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**ORIGINAL ARTICLE**

# The Effect of Thiazolidinediones and SGLT-2 Inhibitors on Minerals,VIT.$D_3$, Blood Glucose and Weight Gain in Wistar Rats Induced with Type 2 Diabetes

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## ABSTRACT

Type 2 diabetes mellitus and osteoporosis are among the most common health problems around the world. Diabetes is usually associated with increase a risk of bone fracture, Recent studies has indicated the harmful effects of some of anti-diabetic drugs on bone metabolism. Therefore, the goal of this study was to investigate the effect of two types of anti-diabetic drugs: Thiazolidinediones (TZDs) (30 mg/kg) and sodium-glucose cotransporter-2 (SGLT-2) inhibitors (10 mg/kg) on Vit.D and bone minerals on serum of laboratory female rats induced with type 2 diabetes mellitus. The study was conducted on animals Faculty of science/ Department of biology/University of kufa. And Central Laboratories / In Medical City. during the period from 27 october 2021 till the end of 22 February 2022. Thirty-two adults female Albino rats (Rattus norvigicus) were randomly divided to two main groups of sixteen animals, The first group was treated for a period of 2 weeks and the second group was treated for a period of 2 months. Each group of them was divided into secondary four groups of four rats including: a control (Co1 and Co2) were fed regular rats pellet, the second group (HFD 1 and HFD 2) were fed with high fat diet (42% lipid, 32%g sucrose, 14% protein), the third group were fed with high fat diet and treated with TZDs (30 mg/kg) and fourth group were fed with high fat diet and treated with SGLT-2i (10 mg/kg). After the end of the treatment period, which is two weeks and three months, the blood was drawn using a method of cardiac puncture technique for estimation of blood glucose, bone minerals in serum which include (calcium, magnesium, inorganic phosphate) and serum Vit.$D_3$ concentration. Body weight gain was significantly increased ($p \leq 0.05$) in animals treated with HFD high-fat diet (uncontrolled diabetic group) and in animals treated with TZDs compared to the control and animals treated with SGLT-2i. Also, the results of this study showing a significant increase ($p \leq 0.05$) on blood glucose in animals fed with HFD compared with other groups and there was a significant decrease ($p \leq 0.05$) in blood glucose in animals group treated with TZDs and SGLT-2i compared with HFD. The diabetic induced group (HFD group) treated for two months showed a significant decrease ($p \leq 0.05$) in vitamin $D_3$ concentrations. The results of calcium concentrations in the blood showed a significant decrease ($p \leq 0.05$) in calcium concentration in the group induced with diabetes and the group treated with TZDs for two months compared to other groups. With regard to magnesium concentrations, the results showed a significant decrease ($p \leq 0.05$) in the group induced with diabetes and the group treated with TZDs for two months compared to other groups. While there was a significant increase ($p \leq 0.05$) in the concentrations of magnesium in the group treated with $SGLT_2$i for two months compared to other groups. Also, the results showed a significant increase in the concentrations of serum inorganic phosphate in the groups treated with high-fat diet (diabetic induced group) for two months and the group treated with SGLT2i for two months, compared to the control group and the groups treated with TZDs. In conclusion: Uncontrolled diabetes mellitus has adverse effects on the concentrations of Vit.D3 and bone minerals. Also, Long-term treatment with Thiazolidinedionse (TZDs) and SGLT-2 inhibitors, despite its control of blood sugar has harmful effects on bone minerals and Vit.D3.

