Review

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Incretins Beyond the Pancreas: Exploring the Impact of GIP and GLP-1/2 on Bone Remodeling

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Incretin hormones, such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 and 2 (GLP-1, 2), belong to the group of gastrointestinal hormones. Their actions occur through interaction with GIP and GLP-1/2 receptors, which are present in various target tissues. Apart from their well-established roles in pancreatic function and insulin regulation, incretins elicit significant effects that extend beyond the pancreas. Specifically, these hormones stimulate osteoblast differentiation and inhibit osteoclast activity, thereby promoting bone anabolism. Moreover, they play a pivotal role in bone mineralization and overall bone quality and function, making them potentially therapeutic for managing bone health. Thus, this review provides a summary of the crucial involvement of incretins in bone metabolism, influencing both bone formation and resorption processes. While existing evidence is persuasive, further studies are necessary for a comprehensive understanding of the therapeutic potential of incretins in modifying bone health.

Keywords: incretins; GIP; GLP-1; bone remodeling; therapeutic potential

Introduction

The process of bone remodeling serves a dual purpose: renewing bone's structural integrity and maintaining crucial calcium homeostasis. Precise control over calcium absorption or excretion occurs in three distinct reservoirs: (1) the gastrointestinal tract, (2) the skeletal system, and (3) the urinary system, all contributing to regulating serum calcium levels [1]. The human intestine has evolved to optimize nutrient absorption, particularly during postprandial periods. Concurrently, skeletal activity shifts from catabolic to anabolic states during this phase. Recognizing the intestine's role in releasing gut hormones that promote anabolism, notably in the skeletal system, has led to the concept of the entero-osseous axis [2]. Contrary to initial beliefs regarding diurnal variation, fasting appears to moderate bone turnover, showing less influence from circadian hormones [1,2].

This article aims to comprehensively review the impact of incretin hormones, such as glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), and glucagon-like peptide-2 (GLP-2), on bone remodeling. While prior research has established the involvement of GIP and GLP-1/2 in bone metabolism [3–10], a deeper understanding of the underlying mechanisms is required. To address these existing gaps, it is imperative to conduct a more thorough investigation into the precise molecular, biological, and metabolic mechanisms through which GIP, GLP-1, and GLP-2 influence bone remodeling, surpassing the current understanding. This comprehensive review seeks to contribute to advancing new therapeutic avenues for bone-related disorders or enhancing existing treatment modalities.

Bone Remodeling

Bone remodeling stands as a crucial physiological mechanism that consistently renews the skeletal system throughout an individual's lifetime. It plays a pivotal role in preserving and bolstering bone strength, replacing primary, immature, and aged bone that may have encountered microdamage or fractures. Moreover, bone remodeling significantly contributes to the maintenance of calcium homeostasis. Annually, approximately 5% of cortical bone and 20% of trabecular bone undergo this process. Notably, trabecular bone, though representing just a quarter of the total bone volume, exhibits a tenfold higher metabolic rate owing to its larger surface area-to-volume ratio [3,4].

Throughout life, this dynamic process remains constant, yet the balance between resorption and formation experiences fluctuations. In healthy individuals, bone formation takes precedence over the initial three decades of life, culminating in the attainment of peak bone mass [4,5]. Subsequently, bone mass maintenance continues for nearly two decades until resorption begins to surpass production, resulting in a reduction in mass.

In contrast, bone modeling, distinct from remodeling, involves the independent functions of osteoblasts and osteoclasts, leading to bone molding or reshaping. This process initiates before birth, exerting its most significant effects during skeletal growth in early adulthood. However, it persists later in life due to mechanical loading, as suggested by previous studies [4,5].

The conventional model of bone remodeling entails a division into five distinct phases, although there is no universally agreed-upon nomenclature for each stage. In this context, we employ the terms "quiescent", "activation", "resorption", "formation", and "mineralization" (Fig. 1). The "quiescent" phase characterizes bone status before the initiation of remodeling. Subsequently, the "activation" phase is triggered by various events, such as microfractures, mechanical loading, or imbalances in calcium levels due to factors like pregnancy or a nutritionally deficient diet. This phase prepares the bone for remodeling by establishing the bone remodeling compartment (BRC) and recruiting osteoclast precursors. Receptor activator of nuclear factor kappa beta ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) activate these precursors, leading to their attachment to the bone's surface. The "resorption" phase commences, wherein osteoclasts initiate the breakdown of the bone structure, releasing growth factors previously bound within the matrix before undergoing apoptosis. Macrophages undertake the task of clearing debris from the resorption pit, facilitating the transition to subsequent developmental phases. In the initial stages, a collagenous matrix known as "osteoid" is deposited to fill the cavity.

