

RESEARCH ARTICLE

The Effect of Liraglutide on Biochemical Markers of Bone Turnover in Rats Treated by Dexamethasone

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ABSTRACT

Background: Glucocorticoids (GCs) are the most common cause of secondary osteoporosis. Chronic uses of GCs have serious adverse effects on bone, including fragility fracture. Liraglutide is a glucagon-like peptide-1 which is a new class antidiabetic drug. Glucagon-like peptide-1 agonist has shown a role in bone physiology and remodeling. Previous studies suggested promising results of using liraglutide on bone health.

Objective: Evaluate the effects of a relatively low dose of liraglutide on bone turnover markers in dexamethasone-treated rats.

Method: Thirty-two male rats were randomly divided into three groups (Three months old; 300 to 325g). The normal control group (n=8) received intramuscular normal saline twice weekly (the vehicle of dexamethasone) and subcutaneous normal saline daily (the vehicle of liraglutide); the negative control group received intramuscular dexamethasone 2.5 mg twice weekly and subcutaneous normal saline (vehicle of liraglutide); liraglutide treatment group received intramuscular normal saline twice-weekly (vehicle of dexamethasone) and subcutaneous liraglutide 75 µg/mL daily. Blood was collected at week 4 and 8, via orbital sinuses. In addition, enzyme-linked immunoassay (ELISA) tests for osteocalcin, alkaline phosphatase, cross-linked C-telopeptide of type I collagen (CTX) and serum tartrate-resistant acid phosphatase 5b were performed.

Results: Dexamethasone causes deterioration of osteocalcin, alkaline phosphatase, cross-linked C-telopeptide of type I collagen and serum tartrate-resistant acid phosphatase 5b compared to the control group. Liraglutide improved week 8 alkaline phosphatase and cross-linked CTX compared to week 4 and dexamethasone group results. Moreover, liraglutide improves Serum tartrate-resistant acid phosphatase 5b compared to the dexamethasone-treated group.

Conclusion: Low dose liraglutide can prevent deterioration of bone formation and bone resorption biochemical parameters after dexamethasone treatment.

Keywords: Alkaline phosphatase, CTX-1, Glucocorticoids, Liraglutide, Osteocalcin, TRAP-5b.

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INTRODUCTION

Glucocorticoids (GCs) are the most common cause of secondary osteoporosis.¹ Chronic uses of GCs have serious adverse effects on bone, including fragility fracture. In addition, GCs are commonly used for many inflammatory and autoimmune and other conditions.^{2,3} Glucocorticoid-induced osteoporosis (GIOP) causes a decrease in bone formation with an early and transient increase in bone resorption. These effects cause a high rate of bone loss due to the shift bone remodeling to a negative manner with increased bone turnover.^{1,3} GCs have direct and indirect bone insulting effects. The direct effects are mediated by inhibition of osteoclast and increase in their lifespan, inhibition of osteoblastogenesis and increase osteoblast apoptosis. Indirect GC effects include an increase in

intestinal and renal calcium loss, physical activity reduction, hypogonadism, increased parathyroid hormone secretion, decreased synthesis of some anabolic proteins such as insulin-like growth factor 1 and growth hormone, and a decrease in the synthesis of type 1 collagen.¹⁻⁴

Liraglutide is glucagon-like peptide-1 (GLP-1), a new class antidiabetic drug. Liraglutide mimics the endogenous GLP-1 effects.^{5,6} These effects promote insulin secretion from b-cells of pancreas in a glucose-dependent manner, thus lowering glucose level, suppressing appetite, and delaying gastric emptying and possible action on weight loss throughout effects on vagal afferent nerves and hypothalamus. Expression of GLP-1 receptors occurs in many tissues and organs; that includes the pancreas, stomach, small intestine, brain, and

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lung.⁷⁻⁹ Bone tissue is also expressed GLP-1 receptor that may play a role in bone physiology and remodeling. A study investigated that GLP-1 has a direct functional role with osteoblast by functional GLP-1 receptors. Evidence suggests that GLP-1 agonists do not affect glycemic control and appetite but may play a role in bone remodeling. Studies showed that incretin mimetics could stimulate osteoblastogenesis.⁸⁻¹¹ Preclinical studies showed that using liraglutide could prevent osteoporosis in different animal models of bone loss.^{8,11,12}

