VITAMIN D3 IMPROVES LIVER FUNCTIONS IN EXPERIMENTALLY INDUCED TYPE II DIABETES MELLITUS IN WISTAR RATS

Noha N. Lasheen¹ and Nashwa El-Khazragy²
1. Lecturer of Physiology, Faculty of Medicine, Ain Shams University.
2. Lecturer of Clinical Pathology, Faculty of Medicine, Ain Shams University Cairo, Egypt.

Abstract

Background and Aim: Vitamin D3 could ameliorate the effects of type 2 diabetes mellitus; however, liver changes in diabetes needs more studies and to highlight changes in liver cell mitochondrial NAD+ and Stearoyl-CoA desaturase 1 (SCD1).

Materials and Methods: Forty adult Wistar rats, of both sexes, were randomly allocated into equal groups of control, vitamin D3 non-diabetic, diabetic, type 2 diabetic and vitamin D3 treated diabetic groups. At the end of the study, all rats were anaesthetized by i.p. Pentoparbitone (40 mg/kg B.W). Fasting blood glucose level was measured by rat tail, then after aortic cannulation, the separated plasma was used for determination of lipid profile, liver functions and insulin level. HOMA-IR and atherogenic indices were calculated. Liver specimens were used for detection of SCD1 gene expression by Real time PCR and mitochondrial NAD+

Results: Glycemic parameters and plasma liver enzymes, liver mitochondrial NAD+ were significantly elevated in the diabetic group compared to the control group, and were significantly lowered in vitamin D3 treated diabetic group compared to the diabetic group. The diabetic group had dyslipidemia compared to the control group, while lipid profile was improved in vitamin D3 treated diabetic group compared to the diabetic group. SCD1 gene expression in liver cells was downregulated in both diabetic groups (untreated and treated) compared to the control group.

Conclusion: Vitamin D3 reversed the damaging diabetic effects on liver functions efficiently, probably by ameliorating oxidative stress, however, it altered SCD1 gene expression and regulated its effect on diabetes to lesser extent.

Introduction:

Diabetes mellitus is a worldwide problem, and increases in incidence and prevalence (Danaei et al., 2011). The relation between vitamin D and type 1 diabetes mellitus has been extensively studied in humans and animals, and it could be due to the immunomodulatory actions of vitamin D (Stene et al., 2000; Gregori et al., 2002; Mathieu et al., 2005; Luong et al., 2005).
For type 2 diabetes mellitus to develop, impaired pancreatic β cell function and insulin resistance are often present. The prevalence of type 2 diabetes rises in obesity, which is often associated with hypovitaminosis D (Palomer et al., 2008). Vitamin D is efficiently deposited in body fat stores decreasing its bioavailability, thereby many obese persons could be chronically vitamin D deficient (Holick, 2004). Zeitz et al. (2003) reported that vitamin D receptor could be expressed in β cells. Thus, vitamin D deficiency could decrease insulin secretion in rats and humans, and its replenishment could improve β-cell function and glucose tolerance (Boucher et al., 1995). Also, it was suggested that vitamin D insufficiency could cause insulin resistance probably by elevated TNF-α level which is a cytokine inversely related to 25(OH) vitamin D (Hotamisligil and Spiegelman, 1994). In addition, Ortlepp et al. (2003) reported that alteration in vitamin D receptor and vitamin D-binding protein could affect glucose tolerance and insulin secretion. Ogunkolade et al. (2002) mentioned that vitamin D could affect type 2 diabetes mellitus through either controlling plasma calcium level, which regulates insulin synthesis and secretion, or directly on pancreatic β-cell function.

In addition, DeLuca and Cantorna(2001) mentioned that liver cells had cytosolic or nuclear and/or membrane-bound vitamin D receptors. However, few researchers studied the impact of vitamin D supplementation on altered liver functions in type 2 diabetes mellitus (Yang et al., 2016).

