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ORIGINAL ARTICLE

Study the Effect of Antidiabetic Drugs on Bone Remodeling Biomarker and Bone Histological Structure in Wistar Rats Induced with Type 2 Diabetes

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ABSTRACT

Diabetes is usually associated with increase a risk of bone fracture. Many anti-diabetic drugs are used and the effects of these drugs on bone metabolism is one of the important issues that should be studied and not neglected. So, 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 bone minerals and Vit.D in laboratory female rats induced with type 2 diabetes mellitus. The study was conduct on animals house in 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). The results of this study indicated that there was a significant increase ($p \leq 0.05$) on bone formation (PINP) and bone resorption biomarker (CTX-1) in the group induced with type 2 diabetes for the two treatment periods (two weeks and two months), and the increase was more significant ($p \leq 0.05$) in the group treated for two months compared to the treated group for two weeks. While the groups treated with TZDs and group treated with SGLT2i for two months showed a significant decrease ($p \leq 0.05$) in bone formation biomarker (PINP) compared with the other treated groups. In addition, the groups treated with TZDs for two months and group treated with SGLT2i for two weeks and two months showed a significant increase ($p \leq 0.05$) in bone resorption biomarker (CTX-1) compared with the other treated groups. The histological examination of the femur bone epiphysis in control1, HFD, HFD+TZDs, HFD+ SGLT-2i which treated for 2 weeks and control 2 which treated for 2 months revealed a normal bone structure. While, HFD groups treated four 2 months revealed that the bone matrix contained irregularly-oriented osteocytes within indistinct lacunae and multiple empty lacuna, increase adiposity of bone marrow and the presence of a resorption cavities in the irregular thin trabecular plates. In addition, groups treated with HFD+TZDs and HFD+ SGLT-2i four 2 months showed the presence of a resorption cavities, an empty lacuna, less dense appearance of bony tissue and osteocyte necrosis.

Conclusion: Uncontrolled diabetes mellitus has adverse effects on bone metabolism**,** Long-term treatment with Thiazolidinedionse (TZDs) and SGLT-2 inhibitors, despite its control of blood sugar, has harmful effects on bone metabolism**.**

Keywords: Type 2 diabetes mellitus, CTX-1, PINP, Thiazolidinediones, SGLT-2 inhibitor.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of inadequate glycaemic control, defined by the American Diabetes Association as a haemoglobin A1C (HgA1c) level ≥6.5%. According to the National Health and Nutrition Examination Survey (NHANES), the worldwide prevalence is estimated to reach nearly 370 million by 2030 [1]. Osteoporosis and DM are frequent conditions and occur simultaneously. Osteoporosis and bone fractures are prevalent in DM. There are more bone fractures in T2DM despite increased BMD, which is due to hypoglycemia, cerebral ischemia, and impaired eyesight complications. Anti- Diabetic drugs also affect the risk of bone fracture [2], there is a complex pathophysiological interaction between them, T2D affects bone metabolism and strength in a direct way, certain antidiabetic medications affect bone metabolism, and there is an association between diabetic complications and risk for falls and subsequent fractures [3].

Type 2 diabetes mellitus (T2DM) is characterized by normal-high bone mineral density (BMD) and increased fracture risk [4]. Patients with diabetes mellitus are at an increased risk of bone fractures. Several groups of effective antidiabetic drugs are available, which are very often given in combination. The effects of these medications on bone metabolism and fracture risk must not be neglected. Commonly used antidiabetic drugs might have a positive, neutral or negative impact on skeletal health [5]. Because the pathophysiology of T2DM is complex and multifactorial, a variety of oral antidiabetic agents (OADs) have been developed based on the underlying mechanisms associated with T2DM.

Detections of bone metabolism have been studied with the biomarkers of enzymes, proteins and by-products during the bone remodeling process [6]. Bone biomarkers are produced from the bone remodeling process included bone formation biomarkers, bone resorption biomarkers and regulators of bone turnover [7]. Carboxy-terminal Crosslinked Telopeptide of type 1 collagen (CTX-1) is a specific and sensitive biomarker of bone resorption that can rapidly indicate the response to bisphosphonate therapy for postmenopausal osteoporosis [8]. Serum intact N-terminal propeptide of type I collagen (P1NP), another marker of bone formation procollagen type I N-terminal peptide is the degradation product during the formation of type I collagen secreted by osteoblasts [9]. P1NP has shown the great potential as a sensitive and stable bone biomarker for the early detection of osteoporosis [10].

