
Week 4	138.40 ± 8.59[a]	177.70 ± 11.96[b]	183.70 ± 14.24[b]	163.00 ± 7.29[ab]
OGTT AUC (mg/dL x 120 min)	21233.40±1679.21[a]	35329.00 ± 1369.67[c]	31208.20 ± 3229.82[bc]	27625.40 ± 1828.19[ab]
Hepatic function (IU/L)				
Plasma AST	88.63 ±15.26[a]	151.04 ± 15.83[b]	90.43 ± 11.72[a]	86.57 ± 25.08[a]
Plasma ALT	43.53 ± 6.95	71.24 ± 3.705[b]	38.85 ± 3.91[a]	51.00 ± 9.51[a]
Plasma lipid (mg/dL)				
TG	51.10 ± 2.79[a]	119.77 ± 39.23[b]	56.13 ± 5.93[a]	53.71 ± 5.46[a]
TC	113.72 ± 4.41	156 ± 6.73 [b]	150.12 ± 7.67[b]	152.16 ± 8.73[b]
HDL-C	42.10 ± 2.83[a]	53.4 ± 7.72[ab]	59.53 ± 5.37[ab]	73.33 ± 8.90[b]

Note: Data were presented as mean±SEM. (n¼8). Values with the different superscript letters were significantly different (P<0.05; ANOVA with post hoc Duncan's multiple range test).

FBG: Fasting blood glucose; OGTT: Oral glucose tolerance test, AUC: area-under-the curve; AST: aspartate transaminase; ALT: alanine transaminase; TG: triglyceride; TC: total cholesterol; HDL-C: high density lipoprotein cholesterol

The FBG level in the DC group was significantly higher than in the NC group. However, the administration of vitamin D3 showed no significant difference compared to that of the DC group in FBG level during the experiment. OGTT was performed to determine glucose homeostasis. The OGTT AUC was higher in the DC group than in the NC group. At the same time, the high-dose vitamin D3 treatment showed a significant decrease in the AUC level compared to the DC group. AST and ALT plasma levels were significantly increased in the DC group compared to the NC group. However, vitamin D3 treatment in the diabetic rats significantly reduced AST and ALT levels, which were comparable to those in the NC group, regardless of the dose. Plasma TG and TC levels were higher in the DC group than in the NC group, and vitamin D3 administration significantly reduced plasma TG levels in diabetic rats. While plasma TC levels were not significantly altered after vitamin D3 administration, plasma HDL-C levels were significantly increased after treatment with high vitamin D3 among type 2 diabetic rats.

Discussion

The current study demonstrated the ameliorating effect of vitamin D3 supplementation on NAFLD. Although vitamin D3 is known to improve insulin signaling,15,16 previous studies reported that vitamin D3 administration showed no significant difference in FBG in diabetic animals.7,15,16 The current study also supported that treatment with Vitamin D3 had no direct effect at the FBG level. However, in a recent study, Benetti E. et al.17 confirmed that vitamin D3 administration improved glucose tolerance in non-diabetic obese rats. Our result showed that an increased vitamin D3 plasma concentration correlated with a reduced OGTT AUC. The result suggests that high-dose vitamin D3 supplementation would be effective in improving insulin sensitivity in T2DM.

Elevated levels of AST and ALT in the blood indicate liver damage, such as B. hepatocellular necrosis.18 Previous studies demonstrated the beneficial effect of vitamin D3 administration on reducing AST and ALT levels in diabetic animals.19,20 In addition, treatment with 2100 IU vitamin D3 for 48 weeks was well tolerated and reduced serum ALT levels in patients with nonalcoholic steatohepatitis.21 Similar results in this study suggest that vitamin D3 treatment may improve hyperglycemia-induced liver dysfunction by attenuating liver damage.

Insulin resistance stimulates the over secretion of VLDL from the liver into the blood and causes hypertriglyceridemia.21 In hypertriglyceridemia, reduced plasma HDL-C levels but increased small dense LDL particles lead to dyslipidemia, which accelerates the deposition of hepatic lipids. Previous studies reported that vitamin D3 normalised serum TG, TC and HDL-C levels in HFD-induced NAFLD rats22 and decreased hepatic TG levels in diabetic rats7. Control studies had significantly lower levels of 25(OH)D3 in patients with NAFLD than in controls.8,25 Researchers believe that lower levels of vitamin D3 in patients with NAFLD may contribute to NAFLD progression. The exact mechanism of vitamin D3 deficiency and NAFLD is not fully established. A reduction in triglycerides and an increase in HDL-cholesterol were observed in the 25 mg calcitriol group compared to the placebo during the 4 weeks of the intervention.23,24 In our research, vitamin D3 treatment significantly reduced plasma TG levels regardless of dose. In addition, vitamin D3 supplementation decreased hepatic TG levels and increased plasma HDL-C levels in a plasma vitamin D3 concentration-dependent manner. Our results indicate that vitamin D3 could improve dyslipidemia and prevent NAFLD in T2DM.

Conclusion

AST and ALT levels in the vitamin D3-treated group of rats suggest that it might improve hyperglycemia-induced liver dysfunction by attenuating liver damage. The present study indicates that treatment with vitamin D3 significantly reduces plasma TG levels, regardless of dose. In addition, vitamin D3 supplementation decreased hepatic TG levels and increased plasma HDL-C levels in a plasma vitamin D3 concentration-dependent manner. Our results demonstrate that vitamin D3 could improve dyslipidemia and prevent NAFLD in T2DM.

