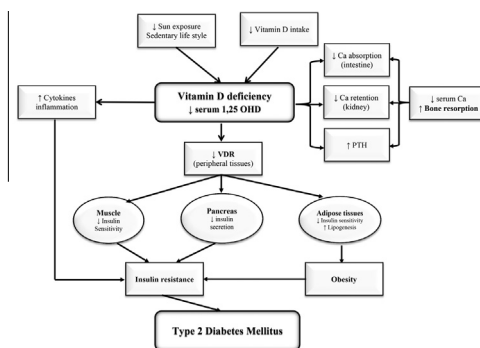


ORIGINAL ARTICLE

Effect of exercise on serum vitamin D and tissue vitamin D receptors in experimentally induced type 2 Diabetes Mellitus

Yosria E. Aly^a, Azza S. Abdou^{a,*}, Mona M. Rashad^b, Menatallah M. Nassef^b^a Department of Physiology, Medical Research Institute, Alexandria University, Alexandria, Egypt^b Department of Applied Medical Chemistry, Medical Research Institute, Alexandria University, Egypt

GRAPHICAL ABSTRACT



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ABSTRACT

This work aimed to study the effect of swimming exercise on serum vitamin D level and tissue vitamin D receptors in experimentally induced type 2 Diabetes Mellitus. Sixty adult male rats were divided into control and diabetic groups. Each was further subdivided into sedentary and exercised subgroups. Diabetes Mellitus was induced by a single intraperitoneal dose of streptozotocin (50 mg/kg) dissolved in cold 0.01 M citrate buffer (pH 4.5). The exercised subgroups underwent swimming for 60 min, 5 times a week for 4 weeks. Serum glucose, insulin,

* Corresponding author. Fax: +20 4283719.

E-mail address: azzasaad_mri@yahoo.com (A.S. Abdou).

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homeostasis model assessment of insulin resistance (HOMA-IR), lipids, vitamin D and tissue Vitamin D receptors (VDR) were evaluated. Significant increase in serum glucose, insulin, HOMA-IR, cholesterol, triglycerides, and low density lipoprotein (LDL) levels in sedentary diabetic rats was detected. On the other hand, high density lipoprotein (HDL), free fatty acids, serum vitamin D and pancreatic, adipose, and muscular VDR showed a significant decrease in the same group. It is evident that all these parameters were reversed by swimming exercise indicating its beneficial role in type 2 Diabetes. In diabetic groups; serum vitamin D was found to be correlated negatively with serum glucose, insulin, HOMA, cholesterol, triglycerides, and LDL and positively correlated with HDL and tissue VDR. In conclusion, Disturbed vitamin D is associated with metabolic impairments in sedentary diabetic rats. Moderate swimming exercise is beneficial in improving these consequences through modulation of vitamin D status. Future studies could be designed to investigate the effect of the combination of vitamin D intake with exercise in diabetic patients.

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Introduction

Type 2 Diabetes Mellitus (DM) is an epidemic non-communicable disease that threatens health and life quality of people. Currently, there are 285 million people worldwide living with diabetes, and 90–95% have type 2 DM. This number is expected to reach 439 million by the year 2030 [1]. Type 2 DM is a multifactorial disease characterized by chronic hyperglycemia, altered insulin secretion, and insulin resistance. It can be also defined by impaired glucose tolerance (IGT) that results from islet β cell dysfunction, followed by insulin deficiency in skeletal muscle, liver and adipose tissues [2].

Apart from its calcemic effects, vitamin D is now known to have many non-calcemic functions through which it may have some roles in several human pathologies including some cancers, autoimmune and metabolic disorders [3]. A novel association with diabetes that has received a considerable attention recently is vitamin D disturbance. Some evidences indicate a high prevalence of vitamin D deficiency worldwide. Vitamin D deficiency is usually caused by low dietary vitamin D intake and reduced cutaneous production of vitamin D [4]. The most abundant form of vitamin D is 25-hydroxyvitamin D (25(OH) D₃), which indicates serum concentrations of vitamin D status. It exerts its biological effects through the active metabolite 1 α -25 dihydroxy vitamin D₃, which serves as a ligand for vitamin D receptors (VDR) [5]. Alvarez and Ashraf [6] demonstrated the expression of (VDRs) in pancreatic, muscle and adipose tissue. It is hypothesized that vitamin D may have a role in the pathogenesis and prevention of type 2 DM [7].

Swimming exercise is widely used in rats as a model for evaluating the effects of aerobic activity in pathological and physiological conditions [8]. The role of regular physical training on cardiovascular and metabolic disorders, including diabetes has been assessed [9]. However, little is known about the effect of exercise on vitamin D status in case of type 2 DM. Therefore, the current study was conducted to evaluate the effect of swimming exercise on serum vitamin D level and tissue vitamin D receptors in experimentally induced type 2 DM Mellitus in rats.