Therefore, the aim of this study was:

1 Induction of type 2 diabetes mellitus in laboratory rats by diet.

2 To investigate the influence of antidiabetic drugs and T2DM on bone metabolism by estimation of bone metabolism biomarker includes: Bone formation markers (Procollagen type 1 amino-terminal propeptide (P1NP)) and Bone resorption markers (Carboxy-terminal cross-linked telopeptide of type 1 collagen (CTX-1)

3 Histological study for the bones.

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 with 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 10mg/20ml respectively from Mazaya Baghdad store, NORMON SPAIN company which is given for experiment animals orally by using gavage, which is given for experiment animals orally by using gavage.

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, Control group (N: 4): fed the standard pellet diet, HFD group (N:4): fed the high fat diet, Thiazolidinedionse (TZDs) (Pioglitazone) group (N=4): fed the high fat diet and treated daily with TZDs and 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 room temperature left for 30 minutes and used to get serum through centrifugation at 6000 rpm for 5 minutes for the biochemical tests.

Biochemical analysis

Bone remodeling Biomarker measurement

1 Estimation of Rat C-telopeptide of type 1 collagen CTX-I in Serum (Bone resorption biomarker): CTX-I levels in the serum was identified by using the enzyme- linked immunosorbent assay (ELISA) methods according to the procedure provided by the Shanghai yl Biont, China. Catalog No: YLA060.

2 Estimation of Rat Procollagen IN-terminal Peptide PINP in Serum (Bone formation biomarker)PINP levels in the serum was identified by using the enzyme- linked immunosorbent assay (ELISA) methods according to the procedure provided by the Shanghai yl Biotec, China. Catalog No: YLA048.

Animal dissection and histological examination: The abdominal cavity of the animals was opened to eradicate the femur bone each rat's femur will be excised and stored in 70% ethanol solution for bone histological preparation, The hematoxyline and eosin microscopic slides were prepared and stained according to [11], for hematoxylin and eosin, the slides were examined under supervision of special histopathologic.

Statistical Analysis: The results of the study were expressed by using (mean±standard error). The Tow Way Anova test was 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 ($p<0.05$). 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.

RESULT

Effects of treatment with anti-diabetic drugs (TZDs and SGLT-2i) on PINP and CTX-1 in laboratory female rats

The effect of treatment duration: The results in table (1) show that PINP concentration was an insignificant decrease ($p>0.05$) in animals treated for two months compared to those treated for two weeks. While CTX-1 concentration was significantly increased ($p\leq0.05$) in animals treated for 2 months compared with animals treated for 2 weeks.

The effect of the type of treatment: Results of current study indicated that there was a significant increase ($p\leq0.05$) in the PINP concentration in animals treated with HFD compared with HFD+TZDs, HFD+SGLT-2i and control groups (Table 2). Also, CTX-1 results in (Table 2) indicated that there was a significant increase ($p\leq0.05$) in animals treated with HFD compared with HFD+TZDs, HFD+SGLT-2i and control groups. The results also indicated that there was a significant decrease ($p\leq0.05$) in the CTX-1 concentration in control group compared with other groups.

Pairwise comparisons and the effect of the interaction between the duration and the type of treatment: The results of pairwise comparisons in finger (1-A) showed a significant increase ($p\leq0.05$) in PINP concentration in treated groups with HFD for the two treatment periods (two weeks and two months) compared with the treated groups HFD+TZDs and HFD+SGLT-2i. Also, the increase was more significant ($p\leq0.05$) in group treated for two month compared to the treated group for two weeks.

The results in the same figure showed changes in the PINP concentration in treated animals according to the interaction between duration and type of treatment, the results showed that there was a significant decrease ($p\leq0.05$) in PINP concentration in HFD+TZDs and HFD+SGLT-2i groups which treated for 2 months compared with other groups. Also the group treated with HFD for two months had the most significant increase ($p\leq0.05$) compared with other groups.

The results of pairwise comparisons in figure (1-B) showed a significant increase ($p\leq0.05$) in CTX-1 concentration in treated groups with HFD, and HFD+TZD for 2 months compared with same groups treated for 2 weeks. The results in the same figure showed changes in the CTX-1 concentration in treated animals according to the interaction between duration and type of treatment, the results showed that there was a significant increase ($p\leq0.05$) in bone resorption biomarker CTX-1 in HFD which treated for 2 weeks and 2 months, HFD_TZDs group treated for 2 months and HFD+SGLT2i groups treated for 2 weeks and 2 months compared with other groups. Also, the results indicated that groups treated with HFD for two months had the most significant increase ($p\leq0.05$) compared with other groups.