**Keywords:** T2DM, Thiazolidinediones, SGLT-2 inhibitor, Calcium, Vit. $D_3$, Magnesium, Inorganic phosphate.

## INTRODUCTION

Patients with diabetes mellitus (DM) have an rise peril of bone fractures. Several types of effective anti-diabetic drugs are available, and they are usually used in combination. The influence of these medicines on bone metabolism and fracture risk should not be underestimated. Anti-diabetic drugs can affect bone health in a positive, neutral, or negative way 1 Due to the intricate and multifaceted pathophysiology of T2DM, oral antidiabetic drugs (OADs) have been progressing to treat the underlying technicality. Among the OAD classes established thus far, the thiazolidinedione (TZD) class is one that primarily targets insulin resistance 2, via peroxisome proliferator–activated receptor (PPAR)-receptor activation 3 Thiazolidinedione use can cause osteoporosis by encouraging adipocyte formation at the expense of osteocytes, leading to not just osteoporosis but also an raise in marrow adiposity and raised adipocytokine degrees, all of which contribute to bone fragility 4. Sodium-glucose co-transporter-2 inhibitors SGLT2i) are oral diabetes treatments that reduce blood glucose degrees without the use of insulin by boosting renal glucose excretion 5. Until used with insulin or insulin secretagogues, they have beneficial effects on blood pressure and weight while avoiding hypoglycemia 6. Sodium-glucose cotransporters are proteins that transport sodium and glucose. These cotransporters are inhibited by SGLT-2i, culminating in a drop in glycemia and a loss of gluco calories in the Urine 7. Several agents are available, each with its own set of properties (the "class effect"). In terms of bone health, (canagliflozin) has been demonstrated to have detrimental impacts on bone density, bone resorption, and hip fracture risk 8**.**

Therefor the aim of this study was estimation of bone minerals in blood includes**:** (Serum Calcium levels, inorganic Phosphate, Magnesium), 1,25- dihydroxyvitamin $D_3$, body weight gain and blood glucose in laboratory rats induced with type 2 diabetes mellitus and treated with TZDs and SGLT2i.

## MATERIALS AND METHODS

**Experimental animals:** The current study will involve 32 healthy adult albino female rats (Rattus norvegicus) females, most of them are beyond the age of eight weeks and weigh between (225±25) g. They should be in decent physical condition. The rats are housed in 48-centimeter-long, 15-centimeter-wide, and 7-centimeter-high plastic cages with metal coverings. Plastic bottles may be used to build a watering difficult with a cork equipped with metal pipes, and sawdust, which should be replenished three times a week, is considered in its care to clean the hatching of the special diet. The animals are kept in a controlled environment with temperatures ranging from 18 to 26 degrees Celsius.

**Induction of type 2 diabetes:** After acclimatization, 32 animals were selected and divided into two group Non Diabetic control group, and Diabetic induced group. Non Diabetic control group animals, were fed a normal pellet diet during the experimental period. And the second group were named Diabetes Induced group animals feed on High Fat Diet. normal pellet diet mix with ( 42% lipid, 32%g sucrose, 14% protein) After two weeks of feeding

on HFD. Diabetic rats have higher blood glucose levels than non-diabetic control rats**.**

**Drug used doses and routs of administration:** In this study, two types of drugs thiazolidinedionse (pioglitazone) and SGLT2 (Dopagliflazone) are used in the form of tablet 30 mg /16 ml, and 10 mg/20ml respectively from Mazaya Baghdad store, NORMON SPAIN company which is given for experiment animals orally by using gavage.

**Measurement of body weights:** The weight of female rats was measured by using sensitive balance before the experiment and after end of the experiment**.**

**Experimental design and blood collection:** Thirty two adults female albino White rats (Rattus norvigicus) were randomly divided to eight groups of animals according to the dosing time: (4) groups will be treated for a period of 2 weeks; (4) groups will be treated for a period of 12 weeks each group of them were divided into secondary four groups of four rats according to type of treatment: 1. Control group (N: 4): fed the standard pellet diet, 2.HFD group (N:4) fed the high fat diet, 3.Thiazolidinedionse (TZDs) (Pioglitazone) group (N=4) fed the high fat diet and treated daily with TZDs and 4. SGLT-2 inhibitors (Dopagliflazone) group (N=4) fed the high fat diet and treated daily with SGLT-2 inhibitors administrations. At the end of experiment (after 2 weeks and 2 months), each animal was anaesthetized by a mix of xylazine (0.2 ml) and ketamine (0.1 ml) and they were scarified. The animals were attached to a piece of cork by using pins and then blood was drawn from the heart directly through the heart puncture to obtain adequate volume of blood (5 ml). Blood sample was put in a tube with no anticoagulant at roomtemperature left for 30 minutes and used to get serum through centrifugation at 6000 rpm for 5 minutes for the biochemical tests.

**Biochemical analysis:** Glucose concentration in the serum was identified via GLUCOSE MR Enzymatic colorimetric method ENDPOINT according to procedure provided by LINEAR CHEMICALS S.L. Joaquim Costa 18 2ª planta. 08390 Montgat, Barcelona, SPAIN - Linearity : Up to 500 mg/dL . QUALITY SYSTEM CERTIFIED ISO 9001 ISO 13485.

