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Effect of 25(OH)D supplementation on adipokines and advanced glycation end products in experimental type 2 diabetic and obesity rat model

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Abstract: Diabetes mellitus is a global health problem and vitamin D deficiency is thougt to be a reasonal factor for development of diabetes. Our aim is to investigate among vitamin D levels and blood glucose, insulin resistance, adiponectin, apelin, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), oxidative stress index (OSI) levels and advanced glycation end products (AGEs) formation in modeled obesity and type 2 diabetes mellitus (T2DM) rats. During the 14-week experiment; Control group was fed standart diet-(12 % of calories of fat), Diabetes group was fed high fat diet (HFD)-at the 4th week Streptozotocin (STZ) (35 mg/kg) was injected, Diabetes/vitamin D group was fed HFD-at the 4th week STZ was injected-between 4th-14th week vitamin D supplement was administered, Obesity group was fed HFD-(40% of calories as fat), Obesity/vitamin D group was fed HFD-between 4th-14th week vitamin D supplement was administered. At 14th week, biochemical parameters, adipokine concentrations, kidney tissue cytokine levels, liver tissue oxidative stress index parameters were measured by using ELISA method and Hematoxylin&Eosin staining of kidney, heart, liver, coronary/renal artery tissues and immunohistochemical staining of kidney tissues were performed. Vitamin D can alleviate glucose and lipid parameters and we think vitamin D may be useful adjuvant agent in obesity and T2DM treatment.

Keywords: Type 2 diabetes mellitus and obesity, adipose tissue, vitamin D

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INTRODUCTION

Diabetes mellitus is an important and a difficult health problem and global prevalence of diabetes is predicted to reach 642 million people by 2040 (Ogurtsova *et al*., 2017). Studies have reported that serum 25(OH)D levels is correlated with enhanced risk of obesity, T2DM and metabolic complications, but some are contradictory. Vitamin D can regulate systemic inflammation through its immunomodulator effect on adipokines and ameliorate glycemic control, insulin sensitivity and endothelial function. Adipokines and adipocytokines have an important role in insulin resistance and cardiovascular complications related with central and visceral obesity. Although insulin resistance is a general condition in obesity, the molecular mechanism of T2DM formation has not been definitely revealed yet. Adiponectin is a protein hormone-adipokine and has significant metabolic and anti-inflammatory effects, can play preventive role in diabetes development. When these biological functions and associations of adiponectin are evaluated, adiponectin can be used as clinical diagnostic marker among T2DM patients (Spranger *et al*., 2003). Apelin is includes in the adipokines family which are bioactive mediators released by adipose tissue. Type 2 diabetes patients with higher apelin levels were compared to the control group, but between type 1 diabetes patients and control group no significant difference was found. It has been reported that apelin has a negative correlation between insulin resistance and hemoglobin-A1c and a positive correlation with insulin sensitivity and it decreases blood sugar by increasing glucose use in the muscles. Because of these properties, apelin can be used as a therapeutic agent in controlling insulin resistance (Boucher *et al*., 2005).

It is possible to say that adipose tissue has an endocrine functions and obesity is an inflammatory disease, inflammatory-induced responses or clinical existance undern pathophysiological factors of obesity (Das *et al*., 2001). AGEs, formed in excess in the hyperglycemic environment in diabetes, demonstrate detrimental effects with an augment in production of inflammatory cytokines and growth factors resulting from vascular pathologies. Low vitamin D level is correlated with glycated hemoglobin and AGE formation. Vitamin D deficiency is thougt to be a reasonal factor for development of diabetes (Zhao *et al*., 2020). Therefore, we aim to evaluate associations among vitamin D levels and blood glucose, insulin resistance, adiponectin, apelin, TNF-α, IL-6, OSI levels and AGEs formation in obesity modeled rats induced by HFD and in type 2 diabetes modeled rats

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induced HFD and low dose streptozotocin administration (HFD/STZ).

MATERIALS AND METHODS

Experimental animals and housing

The 40 male Wistar-albino rats- 3/4 months-200±20 g- were purchased Eskişehir Osmangazi University Animal House and housed 4 rats per cage in an animal room in standard laboratory conditions (20-25 °C-12 hr light-dark cycle) for one week before the start of the study. Our research was started to induce modeled obesity and type 2 diabetes in a non-obese, outbred rat strain that reflects the metabolic characteristics of the human syndrome and is favorable for pharmaceutical research.

