The Impact of Glucagon-Like Peptide-1 on Bone Metabolism and Its Possible Mechanisms

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The impact of antidiabetic drugs on bone metabolism is drawing increasing attention due to the discovery of a correlation between type 2 diabetes mellitus (T2DM) and osteoporosis. Glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1RAs) are a novel and promising class of drugs for T2DM, which may also have clinical applications in bone tissue disorders. This review examines the impact of GLP-1 on bone metabolism, including enhancement of bone mineral density and improvement of bone quality. However, the precise effect of GLP-1 on fracture risk has not been unambiguously defined. This review also summarizes our current understanding of the mechanisms by which GLP-1 affects bone metabolism. GLP-1 may act on bone by promoting bone formation, inhibiting bone resorption, and affecting the coordination of the two processes. We describe molecular pathways and proteins, such as Wnt and calcitonin, that are associated with GLP-1 and bone tissue. The specific processes and related molecular mechanisms of the effects of GLP-1 on bone metabolism need to be further explored and clarified.

Keywords: glucagon-like peptide-1, osteogenesis, bone resorption, osteoporosis, diabetes mellitus

INTRODUCTION

Diabetes mellitus often leads to the development of osteoporosis, a degenerative bone disorder. Both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) can affect bone mineral density (BMD) and the risk of bone fractures (1). GLP-1 receptor agonists (GLP-1RAs) are a novel class of drugs for T2DM (2); they stimulate insulin secretion, increase β cell mass, and suppress glucagon secretion (3–5). Currently, four such drugs available in the United States: exendin-4 or its synthetic version exenatide, liraglutide, albiglutide, and dulaglutide. Various products are in different stages of research and development (2, 6–8). The effectiveness of these drugs has led to increasing interest in the mechanisms underlying their effects on bone metabolism. It has been reported that glucagon-like peptide-1 (GLP-1) can enhance BMD and improve bone quality, and the relationship between GLP-1 and bone fractures is still under investigation. GLP-1 can promote bone formation and inhibit bone resorption, but the specific process and related molecular pathways are still not completely understood (9–11). This review summarizes the current state of research into the impact of GLP-1 on bone metabolism and its possible mechanisms.

THE IMPACT OF GLP-1 ON BONE METABOLISM

The Impact of GLP-1 on BMD
Glucagon-like peptide-1 may enhance BMD. In an animal experiment, GLP-1 and exendin-4 (a GLP-1 mimetic) reversed the decrease in bone mass of femurs and vertebrae in hyperlipidic and
hypercaloric Wistar rats (9). In a clinical trial, 61 T2DM patients were divided into three groups randomly: liraglutide 1.8 mg/day or liraglutide 1.2 mg/day or glimepiride 8 mg/day. This research showed no significant differences in BMD among groups in drug use at 52 or 104 weeks (12). The 24-week use of exenatide in newly diagnosed and treatment-naïve patients with T2DM presented no influence on BMD (13). However, 69 T2DM patients receiving metformin were also given either exenatide (n = 36) or insulin glargine (n = 33). Only patients in the former group experienced significant lowering of body weight and had maintained BMD levels, after 44 weeks of treatment (14). As weight loss results in bone loss (15), and insulin administration may contribute to reduction in bone resorption (16). This result implied that exenatide, in comparison with insulin, may promote BMD while facilitating weight reduction.

The Impact of GLP-1 on Bone Quality
Glucagon-like peptide-1 may improve bone quality. In ovariectomy (OVX)-induced osteoporosis in aged rats, exendin-4 enhanced bone strength and prevented exacerbation in trabecular microarchitecture (10). In another study, the percent bone volume (BV/TV) and trabecular number (Tb.N) were lowered in diabetic and insulin-resistant (IR) rats, while their trabecular separation (Tb.Sp), trabecular bone pattern factor (Tb.Pf), and structure model index increased, indicating an elevated level of anisotropy in trabecular bone and destruction of the normal bone structure. It was found that GLP-1 could reduce these trends and restore normal bone structure (17). In streptozotocin-induced T1DM Swiss TO mice, liraglutide improved mechanical properties in bone tissue, but failed to reverse cortical microstructure degradation or improve whole bone mechanical properties (18). Furthermore, GLP-1 and glucose-dependent insulinotrophic polypeptide double incretin receptor knockout (DIRKO) mice had increased trabecular bone mass and a higher trabecular number compared to wild-type mice, with reduced bone outer diameter, cortical thickness, and cortical area. Mechanical properties of bone matrix were also affected in these mice as evidenced by decreases in yield stress, ultimate stress, and post-yield work-to-fracture in DIRKO mice at the level of whole bone. At the tissue level, the maturity level of collagen was 9% lower in DIRKO mice, which contributed to the lowering of maximum load, hardness, and dissipated energy (19).