Calcium concentration in the serum was identified via CALCIUM OCC TOTAL Colorimetric method ENDPOINT according to procedure provided by the LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN NORMAL Borderline level of calcium. Linearity : Up to 22 mg/dL. The method is based on the specific binding of cresolftalein complexone (OCC), a metallochromic indicator2 , and calcium at alkaline pH with the resulting shift in the absorption wave length of the complex. The intensity of the cromophore formed is proportional to the concentration of total calcium in the sample.

Magnesium concentration in the serum was identified via Magnesium. Colorimetric Assay Kit (Catalog #K385-100; 100 assays; Store at -20°C) BioVision Incorporated 155 S. Milpitas Boulevard, Milpitas, CA 95035 USA. The linear range of the assay is 2-15 nmoles with detection sensitivity~ 40 µM.

System reagent for the quantitative determination of Inorganic Phosphorus in human serum and urine on Beckman Coulter AU analyzers . Kraemer Blvd. Brea, CA 92821, USA. To detect inorganic phosphorous depend on reaction between ammonium molybdate and sulfaric acid by presence phosphorous to formed phospho molybdic complex.

Human Vitamin D3(VD3) ELISA Kit Cat No. MBS264661 Instructions version :09.3.1. Manufactured in an ISO 9001:2015 Certified Laboratory. This kit employs Double Antibody Sandwich Technique. The principle of Double Antibody Sandwich is based on characteristics of the tested antigen with more than two valances which can identify coated antibody and detection antibody at same time

**Statistical Analysis:** The results of the study were expressed by using (mean±standard error). The Tow Way Anova test was also used to study and compare the effect of treatment type and treatment period and the interaction between them. The difference between groups is considered as statistically different when ($p \leq 0.05$). All statistical analysis was performed using SPSS Statistics version 25, Multilingual program, IBM-USA. Whereas the figures built by using EXELL program of Microsoft- Office 2010**.**

## RESULTS

**Effects of treatment with anti-diabetic drugs (TZDs and SGLT-2i) on blood glucose and body weight in laboratory female rats**

**The effect of treatment duration:** Study results in table (1) showed changes in the body weight gain in experimental groups according to duration of treatment, the results indicated that there was a significant increase ($p \leq 0.05$) in body weight gain in animals treated for 2 months compared with animals treated for 2 weeks. The data in the table (1) showed that blood glucose concentration was significantly decreases ($p \leq 0.05$) in animals treated for 2 months compared with animals treated for 2 weeks.

**The effect of the type of treatment:** The results in table (2) showed changes in the body weight gain in experimental groups according to the type of treatment, the results indicated that there was an insignificant rise ($p > 0.05$) in body weight gain in animals treated with HFD and HFD+TZDs compared with control and HFD+SGLT-2i groups.

Results in the same table showed changes in the blood glucose concentration in experimental groups according to the type of treatment, the results indicated that there was a significant increase in the glucose concentration in HFD group compared with control, HFD+TZDs and HFD+SGLT-2i groups.

**Pairwise comparisons and the effect of the interaction between the duration and the type of treatment:** The results of pairwise comparisons in figure (1A) showed a significant decrease on blood glucose in treated groups with HFD, HFD+TZDs and HFD+SGLT-2i for two months compared with the treated groups for two weeks.

The results in the same figure (1 A) showed changes in the blood glucose in treated animals according to the interaction between duration and type of treatment, the results showed that there was a significant increase ($p \leq 0.05$) on blood glucose in HFD groups which treated for 2 weeks and 2 months compared with other groups, and the group treated with HFD for two weeks had the most significant increase compared with other groups. Also, the results indicated that groups treated with HFD+SGLT-2i for two months had the most significant decreases compared with other groups.

Pairwise comparisons in figure (1 B) showed a significant increase ($p \leq 0.05$) on body weight gain in all treated groups for two months compared with the treated groups for two weeks.

Table 1: Comparison of the blood glucose and weight gain according to the duration of treatment with TZDs and SGLT-2i in laboratory female rats induced with type 2 diabetes

| Dependent Variable: Blood glucose and body weight gain | | |
|---|---|---|
| Mean ± Std. Error | | |
| Duration of treatment | Blood glucose (mg/dl) | Body weight gain (g) |
| 2 Weeks (N=16) | 145.31±13.33 * | 17.66±1.68 * |
| 2 Months (N=16) | 113.18±11.03 | 53.62±5.11 |
| Significant | 0.043 | 0.000* |

Results are represented as mean ± SE.
*. The mean difference is significant at the 0.05 level.