Mineralization occurs over a span of 3 to 6 months, with osteoblasts releasing matrix vesicles that create an environment conducive to mineralization by increasing calcium and phosphorus ion levels. During this biological process, certain osteoblasts become entrapped and undergo osteocytogenesis, while others undergo apoptosis or differentiate into bone lining cells [4,6].

Successful bone remodeling requires the complete removal of the existing bone matrix before the initiation of new bone production. However, the degradation of fibrillar collagen type 1, a major component of bone, is limited to a few collagenases, with cathepsin K and matrix metalloproteinase-9 (MMP-9) being notable exceptions. The challenge lies in the densely packed, helical structure of collagen, shielded by surrounding minerals, which makes it relatively inaccessible [4]. To overcome this challenge, osteoclasts adhere to the bone surface and create a sealed zone through cytoskeletal rearrangement, forming an actin ring. This process involves membrane enlargement and convolution, resulting in the formation of a ruffled membrane with finger-like projections. These structural modifications increase the membrane surface area, facilitating interaction with the bone matrix. Acidification of this compartment initiates the demineralization of the bone matrix, exposing the fibrillar collagen. In this acidic environment, cathepsin K, a cysteine protease presents in lysosomes and released through the ruffled border, plays a role in breaking down the collagenous matrix [4,6].

Serum cross-linked C-telopeptide of type I collagen (CTX) and N-telopeptide (NTx), degradation products of cathepsin K and collagen type 1, respectively, have potential as indicators of bone turnover. Their presence in culture media also serves as an indicator of collagen degradation. Among osteoclasts, MMP-9 exhibits the highest expression levels and possesses the capability to degrade demineralized collagen. Beyond its primary role, it appears to be involved in osteoclast recruitment by converting the inactive form of tumor necrosis factor alpha (TNF-α) into the active cytokine, thereby promoting osteoclast production and survival [6].

Incretin

The incretins encompass a group of gastrointestinal hormones that stimulate insulin secretion in response to meal ingestion, their effectiveness being reliant on glucose availability [7–10]. The insulinotropic actions of the extensively studied incretins—glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1)—operate through distinct G-protein-coupled receptors notably expressed on islet β cells. These receptors for GLP-1 and GIP are also present in various cells beyond the islets of Langerhans, exerting indirect metabolic effects [7–10]. As a result, there is substantial interest in understanding the extrapancreatic influences of incretin hormones [7]. This review primarily focuses on scrutinizing GIP, GLP-1, and GLP-2.

GIP and GLP-1, secreted by enteroendocrine K-cells and L-cells respectively, have been extensively studied for their impact on glucose metabolism, notably as mediators of the incretin effect—an increase in insulin secretion observed after oral glucose intake compared to intravenous glucose injection [8,9]. This rationale has sparked significant interest in their application for managing type 2 diabetes, leading to the common prescription of medications like liraglutide and exenatide, which are GLP-1 receptor agonists (GLP-1RAs). Liraglutide, classified as a GLP-1 receptor agonist, is a pharmaceutical agent used in treating type 2 diabetes. It mimics the function of GLP-1 by stimulating insulin release, reducing glucagon synthesis, and slowing gastric emptying. Furthermore, liraglutide has been studied for its potential impact on weight loss and cardiovascular health, in addition to its diabetes management application [9,10]. It is administered via subcutaneous injection and is available under various brand names, which may differ based on the country and specific use [8–10].

In contrast, GLP-2, also secreted by enteroendocrine L-cells in the small intestine, is primarily recognized for its role as an intestinotropic factor [9,10]. While GIP, GLP-