Experimental design

Rats were randomly classified into five groups: Control group (n=8), Diabetes group (n=7), Diabetes/vitamin D group (n=8), Obesity group (n=8), Obesity/vitamin D group (n=8). Control group was fed normal chow diet consisting of 19% protein, 4.5% carbonhdyrate, 4% fat and other groups were fed high fat diet consisting of 26.2% protein, 26.3% carbonhdyrate, 34.9% fat. Streptozotosin (STZ) was supplied from Sigma (Sigma-Aldrich, St. Louis, Missouri, USA) and dissolved in at pH 4.5 sterile sodium citrate buffer (0.1 M) before injection. At the end of 4^{th} week, rats in group 2-3 were administered STZ (35mg/kg) and in other groups the equal volume of citrate buffer as placebo was given through intraperitoneal (IP) injection (fig. 1). After 72 hours, fasting plasma glucose (FPG) was evaluated by glucometer (Accu-check, RocheDiagnostic GmbH, Mannheim, Germany) and 250mg/dl and higher values were confirmed as a determiner of diabetes. Cholecalciferol (Sigma Aldrich, St Louis, MO, USA) 12.5µg (500IU) kg^{-1} body weight, dissolution in 0.3ml olive oil, was started supplement after confirmation of diabetes. Olive oil was used for dissolution of vitamin D and was given orally as a placebo to non-supplemented groups (group 1-2-4). In supplementation groups (group 3-5) oral cholecalciferol was given orally every other day during 10 weeks. The rats weight was measured on the 1^{st} and 98^{th} days. 14^{th} week of the experiment, all rats were anesthetized and sacrificed with IP injection of Ketamine (50mg/kg) and Xylazine (30 mg/kg). Blood samples, liver and kidney tissues were collected and stored at proper conditions.

Laboratory parameters

Fasting serum glucose, insulin levels and calculating HOMA-IR

Serum glucose level was measured by biochemical colorimetric method and insulin levels were quantified by ELISA method in accordance with kit datasheets (Rat insulin ELISA kit, Shanghai YL Biotech Co. Ltd.) and HOMA-IR index was calculated according to in reference to formula,

HOMA-IR= Fasting plasma insulin (FPI) (µIU/l) X Fasting plasma glucose (FPG) (mmol / l) /22.5.

Lipid profile levels

Totalcholesterol, Triglyceride, HDL (Beckman Coulter Uni Cel® DxC600/800 System) levels were detected by biochemical colorimetric method in accordance with kits datasheets.

25 (OH) vitamin D status

Vitamin D (Access 25(OH) Vitamin D Total Kit for Uni Cel DxI System) levels of rats were detected by Chemiluminescence Immunoassay method in accordance with kit datasheet.

Adiponectin and apelin levels

Adiponectin and apelin levels were measured by enzyme-linked immunosorbent (ELISA) method with using commercial adiponectin and apelin ELISA test kits (Shanghai YL. Biotech Co. Ltd.). The specific kit protocol was applied and the absorbance of each well microplate reader using 450 nm as the primary wavelength was measured. The concentration of adiponectin was calculated by comparison to a standard curves consisting of known concentrations of ADP-Acrp 30. Apelin levels were assessed with the same protocol as adiponectin and the concentration of apelin was calculated by comparison to a standard curve consisting of known concentrations of apelin.

Proinflammatory cytokine (TNF-α, IL-6) levels

Renal tissue samples were homogenized in cold 0.9% NaCl solution and centrifuged at 4°C for 10 minutes (15.000xg). TNF-α and IL-6 levels were measured by ELISA method with using commercial TNF-α, IL-6 ELISA test kits (Shanghai YL. Biotech Co. Ltd.).

Histochemical and Immunohistochemical methods

At the end of the experiment, the renal artery, coronary artery, kidney, liver and heart tissues of the control and experimental groups were fixed with 10% buffered formaldehyde for 24 hours. After the fixation process the tissues were held in the increasing degrees of ethyl alcohol series and turned into paraffin blocks after clearing with xylol. Sections of 3-4 µm thickness were taken from each block. After the sections were kept at 65°C for 1 hour, they were dyed with Hematoxylin and Eosin (H&E) technique to be evaluated under a light microscope. Immunohistochemical staining was performed by taking 3-4 µm sections from the kidney tissue blocks of the control and experimental groups on poly L-lysine slides. In order to determine the effects of HFD, T2DM and vitamin D on carboxymethyl lysine protein, which is an advanced glycation end product, Abcam-Mouse monoclonal primary antibody (Cat:ab125145) was stained using indirect immunohistochemical method.

Ethical approval

Eskişehir Osmangazi University Animal Ethics Committee (approval number 128-682).

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STATISTICAL ANALYSIS

To analyze our research data, SPSS Statistics 29.01 package program was used. P<0.05 was regarded as statistically significant. The conformity of data to normal distribution was surveyed by using the Shapiro-Wilk test. Normally distributed data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test. The Kruskal-Wallis test was used for data that were not normally distributed.

RESULTS

Body and heart weights

Rats in both diabetes (P<0.05) and obesity (P<0.0001) groups showed significantly upper body weights to control group. A statistically significant increase in heart weights was observed in obesity groups (P<0.0001, P<0.05) to other groups and decrease in heart weight was observed in obesity/vitamin D group (P<0.05) to obesity group (table 1).

Vitamin D levels and biochemical parameters

Serum 25(OH)D levels didn't differ between the untreated diabetes and obesity groups to the control group. Both vitamin D (P<0.05, P<0.0001) groups showed significantly high 25(OH)D levels to control and untreated groups. In terms of lipid profile values, there were significant differences between obesity (P<0.0001) groups but no differences between diabetic groups were seen in total cholesterol values. In high density cholesterol values, there were significant correlations between 25(OH)D levels and diabetes groups but no correlations between 25(OH)D levels and obesity (P<0.0001) groups were detected. Although we found no cross-associations in regards to triglyceride values between diabetes groups and obesity groups, high triglyceride values were seen in these groups (P<0.0001) to control group (table 2).