Table 2: Comparison of the blood glucose and weight gain according to the type of treatment with TZDs and SGLT-2i in laboratory female rats induced with type 2 diabetes

| Dependent Variable: Blood glucose and body weight gain | | |
|---|---|---|
| Mean ± Std. Error | | |
| Type of treatment (study groups) | Blood glucose (mg/dl) | Body weight gain (g) |
| Control (N=8) | 101.00±9.61 | 30.05±7.16 |
| HFD (N=8) | 199.12±15.21* | 42.25±9.56 |
| HFD_TZDs (N=8) | 112.75±6.99 | 42.28±9.35 |
| HFD_$SGLT_2$i(N=8) | 104.12±9.26 | 28.00±7.28 |
| Significant | 0.000 | 0.486 |

Abrreviation: Co: normal control group, HFD: High fat diet group, HFD+TZDs: Group fed with HFD and treated with TZDs, HFD+SGLT-2i: Group fed with HFD and treated with SGLT-2i

Results are represented as mean ± SE.

*. The mean difference is significant at the 0.05 level.

The results in the same figure (1 B) showed changes in the body weight gain in treated animals according to the interaction between duration and type of treatment, the data indicated that there was a significant increase (p≤0.05) on body weight gain in control, HFD, HFD+TZDs and HFD+SGLT-2i groups which treated for 2 months compared with control, HFD, HFD+TZDs and HFD+SGLT-2i groups which treated for 2 weeks. Also, the two groups treated with HFD and HFD+TZDs for two months had the most significant increase.

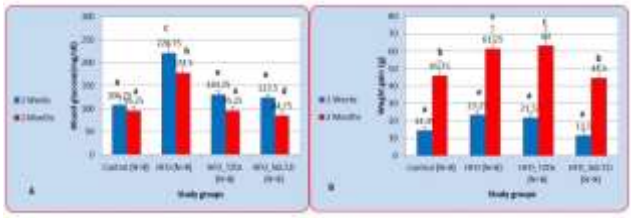

Figure 1: Comparison of the blood glucose (A) and weight gain (B) according to the interaction between the duration and type of treatment with TZDs and SGLT-2i in laboratory female rats

Abrreviation: Co: normal control group, HFD: High fat diet group, HFD+TZDs: Group fed with HFD and treated with TZDs, HFD+SGLT-2i: Group fed with HFD and treated with SGLT-2i

Results are represented as mean ± SE.

Similar letters indicate no significant differences at the 0.05 level.

Different letters indicate a significant difference at the 0.05 level.

**Effects of treatment with anti-diabetic drugs (TZDs and SGLT-2i) on Vitamin $D_3$, calcium, Magnesium and inorganic phosphate in laboratory female rats**

**The effect of treatment duration:** The results in (Table 3) show that Vit.$D_3$, Calcium, and magnesium concentration was significantly decreases (p≤0.05) in animals treated for 2 months compared with animals treated for 2 weeks (Table 3). While inorganic phosphate concentration was insignificantly increase (p>0.05) in animals treated for two months compared to those treated for two weeks.

**The effect of the type of treatment:** The data in (Table 4) exhibited that the Vitamin $D_3$ concentration was an insignificant decrease (p>0.05) in the vitamin $D_3$ concentration in animals treated with HFD compared with HFD+TZDs, HFD+SGLT-2i and control groups. Calcium concentration was significantly decrease (p≤0.05) in animals treated with HFD+TZDs and HFD compared with HFD+SGLT-2i and control groups. While magnesium concentration. the results indicated that there was a significant decrease (p≤0.05) in the magnesium concentration in animals treated with HFD compared with HFD+SGLT-2i and HFD+TZDs and control groups. The results also indicated that there was a significant increase (p≤0.05) in the magnesium concentration in animals treated with HFD+SGLT-2i compared with HFD and HFD+TZDs and control groups. The results of inorganic phosphate concentration indicated that there was a significant increase (p≤0.05) in animals treated with HFD and HFD+SGLT-2i compared with HFD+TZDs and control groups.