Table 1: Comparison of the serum PINP and CTX-1 concentration according to the duration of treatment with TZDs and SGLT-2i in laboratory female rats induced with type 2 diabetes

Dependent Variable: PINP and CTX-1		
Mean ± Std. Error		
Duration of treatment	PINP (ng/dl)	CTX-1 (ng/dl)
2 Weeks (N=16)	385.90±37.84	3.49±0.41
2 Months (N=16)	380.71±61.50	6.34±1.09
Significant	0.904	0.022

Results are represented as mean ± SD.

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

Table 2: Comparison of the serum PINP and CTX-1 concentration 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)	PINP (ng/dl)	CTX-1 (ng/dl)
Control (N=8)	353.89±20.61 a	1.99±0.29 a
HFD (N=8)	644.18±83.50 b	9.19±1.49 b
HFD_TZDs (N=8)	263.90±20.75 a	3.86±0.67 c
HFD_SGLT2i(N=8)	271.25±29.18 a	4.61±0.57 c
Significant	0.000	0.000

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 ± SD.

Similar letters indicate no significant differences at the 0.05 level.

Different letters indicate a significant difference at the 0.05 level.

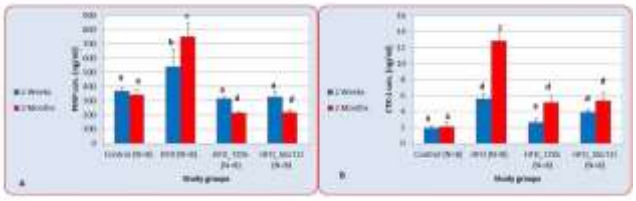

Figure 1: Comparison of the PINP (A) and CTX-1 (B) according to the interaction between the duration and type of treatment with TZDs and SGLT-2i in laboratory female rats

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 ± SD.

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

Similar letters indicate no significant differences at the 0.05 level.

Different letters indicate a significant difference at the 0.05 level.

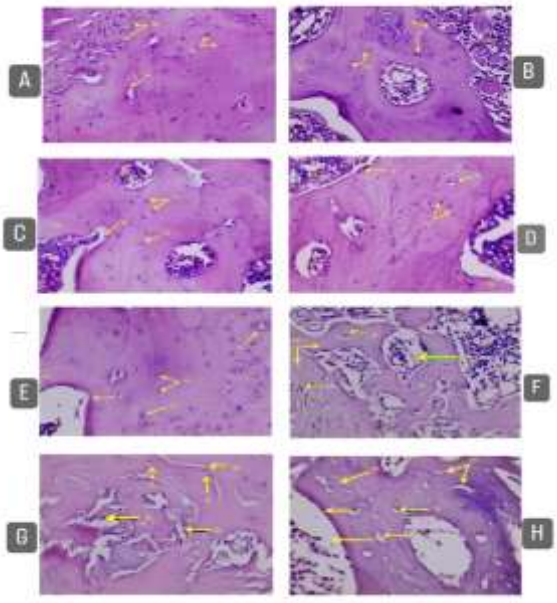

Figure 1: Photomicrograph of the epiphysis for femur bone in control group treated for 2 weeks (A), HFD groups treated for 2 weeks (B), HFD+TZDs groups treated for 2 weeks (C), HFD+SGLT-2i groups treated for weeks 2 (D), control 2 groups treated for 2 months (E), show: the normal histological structure of (H) Haversian canal, (Os) osteocytes, (BM) bone marrow, (Ob) osteoblast and trabecular plates (TP). The epiphysis for femur bone in HFD groups treated four 2 months (F) show: empty lacuna, increase adiposity of bone marrow and the presence of a resorption cavities (RC) in the irregular and thin trabecular plates (TP). In HFD+TZDs groups (G) and HFD+ SGLT-2i groups (H) which treated four 2 months show: empty lacuna, and the presence of a resorption cavities (RC), osteocyte necrosis and less dense appearance of bony tissue.

Effects of treatment with anti-diabetic drugs (TZDs and SGLT-2i) on bone histological structure in laboratory female rats: The histological examination of the femur bone epiphysis in study groups: control 1 (Fig. 1A), HFD (Fig. 2B), HFD+TZDs (Fig. 1C), HFD+ SGLT-2i (Fig. 1D) which treated for 2 weeks and control 2 (Fig. 1E) which treated for 2 months revealed a normal bone structure with a normal cellular endosteum lining the bone surface opposite the bone marrow cavity, and a fibrous periosteum covering the outside surface. In addition, the bone matrix exposed normal osteocytes within their lacunae, normal cement or remodeling lines, normal blood vessels, Haversian canal and trabecular plates.