Blood glucose, Insulin levels and HOMA-IR levels

Our results showed there were significant correlations between blood glucose and 25(OH)D values in diabetic (P<0.0001) groups but this improvement effect of 25(OH)D in obesity groups wasn't observed. Insulin levels were lower in both diabetes and obesity groups (P<0.0001) to control group and in diabetes/vitamin D (P<0.05) group, vitamin D supplementation inhibited the reduction of insulin levels as much as diabetes group. About the correlation between supplementation vitamin D and HOMA-IR values, no significant difference were observed in diabetes groups and obesity groups (fig. 2).

Adiponectin and apelin levels

We demonstrated an association with lower adiponectin levels in diabetes (P<0.0001) and obesity (P<0.05) groups and it may indicate the role of adiponectin in insulin resistance. A siginficant negative correlation between 25(OH)D and adiponectin levels was seen after supplementation vitamin D in diabetes group (P<0.0001). However, there was no distinction found in apelin levels between all groups (fig. 3).

TNF-α and IL-6 levels

Significantly increased values of TNF-α and IL-6 in the diabetes group were observed compared to the control group (P<0.0001). A negative association between TNF-α, IL-6 values, and supplementation 25(OH)D was observed, but this was significant only in the diabetes group (P<0.0001) (fig. 4).

Histochemical results

Kidney tissue

According to the H&E kidney tissue staining results, normal histological kidney tissue was observed in control, obesity and obesity/vitamin D groups (fig. 5 A-C), however in diabetes and diabetes/vitamin D groups, degeneration in tubule structures, glomerular compaction and narrowing of the Bowman's capsule space were noticed (fig. 5 D-E).

Heart tissue

According to the H&E heart tissue staining results, healthy myofibrils with euchromatin nuclei were observed in control group (fig. 6A). Hypertrophic myofibrils were identified in obesity and obesity/vitamin D group (fig. 6 B-C). However, in diabetes and diabetes/vitamin D group, similar thickness myofibrils and nuclei were detected in the control group (fig. 6 2D-E).

Liver tissue

According to the H&E kidney tissue staining results, normal histological liver tissue (fig. 7 3A) was observed in control group. Significant microvesicular adiposity, vacuolar degeneration in cytoplasm and heterochromatin, shrunken nuclei were observed in the obesity group (fig. 7 7B). Cytoplasmic degeneration and microvesicular adiposity continued to be detected in obesity/vitamin D group (fig. 7 3C). In diabetes group, a small amount of microvesicular fat foci were observed in zone 3 of the liver lobule (fig. 7 3D). No degeneration was detected in the diabetes/vitamin D group (fig. 7 3E).

Immunohistochemical results

Kidney tissue

No significant changes were observed between the groups in the kidney sections stained with anti-Carboxymethyl Lysine immunohistochemically. Positive staining in all groups was detected only in the cytoplasm of tubular cells in the corticomedullary junction region. No immunostaining was observed in the cortex and medulla regions (fig. 8 A-B-C-D-E).