**Pairwise comparisons and the effect of the interaction between the duration and the type of treatment:** The results of Pairwise comparisons in (Figure 2) showed that there was a significant decrease (p≤0.05) on vitamin D3 in treated groups with HFD for 2 months compared with that treated for 2 weeks. As for the remaining groups, no change was observed when comparing the two time periods. The results in the same figure showed changes in the vitamin D3 in treated animals according to the interaction between duration and type of treatment, the results showed that there was a significant decrease (p≤0.05) on vitamin D3 concentration in HFD groups which treated for 2 months compared with other groups.

The results of pairwise comparisons in (Figure 2) showed a significant decrease (p≤0.05) on calcium concentration in treated groups with HFD and HFD+TZDs for 2 months in compared with that treated for 2 weeks. In the other groups, the decrease was insignificant between the two time periods. Regarding the interaction between duration and type of treatment, the results showed that there was a significant decrease (p≤0.05) on calcium concentration in HFD and HFD_TZDs groups which treated for 2 months compared with the same groups treated for 2 weeks.

In the same figure the results reveled a significant decrease (p≤0.05) on magnesium concentration in treated groups with HFD for 2 months in compared that treated for 2 weeks. In the other groups, the change was insignificant between the two time periods. The results of interaction between duration and type of treatment revealed a significant decrease (p≤0.05) on magnesium concentration in treated groups with HFD and HFD_TZDs for 2 months in compared with other groups. Also, there was a significant increase (p≤0.05) on magnesium in group treated with HFD_SGLT2i for 2 months comparing with group treated with HFD and HFD_TZDs for 2 months.

Also, the result in the same figure show a significant elevated (p≤0.05) on serum inorganic phosphate in treated groups with HFD and HFD_SGLT2i for 2 months in compared that treated for 2 weeks. In the other groups, the change was insignificant between the two time periods. The results of interaction between duration and type of treatment revealed a significant increase (p≤0.05) on inorganic phosphate concentration in treated groups with HFD and HFD_TZDs for 2 months in compared with other groups. In addition, when compared to the other groups, groups treated with HFD+ SGLT-2i for two months had the most significant increase (p≤0.05).

Table 3: Comparison of the serum calcium, magnesium, inorganic phosphate and Vit.D3 concentration according to the duration of treatment with TZDs and SGLT-2i in laboratory female rats induced with type 2 diabetes

| Dependent Variable: Bone menerals and Vit.D3 in serum | | | | |
|---|---|---|---|---|
| Mean ± Std. Error | | | | |
| Duration of treatment | Serum. Calcium (mg/dl) | Serum Magnesium (mg/dl) | S. inorganic phosphate (mg/dl) | Serum Vit.D3 (ng/ml) |
| 2 Weeks (N=16) | 11.18±0.21 | 2..12±0.14 | 4.32±0.44 | 33.69±1.45 |
| 2 Months (N=16) | 9.58±0.57 | 1.67±0.21 | 5.48±0.45 | 29.27±1.65 |
| Significant | 0.014* | 0.055* | 0.062 | 0.044* |

Results are represented as mean ± SE.

*. The mean difference is significant at the 0.05 level.

Table 4: Comparison of the serum calcium, magnesium, inorganic phosphate and Vit.D3 concentration according to the type of treatment with TZDs and SGLT-2i in laboratory female rats induced with type 2 diabetes

| Dependent Variable: Bone menerals and Vit.D3 in serum | | | | |
|---|---|---|---|---|
| Mean ± Std. Error | | | | |
| Type of treatment (study groups) | Serum. Calcium (mg/dl) | Serum Magnesium (mg/dl) | S. inorganic phosphate (mg/dl) | Serum Vit.D3 (ng/ml) |
| Control (N=8) | 10.77±0.36 a | 1.92±0.24 a | 3.77±0.41 | 33.81±2.22 |
| HFD (N=8) | 8.59±0.86 b | 1.38±0.31 b | 5.35±0.73 | 27.55±2.59 |
| HFD_TZDs (N=8) | 10.19±0.38 c | 1.91±0.22 a | 4.73±0.43 | 32.04±1.92 |
| HFD_SGLT2i(N=8) | 11.97±0.33 a | 2.37±0.20 ac | 5.76±0.83 | 32.51±2.24 |
| Significant | 0.001 | 0.052 | 0.125 | 0.191 |

Abrreviation: Co: normal control group, HFD: High fat diet group, HFD+TZDs: Group fed with HFD and treated with TZDs, HFD+SGLT-2i: Group fed with HFD and treated with SGLT-2i

Results are represented as mean ± SE.

