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The Biochemical Efficiency of Vitamin D on Experimentally Induced Diabetes Mellitus in Rats

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A B S T R A C T

Because of the highly effective endocrinal role of vitamin D (Vit D) as an anti-diabetic, there is a great trend to use of Vit D in both prevention and control of type 2 Diabetes mellitus (T2DM). This is the target of our study in which we have used 40 rats allocated into four groups: a control group, given a normal basal diet, and not treated with any drug. Second group; vitamin D supplemented group given vitamin D Vidrop® (10 IU/kg) by gavage tube daily and given basal diet throughout the experiment. The third group that was a model of T2DM in rats, given a high-fat diet followed by two successive doses of streptozotocin (STZ; 35 mg/kg, i/p), and they were given Vit D Vidrop® (10 IU/kg) by gavage tube after induction of T2DM till the end of the experiment. The fourth group, which was also a model of T2DM, was given a high-fat diet followed by two successive doses of STZ (35 mg/kg, i/p) but not treated with vitamin D. The result shows that: Vitamin D Vidrop® (10 IU/kg/60 days) has significantly decreased levels of fasting blood glucose, improved lipid profile and non-esterified fatty acids (NEFA). So, vitamin D acts by supporting β-cell and increases insulin secretion and sensitivity resulting in a significant reduction of both fasting blood glucose and lipoproteins (except HDL), which is a great result.

Keyword: Diabetes mellitus; Vitamin D; Glucose; Lipid profile

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1. Introduction

As known diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to complete or partial deficiency of insulin (the only hypoglycemic hormone). This chronic hyperglycemia leads to long term microvascular (retinal, glomerular, neural) and macrovascular (atherosclerosis of coronary, cerebral, and peripheral arteries) complications. ADA, (2012). The stage that precedes T2DM is impaired fasting glucose (IFG) in which there is a fear of the development of T2DM. There is a great effort to prevent and treat DM or at least control its complications by lowering blood glucose to normal levels with a focus on vitamin D supplementation to achieve these goals. In studies by (Grimnes et al., 2010; Joergensen et al., (2010), there is a relation betweenvitamin D deficiency and the development of T2DM macrovascular complications. Furthermore, another study has illustrated its beneficial role in glucose metabolism through the vitamin D receptor (VDR) on pancreatic cells through its effect on the synthesis and transport of insulin and it is signaling pathways. In addition, it has a role in improving insulin sensitivity by acting on VDR in the liver and muscle, modulating immune responses and halting systemic inflammation (Gysemans et al., 2005; Zhou et al., 2008; Park et al., 2016). This promising Vitamin is a steroid synthesized in the body through direct exposure to ultraviolet rays (Mohr et al., 2008). It is responsible for normal calcium and phosphorus levels (Holick, 2007a). So, deficiency of this vitamin leads to secondary hyperparathyroidism with the destruction of bone to increase calcium levels. (Holick, 2004; Holick, 2007b; Adams et al., 2010). There is a widespread distribution of VDR across many tissues that explains the broad physiological action of vitamin D (Holick, 2009; Rosen et al., 2012). Additionally, muscle pain and weakness may develop especially in the proximal muscles, and a reduction in their speed (Schott & Wills, 1976; Bischoff-Ferrari et al., 2006; Bischoff-Ferrari, 2012). This study explains how vitamin D supplementation is a strong factor in controlling T2DM and its complications. Vitamin D acts by many steps on beta cells (β-cells) of islets of Langerhans, ending in insulin secretion to control blood glucose levels. The β-cells are capable to raise the released insulin to improve insulin resistance. However, in the end, β-cells begin to die resulting in a decline in insulin level with more elevation of blood glucose which means diabetes (Wimalawansa, 2016). The action of vitamin D is to protect β cells from apoptosis to prevent the progression of DM. We aim to evaluate the effect of vitamin D on experimentally induced type 2 diabetes mellitus in rats.

2. Materials and Methods

2.1. Chemicals

Streptozotocin (STZ) and other reagent kits were purchased from Sigma Chemicals (St Louis, MO, USA). Vitamin D (cholecalciferol) (Vidrop®) was obtained from Medical Union Pharmaceuticals (Abu Sultan, Ismailia, Egypt); the recommended dose is 10 IU/kg (Pittas, 2006).