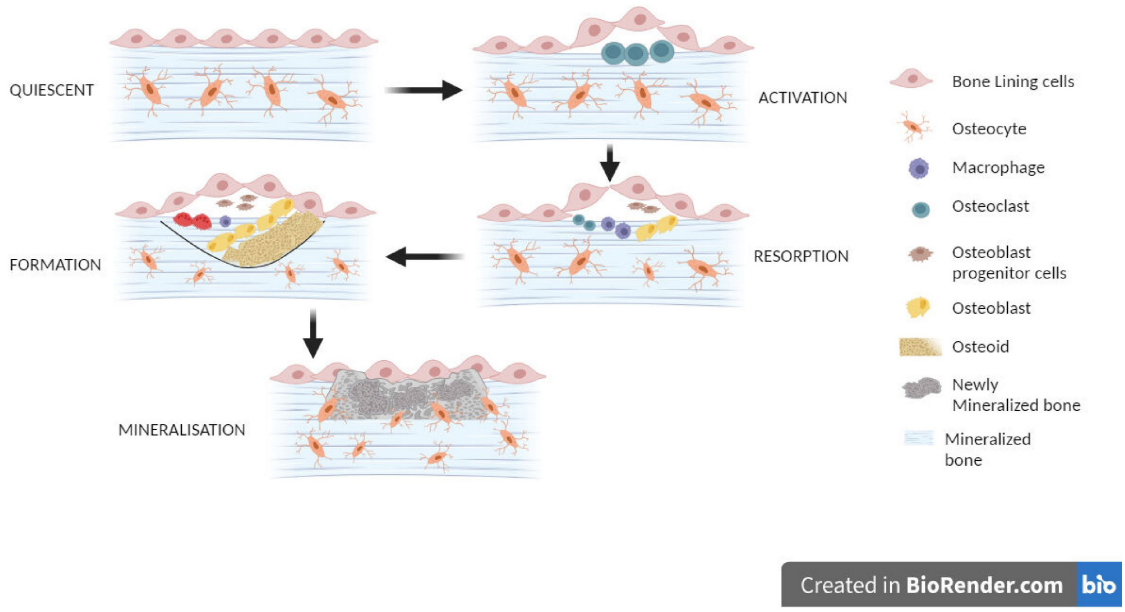

Fig. 1. A short overview of bone remodeling. The figure was drawn with BioRender: Scientific Image and Illustration Software, https://www.biorender.com (Toronto, Ontario, Canada).

1, and GLP-2 are chiefly linked to glucose regulation, new evidence suggests their potential protective effects against bone resorption [9–13]. These effects may stem from their direct actions on bone cells or through indirect mechanisms [9,10].

Incretin in Bone Remodeling

The Impact of GLP-1 on Bone Remodeling

Multiple studies have presented evidence indicating that GLP-1 plays a role in maintaining bone homeostasis, although the exact underlying mechanisms remain unclear. GLP-1 receptors (GLP-1Rs) have been identified in MG63 and immature osteoblastic TE-85 cells, although their presence has not been observed in Saos2 cells [11]. Similarly, in two separate investigations, the existence of GLP-1R was noted in MC3T3-E1 cells, a mouse osteoblast-like cell line [12,13]. Notably, one of these studies revealed a positive correlation between GLP-1R expression and elevated glucose concentrations in the surrounding medium [12]. Another study identified a GLP-1 receptor distinct from the conventional pancreatic-type receptor in MC3T3-E1 cells [14]. However, GLP-1R has not been detected in primary osteoblasts [15] or osteocyte-like cells, such as MLO-Y4 [16]. Conversely, GLP-1R expression has been observed in various cellular components of mice, including bone, bone marrow, primary osteoblasts, and osteoclasts. Additionally, it has been identified in the IDG-SW3 osteocytic cell line but not in the MLO-Y4 osteocytic cell line [17].

In vitro studies have highlighted the importance of GLP-1R activation in bone metabolism. GLP-1 administration exhibited enhanced cell survival and decreased procollagen type 1 N-terminal propeptide (P1NP) secretion in TE-85 and MG-63 cells [11]. Additionally, another study indicated that GLP-1 stimulated the production of c-Fos, a gene crucial for bone cell proliferation and differentiation, particularly in osteoblastic TE-85 cells. Peak expression of c-Fos occurred 60 minutes post-GLP-1 treatment [18].

Furthermore, the GLP-1 receptor agonist (GLP-1RA) exendin-4 was shown to enhance proliferation, differentiation, and mineralization in mouse osteoblast-like MC3T3-E1 cells by activating the mitogen-activated protein kinases (MAPK) pathway [13]. Similarly, in one study, liraglutide augmented the proliferation and differentiation of these cells [13]. However, another investigation involving cell culture in an osteogenic differentiation medium reported that liraglutide hindered differentiation, evidenced by reduced levels of alkaline phosphatase (ALP) and osteocalcin [19]. Regarding bone marrow-derived mesenchymal stem cells (BMSCs), it was observed that GLP-1 inhibited adipogenic development while promoting osteogenic differentiation [15,20]. Exendin-4 and liraglutide administration increased the number of osteoclasts but reduced the resorbed area when administered to mature osteoclasts [16]. Furthermore, GLP-1RAs were found to impact osteoclasts by promoting their development while simultaneously decreasing the resorbed area [17]. Additionally, GLP-1RAs reduced the expression of Sclerostin (SOST)/sclerostin in osteocyte-like MLO-Y4 cells [16].

Several *in vivo* studies conducted on rodents have emphasized the role of GLP-1 in bone metabolism. In mice subjected to ovariectomy, the administration of exendin-4 and liraglutide demonstrated beneficial effects, particularly on trabecular bone, while showing no discernible im-

pact on cortical bone. Notably, exendin-4 resulted in increased calcitonin levels and decreased serotonin levels, whereas liraglutide did not exhibit these effects. Additionally, both exendin-4 and liraglutide promoted osteoclast differentiation while simultaneously reducing the area of bone resorption [17]. In another study involving mice with streptozotocin-induced type 1 diabetes mellitus (T1DM), the short-term administration of liraglutide did not significantly affect bone loss. Micro-computed tomography (micro-CT) evaluations of trabecular bone microstructure and assessments of factors related to bone formation revealed no remarkable impact on these parameters. However, liraglutide was found to enhance the material characteristics of the tissue [21].