*. The mean difference is significant at the 0.05 level

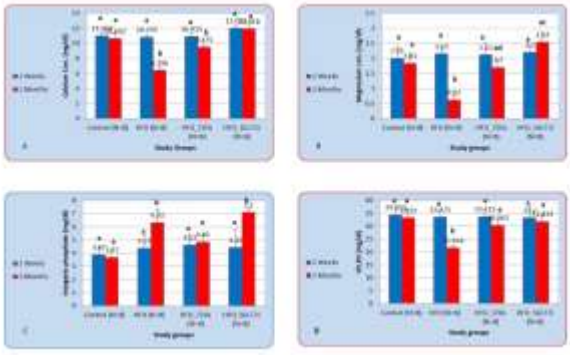

Figure 2: Comparison of the calcium (A), magnesium (B), inorganic phosphate (C) and Vit D3 according to the interaction between the duration and type of treatment with TZDs and SGLT-2i in laboratory female rats

Abrreviation: Co: normal control group, HFD: High fat diet group, HFD+TZDs: Group fed with HFD and treated with TZDs, HFD+SGLT-2i: Group fed with HFD and treated with SGLT-2i

Results are represented as mean ± SE.

Similar letters indicate no significant differences at the 0.05 level.

Different letters indicate a significant difference at the 0.05 level.

## DISCUSSION

The results of current study exposed a significant increase ($p \leq 0.05$) on blood glucose in animals fed with HFD compared with other groups. The main reason for these results was DM is associated with chronic elevation in plasma blood glucose. Persistent high blood glucose induces oxidative stress and toxicity that destroys the few remaining viable pancreatic beta cells, leading to a further deterioration of DM [9]. Chronic hyperglycemia causes severe impairment in lipid, carbohydrate and protein metabolism. Disruption in lipid metabolism results in increased level of very low density lipoprotein (VLDL) and total cholesterol (TC) [10], resulting in the accumulation of VLDL and TC in sub-endothelial and endothelial cell layers.

The results of this study show that there was a significant decrease ($p \leq 0.05$) in blood glucose in animals group treated with TZDs and SGLT-2i compared with control and HFD. Pioglitazone, a thiazolidinedione derivative, is an oral glucose-lowering medication commonly prescribed to treat type 2 diabetes (T2D). Peroxisome proliferator-activated receptors (PPARs) are key transcriptional regulators of lipid and carbohydrate metabolism, energy production and regulating cardiovascular function . Pioglitazone is a high-affinity ligand of PPARγ that activates and translocate PPARγ to the nucleus. Once in the nucleus it forms a complex with the retinoid x receptor alpha in modulating gluconeogenic genes in the liver and induces transcription of the insulin-sensitive glucose transporter, GLUT4 in adipose tissue, enhancing glucose transport and utilization, as well as suppressing hepatic glucose production [11].

Sodium-glucose co-transporter 2 (SGLT-2) is a protein incorporated into the luminal membrane of proximal tubule kidney cells that catalyzes the active transport of glucose, against a concentration gradient, across the luminal membrane by coupling it to sodium transport. Glucose reabsorption by SGLT-2 accounts for approximately 90% of the total amount of glucose reabsorbed. Inhibitors of SGLT-2 have been designed and approved for the treatment of T2DM. Their use results in increased glycosuria and reduces circulating glucose concentrations and hyperglycemia [12]. SGLT2 inhibitors decrease concentrations of plasma glucose by inhibiting proximal tubular reabsorption of glucose in the kidney. The favorable efficacy profile of these drugs in terms of glucose lowering and weight loss needs to be balanced against possible side effects, including an increase in the frequency of treatment-emergent bone fractures reported in clinical studies [13].

The results of this study demonstrated a significant increase ($p \leq 0.05$) in body weight gain in animals treated with HFD high-fat diet (uncontrolled diabetic group) and in animals treated with TZDs compared to the control and animals treated with SGLT-2i. While there was an insignificant decrease ($p > 0.05$) in the weight gain of the animals treated with SGLT-2i compared to the control group.