The Impact of GLP-1 on Bone Fractures
The relationship between GLP-1 and the risk of bone fractures has not been fully elucidated. A population-based cohort study using data from the Clinical Practice Research Datalink database (2007–2012) concluded that GLP-1RAs were not related to a decreased risk of bone fractures, independent of dose accumulation and the type of GLP-1 drug (exenatide or liraglutide) used (20). And a case–control study also presented a same conclusion (21). Similarly, a meta-analysis has studied randomized clinical trials (RCTs) comparing GLP-1RAs and other antidiabetic drugs versus a placebo in T2DM patients (>24 weeks). It revealed that GLP-1RAs failed to reduce the risk of bone fractures compared to other antidiabetic agents [OR = 0.75, 95% confidence interval (CI) 0.28–2.02, P = 0.569]. However, with the lack of data on bone condition, including BMD, microarchitecture, bone quality, calcium, and phosphorus levels at baseline in this study, the results should not be taken as definitive. Furthermore, GLP-1RA is related to decreased body weight, which is unfavorable for bone fractures as weight loss induces mechanical loading and bone mass decrease. These disadvantages can obscure the potential protective effects of GLP-1RA. Additionally, GLP-1RA is associated with gastrointestinal adverse events, which may cause absorption of minerals and nutrients and thereby interfere with the beneficial function of GLP-1RA on bone physiology (22).

It is possible that different GLP-1 agents have divergent effects on bone fractures, as shown in another meta-analysis of RCTs that ended in December 2013. It indicated that liraglutide significantly reduced the risk of bone fractures (OR = 0.38, 95% CI 0.17–0.87), whereas exenatide increased the risk of bone fractures (OR = 2.09, 95% CI 1.03–4.21), compared to placebo or other antidiabetic drugs. Clues exist that may help explain the distinct results of liraglutide and exenatide. First, differences in molecular structure result in dissimilar pharmacokinetic profiles. Because liraglutide shares 97% homology with GLP-1 while exenatide only shares 50% of GLP-1, it is inferred that liraglutide is more likely to mimic the function of endogenous GLP-1. Second, exenatide tends to cause more weight loss and lower glucose control than liraglutide, which may result in a higher risk of bone fractures (11). In summary, current research is inadequate to explain the effects of GLP-1 and related drugs on bone fracture risk. Furthermore, the incidence of bone fracture is only recorded as adverse reactions instead of being part of routine data collection, which may result in less than complete information. The published animal and human studies on the impact of GLP-1 on bone metabolism are described in Table 1.

The Potential Mechanisms of the GLP-1 Effects on Bone Metabolism
The continuous cycle of bone formation and bone resorption in osseous tissue maintains normal bone quality and bone mass. GLP-1 affects both parts of this cycle. Potential mechanisms of GLP-1 are described below and in Figure 1.

Promotion of Bone Formation and Possible Mechanisms
GLP-1 Promotes Bone Formation
Glucagon-like peptide-1 increases the number of osteoblasts. Osteoblast number on the surface of trabecular bone was observed by a histological analysis that demonstrated a significant increase after 16 weeks of exendin-4 use in OVX rats (10). This result was also confirmed in another study (32).

Glucagon-like peptide-1 promotes the expression of genes related to bone formation. Runx2 encodes osteoblast-specific transcription factor 2, which is a transcriptional activator of osteoblast differentiation (33). Furthermore, alkaline phosphatase (ALP), collagen type 1 (Col 1), and osteocalcin (OC) are common bone formation markers (34); all are upregulated. In old rats with OVX-induced osteoporosis, 16 weeks of exendin-4 administration led to a rise in Runx2, ALP, Col 1, and OC mRNA levels

Zhao et al. GLP-1 in Bone Metabolism
TABLE 1 Published animal and human researches on the impact of GLP-1 on bone metabolism.