Fat metabolism changes in type 2 diabetes mellitus were proved to be present (Shulman, 2000). Stearoyl-CoA desaturase (SCD) is the rate limiting enzyme catalyzing the biosynthesis of monounsaturated fatty acids, the substrates for triglycerides synthesis and formation of cholesterol esters and phospholipids. SCD1 is the main SCD isof orm expressed in liver (Dobrzyn and Dobrzyn, 2006). Abnormal monounsaturated fatty acid production could cause many diseases such as obesity, diabetes and cancer. Therefore, it is of value to study SCD1 expression in normal and disease states (Miyazaki et al., 2003). Although many studies are present regarding the effect of type 2 diabetes mellitus on SCD, little is known about the effect of vitamin D on the SCD gene expression in type 2 diabetes mellitus.

On the other hand, oxidative stress could be present in type 2 diabetes mellitus resulting in the development of diabetic complications in many organs (Giacco and Brownlee, 2010). However, changes in mitochondrial NAD+ in liver cells in type 2 diabetes were not extensively studied. Therefore, it is valuable to study the draw backs of diabetes mellitus (type 2) on liver functions and to elucidate the changes in SCD1 gene expression and mitochondrial NAD+ in the liver tissues; in addition to study the possible effects of vitamin D3 in type 2 diabetes mellitus.

Aim of the work:-
This study was performed to demonstrate the effects of vitamin D3 treatment on liver functions in type 2 diabetic rats and to highlight the changes in SCD1 gene expression and mitochondrial NAD+ in liver cells.

Materials and Methods:-
Experimental Protocol:
Animals:
This study was carried out on 40 adult Wistar rats, of both sexes, initially weighing 160-200 gm, purchased from animal farm from Helwan, Cairo, Egypt. Animals were kept in the Medical Research Center Animal House, Faculty of Medicine, Ain Shams University under standard conditions of boarding and feeding, with free access to water, ad libitum, throughout the whole period of the study. Rats were left for 7 days as a period of acclimatization, during which regular normal diets were introduced daily at 8 a.m., in the form of milk, bread and vegetables.

Experimental Protocol:-
Rats were randomly allocated into the following groups:-
Group I: Control group (n= 10): received a single i.p. injection of saline in equivalent dose of Streptozotocin and also equivalent dose of 1, 25(OH)2D3 each 2 days for 8 weeks.

Group II: Vitamin D3 Supplemented Non-Diabetic group (n=10): which received i.p. injection of 5 μg/kg 1, 25(OH)2 D3, each 2 days for 8 weeks, only without induction of diabetes mellitus (Yin et al., 2012).
Group III: Type 2 Diabetic group (n = 10): which were fed high fat diet for 2 weeks followed by single i.p. injection of Streptozotocin (Sigma Co.) in a dose of 35 mg/kg B.W. dissolved in 2 ml of 0.05 M citrate buffer (Srinivasan et al., 2005).

Group IV: Vitamin D3 Treated Diabetic group (n= 10): which were rendered diabetic by the same method as the diabetic group, and then they were treated with i.p. injection of 5 μg/kg 1, 25(OH)\(_2\)D\(_3\), each 2 days for 8 weeks (Yin et al., 2012).

Induction of Diabetes Mellitus Type 2:-
High fat diet, introduced to diabetic groups, was prepared by adding butter, to increase its fat content to 16 -17%. Analytical composition of butter used in the present study was in accordance with Holland and Welch (1992). After Streptozotocin injection into overnight fasted rats, they had free access of food and water, and were given 5% glucose solution after 6 hours of Streptozotocin injection to drink overnight to counter hypoglycemic shock. The diabetic state was assessed by measuring the non-fasting blood glucose levels from rat tail 72hrs after Streptozotocin injection. The rats with blood glucose level above 250 mg/dl were selected for the experiment and considered as diabetics (Zhang et al., 2006).