Increase body weight in groups treated with HFD is expected because obesity is one of the most important risk factors for type 2 diabetes. Barbosa-Da-Silva et al. indicated that the intake of a high fat (HF) diet is an independent risk factor for becoming overweight in rat. Therefore, an HF diet is a suitable model for studying DM2 [14]. Apovian et al. explained that obesity is one of the main risk factors for type 2 diabetes (T2D), representing a major worldwide health crisis. Modest weight-loss (C 5% but \10%) can minimize and reduce diabetes-associated complications, and significant weight- loss can potentially resolve disease [15].

Lang et al. indicated that obesity is associated with a general dysregulation of metabolic homeostasis, resulting in insulin resistance, dyslipidemia, altered regulation of blood pressure, and increased risk for diabetes, cardiovascular disease, chronic kidney disease, and cancer. Therefore, obesity and its comorbidities represent a major Feld of interest for basic science and clinical research [16]. As for the high weight gain of animals treated with TZDs, this result agreed with the results of many studies. The effects of antihyperglycemia therapy on clinical outcomes (including body weight) vary both between and within the drug classes [17]. Weight gain has been associated with both rosiglitazone and pioglitazone treatments. The weight gain appears to be dose-related (1 or 1.5 kg at low doses) and is greater during combination therapy with insulin secretagogues (2 to 3 kg) and remarkably so when TZDs are combined with insulin therapy (3.5 to 6 kg). The weight gain is attributable to several factors: increase in subcutaneous fat mass with either no change or a small decrease in visceral fat mass, fluid retention, and positive calorie balance because of improved glycemic control. Though the increase in subcutaneous fat mass is distressing to patients with T2D who are trying to lose weight, the TZD-mediated changes in fat mass distribution is related to the improvement in insulin resistance and glycemic control [18].

The result of a decrease in body weight in animals treated with SGLT-2i, was agreed with most studies. Ribola et al explained the reason for the decrease body weight as a result of treatment with SGLT-2i by: weight loss can be considered an indirect effect resultant from the renal excretion of glucose. However, it is important to define the etiology of this weight decrease. The resultant weight loss was a combination of many factors including: the caloric deficit caused by glucose elimination induce a higher lipid catabolism with a consequent body mass loss, and the glucose is more concentrated in the glomerular filtrate, promote higher water retention in the urine, which constituted the weight effectively lost [19].

The results of this study showed that the diabetic induced group (HFD group) treated for two months showed a significant decrease ($p \leq 0.05$) in vitamin $D_3$ concentrations compared to other treated groups. In addition, the groups treated with **TZDs** and **$SGLT_2$i** showed an insignificant decrease ($p > 0.05$) in vitamin $D_3$ compared with the control group. As for the results of calcium concentrations in the blood, the results showed a significant decrease ($p \leq 0.05$) in calcium concentration in the group induced with diabetes and the group treated with **TZDs** for two months compared to other groups. With regard to magnesium

concentrations, the results of this study showed a significant decrease ($p \leq 0.05$) in the group induced with diabetes and the group treated with **TZDs** for two months compared to other groups. While there was a significant increase ($p \leq 0.05$) in the concentrations of magnesium in the group treated with **$SGLT_2$i** for two months compared to other groups. Also, the results of the this study showed a significant increase in the concentrations of serum inorganic phosphate in the groups treated with high-fat diet (diabetic induced group) for two months and the group treated with SGLT2i for two months, compared to the control group and the groups treated with TZDs.

Hyperglycemia appears to have a significant impact on the vitamin D-calcium axis through impairing renal calcium absorption. High glycemic levels lead to a reduction in the amount of 1,25(OH)2D3 (1,25-dihydroxyvitamin D) receptors on osteoblasts, limiting the osteoblast's capacity to synthesis osteocalcin in response to 1,25(OH)2D3. However, the effectiveness of vitamin D in reducing T2DM and fracture risk is still unknown [20] Vitamin D, calcium, and parathyroid hormone metabolism disturbances, diabetic nephropathy, and alterations associated with glucose-lowering medication are all metabolic abnormalities that may impair bone strength in T2DM [21]

Pritchard et al. show that the mineral:matrix ratio, which measures tissue mineral content, was found to be 7% higher in T2DM specimens than in non-DM specimens. Their findings of higher mineral content in T2DM men and women are consistent with findings of higher mean calcium content in the proximal femur of T2DM men and women compared to nondiabetic controls. Because increased mineral content in T2DM tissue is associated with lower ductility, the higher mineral content in T2DM tissue is likely to contribute to the increased fracture risk in patients with T2DM [22]. Zhang et al. indicated that no changes in serum calcium concentration in the pioglitazone group compared to the control group were found. Calcium excretion into urine was increased with pioglitazone, which was in accordance with Zhang et al. study in which they examined the effects of pioglitazone on renal calcium excretion [23].