The biomarker is a biological measure that estimates body levels and can evaluate many physiological, pathological, and pharmacological interventions. There are wide ranges of biomarkers used currently. Different biological systems have specific biomarkers that including bone. It is relatively easy to estimate and measure a wide range of biomarkers. These measurements are usually performed routinely in preclinical and clinical studies and evaluations.^{13,14} Bone turnover markers (BTM) reflect either osteoblasts bone formation activities or osteoclast bone resorptions activity. They can be measured and detected in plasma, serum or urine. Bone formation markers include osteocalcin (OC), bone-specific alkaline phosphatase (ALP) and procollagen type I N propeptide (PINP). However, bone resorption markers include protein segments released from the telopeptide end region of type I collagen after its enzymatic degradation, for instance, the cross-linked CTX, N-telopeptide of type I collagen (NTX), deoxypyridinoline (DPD), and the enzyme tartrate-resistant acid phosphatase (TRAP-5b).¹⁴⁻¹⁶

The current study evaluates the effects of a relatively low dose of liraglutide on bone turnover markers in bone formation and resorption level in rats treated with dexamethasone. We hypothesize that a low dose of liraglutide effectively reverses dexamethasone effects in reducing the bone formation and stimulating bone resorption.

MATERIAL AND METHOD

Preparation of Treatment

Dexamethasone injection (Medochemie Ltd, Cyprus) was diluted in normal saline and was administered intramuscularly. Liraglutide (Novo Nordisk, Denmark) was diluted with normal saline and administered subcutaneously with two doses 75 µg/mL.

Animal Experimentation

The national drug control and research center in Baghdad, Iraq, provided 32 three-month-old Wistar male rats (NCDRCR). They were kept in vented plastic cages at the University of Al-Animal Nahrain's Laboratory, Department of Pharmacology, College

of Medicine (Baghdad, Iraq), under regular environmental conditions (12/12 h dark/light cycle, 25°C). They had unrestricted access to normal rat food and tap water throughout the research. The rats were randomly split into three groups (n = 8) after one week of acclimation. For eight weeks, two groups were administered injectable dexamethasone (2.5 mg/kg) twice weekly. In contrast, another group was given an equivolume of normal saline and assigned as a control group. Table 1 summarizes all study and treatment groups used in the current study.

Bone Turnover Marker

The blood of the rats was collected using sterile blood collection tubes containing EDTA via orbital sinus at week 4 of the study and cardiac puncture at sacrifice under anesthesia using intraperitoneal mixture of ketamine 100 mg/kg (Troy Laboratories, Australia) and xylazine 2 mg/kg (IIL India). Blood was centrifuged at 3000rpm for 10 minutes to extract the serum. The serum supernatant for ELISA was aliquoted into microcentrifuge tubes and kept at -80°C after centrifugation.

Bone OC and ALP (bone formation markers) and bone CTX-1 and TRAP-5b (bone resorption markers) were assessed according to the manufacturer's instructions using rat-specific quantitative sandwich ELISA kits (Elabscience Biotechnology Co., Ltd, USA).

Before using, all of the chemicals and the 96-well ELISA microplates were brought to room temperature. The standards were placed in the first seven wells, the blank in the eighth, and 100 µL of serum in each remaining wells. After 90 minutes of incubation at 37°C, a 100 µL biotinylated detection antibody was added to each well, and the plate was replaced in incubator for 60 minutes at 37°C. After a three-step washing procedure, 100 µL of HRP conjugate was added to each well and the plate was placed back in the incubator (at 37°C for 30 minutes). After the last 5 step-washing, 90 µL of substrate reagent was applied, followed by 15 minutes of incubation in the dark. After that, a 50 liter stop solution was added. Finally, a 50 µL stop solution was added to each well, and the plate was read at 450 nm.

Statistical Analysis

The statistical package for social sciences (SPSS) version 20 was used for statistical analysis (IBM, Armonk, USA). The Shapiro-Wilk test was used to determine the data's normality. The parameters were compared among the experimental groups using one-way analysis of variance (ANOVA) with Tukey's post hoc test. The statistical significance level was chosen at p0.05. The mean and standard deviation were used to present all data (SD).