On the day of sacrifice, overnight fasted rats were weighed and anaesthetized by i.p. injection of pentobarbitone, in a dose of 40 mg/kg B.W. Fasting blood glucose (FBG) was measured in a blood drop from the rat tail by GlucoDr™ SuperSensor Test Meter, AGM- 2200, Korea. A midline abdominal incision was made and the abdominal aorta was cannulated to collect aortic blood in 2 tubes; EDTA containing tube for subsequent determination of HbA1c in whole blood sample and a heparinized tube, which were centrifuged and the separated plasma was stored at - 80 °C for later determination of insulin level to calculate homeostasis model assessment of insulin resistance (HOMA-IR), γ-GT activity and levels of AST, ALT in addition to lipid profile. Liver specimens were also stored at -80°C for later determination of mitochondrial NAD’ and SC1 gene expression.

Determination of Plasma Insulin Level:-
was performed according to Flier et al. (1979), using enzyme linked immunosorbent assay (ELISA) technique kits supplied by Insulin ELISA kit supplied by DRG.

Homeostasis Model Assessment- Insulin Resistance (HOMA-IR) = [fasting plasma insulin (μU/mL) x fasting plasma glucose (mmol/L)] /22.5 (Salgado et al., 2010). HbA1c were quantified by HPLC (Jeppsson et al.; 2002).

Liver enzymes levels [Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT)] and Gamma Glutamyl Transferase (γ-GT) activity were determined colorimetric assay according to Reitman and Frankel (1957) and Tietz(1986) using commercial kits from Biolabo (France).

Determination of plasma levels of triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol: were performed according to the method described by Vassault et al. (1986), Rifai et al. (1999) and Fruchart(1982) using kits supplied by Greiner Diagnostic, Germany.

Atherogenic index was calculated according to Grundy et al. (1987) as follows: Atherogenic index = Total cholesterol / HDL-C.

Evaluation of Mitochondrial NAD’inHepatic tissue:-
Mitochondrial isolation from hepatic tissues which were stored frozen at -80°C till the day of NAD’ determination, was performed according to Saleh and Saleh (2010). Hydrolysis of mitochondrial nicotinamide adenine dinucleotide (NAD’) directly reflects mitochondrial pores opening. The NAD’ was measured after perchloric acid extraction. In the case of isolated mitochondria, 0.1 ml of 21% (v/v) perchloric acid was added to 1 mg of protein/ml suspensions. The concentration of NAD’ in the perchloric acid extract of the hepatic mitochondria was measured using an alcohol dehydrogenase reaction. The reaction mixture contained 1000 μl of buffer-substance (0.1 M Tris acetate [pH 8.8] and 0.5 M ethanol), 100 μl of the tissue extract neutralized and 20 μl of alcohol dehydrogenase. The reaction was initiated by enzyme addition and change of absorbance at 340 nm recorded by a spectrophotometer (Behring Werke AG, Marburg, and West-Germany).
RNA preparation and real-time qPCR analysis:-
Total mRNA was extracted from liver tissues using RNeasy kits with spin-column DNase digestion (Qiagen). Purity and concentration were determined with a Nanodrop 1000 spectrophotometer (Thermo Scientific). One μg of RNA was used to synthesize cDNA with a QuantiTect Rev. Transcription Kit (Qiagen) and diluted to 10 ng/μL. Expression of mRNA was determined using RT² qPCR Primer Assay for rat stearoyl-Coenzyme A desaturase 1 (SCD1) catalogue no: 330001 (Qiagen) and SYBR green RT² SYBR Green ROX™ qPCR Mastermix on an Applied Biosystems Step One Plus RT-PCR system. PCR mix for one reaction Component Volume RT² SYBR Green Mastermix was prepared as follows (12.5 μl cDNA synthesis reaction 1 μl RT² qPCR Primer Assay (10 μM stock) 1 μl RNase-free water 10.5 μl Total volume 25 μl. The PCR Cycling conditions were adjusted following the manufacture instruction. Cycling program: Initial activation step for 10 min at 95°C to activate HotStarTaq DNA Polymerase, cycling; (Denaturation for 15 seconds at 94°C, annealing for 30 seconds at 60°C, then extension for 30 seconds at 70°C) X40 cycles. PCR products were quantified fluorometrically using SYBR Green, and normalized to the housekeeping gene rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and relative to the control according to the following formula:

\[
\text{Target amount} = 2^{-\Delta\Delta Ct}
\]
Where \(\Delta\Delta Ct = \{[\text{Ct (target gene)} - \text{Ct (GAPDH)}] - [\text{Ct (control)} - \text{Ct (GAPDH control)}]\}.