Furthermore, studies observed that mice with knockout of the *GLP-1R* gene, as well as animals with knockout of both incretin receptors, demonstrated a decline in bone quality and strength, along with a reduction in cortical area. These mice also exhibited a decrease in collagen maturity [22,23].

In rats with normal physiology, insulin resistance, and type 2 diabetes mellitus (T2DM), GLP-1 administration has demonstrated a positive impact on trabecular bone structure. Additionally, in all three conditions, there was an upregulation of osteoprotegerin and osteocalcin levels, while the increase in RANKL was observed only in T2DM rats [24].

In hyperlipidemic rats showing osteopenia, short-term administration of GLP-1 or exendin-4 restored bone quantity and quality, accompanied by elevated levels of osteocalcin and osteoprotegerin [25].

Rats undergoing ovariectomy and treated with the GLP-1 receptor agonist liraglutide exhibited improvements in trabecular number, volume, thickness, and increased areal bone mineral density (aBMD) compared to a control group [20]. Similarly, exendin-4 treatment resulted in increased aBMD and bone mineral content (BMC) measured by dual-energy X-ray absorptiometry (DXA), improved trabecular structure by micro-CT, and enhanced bone strength. Gene analysis indicated an elevation in bone formation indicators (ALP, osteocalcin, and P1NP) along with a reduction in the bone resorption marker CTX following exendin-4 administration [26]. In Goto-Kakizaki rats with natural diabetes, liraglutide administration restored impaired areal bone mineral density (aBMD). Similarly, exendin-4 treatment improved trabecular structure, aBMD, and bone strength [15]. Furthermore, exendin-4 administration increased femoral aBMD in otsuka long-evans tokushima fatty strain) OLETF rats with type 2 diabetes mellitus and led to decreased sclerostin levels and increased osteocalcin levels [16].

In human studies, findings regarding the impact of GLP-1 agonists on bone health have shown inconsistency. In a randomized controlled study, the administration of liraglutide revealed promising results in mitigating the adverse effects of weight loss on bone health [27]. After an 8-week weight-loss program, 37 women were divided into two groups for a subsequent 52-week weight-maintenance phase: a control group (19 women) and a liraglutide group (18 women). Dual-energy X-ray absorptiometry (DXA) scans revealed a significant decrease in bone mineral content (BMC) in the control group, while no such decline was observed in the liraglutide group. Additionally, the liraglutide group exhibited a substantial increase in P1NP levels, indicative of enhanced bone formation, whereas the control group did not show a similar increase. These findings suggest that liraglutide's protective effects on bone health are likely attributed to promoting bone formation. The study did not find a significant impact on bone resorption, as assessed by plasma CTX [27]. This aligns with a previous investigation where subcutaneous GLP-1 administration showed no effect on CTX levels in a group of seven healthy individuals [28,29].

Several meta-analytical studies have explored the potential relationship between GLP-1 analogs, particularly liraglutide and exenatide, and the risk of fractures in individuals with type 2 diabetes. These investigations did not reveal any significant correlation between the use of GLP-1R agonists and changes in areal bone mineral density (aBMD), a measure of bone density in specific body areas [30]. Moreover, the administration of GLP-1R agonists did not appear to affect the risk of bone fractures [31,32].

However, an alternative meta-analysis of randomized controlled trials suggested that while liraglutide reduced the risk of fragility fractures, exenatide was associated with an increased risk [33]. In summary, multiple studies provide evidence of GLP-1's influence on bone metabolism, including its ability to stimulate osteoblast activity and suppress osteoclasts. The effects of GLP-1R agonists on bone health in patients with type 2 diabetes mellitus have been extensively researched. For instance, in a 52-week clinical study [34], the impact of GLP-1R agonists on bone mineral density was examined, shedding light on the long-term effects of these medications on bone health.

Ongoing research has delved into the role of endogenous gastrointestinal hormones like GIP and GLP-1 in postprandial bone homeostasis, shedding light on their physiological mechanisms and influence on bone metabolism [35]. These investigations have expanded our understanding of how the body maintains bone health after meals, contributing to the broader comprehension of bone metabolism. A meta-analysis of randomized controlled trials has been instrumental in evaluating the risk of bone fracture in patients with type 2 diabetes using glucagon-like peptide-1 receptor agonists. Such analyses offer crucial insights into the safety profile of these medications concerning bone health [36].