Table 1: Design of animal treatment

Groups	Intermuscular	Subcutaneous
Normal control (Con)	Normal saline*	Normal saline**
Negative control (Dex)	Dexamethasone 2.5 mg twice weekly	Normal saline**
Liraglutide (Lrg)	Dexamethasone 2.5 mg twice weekly	Liraglutide 75 µg/mL/day

* Vehicle of dexamethasone administered twice weekly

** vehicle of liraglutide administered once daily

RESULTS

Dexamethasone treatment showed a significantly lower level of OC and ALP while higher CTX-1 and TRAP-5b compared with control group treatment ($p < 0.05$). Both Con and Dex showed no significant difference between week 4 and week 8 values in OC, ALP, CTX-1, and TRAP-5b level ($p > 0.05$) (Figures 1 to 4). Lrg rats showed a significantly higher level of ALP Dex rats ($p < 0.05$) (Figure 2). Lrg rats showed significantly higher CTX-1 and TRAP-5b compared to Dex rats ($p < 0.05$) Figures 3 and 4. Lrg group week 4 and week 8 results were significantly different in ALP and CTX-1 ($P < 0.05$) figs. 2 and 3 while showed no significant differences in OC and TRAP-5b ($p > 0.05$) Figures 1 and 4.

DISCUSSION

Dexamethasone adversely affected all measured parameters compared with the normal control group. Bone formation parameters (OC and ALP) significantly decreased in Dex rats compared to Con rats, while bone resorption parameters

(CTX-1 and TRAP-5b) were significantly increased compared to the Con group. These results indicate that a GIOP model was successfully induced. These results are similar to comparable studies^{11,17-19} Both week 4 and week 8 in Dex group have shown no statistical difference; these may indicate continuous effects of dexamethasone throughout the study period on the expression of these parameters.

Bone formation markers are osteoblast enzymes or by-products of osteoblast activity expressed during their differentiation and development phases.¹⁶ The bone formation markers measured in the current study were osteocalcin and ALP, the most common biochemical markers for bone formation.^{11,20} In the present study, there was a marginal increase of osteocalcin level in Lrg rats compared to Dex rats without reaching a statistically significant level. This may be subjected to continuous use of GC throughout the study, which

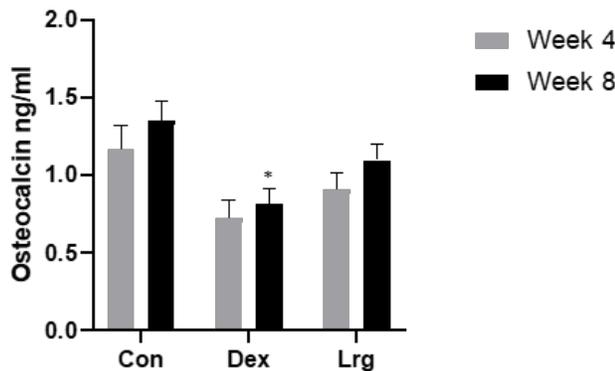


Figure 1: Serum osteocalcin level. All values are expressed as mean \pm SEM. * indicates a significant difference from control group ($p < 0.05$, Repeated measures ANOVA). Con: control group, Dex: dexamethasone-treated group, Lrg: liraglutide treatment group.

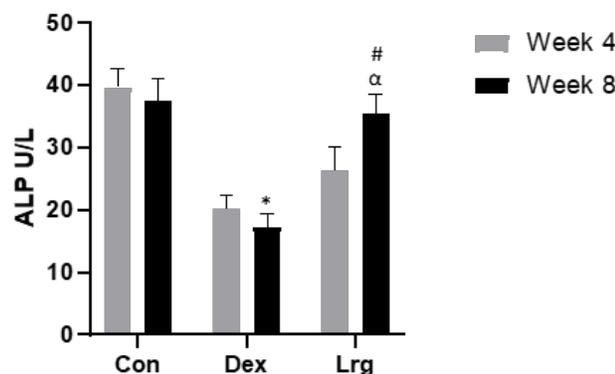


Figure 2: Serum alkaline phosphatase level. All values are expressed as mean \pm SEM. * indicates a significant difference from control group, # indicates a significant difference from the dexamethasone-treated group, α indicates a significant difference from week 4 values ($p < 0.05$, Repeated measures ANOVA). Con: control group, Dex: dexamethasone-treated group, Lrg: liraglutide treatment group.