Material and methods

All procedures were performed in accordance with ethical guidelines of Medical Research Institute, Alexandria University, for the care and use of laboratory animals, (IORG0008812).

Animals and experimental design

The present study was carried out on sixty adult male albino-rats. Rats were obtained from the animal house of Medical Research Institute, Alexandria University. They were housed under controlled environmental conditions: temperature 25 °C with an established photo-period of 12 h light/day. The rats had free access to food and tap water *ad libitum*. Rats were divided randomly equally into 2 main groups:

Group I (control group): 30 males rats which were equally subdivided into the following:

- Group I (a): control sedentary
- Group I (b): control exercised

Group II (diabetic group): 30 males rats that were equally subdivided into the following:

- Group II (a): diabetic sedentary
- Group II (b): diabetic exercised

Induction of type 2 Diabetes Mellitus

To develop a rat model of experimentally-induced type 2 Diabetes Mellitus, which resembles that occurring in human population, overnight fasting rats were injected with a single intraperitoneal (IP) dose of streptozotocin STZ (50 mg/kg) (Sigma, St. Louis, MO, USA) dissolved in cold fresh 0.01 M citrate buffer fresh or frozen in 1 mL aliquots at –20 °C (0.1 mol/L citric acid, 0.1 mol/L sodium citrate), pH 4.5. Diabetes was confirmed 3 days later when blood glucose rose above 126 mg/dL [10].

Swimming exercise protocol

The swimming moderate exercise protocol used included 2 phases: adaptation and training. The adaptation phase included the first three days of training. On the first day, the animals exercised in the pool (area 100 cm², with water depth 35–45 cm at 37 °C) for 15 min. The exercise period was extended by 15 min each day until animals were swimming for 60 min. The training phase consisted of 60 min session, 5 times a week for 4 weeks [11].

At the end of the experiment, weight and length of each rat were estimated with the calculation of anthropometric measurements: body mass index (BMI) and Lee index. Then rats were decapitated, blood samples were collected and centrifuged and sera were separated into 2 aliquots; one part was used immediately for assay of biochemical measurements and the other part was stored at -20°C for the assay of serum vitamin D and insulin hormone. The pancreas, skeletal muscle and adipose tissues were excised from each rat and prepared for vitamin D receptors assay.

Tissue preparation

The excised tissues were rinsed with ice-cold phosphate-buffered saline (PBS) (0.02 mol/L, pH 7.0–7.2) to remove excess blood thoroughly, then minced into small pieces and homogenized in 10 mL of PBS with a glass homogenizer on ice. The resulting suspension was subjected to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifuged for 5 min at 5000 rpm and the supernatant of each was removed and then stored at -20°C until assay of vitamin D receptors.

Methods

The following parameters were determined for each rat.

Anthropometric measurements

- **Body mass index (BMI)** Body mass index (gm/cm^2) = weight (g)/total length² (cm)² [12].
- **Lee index (g/cm)** Lee index = cube root of body weight (g)/nose-to-anus length (cm) [12].

Biochemical measurements

- Serum glucose [13].
- Serum insulin level using rat specific Enzyme linked immune sorbent assay (ELISA) kits purchased from DRG International Inc, USA.
- Assessment of insulin resistance using the homeostasis model assessment (HOMA-IR score) using the equation (fasting glucose (mmole/L) \times fasting insulin ($\mu\text{U}/\text{L}$)/22.5) [14].
- Serum cholesterol level [15].
- Serum triglycerides level [16].
- Serum High Density Lipoprotein (HDL.C) level [17].
- Serum Low Density Lipoprotein (LDL.C) level [18].
- Free Fatty Acid (FFA) level [19].

Vitamin D status assays

Serum vitamin D level and tissue VDR in the pancreas, muscle and adipose tissue of each rat were determined using ELISA kits purchased from Usbn life science Inc., USA.

Statistical analysis

Data were analyzed using IBM Statistical Package for Social Sciences (SPSS) software package version 20.0. Data were first

tested for normality. The quantitative data were normally distributed and expressed in means and standard deviations. Comparison between the different studied groups was analyzed using F test, Analysis of Variance (ANOVA) and Post Hoc test (LSD) (Tukey) for pairwise comparisons. Pearson correlation was also performed between serum vitamin D and the other studied parameters. For all statistical tests, the level of *P* equal to or less than 5% was considered significant.