Fold difference for gene expression was calculated as \(2^{-\Delta\Delta CT}\) using the endogenous control genes (liver). The identity and purity of the amplified product were assessed by melting curve analysis at the end of amplification.

Statistical Analysis:-
All results in the present study were expressed as mean ± SE of the mean. Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL, USA) program, version 20.0 was used. Differences were considered significant when \(P \leq 0.05\).

Ethics Committee:-
This study was approved by the Ethics Committee of Faculty of Medicine, Ain Shams University.

Results:-
The diabetic and vitamin D3 treated diabetic groups had significant rises in fasting blood glucose, fasting insulin, HbA1c levels and HOMA-IR compared to the control group, denoting the occurrence of type 2 diabetes mellitus. However, these glycemic parameters were significantly reduced in vitamin D3 treated diabetic group compared to the diabetic group. Vitamin D3 supplemented group (Positive control group) had insignificant changes in fasting blood glucose, fasting insulin, HbA1c levels and HOMA-IR compared to the control group (negative control), as shown in table (1).

Table 1: Changes in fasting blood glucose (mg%), fasting insulin level (μIU/ml), HOMA-IR and HbA1c level (gm%) in the different studied groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fasting blood glucose (mg%)</th>
<th>Fasting insulin level (μIU/ml)</th>
<th>HOMA-IR</th>
<th>HbA1c (gm%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (10)</td>
<td>95.7 ± 3.03</td>
<td>3.56 ±0.18</td>
<td>0.86 ±0.038</td>
<td>3.19 ±0.11</td>
</tr>
<tr>
<td>Vitamin D3 supplemented group (10)</td>
<td>89.8 ± 2.89</td>
<td>3.6 ±0.21</td>
<td>0.8 ±0.053</td>
<td>3.27 ±0.13</td>
</tr>
<tr>
<td>Diabetic group (10)</td>
<td>189.2 ± 5.02</td>
<td>4.82 ±0.19</td>
<td>2.26 ±0.115</td>
<td>4.33 ±0.13</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin D3 treated diabetic group (10)</td>
<td>110.1 ± 2.43</td>
<td>4.17 ±0.14</td>
<td>1.14 ±0.051</td>
<td>3.93 ±0.16</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P*</td>
<td>&lt;0.001</td>
<td>&lt;0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

In parenthesis is the number of rats studied in each group.
Values are expressed as means ±SEM. NS: Not significant.
P: Significance by LSD at \(P<0.05\) from the control group.
Regarding liver functions, plasma AST and ALT levels and γ-GT activity in plasma were significantly increased in both diabetic (untreated and treated) groups compared to the control group, while they were significantly reduced in vitamin D3 treated diabetic group compared to the diabetic group. On the other hand, no-statistical significance was detected in these parameters in vitamin D3 supplemented group compared to control group, as shown in table (2).

**Table 2:** Changes in AST (IU/mL) and ALT (IU/mL) levels and γ-GT activity (IU/L) in the different studied groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>AST (IU/mL)</th>
<th>ALT (IU/mL)</th>
<th>γ-GT activity (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (10)</td>
<td>18.14</td>
<td>8.85</td>
<td>5.04</td>
</tr>
<tr>
<td></td>
<td>±1.78</td>
<td>±1.19</td>
<td>±0.59</td>
</tr>
<tr>
<td>Vitamin D3 supplemented group (10)</td>
<td>21.64</td>
<td>10.11</td>
<td>6.39</td>
</tr>
<tr>
<td>P</td>
<td>±1.55</td>
<td>±0.56</td>
<td>±0.49</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetic group (10)</td>
<td>36.49</td>
<td>36.79</td>
<td>23.67</td>
</tr>
<tr>
<td>P</td>
<td>±3.01</td>
<td>±5.04</td>
<td>±1.91</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin D3 treated diabetic group (10)</td>
<td>27.4</td>
<td>27.1</td>
<td>11.55</td>
</tr>
<tr>
<td>P</td>
<td>±2.91</td>
<td>±2.93</td>
<td>±1.38</td>
</tr>
<tr>
<td></td>
<td>&lt;0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td></td>
<td>&lt;0.02</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