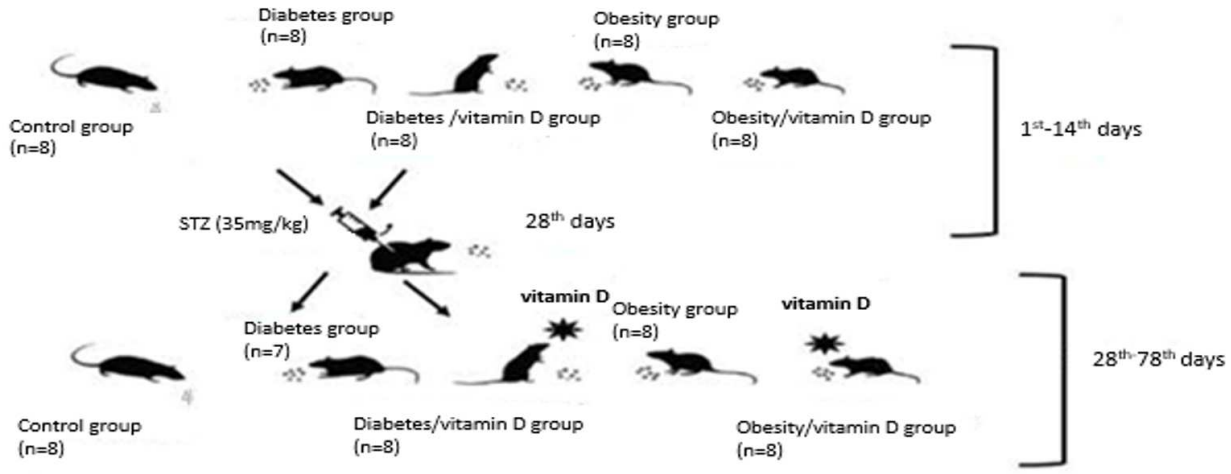


Fig. 1: Experimental design for 14 weeks

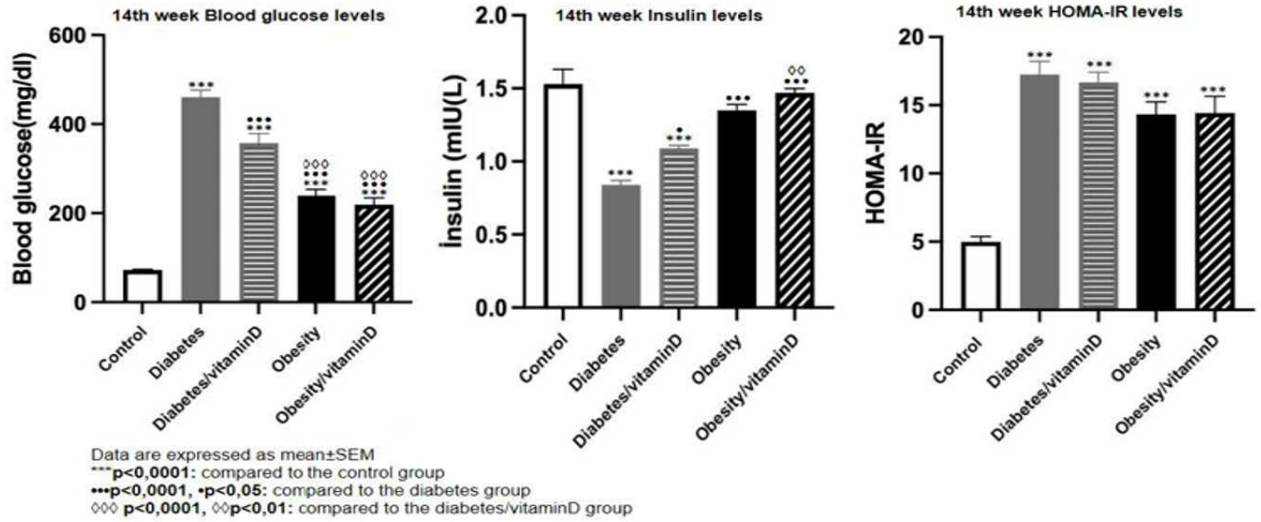


Fig. 2: Blood glucose, insulin, HOMA-IR levels

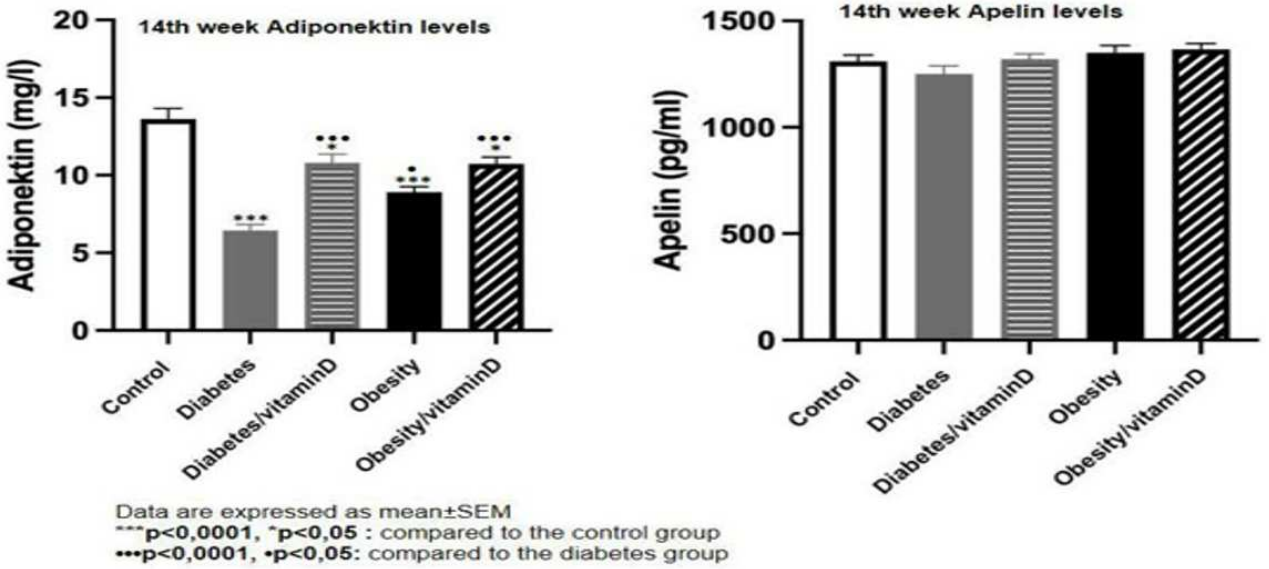


Fig. 3: Adiponectin and Apelin levels

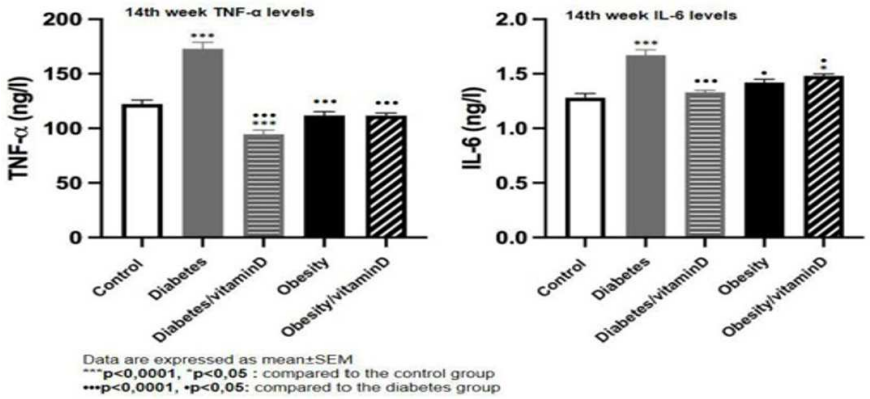
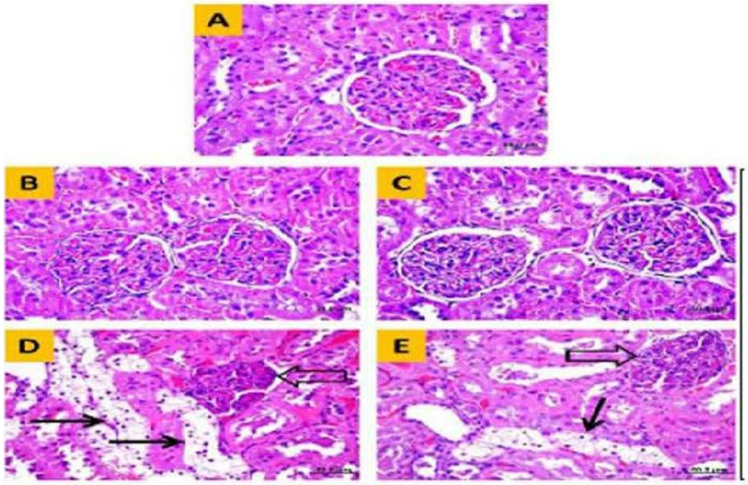
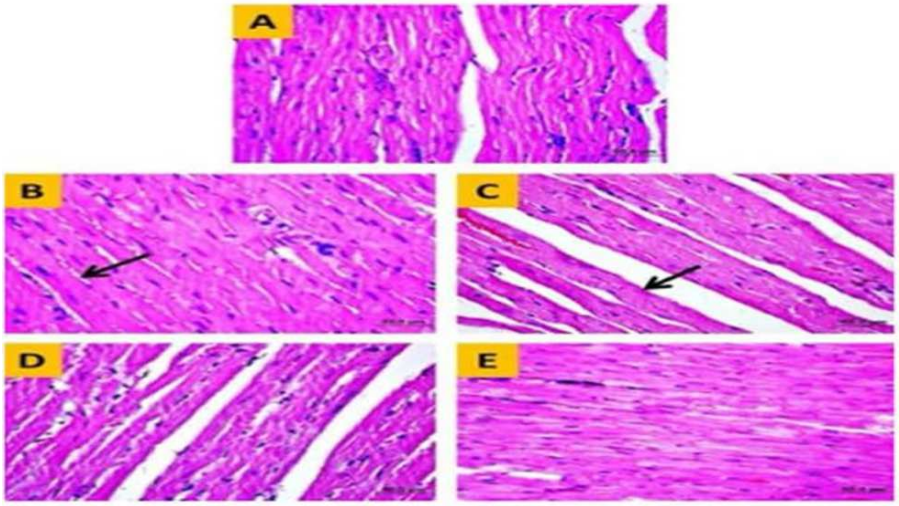


Fig. 4: TNF-α and l IL-6 levels



A: control group
B: obesity group
C: obesity/vitaminD group; the glomerular and tubular structures of kidney tissues have histological appearance