Results

The comparison among the four studied groups (sedentary control, exercised control, sedentary diabetic and exercised diabetic) was done using ANOVA test and the Pairwise comparison between each two groups was done using Post Hoc test (LSD) (Tukey). Findings of anthropometric and biochemical measurements are summarized in Tables 1 and 2. As regards BMI and Lee index, significant differences are noticed ($F = 13.42, 15.45$ and $P \leq 0.001$). Findings of serum glucose level, fasting serum insulin and HOMA-IR demonstrated significant differences among the four groups by ANOVA ($F = 70.11, 241.18$ and 185.18 respectively) with $P \leq 0.001$ (Table 1). In addition, significant differences among the four groups were also noticed in serum cholesterol, triglycerides, HDL, LDL and FFA ($F = 260.49, 51.99, 22.79, 240.86$ and 30.690 respectively) with $P \leq 0.001$ (Table 2). The sedentary diabetic group showed the most significant increase in serum glucose, insulin, HOMA-IR, serum cholesterol, TG and LDL as compared to the other groups. It showed also the most significant decrease in serum HDL and FFA.

As shown in Table 3, significant differences were found between the four groups in serum vitamin D and tissue vitamin D receptors in pancreatic, muscle and adipose tissues (F values = 64.53, 66.17, 40, 74 and 95.13 respectively) with $P \leq 0.001$. The most significant decrease in serum vitamin D and tissue VDR is observed in the sedentary diabetic group as compared to the other groups. The $P1$ – $P6$ values for pairwise comparisons of each two groups are shown in Tables 1–3.

Exercise induced a significant increase in muscle and adipose tissue vitamin D receptors in both diabetic and control groups as compared to sedentary one while a significant increase in serum vitamin D and pancreatic receptors was noticed in the exercised diabetic group only (Figs. 1–4).

Correlations between serum vitamin D and the other studied parameters in both sedentary and exercised diabetic groups are represented in Table 4. Findings showed a positive correlation between serum vitamin D and tissue VDR. On the other hand, negative correlations were noticed between serum vitamin D and the other studied parameters in both diabetic groups.

Discussion

Vitamin D deficiency has reached epidemic proportions worldwide primarily due to the shift to sedentary indoor lifestyles and sun avoidance behaviors. Impacts on calcium metabolism and bone health are well known; however, non-skeletal associations with chronic health problems are recently recognized. Increasing evidence suggested also the relationship between vitamin D and many metabolic diseases including diabetes. Type 2 DM and vitamin D deficiency have risk factors in com-

Table 1 Anthropometric parameters and biochemical measurements in control and diabetic groups (Mean \pm SD).

	Group I (Control) (n = 30)		Group II (Diabetic) (n = 30)		F	P
	Group I (a) Sedentary (n = 15)	Group I (b) Exercised (n = 15)	Group II (a) Sedentary (n = 15)	Group II (b) Exercised (n = 15)		
BMI (g/cm ²)	0.53 \pm 0.06	0.65 \pm 0.06	0.53 \pm 0.06	0.53 \pm 0.07	13.423	< 0.001*
Sig. bet. grps	$P_1 < 0.001^*$, $P_2 = 0.999$, $P_3 = 0.990$, $P_4 < 0.001^*$, $P_5 < 0.001^*$, $P_6 = 0.998$					
Lee index (g/cm)	0.73 \pm 0.040	0.81 \pm 0.04	0.73 \pm 0.04	0.73 \pm 0.03	15.466	< 0.001*
Sig. bet. grps	$P_1 < 0.001^*$, $P_2 = 1.000$, $P_3 = 1.000$, $P_4 < 0.001^*$, $P_5 < 0.001^*$, $P_6 = 1.000$					
Serum glucose (mg/dL)	87.80 \pm 11.06	90.47 \pm 10.67	165.40 \pm 24.76	137.20 \pm 19.13	70.112	< 0.001*
Sig. bet. grps	$P_1 = 0.975$, $P_2 < 0.001^*$, $P_3 < 0.001^*$, $P_4 < 0.001^*$, $P_5 < 0.001^*$, $P_6 < 0.001^*$					
Serum insulin (μ g/L)	9.72 \pm 1.89	10.93 \pm 1.69	29.27 \pm 3.45	22.80 \pm 1.97	241.175	< 0.001*
Sig. bet. grps	$P_1 = 0.506$, $P_2 < 0.001^*$, $P_3 < 0.001^*$, $P_4 < 0.001^*$, $P_5 < 0.001^*$, $P_6 < 0.001^*$					
HOMA-IR	2.11 \pm 0.53	2.43 \pm 0.46	11.94 \pm 2.30	7.70 \pm 1.16	185.175	< 0.001*
Sig. bet. grps	$P_1 = 0.911$, $P_2 < 0.001^*$, $P_3 < 0.001^*$, $P_4 < 0.001^*$, $P_5 < 0.001^*$, $P_6 < 0.001^*$					

F(P). P value for F test (ANOVA), Sig. bet. grps were done using Post Hoc Test (Tukey).

P_1 : P value for comparing between Group I (a) Sedentary and Group I (b) Exercised.

P_2 : P value for comparing between Group I (a) Sedentary and Group II (a) Sedentary.

P_3 : P value for comparing between Group I (a) Sedentary and Group II (b) Exercised.