| Reference | Subjects and design | Main results (BMD, bone quality, and fracture risk) |
|-----------|---------------------|--------------------------------------------------|
| **Animals** |                     |                                                   |
| Yamada et al. (23) | GLP-1r knockout mice vs. WT mice | BMD: GLP-1r−/− mice presented lowered cortical BMD. Bone quality: GLP-1r−/− mice presented diminished bone flexural rigidity. |
| Nuche-Berenguer et al. (17) | T2D rats, IR rats, and normal rats | BMD: GLP-1 had a tendency for increasing BMD in T2D and IR rats though the differences did not reach statistical significance. Bone quality: GLP-1 alleviated enhanced anisotropy and normalized damaged trabecular bone structure in T2D and IR rats (resulted in a reduction in Tb.Sp, Tb.Pf, and SMI). |
| Nuche-Berenguer et al. (24) | T2D rats, IR rats, and normal rats | BMD: exendin-4 had a tendency for increasing BMD in T2D and IR rats though the differences did not reach statistical significance. Bone quality: exendin-4 might normalize the damaged trabecular structure in IR and T2D rats (resulted in a reduction in Tb.Sp, Tb.Pf, and SMI). |
| Nuche-Berenguer et al. (9) | Wistar HL rats with GLP-1 vs. with exendin-4 vs. with saline vs. normal rats | BMD: GLP-1 and exendin-4 improved lowered BMC and BMD of the femur and lumbar spine of HL rats. |
| Kim et al. (25) | 4-week-old male T2D OLETF rats with saline vs. OLETF rats with exendin-4 (5 nmol/kg twice a day for 3 weeks) vs. LETO control rats with saline | BMD: exendin-4 increased BMD of the femurs in OLETF rats compared to the other two groups. |
| Ma et al. (13) | 12-month-old female Sprague-Dawley rats | BMD: exendin-4 increased BMC (10 μg/kg/day) and even increased BMD dose dependently of the femur and lumbar spine. Bone quality: exendin-4 (3 μg/kg/day) improved bone trabecular microarchitecture similarly, as compared to estradiol. It also improved bone strength of OVX rats. |
| Mabileau et al. (26) | Male GLP-1r knockout mice vs. control wild-type mice | Bone quality: GLP-1r knockout mice presented damaged bone strength and quality. |
| Sun et al. (27) | 2-week-old male diabetic GK rats and age-matched male Wistar rats | BMD: liraglutide reversed the lowered cortical and trabecular BMD in GK rats. Bone quality: liraglutide ameliorated abnormal cortical and trabecular bone microarchitecture in GK rats. |
| Mansur et al. (18) | STZ-induced diabetic male Swiss TO mice | Bone quality: liraglutide improved mechanical properties in bone tissue, but there were no significant results in reversing cortical microstructure degradation or improving whole bone mechanical properties. |
| Sun et al. (28) | 5-month-old female non-diabetic and OVX Wistar rats | BMD: exendin-4 increased BMD. Bone quality: exendin-4 improved trabecular structure and reduced trabecular spacing both in the femur and lumbar vertebrae. Fracture risk: exendin-4 might have little influence on the mechanical resistance to fracture in the femur. |
| Lu et al. (29) | 5-month-old female Wistar rats | BMD: liraglutide increased BMD both in the femur and lumbar vertebrae. Bone quality: liraglutide improved trabecular structure and reduced trabecular spacing both in the femurs and lumbar vertebral. |
| Pereira et al. (30) | 12-week-old ovarioctomized female C57BL/6NCrl mice | BMD: both drugs improved trabecular bone mass. Bone quality: both drugs improved bone structure and connectivity, but had no effect on cortical architecture. Both increased osteoclast surfaces but only exenatide enhanced osteoclast number in vivo. |
| Mieczkowska et al. (19) | 26-week-old DIRKO mice and wild-type control mice | BMD: DIRKO mice showed higher trabecular bone mass and lower cortical bone mass. Bone quality: DIRKO mice presented increased trabecular number but reduced cortical strength. They also showed decreased collagen maturity at the tissue level and resulted in exacerbation in bone mechanical response. |
| **Human** |                     |                                                   |
| Bunck et al. (14) | 69 metformin-treated T2DM patients | BMD: exenatide was not related to significant changes of total BMD and serum markers of bone metabolism, despite a significant body weight decrease. And there was no difference between two groups in the endpoint BMD. |
| Mabileau et al. (22) | A meta-analysis, 28 RCTs were identified | Fracture risk: the administration of GLP-1RA was not associated with reduced fracture risk compared to the use of other antidiabetic drugs. |
Glucagon-like peptide-1 (GLP-1) promotes bone formation and inhibits bone resorption. For bone formation, GLP-1 results in increasing osteoblast number, gene expression related to bone formation, and serum level of bone formation markers. GLP-1 might bind to its receptor on osteoblasts and its function is possibly mediated by mitogen-activated protein kinase (MAPK) pathways, Wnt pathways, or c-fos transcription promotion. GLP-1 also turns mesenchymal stem cell (MSC) differentiation from adipocytes toward osteoblasts.