D: diabetes group
E: diabetes/vitaminD group; the glomeruli (thick arrow) and some tubules (thin arrow) appear to be degenerated

Fig. 5: Histological kidney tissue sections of all groups



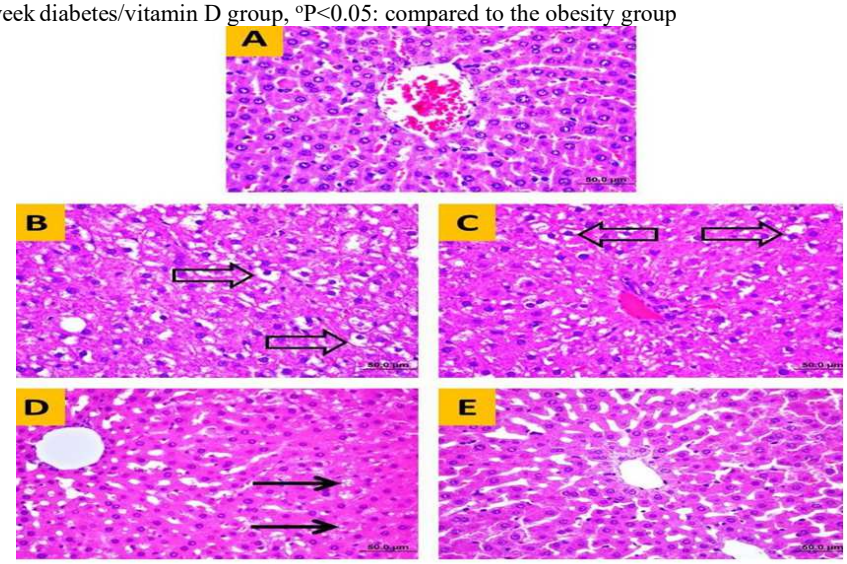
A: control group; typical cardiac muscle fibers are observed
B: obesity group; cardiac muscle fibers are thickened
C: obesity/vitaminD group; cardiac muscle fibers are thickened
D: diabetes group; cardiac muscle fibers have a near-control appearance
E: diabetes/vitaminD group; cardiac muscle fibers have a near-control appearance