P_4 : P value for comparing between Group I (b) Exercised and Group II (a) Sedentary.

P_5 : P value for comparing between Group I (b) Exercised and Group II (b) Exercised.

P_6 : P value for comparing between Group II (a) Sedentary and Group II (b) Exercised.

* Statistically significant at $P \leq 0.05$.

Table 2 Lipid profiles in control and diabetic groups (Mean \pm SD).

	Group I (Control) (n = 30)		Group II (Diabetic) (n = 30)		F	P
	Group I (a) Sedentary (n = 15)	Group I (b) Exercised (n = 15)	Group II (a) Sedentary (n = 15)	Group II (b) Exercised (n = 15)		
Cholesterol (mg/dL)	91.33 \pm 11.90	73.27 \pm 8.93	174.4 \pm 14.53	154.8 \pm 10.72	260.497	< 0.001*
Sig. bet. grps	$P_1 < 0.001^*$, $P_2 < 0.001^*$, $P_3 < 0.001^*$, $P_4 < 0.001^*$, $P_5 < 0.001^*$, $P_6 < 0.001^*$					
Triglycerides (mg/dL)	81.40 \pm 17.2	53.47 \pm 7.24	118.0 \pm 18.74	76.33 \pm 11.43	51.994	< 0.001*
Sig. bet. grps	$P_1 < 0.001^*$, $P_2 < 0.001^*$, $P_3 = 0.769$, $P_4 < 0.001^*$, $P_5 < 0.001^*$, $P_6 < 0.001^*$					
HDL (mg/dL)	25.0 \pm 3.72	36.07 \pm 6.23	21.67 \pm 4.19	32.47 \pm 6.73	22.797	< 0.001*
Sig. bet. grps	$P_1 < 0.001^*$, $P_2 = 0.334$, $P_3 = 0.002^*$, $P_4 < 0.001^*$, $P_5 < 0.001^*$, $P_6 = 0.268$					
LDL (mg/dL)	50.05 \pm 12.83	26.51 \pm 9.05	129.13 \pm 14.69	107.3 \pm 10.47	240.857	< 0.001*
Sig. bet. grps	$P_1 < 0.001^*$, $P_2 < 0.001^*$, $P_3 < 0.001^*$, $P_4 < 0.001^*$, $P_5 < 0.001^*$, $P_6 < 0.001^*$					
FFA (mmol/L)	4.85 \pm 1.26	7.80 \pm 2.21	2.29 \pm 0.78	4.65 \pm 1.70	30.687	< 0.001*
Sig. bet. grps	$P_1 < 0.001^*$, $P_2 < 0.001^*$, $P_3 = 0.987$, $P_4 < 0.001^*$, $P_5 < 0.001^*$, $P_6 = 0.001^*$					

F(P). P value for F test (ANOVA), Sig. bet. grps were done using Post Hoc Test (Tukey).

P_1 : P value for comparing between Group I (a) Sedentary and Group I (b) Exercised.

P_2 : P value for comparing between Group I (a) Sedentary and Group II (a) Sedentary.

P_3 : P value for comparing between Group I (a) Sedentary and Group II (b) Exercised.

P_4 : P value for comparing between Group I (b) Exercised and Group II (a) Sedentary.

P_5 : P value for comparing between Group I (b) Exercised and Group II (b) Exercised.

P_6 : P value for comparing between Group II (a) Sedentary and Group II (b) Exercised.

* Statistically significant at $P \leq 0.05$.

Table 3 Serum vitamin D, pancreatic VDR, skeletal muscle VDR and adipose VDR in control and diabetic groups (Mean \pm SD).

	Group I (Control) (n = 30)		Group II (Diabetic) (n = 30)		F	P
	Group I (a) Sedentary (n = 15)	Group I (b) Exercised (n = 15)	Group II (a) Sedentary (n = 15)	Group II (b) Exercised (n = 15)		
Serum VD (nmol/L)	9.59 \pm 0.79	9.79 \pm 2.60	3.27 \pm 0.11	6.12 \pm 1.38	64.526	< 0.001*
Sig. bet. grps	$P_1 = 0.903, P_2 < 0.001^*, P_3 < 0.001^*, P_4 < 0.001^*, P_5 < 0.001^*, P_6 = 0.001^*$					
Pancreatic VDR (ng/mL)	19.48 \pm 5.02	22.36 \pm 2.79	6.98 \pm 2.11	13.10 \pm 2.29	66.169	< 0.001*
Sig. bet. grps	$P_1 = 0.086, P_2 < 0.001^*, P_3 < 0.001^*, P_4 < 0.001^*, P_5 < 0.001^*, P_6 = 0.001^*$					
Skeletal muscle VDR (ng/mL)	23.87 \pm 2.92	30.07 \pm 3.37	16.60 \pm 4.22	21.16 \pm 2.99	40.739	< 0.001*
Sig. bet. grps	$P_1 = 0.048^*, P_2 < 0.001^*, P_3 < 0.001^*, P_4 < 0.001^*, P_5 < 0.001^*, P_6 = 0.001^*$					
Adipose VDR (ng/mL)	21.67 \pm 2.09	23.68 \pm 2.40	12.19 \pm 1.26	16.30 \pm 2.33	95.130	< 0.001*
Sig. bet. grps	$P_1 < 0.001^*, P_2 < 0.001^*, P_3 = 0.143, P_4 < 0.001^*, P_5 < 0.001^*, P_6 = 0.003^*$					