In parenthesis is the number of rats studied in each group.
Values are expressed as means ±SEM.
P: Significance by LSD at P<0.05 from the control group.
P*: Significance by LSD at P<0.05 from the diabetic group.
NS: Not significant.

The diabetic group showed significantly elevated plasma levels of triglycerides, total cholesterol and LDL-Cholesterol, accompanied by significantly reduced plasma HDL-Cholesterol compared to the control group, whereas vitamin D3 treated diabetic group had significantly increased only plasma total cholesterol and LDL-Cholesterol levels compared to the control group. However, vitamin D3 treated diabetic group showed significantly decreased plasma levels of triglycerides, total cholesterol and LDL-Cholesterol accompanied by a significant rise of plasma HDL-Cholesterol compared to the diabetic group. Although atherogenic index was significantly elevated in untreated and treated diabetic groups compared to the control group, it was significantly decreased in vitamin D3 treated diabetic group compared to the diabetic group. Non-significant changes in lipid profile were present in vitamin D3 supplemented group compared to the control group, as shown in table (3) and fig.(1).

**Table 3:** Lipid profile changes in the different studied groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Triglycerides (mg/dl)</th>
<th>Total Cholesterol (mg/dl)</th>
<th>LDL-Cholesterol (mg/dl)</th>
<th>HDL-Cholesterol (mg/dl)</th>
<th>Atherogenic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (10)</td>
<td>40.18 ± 2.82</td>
<td>79.06 ± 3.42</td>
<td>34.53 ± 1.78</td>
<td>39.96 ± 2.24</td>
<td>2.002 ± 0.079</td>
</tr>
<tr>
<td>Vitamin D3 supplemented group (10)</td>
<td>39.86 ± 2.45</td>
<td>73.41 ± 2.12</td>
<td>32.11 ± 1.93</td>
<td>37.39 ± 1.55</td>
<td>1.987 ± 0.083</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetic group (10)</td>
<td>54.05 ± 3.55</td>
<td>104.56 ± 3.87</td>
<td>68.86 ± 3.84</td>
<td>30.24 ± 1.94</td>
<td>3.6 ± 0.278</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin D3 treated diabetic group (10)</td>
<td>41.54</td>
<td>93.46 ± 4.28</td>
<td>50.49 ± 2.47</td>
<td>38.44 ± 2.55</td>
<td>2.432 ± 0.159</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.02</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
In parenthesis is the number of rats studied in each group. Values are expressed as means ±SEM. NS: Not significant. 
P: Significance by LSD at P<0.05 from control group. P*: Significance by LSD at P<0.05 from the diabetic group.

**Fig. 1:** Lipid profile changes in the different studied groups.
a: Significance by LSD at P<0.05 from control group.
P*: Significance by LSD at P<0.05 from the diabetic group.
Similar levels of SCD1 gene expression was detected in Vitamin D3 supplemented group and control group with insignificant differences. However, the diabetic group showed downregulated SCD1 mRNA by 10 folds less than control group denoting that SCD1 mRNA levels and the enzyme activity in liver were strongly downregulated in both diabetic groups (treated and untreated). In addition, in vitamin D3 treated diabetic group, SCD1 mRNA level was significantly decreased compared to control group; however, it was significantly elevated compared to the diabetic group, as shown in table (4).