HFD groups treated four 2 months (Fig. 1F) revealed that the bone matrix contained irregularly-oriented osteocytes within indistinct lacunae and multiple empty lacuna, increase adiposity of bone marrow and the presence of a resorption cavities in the irregular thin trabecular plates.

In groups treated with HFD+TZDs and HFD+ SGLT-2i four 2 months (Fig. 1G) and (Fig. 1H) respectively, the photomicrograph show an empty lacuna, and the presence of a resorption cavities, osteocyte necrosis and less dense appearance of bony tissue.

DISCUSSION

The results of this study indicated that there was a significant increase ($p \leq 0.05$) on bone formation (PINP) and bone resorption biomarker (CTX-1) in the group induced with type 2 diabetes for the two treatment periods (two weeks and two months), and the increase was more significant ($p \leq 0.05$) in the group treated for two months compared to the treated group for two weeks. While the groups treated with TZDs and group treated with SGLT2i for two months showed a significant decrease ($p \leq 0.05$) in bone formation biomarker (PINP) compared with the other treated groups. In addition, the groups treated with TZDs for two months and group treated with SGLT2i for two weeks and two months showed a significant increase ($p \leq 0.05$) in bone resorption biomarker (CTX-1) compared with the other treated groups .

T2DM may wreak havoc on bone homeostasis by inhibiting osteoblast development and speeding up osteoblast death, which is shown as a lower formation signal [12]. Adiposity, insulin resistance, fatty acid content, and hormones all contribute to bone fragility in diabetics. In diabetics, marrow fat may have a deleterious impact on bone fragility. The buildup of marrow fat disrupts the equilibrium of bone turnover, resulting in a reduction in bone volume and quality [13]. The researchers looked at morphological and biochemical skeletal characteristics in T2DM patients and discovered that postmenopausal women with T2DM had lower levels of the bone formation marker PINP than controls. Some studies have found that people with T2DM had lower levels of the resorption marker CTX (serum C-terminal telopeptide from type 1 collagen) [14].In Japanese individuals with type 2 diabetes mellitus, pioglitazone causes an imbalance in bone metabolism by increasing bone resorption and inhibiting bone formation [15]. The fact that TZDs stimulate adipogenesis and block regulators of bone differentiation is an essential mechanistic reason for their effect on bone. It has been observed that TZD therapy, namely pioglitazone, may cause bone loss in the complete body of older women with T2DM. Despite the many benefits of TZD for the treatment of T2DM, the benefits and risks of pioglitazone therapy in T2DM patients at high risk of fracture must be carefully addressed [12]. Bone development is harmed by inhibiting the differentiation of osteoblast precursors. TZDs also enhance bone resorption by increasing bone marrow obesity, lowering aromatase activity, and boosting osteoclast differentiation. When administered as antidiabetic medications (PPAR-activators), TZDs transform mesenchymal stem cells to adipocytes, inhibiting the growth of osteoblasts [16].Without affecting bone resorption, pioglitazone therapy affects bone production. PPAR activation generated by TZDs affects the level of numerous bone turnover indicators and increases the risk of fracture, according to a growing body of data from preclinical and clinical investigations [17]. Bone resorption

indicators rise with TZD treatment but fall with SGLT2i, whereas both resorption and formation markers rise with SGLT2i. Patients on TZD combination therapy had substantial cortical bone loss [12]. Alba et al. indicated that The observed weight loss may be linked to an increased risk of fractures after using SGLT2 inhibitors. Weight loss has been linked to lower bone mineral density and higher bone turnover indicators [18]**.** BTM alterations following a single agent's administration have been reported in different ways. Bone resorption indicators rise with TZD therapy, but formation markers fall, whereas SGLT2i treatment increases both resorption and formation markers. [19]. Jackuliak et al showed that dapagliflozin had no effect on markers of bone formation and resorption or BMD [16].