F(P). P value for F test (ANOVA), Sig. bet. grps were done using Post Hoc Test (Tukey).

P_1 : P value for comparing between Group I (a) Sedentary and Group I (b) Exercised.

P_2 : P value for comparing between Group I (a) Sedentary and Group II (a) Sedentary.

P_3 : P value for comparing between Group I (a) Sedentary and Group II (b) Exercised.

P_4 : P value for comparing between Group I (b) Exercised and Group II (a) Sedentary.

P_5 : P value for comparing between Group I (b) Exercised and Group II (b) Exercised.

P_6 : P value for comparing between Group II (a) Sedentary and Group II (b) Exercised.

* Statistically significant at $P \leq 0.05$.

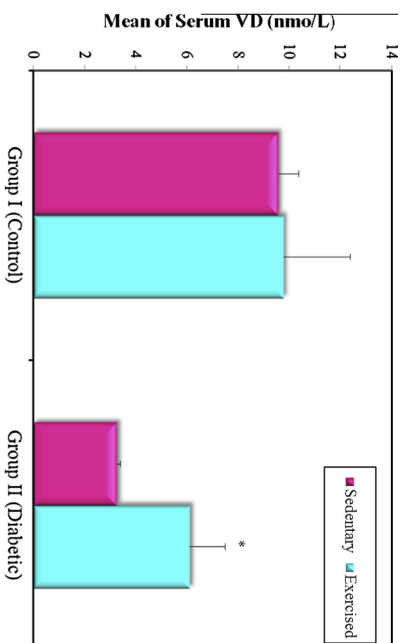


Fig. 1 Serum vitamin D (nmol/L) in control and diabetic rats. * : Statistically significant at $P \leq 0.05$.

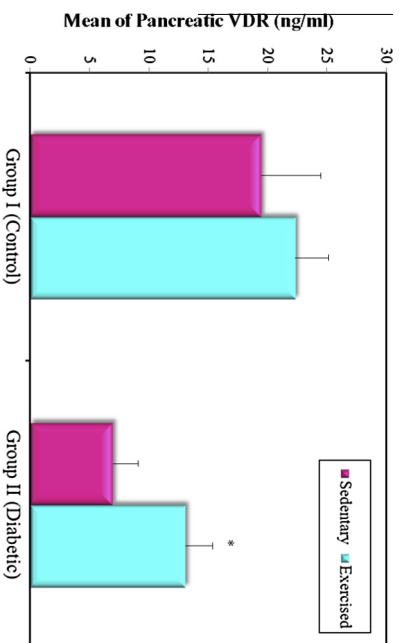


Fig. 2 Pancreatic vitamin D receptor (VDR) (ng/mL) in control and diabetic rats. * : Statistically significant at $P \leq 0.05$.

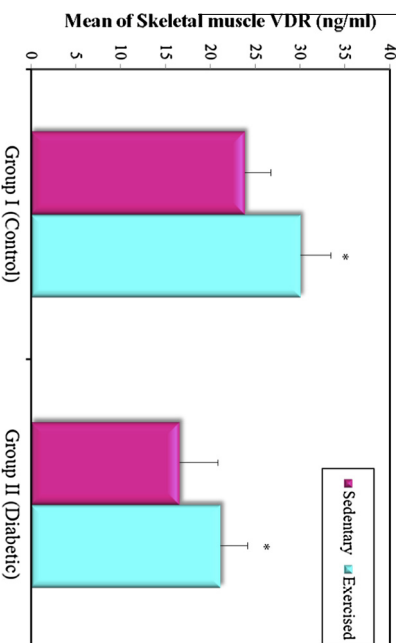


Fig. 3 Skeletal muscle vitamin D receptor (VDR) (ng/mL) in control and diabetic rats. * : Statistically significant at $P \leq 0.05$.

mon conditions such as obesity, aging and low physical activity [20].