For bone resorption, GLP-1 results in decreasing osteoclast number and serum level of bone resorption markers. But GLP-1 might act through a calcitonin-dependent way in thyroid C cells. Furthermore, GLP-1 increases OPG expression while decreases receptor activator for nuclear factor-κB ligand (RANKL) expression. GLP-1 helps to maintain the balance between bone formation and bone resorption.
Glucagon-like peptide-1 is associated with increased serum levels of bone formation markers. The use of exendin-4 in old OVX rats increased the serum levels of several bone formation markers, including ALP, OC, and N-terminal propeptide of type I procollagen (P1NP) (10). After 52 weeks of treatment with liraglutide, healthy obese women experienced serum P1NP level increases of 16% on average (31).

**GLP-1 Promotes Bone Formation by Lowering Glucose Level**

It was indicated that hyperglycemia was negatively associated with lumbar BMD (36). It has been demonstrated that endogenous GLP-1 could help maintain a normal glycemic level acting through GLP-1 receptors (37). GLP-1 controls the glucose level by stimulating insulin secretion, inhibiting glucagon secretion, and modulating gastric emptying, thus contributing to an enhancement of bone formation (3–5).

**The Expression of GLP-1 Receptor on the Osteoblast Function**

It has been established that GLP-1 is involved in important physiological processes through binding to the GLP-1 receptor, which is a cAMP-linked G protein-coupled receptor found in the pancreas and many extra pancreatic tissues. In a study focused on the widely studied mouse osteoblastic MC3T3-E1 cell, the expression of GLP-1 receptor was discovered to be regulated in accordance with glycemic level (38). In another study, it was established that GLP-1 receptors varied in their expression in different stages of osteoblastic cell lines. It can be deduced that the expression level of GLP-1 receptor on osteoblasts decreases with the process of osteoblast maturation (39). Furthermore, an enhanced expression of the GLP-1 receptor gene was observed during the osteogenic differentiation process of adipose-derived stem cells, which have the potential for multiple types of differentiation, including osteogenic differentiation. This result demonstrates that GLP-1 may play a role in the osteogenic differentiation of bone tissue (40).

It is notable that in a study performed by Bernardo, some human GLP-1 could bind to a functional receptor in rat MC3T3-E1 cells with a dependence on time and temperature, but independent of the type of cAMP-linked G protein receptor. To be more specific, the GLP-1 receptor with an attached GLP-1 molecule had the function of instantly hydrolyzing glycosylphosphatidylinositolositos (GPIs), which generated short-lived inositolphosphoglycans (IPGs), and promoted phosphatidylinositol-3 kinase (PI3K) and mitogen-activated protein kinase (MAPK) activities (35). Another study also demonstrated that GLP-1 participated in the same process in liver and muscle (41). Exendin-4 could also induce the hydrolysis of GPIs but was delayed compared to the natural human GLP-1 amide (35). These results show that GLP-1 might act directly through the GPI/IPG-coupled receptor on osteoblastic MC3T3-E1 cells.

**The Pathways of GLP-1 in Promoting Bone Formation**

Wnt pathways may play a significant role in how GLP-1 promotes bone formation. It has been reported that the Wnt pathway was impaired in diabetic rats and that exendin-4 treatment enhanced bone formation (24). The canonical Wnt pathway, which includes low density lipoprotein receptor-related protein 5/6, β-catenin, GSK-3β, and T cell factor (42), promotes osteoblast differentiation and maturation (43); GLP-1 is a direct activator of this pathway (44). Sclerostin, encoded by the SOST gene, is secreted by osteocytes and suppresses bone formation. It binds to bone morphogenetic proteins and negatively affects bone formation by inhibiting ALP activity, type I collagen synthesis, and mineralization (45). Sclerostin also inhibits the Wnt/β-catenin signaling pathway (46). Furthermore, it has been observed that exendin-4 reduced the mRNA and protein levels of SOST/sclerostin in osteocyte-like MLO-Y4 cells and also decreased the serum sclerostin level in T2DM Otsuka Long–Evans Tokushima Fatty rats. The study further proposed that exendin-4 might bind to GLP-1 receptor, mediated by protein kinase A (PKA), and act on the Wnt/β-catenin pathway in osteocytes in order to reduce sclerostin expression and promote bone formation (25).

There are other possible pathways by which GLP-1 could affect bone formation. It has been proposed that GLP-1 could promote c-Fos transcription in osteoblasts in combination with ATP, facilitating the participation of GLP-1 in bone turnover (47). It has been shown that exendin-4 enhanced the proliferation and differentiation of osteoblasts partly mediated by MAPK pathways, including ERK1/2, p38, and JNK pathways (48). It was also proposed that liraglutide regulated MC3T3-E1 cells differentiation mediated by adenosine monophosphate-activated protein kinase/mammalian target of rapamycin signaling (49).