**Table 4:** Changes of liver cell mitochondrial NAD$^+$ (nmol/mg tissue protein) and liver cell SCD1 gene expression [(fold)/ul] in the different studied groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mitochondrial NAD$^+$ (nmol/mg tissue protein)</th>
<th>SCD1 Gene expression (fold)/ul</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (10)</td>
<td>7.52 ±0.97</td>
<td>0.768 ±0.067</td>
</tr>
<tr>
<td>Vitamin D3 supplemented group (10)</td>
<td>8.18 ±1.14</td>
<td>0.865 ±0.031</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetic group (10)</td>
<td>15.97 ±1.16</td>
<td>0.065 ±0.017</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>NS</td>
</tr>
<tr>
<td>Vitamin D3 treated diabetic group</td>
<td>10.48 ±1.14</td>
<td>0.412 ±0.051</td>
</tr>
<tr>
<td>(10)</td>
<td>P</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>P*</td>
<td>NS</td>
</tr>
</tbody>
</table>

In parenthesis is the number of rats studied in each group. Values are expressed as means ±SEM. P: Significance by LSD at P<0.05 from control group. P*: Significance by LSD at P<0.05 from the diabetic group. NS: Not significant.

Liver cell mitochondrial NAD$^+$ was significantly elevated in the diabetic group compared to the control group, and was insignificantly changed in vitamin D3 treated diabetic and vitamin D3 supplemented groups compared to the control group. On the other hand, there was significant decline in liver cell mitochondrial NAD$^+$ in vitamin D3 treated diabetic group compared to the diabetic group, as shown in table (4).

**Discussion:**

Induction of type 2 diabetes mellitus in rats was confirmed by assessment of biochemical indicators which includes: hyperglycemia, hyperinsulinemia and poor glycemic control. Poor glycemic control was evaluated by HbA1c, and elevated HOMA-IR as indicator for insulin resistance (Ahmad et al., 2016).

Vitamin D3 treated diabetic group showed reduction in hyperglycemia, hyperinsulinemia and insulin resistance compared to the diabetic group. These findings agree with the study of Talaei et al. (2013), who found that vitamin D3 supplementation could reduce insulin resistance in Type 2 diabetic patients. Obese persons, who might be diabetics, could have lowered circulating 25(OH) D levels (Bell et al. 1985; Wortsman et al. 2000), which could be due to the storage of vitamin D3 and 25 (OH) vitamin D3 in adipose tissue (Wortsman et al. 2000). Thus, Borissova et al. (2003) suggested that vitamin D3 supplementation could treat type 2 diabetes mellitus during the winter.

However, Vitamin D injection (Heshmat et al., 2011) and vitamin D intake did not change the glycemic control in the diabetics (Behradmanesh et al., 2011). Also, Boucher et al. (1995) found that vitamin D caused elevated blood glucose level in diabetic patients compared to the pre-treatment value 8-12 weeks after a single vitamin D injection in a dose of 2500 mg, they attributed the failure to correct diabetes by vitamin D in their study to the moderate increase of 25(OH) plasma vitamin D levels to be only 25 nmol/L.

These protective vitamin D3 effects in diabetes mellitus could be due to its anti-inflammatory properties, or it might affect calcium and phosphorus metabolism facilitating insulin secretion and synthesis via β-cell calcium-dependent...
endopeptidases (Chiu et al., 2004). Calcium, also, is essential for \(\beta\)-cell glycolysis, which plays a role in signaling circulating glucose concentration (Boucher, 1998). Moreover, vitamin D could upregulate the insulin receptor gene (Maestro et al., 2002). These mechanisms could be supported by presence of vitamin D receptors on pancreatic \(\beta\) cells (Zittermann, 2006), and in skeletal muscle (Simpson et al., 1985), expression of 1\(a\) hydroxylase in pancreatic \(\beta\) cells (Bland et al., 2004) and presence of vitamin D response element insulin receptor genes transcription (Maestro et al., 2002).

In the current study, the diabetic group was dyslipidemic evidenced by elevated plasma levels of triglycerides, total cholesterol, LDL-Cholesterol and atherogenic index accompanied by reduced plasma HDL-Cholesterol compared to the control group. Similar findings were reported in Ahmad et al. (2016). Also, Kudchodkar et al. (1988) reported that elevated plasma triglycerides levels might be commonly present in diabetes mellitus, and could be due to deficient lipoprotein lipaseactivity (Bruan and Severson, 1992). Thus, Lopes-Virella et al. (1983) found that insulin treatment in diabetics lowered plasma triglycerides levels by normalizing lipoprotein lipase levels.