Results of the present study showed impaired vitamin D status in sedentary groups as compared to exercised ones. Lower serum vitamin D levels were found in sedentary diabetic rats as compared to other groups. This hypo-vitaminosis D could have a role in impaired insulin action associated with

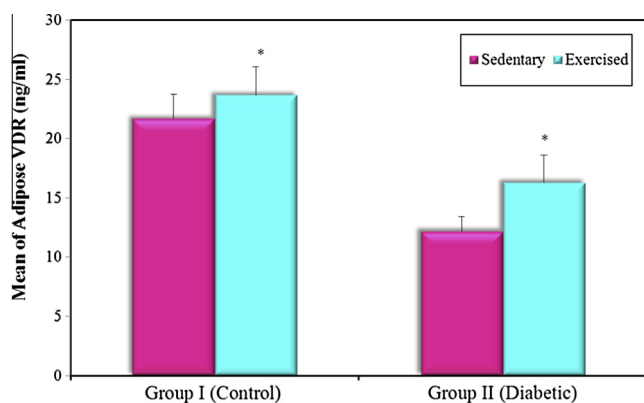


Fig. 4 Adipose tissue vitamin D receptor (VDR) (ng/mL) in control and diabetic rats. *: Statistically significant at $P \leq 0.05$.

type 2 DM. This is clearly evident from the negative correlations obtained between serum vitamin D levels and serum glucose, insulin, HOMA.

Vitamin D may have beneficial effects on insulin action either directly by stimulating the expression of insulin receptors and enhancing insulin responsiveness for glucose transport or indirectly via its role in regulating extracellular calcium ensuring normal calcium influx through cell membranes and adequate intracellular cytosolic calcium pool. Calcium is essential for insulin-mediated intracellular processes in insulin-responsive tissues such as skeletal muscle and adipose tissue [21].

Vitamin D deficiency may influence insulin secretion and sensitivity via its effects on intracellular calcium. The elevated intracellular calcium impairs post-receptor binding insulin action, such as the dephosphorylation of glycogen synthase and of insulin regulatable GLUT-4 [22]. Vitamin D deficiency also results in elevated parathyroid hormone (PTH) which in turn is known to elevate intracellular calcium. Sustained elevations of intracellular calcium may inhibit insulin-target cells from sensing the brisk intracellular calcium fluxes necessary for insulin action, such as glucose transport. In addition, Vitamin D deficiency may result in increased insulin resistance due to the expression of pro-inflammatory cytokines involved in

insulin resistance such as interleukins, IL-1, IL-6, and TNF- α [23].

The identification of Vitamin D receptors in several tissues provides a direct pathway for Vitamin D to impact upon structure and function of these tissues [24].

In the present work, vitamin D deficiency in the sedentary diabetic group is associated with deficient tissue vitamin D receptors (pancreas, muscle and adipose tissue). This finding indicates that these tissues could be target organs for vitamin D.

The potential influence of vitamin D on glucose homeostasis was explained by the presence of specific vitamin D receptors. The presence of these receptors and vitamin D-binding proteins (DBP) in pancreatic tissue as well as the relationship between certain allelic variations in the VDR and DBP genes with glucose tolerance and insulin secretion has further supported this hypothesis. Pancreatic β -cells express not only VDR but also the pivotal enzyme 1α -hydroxylase which catalyzes the conversion of 25(OH) D_3 to 1, 25-dihydroxyvitamin D (1, 25(OH) $_2D_3$) [24].

According to this, Palomer et al. [3] reported that the mechanism of action of vitamin D in type 2 DM is thought to be mediated not only through regulation of plasma calcium levels, which regulate insulin synthesis and secretion, but also through a direct action on pancreatic β -cell function.

VDRs are also expressed by both human skeletal muscle and adipose tissue which are the main determinants of peripheral insulin sensitivity. These tissues were also shown to express the 1α -hydroxylase gene in male Wistar rats [25]. The significant decrease in muscular and adipose tissue VDR obtained in the present study is associated with deficient serum vitamin D levels in the sedentary diabetic group.

Dirks-Naylor and Lennon-Edwards [26] also reported that the direct effects of vitamin D on muscle have to be connected with VDR. Vitamin D affects muscle function through the binding of 1, 25(OH) $_2D_3$ to its receptor resulting in muscle growth as well as other adaptations. The low vitamin D status is associated with loss of handgrip strength and impaired lower extremity function with increased risk of falls [26].

An important mediator of vitamin D action is VDR gene which functions as a transcription factor when bound to 1, 25(OH) $_2D_3$. It is proposed that genetic changes or alterations

Table 4 Correlation between serum vitamin D with different parameters in diabetic groups.

	Vitamin D			
	Group II (a) sedentary		Group II (b) exercised	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Glucose	-0.618*	0.014	-0.595*	0.019
Insulin	-0.544*	0.036	-0.603*	0.017
HOMA	-0.614*	0.015	-0.610*	0.016
Cholesterol	-0.592*	0.020	-0.580*	0.023
Triglycerides	-0.568*	0.027	-0.633*	0.011
HDL	0.538*	0.039	0.666*	0.007
LDL	-0.655*	0.008	-0.697*	0.004
Pancreatic VDR	0.586*	0.022	0.727*	0.002
Skeletal muscle VDR	0.534*	0.040	0.741*	0.002
Adipose VDR	0.623*	0.013	0.708*	0.003

r: Pearson coefficient.