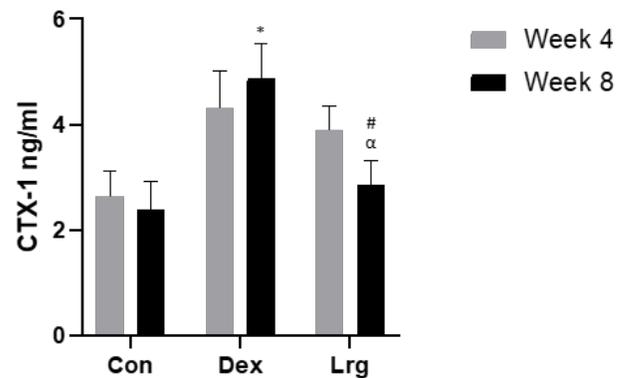


Figure 3: Serum cross-linked C-telopeptide of type I collagen. All values are expressed as mean \pm SEM. * indicates a significant difference from control group, # indicates a significant difference from the dexamethasone-treated group, α indicates a significant difference from week 4 values ($p < 0.05$, Repeated measures ANOVA). Con: control group, Dex: dexamethasone-treated group, Lrg: liraglutide treatment group.

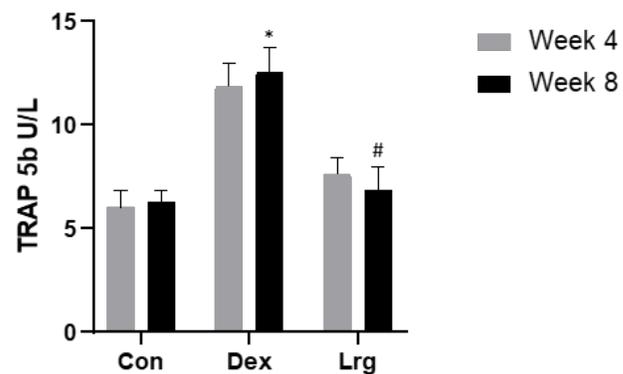


Figure 4: Serum tartrate-resistant acid phosphatase 5b. All values are expressed as mean \pm SEM. * indicates a significant difference from control group, # indicates a significant difference from the dexamethasone-treated group ($p < 0.05$, Repeated measures ANOVA). Con: control group, Dex: dexamethasone-treated group, Lrg: liraglutide treatment group.

negatively affects osteocalcin levels. Moreover, more than eight weeks of treatment may be required to reflect a clearer image of liraglutide effects on osteocalcin level.

ALP Enzyme is present in osteoblast plasma membranes.¹⁶ In the current study, ALP was increased in Lrg rats compared to the Dex group and its week 4 level, indicating that liraglutide increased osteoblastic activity. These results may suggest that liraglutide stimulates bone formation after GC treatment and elevations of these parameters indicate an increase in bone turnover and maybe mineralization.

CTX-1 is a sensitive marker of bone resorption that responds promptly to changes in bone metabolism. Liraglutide treatment increases the CTX compared with Dex rats; this indicates that liraglutide attenuates the stimulated bone GC bone resorption. Moreover, liraglutide significantly decreases CTX at week 8 compared to week 0. These changes may reflect that liraglutide decreases bone resorption induced by GC.¹³ CTX-1 results of the current study were agreed with comparable studies which investigated the effects of liraglutide in different osteoporosis models.^{8,11,12}

TRAP 5b is a unique and sensitive osteoclast marker enzyme. The osteoclast-specific isoform 5b is detectable in serum, and its concentration is regarded as a particular indicator of bone resorption.⁴ Furthermore, there is a relationship between the quantity of TRAP circulating and the amount of bone resorbed, reflecting the bone resorption rate. The present study showed that TRAP-5b decreased significantly compared to Dex group. However, there were no significant differences in Lir rats in week 4 compared with week 8. These TRAP-5b results may indicate the bone resorption inhibition throughout using liraglutide started earlier than 4 weeks.

There are many limitations in the current study. The measured parameters are only bone turnover markers without other bone screening parameters such as bone mineral density, bone biomechanics, or histomorphometry. The study period of 8 weeks may be insufficient for such a model. No dynamic results on bone were investigated.

CONCLUSION

A low dose of liraglutide may promote bone formation and inhibit bone resorption, shown by the improvement of ALP and inhibition of CTX-1 and TRAP 5b after 8 weeks of treatment liraglutide may prevent glucocorticoid-induced osteoporosis. Effects of liraglutide after 8 weeks of treatment more than short term 4 weeks treatment. Liraglutide may become a new drug to prevent bone loss.

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