Fig. 6: Histological heart tissue sections of all groups

Table 1: Comparison of body weights (1st-14th week) and heart weights (14th week) of all groups.

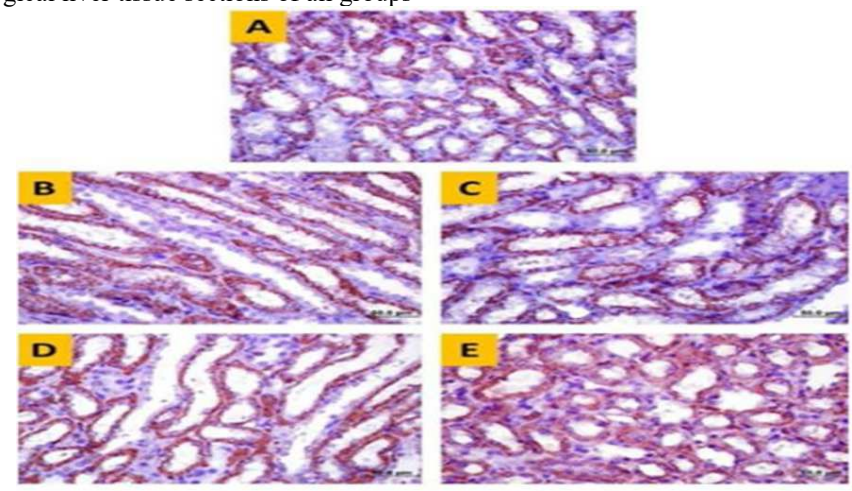
Parameters	1st week BW	14th week BW	14th week HW	P
Control	217.87±9.97	315.62±16.76	1.22±0.88	
Diabetes	220.71±12.92	379±8.25 *	1.43±0.15	<0.05
Diabetes/vitaminD	220±7.55	386.14±9.52 *	1.59±0.08	<0.05
Obesity	225.62±9.46	675.25±12.72 ***•••$^{◊◊◊}$	2.07±0.09***•••$^{◊}$	<0.05, <0.0001
Obesity/vitaminD	226.25±14.41	650.37±12.57 ***•••$^{◊◊◊}$	1.65±0.03 $^{*°}$	<0.05, <0.0001

Data are expressed as mean ± SEM. Different superscripts (*, $^{•,◊}$) within the columns represents significantly different results to different groups. 1st week body weight comparisons show that the distribution of rats between all groups is homogeneous. * P<0.05, ***P<0.0001: compared to the 14th week control group, •••P<0.0001: compared to the 14th week diabetes group, $^{◊}$P<0.05, $^{◊◊◊}$P<0.0001: compared to the 14th week diabetes/vitamin D group, $^{°}$P<0.05: compared to the obesity group



A: control group; typical liver histology is seen.
B: obesity group &C: obesity/vitaminD group; vacuolization in the cytoplasm of some liver cells, shrinkage in their nuclei and chromatin condensation are observed (thick arrow).
D: diabetes group; microvesicular lubrication is observed in the cytoplasm of hepatocytes located at the periphery of the lobule.
E: diabetes/vitaminD group; hepatocytes have an appearance similar to control.

Fig. 7: Histological liver tissue sections of all groups



A: control group
B: obesity group
C: obesity/vitaminD group
D: diabetes group
E: diabetes/vitaminD group
No significant immunohistochemical differences are observed in the sections of the groups.
The immunoreaction is clearly observed in the cytoplasm of tubular cells in the corticomedullary region in all groups.

Fig. 8: Histological sections of kidneys of all groups stained immunohistochemically with anti-carboxymethyl lysine

Table 2: Comparison of vitamin D and biochemical parameters levels of all groups

Parameters	25(OH)D	TC	HDL	TG	P
Control	20.48±0.99	47.12±3.18	38.37±1.29	35.50±1,88	
Diabetes	20.87±1.34	70.14±2.05 ***	27.85±1.38**	148±1.91***	<0.05, <0.01
Diabetes/vitaminD	33.03±1.75 **•	63±2.3 **	45.14±1.7 •••	121.1±2.5 ***	<0.05,<0.01,<0.0001
Obesity	26.59±1.97	77.62±2.86 ***	51±2.22 ***•••	240.71±21.13 ***•••◊	<0.05, <0.0001
Obesity/vitaminD	38.98±2.2***•••○	58.12±1.38 *○○○•	47±1.79 *•••	211.85±13.45 ***◊◊◊	<0.05, <0.0001