* Statistically significant at $P \leq 0.05$.

of VDR gene might contribute to the development of Diabetes Mellitus by at least four different pathways: alteration in calcium metabolism, modulation of adipocyte function, modulation of insulin secretion and modification of cytokine expression [3].

It has been reported that supplementation with vitamin D in deficient rabbits that present with impaired insulin secretion could correct this defect [6]. Moreover, Al-Sofiani et al. [27] demonstrated that vitamin D supplement for 12 weeks increased serum vitamin D concentrations and improved β -cell activity in vitamin D-deficient type 2 DM which suggests the possible role of vitamin D in the improvement of insulin secretion and sensitivity.

On the other hand, Haroon et al. [28] have reported that the currently available evidence based on randomized controlled trials and longitudinal studies suggests that vitamin D supplementation might not improve hyperglycemia, beta cell secretion or insulin sensitivity in patients with established type 2 DM. Factors related to vitamin D and diabetes may be attributed; One factor linked to vitamin D is related to its dosing. Sub optimal dosing of vitamin D may be one potential reason. The dose of vitamin D used may not have been adequate; most studies used daily doses of less than 2000 IU and daily doses up to 5000 IU may be essential to raise serum 25(OH) D_3 levels above the 75-nmol/L level. The appropriate dose of vitamin D that can achieve non-skeletal benefits still remains unclear. As observed in some studies, supra-physiological dosing of vitamin D may have been harmful. Genetic factors related to vitamin D metabolism might play a role. It is likely that some ethnic groups might have a lower sensitivity to the effects of vitamin D and PTH [28].

Our findings demonstrated also the association between vitamin D deficiency and dyslipidemia in sedentary diabetic groups. Bellan et al. [29] suggested that lipid profile clearly reflected vitamin D status, and high 25(OH) D_3 levels were significantly associated with higher levels of HDL cholesterol. This seems to confirm that vitamin D status is inversely related to atherogenic dyslipidemia and indicates that vitamin D may be independently protective against the atherogenic profile in diabetic patients.

Two main mechanisms have been postulated for vitamin D mediated reduction in serum triglycerides. The first mechanism is that vitamin D increases serum calcium by enhancing intestinal calcium absorption. This calcium could then reduce serum triglycerides by reducing hepatic triglyceride formation and secretion. The second mechanism is that vitamin D has a suppressive effect on serum PTH concentration. As plasma post-heparin lipolytic activity is reduced by elevated PTH concentration, low serum PTH may reduce serum triglycerides via increased peripheral removal. Other two mechanisms may be also implicated: Vitamin D may regulate triglyceride metabolism by causing the expression of VLDL cholesterol receptors in some types of cell. Another possible mechanism to explain the association between 25-hydroxyvitamin D and triglycerides would be through insulin resistance: when vitamin D deficiency is present, the risk of insulin resistance increases and this is associated with an elevation of levels of VLDL cholesterol and triglycerides [30].

The results of this study showed that low vitamin D levels are associated with diabetes, independently of BMI. Our findings demonstrated that there is no change in markers of

adiposity (BMI and Lee index) in the sedentary diabetic group. In addition, these were not correlated with vitamin D levels.

Contrary to this, the association of obesity with low vitamin D was previously reported. The high content of body fat acts as a reservoir for lipid soluble vitamin D and increases its sequestration, thus determining its low bioavailability. Moreover, the synthesis of 25-hydroxyvitamin D by the liver may occur at a lower rate in obese subjects due to hepatic steatosis. An alternative explanation is that higher leptin and interleukin 6 circulating levels, mostly secreted by adipose tissue, may have inhibitory effects on 25(OH) D_3 synthesis via their receptors [31].

Experimental studies showed that 1, 25(OH) $_2D_3$ has an active role in adipose tissue on vitamin D by modulating inflammation, adipogenesis and adipocyte secretion. Some of the physiological functions of 1, 25(OH) $_2D_3$ (1,25-dihydroxycholecalciferol or calcitriol) via its receptors within the adipose tissue have been reported. The presence of 1,25(OH) $_2D_3$ inhibited chemokine and cytokine secretion in human adipocytes. 1,25(OH) $_2D_3$ strongly inhibits the activation of the NF- κ B and MAPK signaling pathways, which prevents gene transcription of the proinflammatory factors [32].

Exercise and physical activity are considered as the most effective, non-pharmacological interventions in metabolic diseases as diabetes. However, little is known about the underlying mechanism and the role of vitamin D coordinating these adaptations.