References

1. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 2018;138:271–81
2. Bril F, Cusi K. Nonalcoholic fatty liver disease: the new complication of type 2 diabetes mellitus. Endocrinol Metab Clin North Am 2016;45:765–81
3. Loomba R, Abraham M, Unalp A, Wilson L, Lavine J, Doo E, Bass NM. Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. Hepatology 2012;56:943–51
4. Sanders FW, Griffin JL. De novo lipogenesis in the liver in health and disease: more than just a shunting yard for glucose. Biol Rev Camb Philos Soc 2016;91:452–68
5. Farzanegi P, Dana A, Ebrahimpoor Z, Asadi M, Azarbayjani MA. Mechanisms of beneficial effects of exercise training on non-alcoholic fatty liver disease (NAFLD): roles of oxidative stress and inflammation. Eur J Sport Sci 2019;19:994–1003
6. Garbossa SG, Folli F. Vitamin D, Sub-inflammation and insulin resistance. A window on a potential role for the interaction between bone and glucose metabolism. Rev Endocr Metab Disord 2017;18:243–58
7. Lips P, Eekhoff M, van Schoor N, Oosterwerff M, de Jongh R, Krul-Poel Y, Simsek S. Vitamin D and type 2 diabetes. J Steroid Biochem Mol Biol 2017;173:280–5
8. Duan P, Hu C, Quan C, Yu T, Zhou W, Yuan M, Shi Y, Yang K. 4-Nonylphenol induces apoptosis, autophagy and necrosis in sertoli cells: involvement of ROS-mediated AMPK/AKT-mTOR and JNK pathways. Toxicology 2016;341:28–40
9. Yin Y, Yu Z, Xia M, Luo X, Lu X, Ling W. Vitamin D attenuates high fat diet-induced hepatic steatosis in rats by modulating lipid metabolism. Eur J Clin Invest 2012;42:1189–96
10. Ning C, Liu L, Lv G, Yang Y, Zhang Y, Yu R, Wang Y, Zhu J. Lipid metabolism and inflammation modulated by vitamin D in liver of diabetic rats. Lipids Health Dis 2015;14:31
11. Barchetta I, Angelico FD, Ben M, Baroni MG, Pozzilli P, Morini S, Cavallo MG. Strong association between non alcoholic fatty liver disease (NAFLD) and low 25(OH) vitamin D levels in an adult population with normal serum liver enzymes. BMC Med 2011;9:85
12. Targher G, Scorletti E, Mantovani A, Byrne CD. Nonalcoholic fatty liver disease and reduced serum vitamin D(3) levels. Metab Syndr Relat Disord 2013;11:217–28
13. Song Y, Wang L, Pittas AG, Del Gobbo LC, Zhang C, Manson JE, Hu FB. Blood 25-hydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. Diabetes Care 2013;5:1422–8
14. Zhang M, Lv XY, Li J, Xu ZG, Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. Exp Diabetes Res 2008;2008:704045
15. Grober U, Holick MF. Diabetes prevention: vitamin D supplementation may not provide any protection if there is no evidence of deficiency. Nutrients 2019;11:2651 [CrossRef[10.3390/nu11112651]
16. Yuan Y, Das SK, Li M. Vitamin D ameliorates impaired wound healing in streptozotocin-induced diabetic rats by suppressing NF-kappaBmediated inflammatory genes. Biosci Rep 2018;38:BSR2017129
17. Lee TI, Kao YH, Chen YC, Tsai WC, Chung CC, Chen YJ. Cardiac metabolism, inflammation, and peroxisome proliferator-activated receptors modulated by 1,25-dihydroxyvitamin D3 in diabetic rats. Int

J Cardiol 2014;176:151–7
18. Benetti E, Mastrocola R, Chiazza F, Nigro D, D'Antona G, Bordano V, Fantozzi R, Aragno M, Collino M, Minetto MA. Effects of vitamin D on insulin resistance and myosteatosis in diet-induced obese rats. PLoS One 2018;13:e0189707
19. Thapa BR, Walia A. Liver function tests and their interpretation. Indian J Pediatr 2007;74:663–71
20. Elattar S, Estaphan S, Mohamed EA, Elzainy A, Naguib M. The protective effect of 1alpha, 25-dihydroxyvitamin d3 and metformin on liver in type 2 diabetic rats. J Steroid Biochem Mol Biol 2017;173:235–44
21. Hamden K, Carreau S, Jamoussi K, Miladi S, Lajmi S, Aloulou D, Ayadi F, Elfeki A. 1Alpha,25 dihydroxyvitamin D3: therapeutic and preventive effects against oxidative stress, hepatic, pancreatic and renal injury in alloxan-induced diabetes in rats. J Nutr Sci Vitaminol 2009;55:215–22
22. Subramanian S, Chait A. Hypertriglyceridemia secondary to obesity and diabetes. Biochim Biophys Acta 2012;1821:819–25
23. El-Sherbiny M, Eldosoky M, El-Shafey M, Othman G, Elkattawy HA, Bedir T, Elsherbiny NM. Vitamin D nanoemulsion enhances hepatoprotective effect of conventional vitamin D in rats fed with a high-fat diet. Chem Biol Interact 2018;288:65–75
24. Lorvand Amiri H, Agah S, Mousavi SN, Hosseini AF. Shidfar F. regression of non-alcoholic fatty liver by vitamin D supplement: a doubleblind randomized controlled clinical trial. Arch Iran Med 2016;19:631–
25. Nelson JE, Roth C, Wilson LA, Yates KP, Aouizera B, MorganStevenson V, Whalen E, Hoofnagle A, Mason M, Gersuk V, Yeh MM, Kowdley KV. Vitamin D deficiency is associated with increased risk of non-alcoholic steatohepatitis in adults with non-alcoholic fatty liver disease: possible role for MAPK and NF-kappaB? Am J Gastroenterol 2016;111:852–63