Data are expressed as mean ± SEM. Different superscripts (*, •, ◊, ○) within the columns represents significantly different results to different groups. * P<0,05, ***P<0,0001: compared to the control group, •••P<0.0001: compared to the diabetes group, ◊P<0.05, ◊◊◊P<0.0001: compared to the diabetes/vitamin D group, ○P<0.05, ○○○P<0.0001: compared to the obesity group, TC: Total cholesterol, HDL: High density lipoprotein, TG: Triglyceride, [25(OH)D: ng/ml-TC, HDL, TG: mg/ml]

DISCUSSION

Current research has shown the relation between T2DM and vitamin D, emphasizing that vitamin D is essential for blood glucose and insulin levels. Vitamin D reduced blood glucose and insulin levels in T2DM modeled rats (Anwar *et al*., 2013; De Souza *et al*., 2005). Another study revealed that simultaneoous administration of alfacalcidol (vitamin D analog) and metformin had a greater antidiabetic effects than the implemantation of metformin alone (Abdel *et al*., 2019). Preclinical trials have shown the positive effect of vitamin D on type 2 animal models. Several recent meta-analysis studies have also supported the recovery effect of vitamin D and blood glucose consistent with animal studies (Upreti *et al*., 2018). Our results have supported the direct effect of cholecalciferol supplementation on fasting blood glucose with a reduction of 22% in diabetes group and 9% in obesity group. The antidiabetic effect of vitamin D is through stimulation of beta cell calcium-dependent endopeptidase, which promotes the transformation of proinsulin to insulin by increasing intracellular calcium concentration and insulin secretion, expression of insulin receptors and/or activation of peroxisome proliferator-activated receptor-delta (PPAR-δ) and suppression of local pancreatic renin-angiotensin-aldosterone system (RAAS) (Safarpour *et al*., 2020). Vitamin D supplementation has been reported to reduce blood glucose, ketoacidosis, proinflammatory IL-6 and HOMA-IR in type 2 diabetic rats and therefore it is possible to say vitamin D helps control the glycemic response and ameliorate insulin sensitivity (Alvarez *et al*., 2010; Chou *et al*., 2015). The possible effect of vitamin D on glucose homeostasis has been clarified by presence of specific vitamin D receptors (VDR). In pancreatic tissue the relation with between glucose tolerance and insulin secretion in VDR and DBP genes and some allelic variations further support this hypothesis. Pancreatic β-cells express VDR and pivotal enzyme 1α-hydroxylase, which promotes the transformation of 25(OH)D3 to 1,25-dihydroxyvitamin D (1,25(OH)2D3) (Yosria *et al*., 2016). Low vitamin D levels affect insulin secretion and sensitivity due to its effects on intracellular calcium, and that increase of intracellular calcium may inhibit intracellular calcium flows required for insulin action (Pajor *et al*., 2019). VDR gene genetic modifications may lead to development of T2DM in different ways: Alteration in calcium metabolism, modulation of adipocyte function and insulin secretion, modification of cytokine expression has been suggested (Yosria *et al*., 2016). The impact of vitamin D on insulin resistance remains controversial. Experimental data have shown that vitamin D is important for glucose-dependent insulin secretion, increases insulin resistance and produces anti-inflammatory effects. However, most randomized controlled trials in healthy or prediabetic individuals have also failed to show a strong correlation between the incidence of insulin resistance and diabetes. Vitamin D deficiency in insulin-resistant type 2 diabetic and normal individuals and vitamin D status did not affect insulin resistance has been previously demonstrated. No association was found between vitamin D deficiency and insulin resistance or beta cell function. In T2DM obese patients, vitamin D supplementation has showed a significant decrease in HbA1c but no changes in other glucose indices (FBG, insulin, and IR) observed (Pittas *et al*., 2007). Our results have confirmed the strong correlation between vitamin D supplementation and insulin levels in rats but we did not observe any significant influence of vitamin D on insulin resistance. The expression and activity of SIRT1 is decreased in some chronic diseases such as hunger, calorie restriction and diabetes. Accordingly, SIRT1 activation has been reported to improve glucose indices and mitochondria function (Upreti *et al*., 2018). In addition, vitamin D can increase the level of human endothelium SIRT1, and the insulin sensitizing effect of vitamin D is thought to be possibly related to SIRT1 (Safarpour *et al*., 2020). In a study targeting the effect of vitamin D on left ventricular hypertrophy (LVH) in type 2 diabetic rats, vitamin D supplementation significantly increased insulin levels and reversed LVH compared to the model group. This effect has been reported to include stimulation of insulin secretion and reduction of blood glucose level through 1.25-(OH)2D3-receptor expression (Lai *et al*., 2013). In our heart weight findings, we observed a significant decrease in obesity/vitamin D group compared to the model group. But, we did not observe the same effect between diabetes model group and diabetes/vitaminD group. When serum vitamin D levels are associated with dyslipidemia, high 25(OH)D3 levels have positive enhancing effects on HDL cholesterol, and vitamin D levels are inversely related to atherogenic dyslipidemia