It was reported that regular physical activity increases the physical strength of diabetic patients, controls blood glucose, and prevents the progression from impaired glucose tolerance to type 2DM. Physical activity also enhances insulin sensitivity in the liver, resulting in reduced glucose production and output in the presence of insulin. Due to this connection, the American Diabetes Association (ADA) recommends aerobic exercise of medium intensity such as 150 min of walking per week, or 75 min of high intensity aerobic exercise per week for patients with type 2 DM [33].

It has been reported that regular physical activity is the most effective method for improving insulin resistance. Aerobic exercise was reported to have a positive effect on the insulin resistance index by inducing the oxidation of fatty acids as well as an increase in insulin sensitivity [33].

Improving vitamin D status with modest lifestyle modifications was recently suggested. The National Health and Nutrition Examination Survey (NHANES III) reports indicated that physical activity is related to serum 25(OH) D_3 either due to enhanced vitamin D metabolism or increased sun exposure. Wanner et al. [34] reported significantly higher levels of 25(OH) D_3 in those who exercised outdoors than in those who exercised indoors.

The present study revealed that swimming exercise in diabetic rats was effective in improving vitamin D status resulting in significantly higher serum vitamin D levels associated with increased vitamin D receptors in muscle, pancreas and adipose tissue. The obtained inverse correlations between vitamin D levels with serum glucose, insulin and HOMA indicate that moderate exercise could enhance vitamin D formation which in turn increases insulin sensitivity, reduce glucose and insulin levels.

Muscular atrophy is a well-known complication of chronic human diabetes and commonly affects the lower limb muscles.

Swimming exercise ameliorates the atrophy of muscles by suppressing autophagy. Regular physical activity leads to a number of adaptations in skeletal muscle that allow the muscle to more efficiently utilize substrates for ATP production and thus become more resistant to fatigue. Chronic physical activity (i.e., exercise training) increases glucose transporter 4 (GLUT4) protein levels and mitochondrial enzyme content, and alters fiber type in skeletal muscle [9].

The present study demonstrated also a significant decrease in lipid profile parameters (TC, TG, LDL-C) with significantly increased levels of HDL-C in diabetic rats with swimming exercise. Additionally, the increased vitamin D levels and VDR were negatively correlated with TC, TG and LDL-C and positively correlated with HDL-C plasma levels.

There are three possible mechanisms by which increased vitamin D-VDR axis by exercise could improve lipid profiles: first: vitamin D-induced suppression of PTH secretion, and so increase in lipolysis; second: vitamin D can trigger a decrease in serum triglycerides levels by reducing the hepatic triglyceride formation and secretion and third: vitamin D might improve insulin secretion and insulin sensitivity, thereby indirectly influencing lipid metabolism [30].

Some cross-sectional studies indicate that hypo-vitaminosis D is associated with higher serum levels of inflammatory biomarkers, such as IL-6. The relationship between vitamin D and low-intensity chronic inflammation with insulin resistance in type 2 DM can be mediated in part by the immunomodulating properties of the $1, 25(\text{OH})_2\text{D}_3$, which is able to downregulate the production of pro-inflammatory cytokines. $1, 25(\text{OH})_2\text{D}_3$ may induce its biologic responses through a high-affinity intracellular VDR and so, lacking of the VDR induced the pro-inflammatory responses followed by high intrinsic NF- κB activity [31,32]. Therefore, vitamin D may also function to reduce the risk of diabetes by acting to reduce inflammatory responses.

Moreover, it was reported that exercise training is associated with systemic anti-inflammatory effects, with a reduction in pro-inflammatory markers such as IL-6 and TNF- α in plasma [35]. Therefore, improvement of the studied metabolic parameters in diabetic rats by exercise could be due to the anti-inflammatory response obtained by the modulated vitamin D and enhancement of its VDR. However, the present study did not investigate how inflammation is related to the improved vitamin D status. This is likely to be studied in the near future.

Conclusions

In conclusion, data of the present study demonstrate hypo-vitaminosis D in sedentary type 2 DM groups. Current findings support the importance of vitamin D in contributing to metabolic homeostasis and suggesting its possible role in regulation of glucose homeostasis, insulin and lipid metabolism. The interaction of non-skeletal VDR with type 2 DM pathogenesis is also suggested. Moderate swimming exercise may be beneficial in improving serum vitamin D and its receptors. Type 2 DM patients should maintain serum vitamin D at normal levels by regular monitoring. An aquatic exercise program with an appropriate intensity should be also recommended in those subjects.

Future studies could be designed to investigate the effect of the combination of vitamin D intake with exercise in diabetic patients. Investigation of the role of vitamin D in diabetes using inflammation as the main outcome is needed to provide a more pathophysiological link. Moreover, it is important to examine more genetic polymorphism on a larger sample size to identify individuals that are more susceptible to vitamin D deficiency.

Conflict of Interest

The authors have declared no conflict of interest.

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