2.2. Animals

A total of 40 male rats (two months in age) were obtained from the Faculty of Science, kept in rat stainless steel cages in a well-ventilated room, with a temperature range of 25-27ºC, with available water and dry ration. They spent two weeks to adapt with new circumstances. We had four groups of rats (10 per each): the control group; they received normal basal diet and were not treated with any drug. The second group; vitamin D supplemented group; they given vitamin D (Vidrop®) (10 IU/kg) daily with gavage tube and received the normal basal diet throughout the experiment. The third group; T2DM, given high-fat diet (HFD) then two successive doses of STZ (35 mg/kg, i/p) and given Vit D (Vidrop®) (10 IU/kg) by gavage tube (three days after the induction of T2DM) daily. The fourth group was also T2DM that received HFD followed by two successive doses of STZ (35 mg/kg, i/p) but not treated with vitamin D. The experimental period continued for two

months. We measured fasting blood sugar before and after induction. The high fasting blood sugar level (>200 mg/ dl) marked the development of T2DM.

2.3. Samples

The blood samples (3 ml) were obtained from the orbital venous sinus of overnight-fasting (10–12 hour) control; vitamin D supplemented group, diabetic treated, and diabetic non-treated rats. Samples were collected in serum tubes. Then centrifuged for 10 minutes. The serum glucose was assayed according to (Trinder, 1969), non-esterified fatty acid (NEFA) was determined according to (Schuster, 1979), cholesterol was assayed according to (Richmond, 1973), triacylglycerol (TAG) was evaluated according to Fassati & prencip (1982), HDL-c was assayed according to Burstein et al., (1970), LDL-c and VLDL-c were measured according to Friedewald, (1972).

2.4. Statistical analysis

The results are expressed as the mean ± SD. The statistical analysis of variance (ANOVA) was performed using Duncan's multiple range tests (SPSS Inc). *P* values < 0.05 were considered statistically significant.

4. Results

4.1. Changes in serum glucose

The data summarized in **Table 1** shows the normal level of fasting blood glucose in the control group supplemented with Vitamin D (Vidrop®) orally 10 IU/Kg for 60 days in normal rats that received the normal basal diet, resulted in normal levels of fasting blood glucose. Whileadministering vitamin D (Vidrop®) orally 10 IU/Kg for 60 days after injection of STZ 35 mg/kg in rats that received HFD has controlled glucose levels in this diabetic group. The diabetic group, injected with STZ 35 mg/kg, received HFD and was not treated, showed high fasting blood glucose levels.

4.2. Serum lipid profile levels

The data summarized in **Tables 2, 3, and 4** revealed normal levels of cholesterol, TAG, HDL-c, LDL-c, VLDL-c in the control group. The vitamin D supplemented group, not injected by STZ and receiving normal diet for 60 days, had significantly low lipid profile levels while increasing HDL-c level. Regarding the vitamin D treated group after injection with STZ that received HFD for 8 weeks, the results revealed significant decreases in lipid profile except for HDL-c level in this diabetic group. The fourth group injected with STZ plus HFD and not treated showed an increase in lipid profile levels except HDL-c after eight weeks.

4.3. Serum Non-esterified fatty acids (NEFA)

The data summarized in the **Table 5** showed the normal level of NEFA in the control group. While the vitamin D treated group with Vidrop® 10 IU/Kg and not injected with STZ that received a normal basal diet showed a decrease in NEFA level. Significant control of NEFA level in the diabetic treated group by vitamin D 10 IU /kg for 60 days. With an obvious increase in the level of NEFA in the diabetic not treated group, which was injected by STZ and received HFD for 60 days.

Table 1. Shows blood glucose levels in rat groups.

Groups	Glucose (mg/dl)
Control	89.64±3.49^{c}
Vitamin D	92.63±4.73^{c}
Diabetes+ Vitamin D	130.4±4.06^{b}
Diabetes	203.6±11.33^{a}

Values are means ± SD.
Means with different letters are significantly different ($P < 0.05$)

Table 2. Shows cholesterol and triacylglycerol (TAG) levels in rat groups

Groups	Cholesterol (mg/dl)	Triacylglycerol (TAG) (mg/dl)
Control	86.95 ± 3.89 ^{cd}	58.68 ± 5.29 ^{c}
Vitamin D	77.69 ± 3.11^{d}	62.05 ± 2.61 ^{c}
Diabetes + Vitamin D	115.39 ± 4.52 ^{b}	91.62 ± 2.83 ^{b}
Diabetes	151.25 ± 5.89 ^{a}	124.66 ± 2.39 ^{a}

Values are means ± SD.
Means with different letters are significantly different ($P < 0.05$).

Table 3. Shows VLDL-c and LDL-c levels in rat groups

Groups	VLDL-c (mg/dl)	LDL-c (mg/dl)
Control	11.73±1.05^{c}	51.52±3.99^{cd}
Vitamin D	12.62±0.42^{c}	46.11±2.66^{d}
Diabetes+ Vitamin D	17.71±0.76^{b}	68.32±6.90^{bc}
Diabetes	24.94±0.48^{a}	111.09±4.87^{a}

Values are means ± SD.
Means with different letters are significantly different ($P < 0.05$).