Secondary hyperparathyroidism, increased osteoclastic activity, and decreased bone mass are all symptoms of vitamin D insufficiency. Falls, hip fractures, vertebral fractures, and severe osteoporotic fractures have all been linked to antidiabetic drugs [24]. Vitamin D is a versatile hormone that plays a critical role in a variety of biological activities; hence, changes in its levels have an impact on a variety of processes at the cellular, molecular, and genetic levels, resulting in further skeletal issues [25]. Vitamin D insufficiency may have a role in type 2 diabetes etiology, and epidemiological data relates it to insulin resistance [26]. In both experimental and epidemiological investigations, vitamin D insufficiency has been linked to lower insulin secretion, insulin resistance, and type 2 diabetes. 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) enhances the secretion of insulin in the pancreatic -cell, according to animal research. Vitamin D insufficiency is linked to an increase in inflammatory markers, suggesting that the link between vitamin D deficiency and insulin resistance may arise through inflammation. Furthermore, vitamin D-related gene polymorphisms may lead to glycemic control problems and type 2 diabetes [27]. Vitamin D decreases insulin resistance in the surrounding tissues, lowering excess insulin release in response to an increase in blood sugar caused by insulin resistance and increasing insulin sensitivity. Vitamin D insufficiency has been linked to metabolic syndrome and type 2 diabetes, as well as insulin resistance and cell dysfunction. [28].

Magnesium is an important intracellular cation that plays a key role in a variety of biological activities. It's a neuromuscular excitability, cell permeability, and ion channel and mitochondrial activities regulator that's vital for cell proliferation and death [29] In diabetic individuals, magnesium (Mg) shortage is a prevalent concern. Mg deficiency has been linked to an increased risk of diabetes mellitus (DM) and diabetic complications [30]. In individuals with T2DM complications, serum magnesium content was decreased. Serum magnesium and glycemic control were found to have a negative relationship. This suggests that magnesium plays a critical role in metabolic dysfunction in diabetics [31].

SGLT2 inhibitors raise phosphate levels in the blood, most likely due to enhanced tubular reabsorption, which has the potential to harm bone [32]. Increased renal phosphate reabsorption has resulted in a slight rise in serum phosphate concentration when SGLT2 inhibitors are used. In reality, drug-induced decreases in proximal sodium transport result in an increase in sodium available for resorption with phosphate via the Na+-PO4 co-transporters in the proximal tubules [33]. SGLT2 inhibitors may enhance the electrochemical gradient for sodium in the proximal renal tubule, which provides energy to stimulate phosphate reabsorption via type II sodium-phosphate cotransporters, which are also situated in the proximal renal tubule. Taylor et al hypothesized that Phosphate reabsorption causes the release of fibroblast growth factor 23 (FGF-23), which inhibits the formation of 1,25-dihydroxyvitamin D, lowering calcium absorption and increasing parathyroid hormone output (PTH) [13].

SGLT2 inhibitors can also increase serum magnesium levels in diabetic patients [34]. The SGLT2inhibitor-induced serum magnesium concentration rise has been linked to a variety of pathways, although the exact mechanism is yet unclear [35]. One of the most likely is the attenuated magnesium wasting induced hypomagnesemia associated with diabetes mellitus—in diabetes patients, increased magnesiuria may be the result of reduced transient receptor potential ion channel 6 (TRPM6) activity in the distal convoluted tubules due to insulin resistance. [36]

From the results of this study, it can be concluded that uncontrolled diabetes mellitus has adverse effects on the concentrations of Vit.D3 and bone minerals. Also, Long-term treatment with Thiazolidinedionse (TZDs) and SGLT-2 inhibitors, despite its control of blood sugar has harmful effects on bone minerals and Vit.D3.

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