**Suppression of Bone Resorption and Possible Mechanisms**

**GLP-1 Inhibits Bone Resorption**

Glucagon-like peptide-1 has an impact on the number and functioning of osteoclasts. Osteoclast number on the surface of trabecular bone was observed by histological analysis, which demonstrated a significant decrease after 16 weeks of exendin-4 use in OVX rats (10). When ovariectomized mice were treated with liraglutide, exendin-4, or saline, it was observed that the osteoclast number was increased only in the mice that received exendin-4 and a high dose of liraglutide. Although the result was contrary to a previous study, which might partly result from different animal species, assessment method for osteoclast number and drug dose, the bone resorption efficiency of osteoclasts declined in this experiment, leading to decreased bone resorption overall (30).

Glucagon-like peptide-1 administration is associated with reduced serum levels of bone resorption markers. In old rats with OVX-induced osteoporosis, exendin-4 decreased the concentration of C-terminal cross-linked telopeptides of type
GLP-1 May Inhibit Bone Resorption

The hypothesis that GLP-1 may inhibit bone resorption was supported by the observation that GLP-1 receptor expression level on thyroid C cells is high, whereas in osteoclasts, the increase in urinary DPD concentration was alleviated. Therefore, it can be concluded that GLP-1 inhibits bone resorption in a calcitonin-dependent way (23).

The results from preclinical studies have not necessarily extended to success in clinical studies. In rodents, the GLP-1 receptor expression level on thyroid C cells is high, whereas in humans, the expression of the receptor is lower. The sensitivity of the response of human TT thyroid C cells to GLP-1 that resulted in producing cAMP and releasing calcitonin was different from the rat C cell lines MTC 6–23 and CA-77. Human TT thyroid C cells were unexpectedly less responsive to GLP-1 (52). In general, GLP-1 may inhibit bone resorption via a calcitonin-dependent pathway, but this hypothesis still needs to be explored further.

The Impact of GLP-1 on the Balance between Bone Formation and Bone Resorption

Normal bone metabolism in humans involves both bone formation and bone resorption in a balanced state of equilibrium. These dynamic processes involve the bone multicellular unit composed of osteoblasts, osteoclasts, and osteocytes within bone matrix (53). Osteoblasts and adipocytes are derived from mesenchymal stem cells (MSCs) (54). Liraglutide has been found to influence MSC differentiation toward osteoblasts rather than adipocytes (29). In a further exploration of the molecular mechanisms of this effect, it was revealed that GLP-1 increased hMSC proliferation, inhibited the process of their early adipogenesis, and reduced cell death in them. In this study, it was also pointed out that two potential signaling pathways involved in hMSC differentiation into adipocytes might be the targets for GLP-1: MAPK and PKC pathways (55).

Furthermore, investigation of the relevant mechanisms have indicated that GLP-1 acts on bone tissue by promoting bone formation and inhibiting bone resorption. Furthermore, the homeostasis of bone formation and resorption is essential to bone health and might be maintained by GLP-1 in normal bones and restored by GLP-1 in unhealthy bones. However, the specific molecular mechanisms responsible for the effects of GLP-1 have still not been fully elucidated. Therefore, although several studies have been conducted, additional multiple-centered RCTs are needed to analyze different parts of bone tissue in patients with different metabolic statuses, being treated with different versions of GLP-1 RAs. Elucidating the specific processes and associated molecular pathways will aid in clarifying the impact of GLP-1 on bone metabolism and the mechanisms involved.

CONCLUSION AND EXPECTATIONS

Many scientific investigations have focused on the impact and mechanisms of therapies based on insulin-stimulating hormones, such as GLP-1. The evidence indicates that such therapies may enhance BMD and improve bone quality, but the relationship between GLP-1 and bone fractures is still controversial. Further investigations of the relevant mechanisms have indicated that GLP-1 acts on bone tissue by promoting bone formation and inhibiting bone resorption. Furthermore, the homeostasis of bone formation and resorption is essential to bone health and might be maintained by GLP-1 in normal bones and restored by GLP-1 in unhealthy bones. However, the specific molecular mechanisms responsible for the effects of GLP-1 have still not been fully elucidated. Therefore, although several studies have been conducted, additional multiple-centered RCTs are needed to analyze different parts of bone tissue in patients with different metabolic statuses, being treated with different versions of GLP-1 RAs. Elucidating the specific processes and associated molecular pathways will aid in clarifying the impact of GLP-1 on bone metabolism and the mechanisms involved.

AUTHOR CONTRIBUTIONS

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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