Moreover, studies have explored the inhibitory effects of GIP on bone resorption, independent of insulin and glycemia, contributing to our evolving knowledge about the intricate interactions between gut hormones and bone

metabolism [37,38]. Together, these research efforts contribute significantly to elucidating the relationship between GLP-1 receptor agonists, GIP, and bone health, offering valuable insights for clinicians and researchers in this field.

Regarding available medications, exenatide is offered in two formulations: immediate-release (Byetta) and extended-release (Bydureon). Additionally, many other GLP-1 receptor agonists are accessible, each with distinct properties and characteristics:

- Dulaglutide, an agonist for the GLP-1 receptor, administered once a week via injection.

- Albiglutide, a GLP-1 agonist administered through weekly injections for the treatment of type 2 diabetes.

- Semaglutide, available in oral and injectable forms, with the injectable version given once a week.

- Lixisenatide, an injectable medication functioning as a GLP-1 agonist, primarily used in the management of type 2 diabetes [39].

The Impact of GLP-2 on Bone Remodeling

The initial human trial conducted in 2001 revealed that a 5-week treatment regimen using natural GLP-2 resulted in a significant increase in spinal areal bone mineral density (aBMD) among individuals with short bowel syndrome (SBS) lacking terminal ileum and colon [40,41]. Following this, Nissen *et al*. [28] conducted a study where they demonstrated that subcutaneous delivery of GLP-2 to healthy postmenopausal women led to a decrease in bone resorption, measured through CTX levels. The reduction in bone resorption was observed to be dose-dependent, with doses of 200, 400, and 800 µg resulting in varying degrees of reduction. Notably, the researchers observed that bone formation, assessed by osteocalcin levels, remained unaffected by the administration of GLP-2. Additionally, subcutaneous administration of GLP-2 at bedtime effectively suppressed nighttime bone resorption, evidenced by the reduction in CTX levels [42].

In a 14-day study, participants tolerated the administration of GLP-2 through nighttime injections at doses of 1.6 mg and 3.2 mg quite well. This treatment led to a decrease in CTX levels while showing no impact on markers associated with bone production, such as osteocalcin and P1NP [43]. Following four months of administration, GLP-2 medication showed a significant and dose-dependent increase in hip areal bone mineral density (aBMD), amounting to approximately 1%. Importantly, there were no indications of GLP-2 antibodies or tachyphylaxis observed during this period [44].

Contrary to earlier research demonstrating a positive impact of GLP-2 on aBMD in patients with Short Bowel Syndrome (SBS) [41], it was revealed that the decrease in CTX levels following the administration of exogenous GLP-2 is reliant on the presence of a functional small intestine [45,46]. This suggests that the effect of GLP-2 on CTX levels is indirectly mediated through its interaction with the intestine. Additionally, it was discovered that GLP-2 resulted in a reduction in parathyroid hormone (PTH) levels among individuals without any intestinal impairments, suggesting that PTH might serve as a plausible intermediary in the GLP-2-induced decline in CTX [45,46].

A study investigated the impact of high concentration, delivered through intravenous injection, versus sustained exposure, administered via subcutaneous injection, of GLP-2 in a cohort of healthy individuals [47]. The researchers found that prolonged exposure led to greater efficacy in reducing circulating CTX levels compared to acute exposure to high concentrations. Prior administration of the dipeptidyl peptidase-4 (DPP-4) inhibitor sitagliptin increased plasma concentrations of GLP-2, but this elevation did not further affect CTX levels [47].

While GLP-2 has demonstrated influence on osteoclast activity, it's important to note that the GLP-2 receptor (GLP-2R) hasn't been detected in human osteoclasts or other bone cells. However, the presence of this receptor has been identified in TE-85 and MG-63 cell lines, representing immature human osteoblasts [18].

To summarize, GLP-2 demonstrates a significant ability to suppress bone resorption without affecting bone production, ultimately leading to a visible enhancement in bone mineral density. Current research suggests that higher-than-physiological levels of exogenous GLP-2 alone can decrease bone resorption, notably indicated by CTX levels. However, the exact mechanism by which GLP-2 influences bone metabolism remains undisclosed. Potential mechanisms of action could involve direct interaction with bone cells or indirect modulation, possibly through involvement with other intestinal components.

In a recent preliminary study, the subcutaneous administration of GIP and GLP-2 effectively inhibited nighttime bone resorption in postmenopausal women. These findings underscore the potential of these gut hormones in modulating bone metabolism in this population, illuminating potential therapeutic avenues [47,48].

Furthermore, a randomized, placebo-controlled, crossover study delved into the separate effects of GLP-2 and GIP on bone turnover in healthy young men. This research revealed distinct influences of these hormones on bone health, contributing to our understanding of their roles in bone metabolism [47–49].

Additionally, a study explored the diurnal variation of bone formation in adult patients with type 2 diabetes. Their findings highlighted an altered diurnal pattern in bone formation in this patient group, emphasizing the importance of comprehending the impact of metabolic conditions on bone health [50].