The histological examination of the femur bone epiphysis in control1, HFD, HFD+TZDs, HFD+ SGLT-2i which treated for 2 weeks and control 2 which treated for 2 months revealed a normal bone structure. While, HFD groups treated four 2 months revealed that the bone matrix contained irregularly-oriented osteocytes within indistinct lacunae and multiple empty lacuna, increase adiposity of bone marrow and the presence of a resorption cavities in the irregular thin trabecular plates. In addition, groups treated with HFD+TZDs and HFD+ SGLT-2i four 2 months showed the presence of a resorption cavities, an empty lacuna, less dense appearance of bony tissue and osteocyte necrosis.T2D patients have normal or high BMD, but they are more likely to fracture because to changes in bone microarchitecture and a local humoral milieu that encourages osteoclast activity. In both kinds of diabetes, chronic hyperglycemia leads to non-enzymatic collagen glycation [20].Despite having greater BMD values, patients with T2DM had a higher fracture risk. This seemingly contradictory findings is based on the fact that altered microarchitecture, AGE buildup (mostly pentosidine), and altered bone turnover (particularly decreased osteoblastic differentiation and activity) all have a major impact on bone quality. All of these variables have a negative impact on the mechanical properties of the skeleton and promote bone fragility in a way that both BMD measurement and algorithms based on clinical risk factors fail to capture. Extra skeletal variables, such as frailty, muscular weakness, illness consequences, and certain anti-diabetic medication, on the other hand, result in a higher incidence of falls. The much greater fracture rate of these individuals is due to a combination of bone fragility and an increased likelihood to fall [21]. Even after accounting for possible confounders such as BMI and falls, persons with type 2 diabetes mellitus (T2DM) had higher fracture risks than people without T2DM. As a result, T2DM may change features of bone quality, such as material characteristics or microarchitecture, resulting in increased fragility regardless of bone quantity [22]T2DM specimens demonstrated reduced separation intervals between trabecula in microarchitectural investigations than non-DM specimens. T2DM specimens also showed a somewhat higher number of trabecula (+ 20%, P = 0.059) and a marginally higher connective density (+ 96%, P = 0.061) than non-DM specimens. The T2DM specimens had the same BV/TV = bone volume fraction Total as the non-DM counterparts. (+ 24%, P = 0.125) [22]. The musculoskeletal system can be affected by long-term usage of anti-diabetic medications. As a result, despite its high hypoglycemic efficacy, thiazolidinediones create stem cell differentiation problems, particularly in women, due to estrogen reduction as they age. According to recent research, the SGLT2i family also causes bone tissue damage. Though it only applies to canagliflozin, which causes more fractures than dapagliflozin and empagliflozin. The danger of hypoglycemia, which can lead to falls and fractures, is the major downside of these medications [23].Because they decrease osteogenesis and increase apoptotic osteocyte death, TZDs have been shown to have negative impacts on bone health in studies. These findings imply that TZDs should be used with caution in diabetic individuals who are at risk of osteoporosis [24] Over expression of PPAR- in totipotent mesenchymal stem cells resulted in preferential differentiation of these cells into adipocytes and suppression of development into steoblasts when thiazolidinediones are used [15]. Pioglitazone controls osteocyte activity and increases obesity in the bone marrow of the vertebral body. Increased bone weakening and a lack of bone growth may be explained by marrow adipocyte proliferation at the expense of osteoblast development. Increased bone adiposity might be misinterpreted for bone loss. Finally, PPAR activation might boost osteoclastic differentiation and activity[25] .The osteocyte, a type of long-lived cell buried inside bone tissue that can sense mechanical pressure on the skeleton and orchestrate bone remodeling by directing the creation and activity of osteoclasts and osteoblasts, is a crucial biological regulator of bone metabolism. TZDs have also been shown to cause osteocyte apoptosis in experimental animals, which has been linked to fast changes in bone remodeling, including greater bone breakdown and decreased bone production, as well as the emergence of microcracks [26]. Both isoforms of the PPARg gene are expressed in bone, and TZDs activate them. PPARg2 activation influences mesenchymal stem cell lineage allocation, inhibiting osteoblast differentiation and promoting marrow adipocyte proliferation. The PPARg1 gene is activated, which boosts osteoclast production and activity [26].

Mabilleau et al.. indicate that In vitro treatment of mesenchymal progenitor cells with rosiglitazone (1 mM) resulted in a 27% increase in adipogenesis and a 47% drop in osteoblastogenes, lowering the number of cells required for bone production. Treatment of mesenchymal cells with TZDs resulted in enhanced adipogenesis and decreased transcriptional activity in these cells in tissue culture [27]

Due to their mechanism of action and excretion of sodium in the urine, concerns have been raised about a potential negative effect of SGLT-2 inhibitors on skeletal physiology. These concerns aroused from two reasons: firstly, the skeleton represents a substantial reservoir of sodium that can be exchanged when needed and approximately one-third of total sodium is found in the skeleton [27]. Second, chronic hyponatremia has been linked to an increase in the number and activity of osteoclasts, perhaps due to processes involving arginine vasopressin-dependent and independent pathways, and therefore an increased fracture risk. [28] By resisting hyperglycemia and hyperinsulinemia, dapagliflozin preserves diabetic bones. Blood glucose levels were reduced after treatment with Dapagliflozin, which inhibited calcium absorption induced by increased insulin production, preventing the requirement for bone to release calcium to combat hypocalcemia [29].

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

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