Treatment of type 2 diabetes mellitus by vitamin D, in this study, ameliorated the diabetes mellitus induced dyslipidemia, manifested by lowered plasma levels of triglycerides, total cholesterol, LDL-Cholesterol and atherogenic index together with higher plasma HDL-Cholesterol compared to the diabetic group. When comparing vitamin D3 treated diabetic group and the control group, only total cholesterol and LDL-Cholesterol were reduced. These findings were similar to the study of Al-Daghri et al. (2012), who found that vitamin D3 administration daily for 18 months caused lowered levels of total cholesterol and LDL cholesterol, without changes in levels of triglycerides and HDL cholesterol. Similarly, Shehab et al. (2012) found significant correlations with total cholesterol, LDL-cholesterol with vitamin D. This lipolytic actions of vitamin D3 could be due to upregulation of lipolytic enzymes hormone sensitive and lipoprotein lipases induced by 1, 25(OH)2D (Huang et al., 2013).

However, the lipid lowering effect of vitamin D3 in the diabetic group, in the present study, disagree with the study of Breslavsky et al. (2013), who found no changes in levels of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides in type 2 diabetics despite vitamin D administration for 12 months. The discrepancy of vitamin D effects on lipid profile in type 2 diabetes mellitus was attributed to the requirement of 25(OH)D levels to be higher than 28-32 ng/ml in the type 2 diabetic persons to induce such possible extra-skeletal benefits of vitamin D (Hossein-nezhad and Holick, 2013).

The diabetic group had liver cell injury, evidenced by elevated plasma levels of AST, ALT and plasma \(\gamma\)-GT activity, which were in accordance to Hamadi et al. (2012). This damage could be due to the oxidative stress present in the diabetic group (Kakkar et al., 1998). Tangvarasittichai (2015) reported that increased glucose, free fatty acids (FFA) and insulin levels caused reactive oxygen species overproduction, increased oxidative stress and activate stress transduction factor pathways resulting to inhibited insulin activity and secretion causing the onset of type 2 diabetes mellitus.

Vitamin D3 treated diabetic group had lowered AST, ALT and \(\gamma\)-GT activity compared to the diabetic group, denoting hepato-protective effects of vitamin D3 which were similar to the study of George et al. (2012). They reported that the restoring effect of vitamin D3 could be mediated through vitamin D receptor modulation, thereby improving signal transduction and controlling free radicals in the liver of diabetic rats, suggesting a potential role for vitamin D3 in the treatment of diabetes-associated hepatic complications. In addition, the active vitamin D’s roles of improving the hepatic steatosis and hepatic insulin resistance could be mediated through its receptor-activated peroxisome proliferator-activated receptors-\(\alpha\) (PPAR-\(\alpha\)) signaling pathway (Yin et al., 2012; Zhao et al., 2013).

The protective vitamin D3 effect against liver cell injury observed in the current study may be attributed to its lipid lowering effect. Similar explanation was reported by Yin et al. (2012), who found that vitamin D3 preventing effect against hepatic steatosis was due to inhibited lipogenesis and the elevated FFA oxidation in rat liver.

Liver cell mitochondrial NAD\(^{+}\), as an indicator of oxidative stress, was significantly elevated in the diabetic group compared to the control group. This new finding in type 2 diabetes mellitus could explain the oxidative stress present in diabetes mellitus, which was found in the study of Kakkar et al. (1998). They, also, suggested that oxidative stress could be the cause of development and progression of diabetes mellitus. The elevated NAD\(^{+}\) in liver cells of diabetic group could be due to transient upregulated mitochondrial oxidative capacity in the liver, similar to
the study on obese insulin-resistant persons with or without fatty liver resulting to oxidative stress (Koliaki and Roden, 2016).