These studies collectively provide valuable insights into the complex interplay between gut hormones, bone metabolism, and metabolic disorders, offering promising avenues for further investigation and potential therapeutic interventions.

The Impact of GIP on Bone Remodeling

The expression of the gastric inhibitory polypeptide receptor (GIPR) has been noted in cell lines derived from both osteoblasts and osteoclasts [11,12], along with primary cultures of osteoclasts and osteoblasts in mice [51]. Interestingly, GIPR expression was observed to increase in response to elevated glucose concentrations in the surrounding medium. Moreover, the presence of GIPR has been confirmed in human BMSC [12]. There appears to be variability in the maturity levels among osteoblastic cell lines, potentially linked to the expression of GIPR. Furthermore, the effects of GIP stimulation on bone markers such as ALP, P1NP, and cell viability exhibit variability across different cell lines [12].

GIP has shown the ability to elevate intracellular calcium levels ($[Ca^{2+}]i$) and cyclic adenosine monophosphate (cAMP), along with enhancing the expression of procollagen type 1 N-terminal propeptide (P1NP) and ALP activity [12]. Additionally, administration of [specific intervention] has been observed to upregulate c-Fos, a crucial regulator involved in the processes of bone cell proliferation and differentiation [12].

Additionally, research has indicated that GIP enhances collagen maturation and increases fibril width, a process dependent on cyclic adenosine monophosphate (cAMP) [52,53]. Notably, GIP exhibits an inhibitory effect on PTH-induced bone resorption, observed in primary murine osteoclast cells [51]. Another study has highlighted GIP's capability to reduce the production and resorption of osteoclasts in primary cultures of both human and murine origin. Importantly, this decrease in differentiation is not influenced by the traditional adenylyl-cyclase-cAMP-dependent protein kinase (PKA) pathway. Administration of GIP resulted in a reduction in the elevation of intracellular calcium concentration ($[Ca^{2+}]i$) induced by RANKL. Additionally, GIP decreased the activity of calcineurin and impeded the translocation of Nuclear Factor of Activated T Cells 1 (NFATC1), a downstream target of RANKL crucial in the final stages of osteoclast development [53].

The initial *in vivo* investigation supports the beneficial influence of endogenous GIP on bone density in rats undergoing ovariectomy (OVX) [54]. Peptides resistant to dipeptidyl peptidase-4 (DPP-4) indicate either anabolic or anti-resorptive effects. For instance, the utilization of an N-terminally modified GIP agonist (N-AcGIP) exhibits enhanced cortical bone characteristics in rats and reduces osteoclast-induced bone resorption in mice subjected to ovariectomy. In a murine experimental model of T1DM, it was observed that brief treatment with Dipeptidyl peptidase IV-resistant GIP prevented a decline in bone formation characteristics. Previous studies also indicated that this specific therapy has the potential to enhance mechanical properties at the tissue level [27].

Furthermore, a research investigation conducted on a murine model exhibiting type 2 diabetes mellitus (T2DM) noted that a peptide hybrid comprising GIP and oxyntomodulin, which stimulates GIP, GLP-1, and glucagon receptors, exhibits augmented cortical bone strength [55].

There are two distinct types of GIPR knockout mice characterized by the extent of exon deletion. Both genotypes display altered bone characteristics; however, there is conflicting evidence regarding some outcomes. The initial GIPR knockout mouse, with exon 4-5 deleted, showed reduced bone formation parameters, including aBMD, osteocalcin BMC, ALP, and trabecular bone volume [56,57]. The double incretin knockout (KO) animal, involving simultaneous knockout of the GIPR and the GLP-1R, displayed diminished cortical characteristics and decreased bone strength [57]. The presence of a congenital defect in the GIP hormone aligns with significant involvement in bone metabolism. This deficiency correlates with reduced bone volume and trabecular count, along with an increase in osteoclast surfaces [58].

Conversely, an upregulation of GIP is associated with enhanced bone production, characterized by elevated bone mass, increased osteoblast count, higher levels of osteocalcin, and suppressed bone resorption, evident through reduced pyridinoline crosslinks and decreased osteoclast population [59].

In a preliminary human study, short intravenous GIP treatment did not affect bone resorption. However, subsequent research revealed a 50% decrease in CTX after oral glucose intake and a 30% reduction after intravenous glucose administration. Moreover, higher levels of incretin hormones correlate with greater declines in CTX levels [60,61]. Two consecutive experiments demonstrated that GIP infusion directly inhibits CTX levels irrespective of blood glucose levels, and this effect is not influenced by insulin [62].

A loss-of-function polymorphism (E354Q) in the *GIPR gene* is associated with a decline in areal bone mineral density (aBMD) in DXA scans conducted on 1424 perimenopausal women over ten years. Additionally, a fracture analysis over 16 years revealed a 50% increased fracture risk in individuals carrying this mutation [63]. The cumulative evidence from studies conducted on both human subjects and rodents strongly indicated that GIP plays a pivotal role as a direct regulator of bone metabolism. It has been observed to have direct anabolic effects on osteoblasts, promoting bone formation, while also exhibiting anti-resorptive actions on osteoclasts, thereby inhibiting bone breakdown.

The investigation into GIP's effects on bone metabolism revealed that its actions can be diminished by the selective GIP receptor antagonist [64,65], shedding light on the potential targeting of GIP to influence bone metabolism [65]. Additionally, a study explored the differential impact of intravenous and oral glucose administration on bone turnover markers in healthy male subjects [60]. Understanding how glucose administration affects

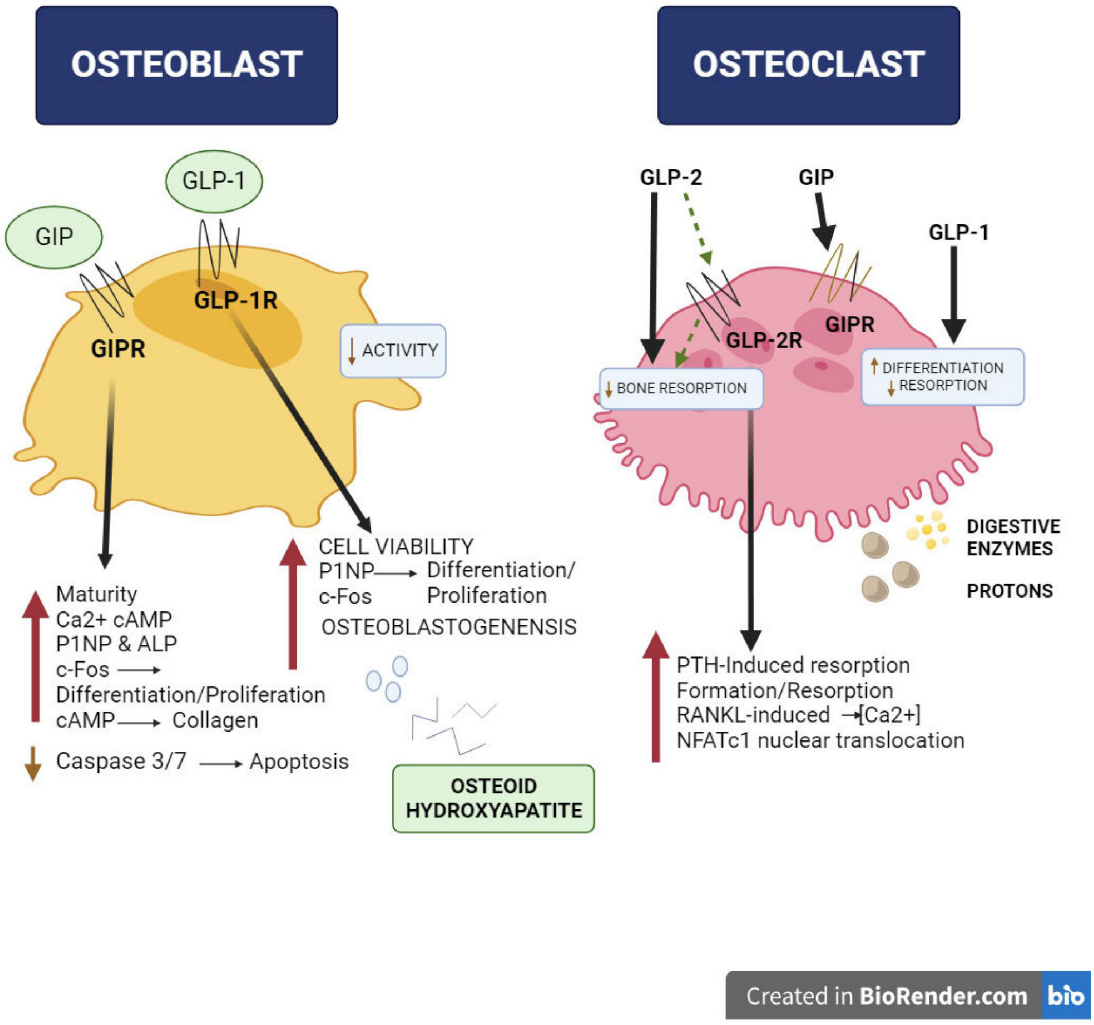

Fig. 2. GLP-1, GLP-2 and GIP, in Bone Metabolism. GLP-1, GLP-2 and GIP have been identified as potential regulators of bone metabolism. Their effects on osteoblast and osteoclast activity can be categorized as either anabolic or catabolic. The figure was drawn with BioRender: Scientific Image and Illustration Software, https://www.biorender.com (Toronto, Ontario, Canada). GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2; GLP-1R, GLP-1 receptor; P1NP, procollagen type 1 N-terminal propeptide; cAMP, cyclic adenosine monophosphate; ALP, alkaline phosphatase; GIPR, gastric inhibitory polypeptide receptor; RANKL, receptor activator of nuclear factor kappa beta ligand; NFATC1, Nuclear Factor of Activated T Cells 1.

bone turnover provides crucial insights into the interplay between metabolic factors and bone health [60,61]. These studies collectively advance our understanding of the intricate interactions among GIP, glucose metabolism, and bone homeostasis, offering potential pathways for further research and the development of therapeutic strategies.