and have been reported to be an independent protective factor against the atherosclerotic profile of diabetic patients (Sharif-Askari *et al*., 2020). In an analysis of 3788 type 2 diabetes patients, vitamin D deficiency has shown parallelism with low HDL levels (Jiang *et al*., 2018). Consistent with these data our findings have supported the positive effect of vitamin D on HDL in type 2 diabetes. However, we could not observe ameliorative effect of vitamin D on HDL levels in obesity group. In another meta-analysis study, vitamin D has decreased serum levels of TC, TG and LDL but failed to increase serum HDL levels in T2DM patients (Jafari *et al*., 2016). In terms of our total cholesterol data, a significant increase was observed between control and other groups. Although no significant difference on total cholesterol levels in diabetes-diabetes/vitamin D group, positive effect of Vitamin D in obesity-obesity/vitamin D group was observed. One study investigated the effects of alphacalcidol in combination with metformin and alone. Alphacalcidol significantly improved glucose homeostasis and lipid profile parameters with a neutral effect on calcium and phosphorus levels and vitamin D3 analogs have been stated to regulate glucose parameters and lipid metabolism in a diabetic rat model. Its combination with metformin has been reported to be an additional protective factor (Abdel *et al*., 2019). In another study that has supported our findings; vitamin D supplementation has improved total cholesterol in rats with visceral obesity. Vitamin D level has demonstrated a negative correlation with VDR, TC, TG, and LDL-C and a positive correlation with HDL-C plasma levels (Jiang *et al*., 2018). The possible mechanism for vitamin D-mediated reduction in serum triglyceride is that vitamin D increases intestinal calcium absorption and serum calcium. It has been argued that this calcium can reduce serum triglycerides by decreasing hepatic triglyceride formation and secretion. Three possible mechanisms by which vitamin D can improve lipid profiles have been reported: suppression of vitamin D-induced PTH secretion and increase in lipolysis; vitamin D may lead to a decrease in serum triglyceride levels by reducing hepatic triglyceride formation and secretion, and vitamin D may increase insulin secretion and insulin sensitivity and affect lipid metabolism (Bolivar-Contreras *et al*., 2021). Serum triglyceride levels were evaluated in our study, but a positive effect of Vitamin D was not observed. We believe that experimental studies are needed with higher number of subjects, dose and duration. It has been reported that Vitamin D can reduce insulin sensitivity and suppress systemic inflammation by changing production and effects of proinflammatory cytokines (Sung *et al*., 2012).

Intra-abdominal adipose tissue in abdominal obesity, widespread in diabetic patients, is a crucial determinant of a low-level chronic inflammatory state. Accordingly, it has been shown that IL-6, TNF-α and CRP levels increase in these individuals and this chronic inflammation has been associted with insulin resistance, obesity and cardiovascular diseases (Das *et al*., 2001). High serum concentration of IL-6 can increase the CRP levels. It has been stated that CRP level, which increases in paralel with the increase in BMI, may be indirectly related to the increase in serum concentrations of cytokines IL-6 and TNF-α (Visser *et al*., 1998). In our results, increased proinflammatory cytokine levels were detected in diabetic rats, but this increase was not observed in obesity group. We found the positive effects of vitamin D supplementation on TNF-α and IL-6 levels in diabetes model. It has been reported that Adiponectin has beneficial effects on atherosclerosis and insulin resistance (Spranger *et al*., 20003). Adiponectin levels decreased in monkeys with obesity and insulin resistance (Hotta *et al*., 2001). In our results, adiponectin levels decreased significantly in diabetes and obesity rats, this decrease significantly increased in diabetes/vitamin D group and no significant difference was observed in obesity/vitamin D group. Consistent with our findings, it has been reported that individuals with high adiponectin levels have a lower risk of developing Type 2 diabetes than those with low adiponectin levels (Yamauchi *et al*., 2003). It has been suggested that TNF-α and IL-6 correlate with insulin resistance through adiponectin. The parallelism of TNF-α and IL-6 levels with adiponectin levels revealed in our study confirmed the relationship between insulin resistance and adiponectin. Apelin is an adipokine with anti-inflammatory and anti-atheromatous properties. Apelin increases glucose uptake and suppresses lipolysis depending on AMPK (AMP-activated protein kinase) and apelin-deficient mice show insulin resistance following high-fat diet feeding. This suggests that apelin increases glucose homeostasis and insulin sensitivity. Apelin also participates in inflammatory responses in obese people. Apelin level is positively correlated with TNF-α and associated with cardiovascular physiopathological processes has been reported. Moreover, positive effects of the therapeutic administration of apelin on cardiac function, hyperglycemia, insulin resistance, dyslipidemia, endothelial function, inflammation and glucose metabolism in type 2 diabetic rats have been demonstrated (Boucher *et al*., 2005). Although the relationship between apelin and T2DM was stated in studies, no relationship was observed between the diabetes and obesity groups in our study. In our study, the formation of advanced glycation end products was evaluated in kidney tissue, but no significant difference was found between the groups. We think that experiment period of at least 20 weeks is more meaningful for this evaluation.

CONCLUSİON

These findings suggest that Vitamin D supplementation had a curative effect on blood glucose and insulin levels. They have strengthened our opinion that vitamin D can be an essential adjuvant supplement and increase the impact

of antihyperglycemic drugs administered in type 2 diabetes mellitus.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest concerning this article.

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