Table 4. Shows HDL-c levels in rat groups

Groups	HDL-c (mg/dl)
Control	23.67±1.17^{a}
Vitamin D	22.21±1.08^{a}
Diabetes+ Vitamin D	22.76±0.79^{a}
Diabetes	15.22±1.74^{b}

Values are means ± SD.
Means with different letters are significantly different ($P < 0.05$).

Table 5. Shows serum non-esterified fatty acids (NEFA) levels in rat groups

Groups	NEFFA (mmol/ml)
Control	12.10±0.53^{bc}
Vitamin D	11.53±0.58^{bc}
Diabetes + Vitamin D	15.05±0.54^{b}
Diabetes	29.22±2.25^{a}

Values are means ± SD.
Means with different letters are significantly different ($P < 0.05$).

5. Discussion

Vitamin can affect development and treatment of T2DM (Forouhi et al., 2008). The results revealed that Vit D supplementation regulates glucose levels, as shown in the **Table 1**. These results agree with those obtained by (Cade &Norman, 1987; Kumar et al., 2010) who reported that vitamin D is good for insulin secretion and glucose tolerance and restores homeostasis in STZ-induced diabetic rats. Also, another study by (Holick, 2008) showed that a low blood level of vitamin D is associated with an increased risk of T2DM due to decreased insulin sensitivity and increased fasting blood sugar (FBS). Also, Need et al., (2005) and Johnson et al., (2010) mentioned that normal vitamin D levels are associated with increased insulin secretion with resultant normal fasting blood glucose levels in adults. A study by (Palomer et al., 2008; Takiishi et al., 2010) reported that Vitamin D supplementation improves hyperglycemia and insulin secretion in patients with T2DM. Also, the study of (Boucher, 2011) showed that the mechanism of action of vitamin D in T2DM not only through the regulation of calcium role in pancreatic β-islet cells, which regulates insulin synthesis and secretion but also by direct action on pancreatic β-cell-mediated by the binding of 1,25- (OH) 2 D to its receptor VDR on pancreatic β-cells in which there are vitamin D response elements (VDRE) in insulin gene and increase transcription and translation of the insulin gene caused by 1, 25-(OH) 2D further supports a direct effect of vitamin D on insulin

synthesis, secretion sensitivity and control of inflammation with reduction of hyperglycemia. Also, studies by (Takiishi et al., 2010; Sentinelli et al., 2016; Sergeev, 2016) revealed that T1D and T2DM patients suffer from hypovitaminosis D. In addition to that, Vitamin D supplementation normalizes the lipid profile as shown in the **Tables 2, 3, and 4** that revealed that injection of STZ led to significant increases in lipid profile except HDL-c in diabetic group compared to Vitamin D treated group. These findings come in accordance with those obtained by (Gamier et al., 1990), that observed increased levels of serum TAG while the HDL-c was reduced and the ratio of total cholesterol over HDL-c was higher in both T1DM and T2DM patients as compared with the controls. Also, a study by (Tacconi et al., 2000) observed increased TAG in rats after induction of diabetes by STZ. This occurs as diabetic mice fail to utilize glucose associated with increased lipolysis due to the lack of insulin hormone. However, a massive accumulation of glucose leads to fatty liver (Bayramoglu et al., 2014).

Furthermore, the overproduction of serum fatty acids by STZ-induced diabetics facilitates the conversion of excessive fatty acids in the liver into cholesterol (Saravanan & Ponmurugan, 2012). Normal blood insulin levels with normal glucose are closely related to blood TAG and total cholesterol (TC); the data confirmed the hypolipidemic and hypoglycemic effects of vitamin D on experimentally diabetic rats. In the study by (Karhapää, et al., 2010) showed a positive relation between 1, 25 (OH) 2D and HDL-c in type 2 diabetes. Recently, a study by (Jorde et al., 2013) observed that vitamin D produces an obvious drop in LDL-c level which contradicts a previous finding by Wang et al., (2012) who reported that there is little effect of vitamin D on levels of LDL-c and supports a protective effect of vitamin D on serum lipid profile. Furthermore, vitamin D benefits non-esterified fatty acids (NEFA) as shown in the **Table 5**. These results come in accordance with those obtained from (Jorde et al., 2010), that found favorable readings of TAG, NEFA, and HDL-c in those with perfect vitamin D levels. Overexpression of VDR in mouse adipose tissue leads to decreased fatty acid β-oxidation and lipolysis (Wong et al., 2011). Vitamin D plays a great role in maintaining the normal level of blood glucose by increasing insulin hormone production from β-cells of islets of Langerhans that assists in the correction of insulin resistance, which is the base of T2DM (Wimalawansa et al., 2016).

6. Conclusion

Vitamin D improves the biochemical events in diabetics through a positive action on β-cell function with increased insulin production, which is the only way to get normal fasting blood glucose and lipid levels.

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