Exploring Therapeutic Possibilities for Bone Disease; Osteoporosis

Osteoporosis is a pathological condition characterized by reduced bone density and an increased susceptibility to fractures. It presents a growing challenge for healthcare systems and carries significant economic implications, as decreased bone strength elevates the risk of fractures, a leading cause of morbidity. Fractures often necessitate hospitalization and immobilization, potentially leading to complications. Moreover, the recovery process typically involves slow and incomplete restoration of function [64,66]. Factors contributing to decreased bone mass and structural changes include both internal factors, such as reduced estrogen levels in postmenopausal women (seen in primary osteoporosis), and external factors like malnutrition. This imbalance in the bone remodeling mechanism significantly increases the risk of pathological fractures [66–68].

The current treatment options for osteoporosis are limited in their effectiveness and often are associated with unwanted side effects. Consequently, ongoing research focuses on developing innovative drugs that target specific molecules crucial for maintaining bone homeostasis. Examples include inhibitors of the Wnt signaling pathway,

like sclerostin and dickkopf-1, as well as inhibitors of cathepsin K, a protein essential for the resorption process produced by osteoclasts [69].

Research has explored the potential therapeutic use of GLP-2 for osteoporosis, leading to human studies investigating the immediate and long-term effects of daily GLP-2 injections on bone remodeling [43,47,66–68,70]. Despite effectively suppressing osteoclast activity and showing significant improvement in areal bone mineral density (aBMD) over a four-month treatment period, GLP-2 has not yet been successfully introduced as a novel pharmaceutical option for managing osteoporosis. However, it's important to note that a GLP-2 analog called teduglutide received the United States Food and Drug Administration (FDA) approval in 2012 for the treatment of short bowel syndrome (SBS) due to its beneficial impact on intestinal functionality [70].

The exploration of GLP-1-based medications' potential impact on bone metabolism holds promise for expanding their therapeutic applications or developing drugs with a more specific focus on bone health. GLP-1 agonists have known effects on appetite regulation through hypothalamic modulation, which could potentially influence their efficacy as therapeutic agents for osteoporosis, considering the significant impact of food intake on skeletal health.

An alternative approach in developing pharmaceuticals targeting the gut-bone axis involves targeting multiple receptors rather than a single receptor. Employing a multi-agonism approach may yield synergistic outcomes, as evidenced in various studies focusing on managing metabolic disorders, including type 2 diabetes mellitus (T2DM) [55, 71–73]. This strategy can pave the way for more effective and targeted therapeutic interventions in bone metabolism and related disorders.

The gastrointestinal tract's role in regulating bone tissue balance is evident from various substances originating in the gut that impact both bone production and resorption processes (Fig. 2). Current therapeutic choices for osteoporosis are limited. The promising characteristics of G-protein coupled receptors (GPCRs) as viable drug targets have spurred significant interest in the emergence of novel pharmaceuticals. Specifically, there's a growing interest in developing drugs that target gut hormone receptors, presenting a potential avenue for managing osteoporosis more effectively.

Conclusions and Future Perspective

The link between metabolic hormones, notably incretins, and bone remodeling has gained significant attention. The role of incretins in preserving bone mass and enhancing bone quality and strength is increasingly recognized. However, the challenge ahead lies in unraveling the precise mechanisms through which incretins exert their influence on skeletal physiology. A comprehensive understanding of these mechanisms is crucial to grasp the intricate interplay between metabolic regulation and bone health.

Future research should prioritize uncovering the signaling pathways, cellular interactions, and molecular mechanisms that underlie how incretins impact bone tissue. This knowledge will not only advance our comprehension of bone physiology but also pave the way for the development of innovative therapeutic approaches. Ultimately, this could significantly improve the lives of individuals affected by bone-related ailments and metabolic disorders. While the association between incretins and bone health is evident, deeper exploration into the molecular complexities of this relationship is essential to fully harness its potential in enhancing skeletal health.

Author Contributions

HZ, JS and ZR designed the research study; HZ and JS collected and analyzed the data. ZR has been involved in drafting the manuscript and all authors have been involved in revising it critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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