The vitamin D3 treatment had decreased mitochondrial NAD$^+$ in treated diabetic group in the present study. This is in accordance to the study of Chang and Kim (2016), who found that 1,25(OH)2D treatment elevated NAD-to-NADH ratio, and suggested that vitamin D could cause fat mobilization and reducing intracellular fat accumulation and increase lipolysis.

The elevated mitochondrial NAD$^+$ could be linked to higher plasma levels of liver enzymes present in the diabetic group. Maxwell et al. (1997) reported that diabetes mellitus could cause oxidative damage resulted from antioxidant protection defects, thereby many diabetic complications could be developed. Also, Alam et al. (2014) found that the antioxidant treatment of diabetic rats, which was quercetin, had lowered liver enzymes and oxidative stress markers. Thus, the hepato-protective effects of vitamin D3 in diabetes mellitus, observed in this study, assure the antioxidant and lipolytic effects of vitamin D3.

In addition, both diabetic and vitamin D3 treated diabetic groups showed downregulation of SCD1 m-RNA levels in liver cells. SCD1 enzyme has an important role in lipid metabolism and energy expenditure in mammals (Wang et al., 2008). SCD genes are regulated at the transcriptional level by the diet, hormones such as insulin and temperature (Wang et al., 2005). This could explain the changes present in SCD1 m-RNA levels in liver cells in both diabetic groups (treated and untreated). Moreover, Popeijus et al. (2008) reported that raised, rather than reduced, SCD1 mRNA levels could be negatively associated with insulin resistance.

Droge(2002) mentioned that SCD-1 could cause fat storage when its activity is elevated, and towards oxidation, when become less active. Hence elevated fatty acid oxidation could raise toxic free radicals levels, the presence of SCD1 downregulation and higher mitochondrial NAD$^+$ in liver cells, in this study, could be linked together.

It was reported by Ntambi et al.(2002) that diabetic dyslipidemia could be attributed to downregulated SCD1 m-RNA levels in liver cells. Mice with whole-body SCD1 deficiency were lean, and developed severe hypercholesterolaemia and a marked decrease in HDL cholesterol levels when they were fed a low-fat, high-carbohydrate diet (Flowers et al., 2006). Also, the downregulated SCD1 in liver cells could be linked to the insulin resistance in the diabetic group. Roden(2006) reported that the hepatic SCD1 activity index was correlated with insulin sensitivity. Stefan et al. (2008) mentioned that SCD1 could affect insulin sensitivity via regulation of fat accumulation in the liver. High liver fat could cause hepatic and skeletal muscle insulin resistance (Stefan et al., 2006), thus hepatic SCD1 activity could regulate skeletal muscle insulin sensitivity (Stefan et al., 2008).

In this study, the novel finding of upregulated SCD1 m-RNA levels in liver cells in vitamin D3 treated diabetic group compared to the diabetic group, could explain the decreased insulin resistance. This suggestion could be supported by high SCD1 activity protected both from obesity and from palmitate-induced insulin resistance and lipotoxicity (Busch et al., 2005). Moreover, Foster (2004) reported that a marked stimulation of the transcription factor carbohydrate-responsive element binding protein were present in the fed state activating the genes of fatty acid and triglyceride synthesis. Thus, SCD-1 is lipogenic via more than one pathway. In the fasting state, SCD-1 levels decreased due to insulin decrease and glucagon increase. This, also, could explain the presence of downregulated SCD1 in liver cell in the vitamin D3 treated diabetic group compared to the control group.

Therefore, the vitamin D3 ameliorated liver damage, dyslipidemia, downregulation of liver cell SCD1 and controlled elevated liver cell mitochondrial NAD$^+$ in type 2 diabetes mellitus.

**Conclusion:**
Diabetes mellitus resulted in deteriorated liver functions in diabetic rats and downregulation of SCD1 m-RNA levels in liver cells. Such changes could be caused by oxidative stress and were alleviated by vitamin D3 treatment.

**Conflict of Interest:**
The authors declared no conflict of interest in this study.

**Funding:**
This study was funded only by the authors, without any source of funding from any institute.
References:


