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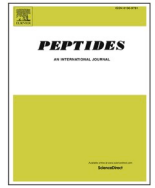
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Gut hormone analogues and skeletal health in diabetes and obesity: Evidence from preclinical models

Guillaume Mabileau^{a,b,*}, Béatrice Bouvard^{a,c}

^a Univ Angers, Nantes Université, ONIRIS, Inserm, RMeS, UMR 1229, SFR ICAT, Angers F-49000, France

^b CHU Angers, Département de Pathologie Cellulaire et Tissulaire, UF de Pathologie osseuse, Angers F-49933, France

^c CHU Angers, Service de Rhumatologie, Angers F-49933, France

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ABSTRACT

Diabetes mellitus and obesity are rapidly growing worldwide. Aside from metabolic disturbances, these two disorders also affect bone with a higher prevalence of bone fractures. In the last decade, a growing body of evidence suggested that several gut hormones, including ghrelin, gastrin, glucose-dependent insulinotropic polypeptide (GIP), glucagon, and glucagon-like peptide-1 and 2 (GLP-1 and GLP-2, respectively) may affect bone physiology. Several gut hormone analogues have been developed for the treatment of type 2 diabetes and obesity, and could represent a new alternative in the therapeutic arsenal against bone fragility. In the present review, a summary of the physiological roles of these gut hormones and their analogues is presented at the cellular level but also in several preclinical models of bone fragility disorders including type 2 diabetes mellitus, especially on bone mineral density, microarchitecture and bone material properties. The present review also summarizes the impact of GLP-1 receptor agonists approved for the treatment of type 2 diabetes mellitus and the more recent dual or triple analogue on bone physiology and strength.

1. Introduction

Bone tissue is a living mineralized material, composed of an organic layer, mainly composed of type I collagen, and a mineral phase mostly composed of poorly crystalline hydroxyapatite crystals [1]. Bone tissue is constantly remodeled in mass, architecture and material properties to adapt to growth, ageing and mechanical stress [2]. Bone is also a tissue with multiple functions: (i) it acts as a calcium, phosphate and sodium reservoir and plays a central role in the calcium/phosphate homeostasis [3]; (ii) it has a strong biomechanical function as its whole micro-architecture, from the molecular to the anatomical level, is controlled by mechanical strains [1]. To maintain a suitable “bone health”, bone is continuously remodeled by bone cells. Osteoblasts, derived from the mesenchymal lineage, are responsible for depositing and mineralising the bone matrix; osteoclasts, derived from a hematopoietic precursor, are responsible for bone resorption, and osteocytes, derived from osteoblasts, sense mechanical stress and orchestrate bone remodelling [4]. However, when bone remodelling is altered, bone tissue can sustain some macroscopical fracture with the consequence on the quality of life of affected individuals [5]. Resistance to fracture is determined by two main factors represented by bone quantity and

quality. Bone quality is an umbrella term encompassing the bone microarchitecture and material quality. Alterations of bone quantity and/or quality leads to the development of bone fragility.

Diabetes and changes in body composition caused by obesity or excessive weight loss affect bone strength. Diabetes mellitus and obesity are global health issues that have a significant impact on national economies by reducing productivity and life expectancy while increasing disability and healthcare costs [6,7]. In 2021, it was estimated that there were 537 million people worldwide suffering from diabetes mellitus and around 650 million people affected by obesity [6, 8]. Unfortunately, projections for the next decade estimate even higher numbers, with approximately 643 million individuals with diabetes mellitus and one billion individuals with obesity.

Diabetes mellitus is characterized by either a decrease in production of insulin (type 1 diabetes mellitus – T1DM) or by resistance to insulin (type 2 diabetes mellitus – T2DM) that ultimately result in higher circulating glucose concentrations. Bone fractures are a significant health problem for people with diabetes mellitus, although it is often neglected or not a primary concern of physicians. The etiology of bone fractures in diabetes mellitus has been the subject of several reviews, and it is known that they can occur due to an increased risk of falls, low

* Correspondence to: Inserm UMR_S 1229 RMeS, REGOS Team, Institut de Biologie en Santé, Université d'Angers, 4 rue Larrey, Angers F-49933, France.

E-mail address: guillaume.mabileau@univ-angers.fr (G. Mabileau).

bone quality, and low bone turnover [9–11]. Prevention and management of fractures in diabetic patients involve improving glycemic control and reducing falls. The Bone and Diabetes Working Group of the International Osteoporosis Foundation has proposed an algorithm for the identification and management of diabetic patients at increased risk of fracture [12,13]. However, the optimal approach to fracture prevention in patients with diabetes, taking into account the structural determinants (microstructure, material properties, ...), has not yet been established.

The interaction between obesity and bone metabolism is complex and not fully understood. Even though the majority of the studies suggest that obesity has a favorable effect on bone density, it is unclear what the effect of obesity is on skeletal microarchitecture. Additionally, the effects of obesity on skeletal strength might be site dependent as obese individuals are at higher risk of certain fractures such as the lower limbs, proximal humerus and ankle [14–16].

Several new treatments for type 2 diabetes mellitus and obesity are currently developed and based on mimetism with gastrointestinal hormones. Indeed, the gastrointestinal epithelium is a single layer of different cell types, including enteroendocrine cells (EECs). EECs, which account for only about 1% of the epithelial cell population, represent the largest endocrine organ in the body. Among the bioactive peptides secreted by the EECs, several hormones have emerged as important modulators of energy metabolism. Interestingly, some of these hormones have also been reported to modulate bone metabolism, at least in bone cell culture in vitro, or in preclinical animal models. Among them, incretins are hormones that are secreted from the intestine in response to glucose and stimulate insulin release in a glucose-dependent manner [17]. The two main incretins identified are glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1).

Dysregulation of incretin secretion and actions are noted in diseases such as obesity and diabetes, and GLP-1 has been the basis for the development of GLP-1 receptor agonists, which are approved for the treatment of type 2 diabetes mellitus and obesity. More recently, double and triple analogues of gut hormones are being specifically developed for the treatment of type 2 diabetes mellitus, obesity or bone fragility.

Recent research in preclinical animal models has shown potential beneficial effects of several gut hormone analogues in bone physiology. The goal of the present review is to propose an up-to-date comprehensive summary of the actions of gut hormone and their analogues on bone physiology and strength.

2. Gastric hormones

2.1. Ghrelin

Ghrelin is a peptide hormone known for its role in stimulating appetite and feeding behavior, energy homeostasis, growth hormone secretion and carbohydrate metabolism. It is secreted by closed-type EEC X/A cells (P/D1 cells in humans) of the gastric fundus/body during fasting by action of the sympathetic nervous system [18,19]. Its secretion is suppressed following food ingestion [20]. To activate its only known receptor, GHSR1a, ghrelin requires acylation at its serine 3 residue by the ghrelin O-acyl-transferase, a member of the membrane-bound O-acyltransferase family within the X/A cell [21]. Ghrelin is also secreted in its nonacylated form, which is not biologically active and has an unclear physiological function [18]. GHSR1a is expressed predominantly in the pancreatic islets, adrenal gland, thyroid, myocardium and in the CNS, including the anterior pituitary gland, hypothalamic arcuate nucleus, hippocampus and brain stem [22].

The link between ghrelin and skeletal physiology was first established by Larsson et al. who showed that gastric fundal extracts were capable of inducing a rise in intracellular calcium in human, rat and mouse osteoblasts [23]. To date, the expression of GHSR1a in osteoblasts and osteoclasts remains controversial. However, in osteoblast cultures from different species, ghrelin stimulates osteoblast

proliferation and differentiation, and reduces apoptosis through activation of MAPK, PI3K, beta-catenin and Gi/cAMP pathways [24–28]. In osteoclast cultures, ghrelin increases osteoclast resorption in rat but not in mouse cultures [29]. Interestingly, ghrelin-null and ghrelin-receptor-deficient mice have no differences in bone mineral density (BMD) when compared with WT littermates [30,31] confirming that gastric hormones do not modulate bone turnover markers. As ghrelin is one of the growth hormone secretagogues, one could wonder whether the lack of effects of ghrelin in KO animals could be due to compensatory effects of GH or other GH secretagogue. In an elegant study, Fukushima and colleagues infused ghrelin into SD rats but also into severely GH-deficient animals (SDR rats) [26]. Interestingly, in both animal strains, ghrelin augmented BMD suggesting that the classical GH-IGF1 pathway was not involved in this anabolic effect. Furthermore, Choi and colleagues investigated the effects of chronic intracerebroventricular injection of ghrelin on bone metabolism in SD rats and showed an anabolic effect of exogenous ghrelin on bone mass, although the exact central mechanism could not be determined [32]. Interestingly, similar experiments performed in C57BL/6 J mice failed to evidence any effects of central ghrelin on bone metabolism [33]. In humans, no strong positive association was found between serum acylated ghrelin and BMD in post-menopausal women, girls with anorexia nervosa or overweight boy [34–38]. Despite positive effects in osteoblasts stimulated by exogenous ghrelin in rodents, the lack of consistent effects in preclinical models and human studies remain inconclusive regarding a possible role of ghrelin in controlling BMD and bone strength. Fig. 1 recapitulates the bone action of ghrelin.

2.2. Gastrin

Gastrin is a peptide hormone primarily responsible for enhancing gastric mucosal growth, gastric motility, secretion of histamine by ECL cells and hydrochloric acid (HCl) by parietal cells [39]. It is secreted by G cells, localized in the gastric antrum and duodenum. Gastrin is released primarily in response to vagal and gastrin-releasing peptide (GRP) stimulation secondary to the ingestion of peptides, amino acids, gastric distention, and an elevated stomach pH [40]. Conversely, gastrin release is decreased in response to paracrine inhibition by somatostatin and decreased stomach pH. Also, and in parallel with hormonal regulation of calcium homeostasis, extracellular calcium and hypercalcemia stimulate gastrin secretion through activation of the CaSR expressed by G cells [41]. Gastrin acts through the CCKB receptor (CCKBR) expressed mostly in the stomach, pancreas and brain. However, conditions with hypergastrinemia have already been linked to skeletal metabolism. Total or segmental gastrectomy, and long-term use of proton pump inhibitors in humans induces loss of bone density and bone fragility [42]. These conditions are associated with hypergastrinemia and hypochlorhydria. Consistent with these observations, deletion of the *Cckbr* gene, which encodes the gastrin receptor, also results in hypochlorhydria, secondary hyperparathyroidism and ultimately to osteoporosis and bone fragility, and hypergastrinemia [43]. Recently, Mihara and colleagues also reported changes of bone material properties in *Cckbr* KO mice with alteration of hydroxyapatite crystallite orientation within the bone matrix that could reduce the bone toughness [44]. As elevated circulating histamine in systemic mastocytosis predisposes to osteoporosis, one could wonder whether gastrin-mediated histamine secretion from ECL cells could indirectly affect calcium homeostasis and thus bone metabolism. In H^+/K^+ ATPase beta subunit KO mice, a model that mimics the action of proton pump inhibitors, administration of netazepide, a selective CCKBR antagonist, did improve bone strength and rescued bone fragility [45]. In the same animal model, administration of cetirizine, a selective histamine 1 receptor (H1R) antagonist, did not rescue the osteoporotic phenotype, suggesting that bone fragility was not due to H1R activation [46]. However, osteoclasts and osteoblasts express both the H1R and the H2R [47] and as such, action of histamine on H2R could still represent a mechanism. Furthermore, gastric calcium

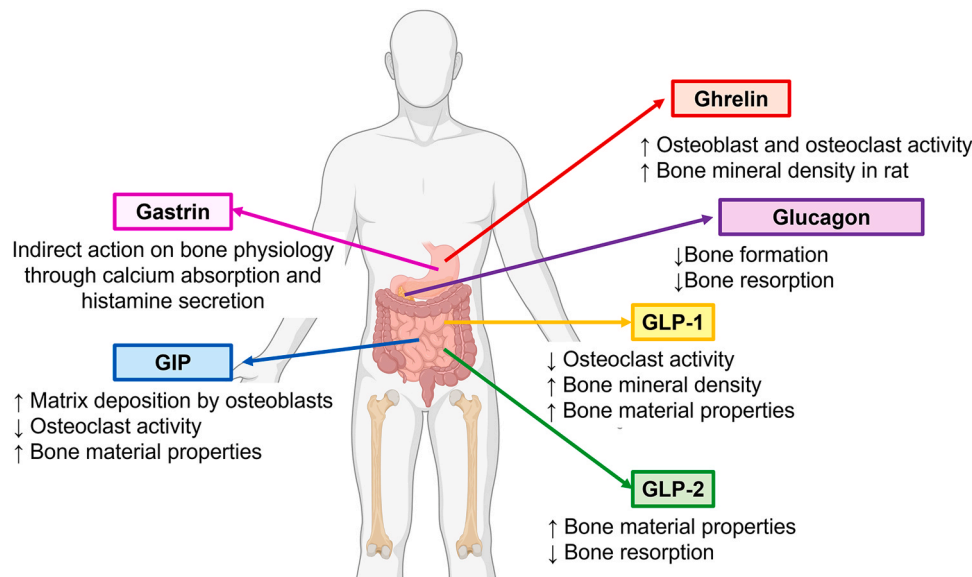


Fig. 1. Summary of action of gastric and gut hormones on bone physiology.

solubility is pH-dependent and impaired gastric acidification as seen in hypochlorhydria, induces calcium malabsorption, which negatively affects bone properties through secondary hyperparathyroidism. Netazepide administration significantly reduced intragastric pH [45] and the observed improvement in bone strength might simply result of better calcium absorption. The effects of gastrin on skeletal physiology remains to be fully elucidated but first evidence suggest indirect effects through calcium absorption and possibly histamine secretion.

3. Pancreatic and gut hormones

3.1. Glucose-dependent insulinotropic polypeptide (GIP)

GIP, or glucose-dependent insulinotropic polypeptide, is a hormone that is primarily produced and secreted by intestinal K-cells, which are located in the proximal regions of the small intestine. K-cells are specialized cells that are highly polarized, with their GIP-containing secretory granules concentrated at the basal pole of the cell [48,49]. Upon stimulation, GIP is released through the basolateral membrane [48,49]. The secretion of GIP from K-cells is regulated by various factors. Neural stimuli, hormones, and the contents present in the intestine can all influence GIP secretion [50]. The close association of K-cells with the capillary network in the lamina propria allows GIP to rapidly enter the bloodstream after its secretion. In addition to affecting GIP secretion, intraluminal contents also play a role in GIP expression. Both glucose and lipids have been shown to be potent stimulators of GIP gene transcription [51,52].

The GIP receptor (GIPr) is expressed in various tissues, including bone. A functional GIPr has been found in human and murine osteoblast-like cells. Upon activation, it increases intracellular cAMP and calcium levels, resulting in the expression and deposition of type I collagen [53, 54]. Additionally, GIP accelerates post-translational enzymatic collagen crosslinking by increasing lysyl oxidase activity in a dose-dependent manner [54]. This allows for greater deformation of the extracellular matrix. The expression of GIPr in murine osteoblasts is enhanced by glucose treatment in the presence of BMP-2 [55]. A functional GIPr has also been discovered in human and rodent osteoclasts [56–58]. Activation of the GIPr in rodent osteoclasts leads to a dose-dependent reduction in the number of newly formed osteoclasts and inhibits osteoclast-mediated bone resorption by suppressing intracellular calcium oscillation and calcineurin activation [56]. This effect is consistent with the rapid decrease in bone remodeling marker of resorption

observed in humans after exogenous GIP infusion [59–62]. Whole-body depletion of GIPr, as observed in skeletally mature *Gipr* KO mice, has been associated with changes in bone material properties, such as significant reductions in tissue mineralization and enzymatic crosslinking, ultimately resulting in bone fragility [63,64]. The GIPr is also expressed by myeloid lineages, including monocytes and macrophages, as well as some bone marrow T cells [65,66]. The close vicinity between bone marrow and bone cells in the trabecular spaces may also indirectly influence the bone phenotype. Evidence from genetic studies in human initially suggested a detrimental effects of the missense loss-of-function GIPR variants on bone mass. Indeed, the GIPR Glu354Gln (rs1800437) variant has been associated with lower BMD at the femoral neck and total hip, as well as an increased risk of non-vertebral fractures in a small prospective cohort of ~1424 perimenopausal women [67]. Morris et al. identified new loci associated with lower eBMD, an estimated BMD obtained with heel ultrasound rather than conventional dual-energy x-ray absorptiometry, including GIPR Arg190Gln (rs 139215588) and Glu288Gly (rs 143430880) variants in a cohort of 426,824 individuals [68]. However, recent evidence from a large meta-analysis of up to 1.2 million participants challenges this view and did not find association between the three above GIPR variants and fracture risk or BMD assessed by DXA, but rather only an association between the rare GIPR variant Glu288Gly and eBMD [69]. None of these genetical studies investigated bone microstructure or bone material properties. In GIP-GFP knock-in mice, where the *Gip* gene is invalidated by the insertion of a GFP cassette, a significant reduction in BMD is observed [70]. Consistent with this finding, GIP overexpression resulted in higher BMD, suggesting higher trabecular bone mass in the appendicular skeleton [71]. This finding is also supported in humans, where the GIP Gly103Ser (rs2291725) variant was associated with lower BMD [72]. Taken together, rodent and human data suggest that the GIP/GIPr pathway appears to be important for maintaining optimal BMD and quality, allowing bone tissue to resist fracture.

3.2. Glucagon-like peptide-1 (GLP-1)

GLP-1, the second incretin hormone, is produced through post-translational processing of the glucagon gene. In the intestine, this process gives rise to glucagon, GLP-1 and GLP-2 [73]. GLP-1 is secreted by enteroendocrine L-cells, which are distributed throughout the small intestine and colon [74]. The GLP-1 receptor (GLP-1r) also belongs to the class B G protein-coupled receptor and is expressed in various

tissues, including the endocrine pancreas, gastro-intestinal tract, lung, heart, kidney, and several regions of the brain. However, its expression in the osteoblast/osteocyte lineage remains controversial due to the use of poorly-characterized cell lines [75], the selection of non-specific primer pairs [76], or the use of non-selective anti-GLP-1r antibodies [55]. The expression in osteoblast cell lines, such as the widely used murine MC3T3-E1 osteoblastic cell line, has not been found [77,78]. Nuche-Berenguer and coworkers suggested that GLP-1 acts in osteoblasts through a second receptor, distinct from the known GLP-1r, activating the PI3K and MAPK pathways instead of the conventional cAMP [78]. This has also been observed in the liver and skeletal muscle [79,80]. Nevertheless, *Glp1r* expression has been found in murine osteoclasts, where it directly reduces osteoclastogenesis by inhibiting the MAPK and NF- κ B pathways [81]. This is important because the anti-resorptive action of GLP-1 was initially thought to be exerted through stimulation of calcitonin release from rodent thyroid C-cells [82]. *Glp1r* KO mice develop bone fragility with aging and are associated with a reduction in bone mineral density and alteration of bone material properties, primarily lower enzymatic collagen crosslinking [82,83]. This phenotype suggests a role of the GLP-1/GLP-1r pathway in maintaining optimal bone strength. However, as GLP-1 is an incretin hormone and potentiates the release of insulin in the presence of glucose, we cannot rule out that the observed skeletal phenotype may be due to lower insulin secretion in response to meal. Several single-nucleotide polymorphisms (SNPs) of the GLP-1r have been studied in relation to bone mineral density in postmenopausal women. The GLP1R Arg44His (rs2295006) variant is negatively associated with lumbar spine and total hip BMD [84].

3.3. Glucagon-like peptide-2 (GLP-2)

GLP-2 is produced in enteroendocrine L-cells through post-translational processing of the glucagon gene. Its primary effects in the intestine include promoting epithelial proliferation, inhibiting intestinal cell apoptosis, enhancing barrier function, and increasing digestion, absorption, and blood flow [85]. In addition to the gut and central nervous system [86], *Glp2r* transcripts have also been found in the osteoblastic cell line MC3T3-E1 where its activation led to an increase in intracellular cAMP and activation of CREB, AMPK α 2, and STAT2, ultimately accelerating enzymatic collagen crosslinking [77]. Deleting GLP-2r, as done in whole-body *Glp2r* KO animals, is associated with a skeletal deficit caused by increased bone turnover [87]. However, deleting *Glp2r* only in POMC neurons does not result in changes in BMD [88], indicating that these neurons in the CNS are not necessary for bone action. Interestingly, in humans administration of exogenous GLP-2 is accompanied by a reduced secretion of parathyroid hormone (PTH) [61]. In order to verify whether reduction in PTH exerts the positive effects of GLP-2 on bone turnover, Skov-Jeppesen and colleagues investigated the response to exogenous GLP-2 administration in female patients with hypoparathyroidism due to total thyroidectomy [89]. In this specific population, administration of exogenous GLP-2 failed to modulate bone turnover markers suggesting that the effects of GLP-2 were due to reduction of PTH secretion [89]. Various analogues of GLP-2 have been developed and administering \sim 13 nmol/kg/day of [Gly²]-GLP-2 to osteoporotic mice improves post-yield strain and toughness [90]. This increased mechanical resistance is attributed to changes in bone material properties, particularly an increase in enzymatic collagen crosslinking and a reduction in tissue hypermineralization, without alterations in bone density or microstructure [90]. In osteoporotic rodents, administering GLP-2 at a dose of \sim 42 nmol/kg/day increases bone mineral density and improves bone microstructure [91]. However, bone material properties and strength were not investigated in this model. In SAMP6 mice, which develop senile osteoporosis, subcutaneous administration of GLP-2 increases bone density and improves trabecular bone microstructure in the appendicular skeleton, but not cortical bone [92]. In healthy

individuals, postmenopausal women and T2DM patients, subcutaneous administration of exogenous GLP-2 leads to lower circulating levels of bone resorption and bone formation markers [60–62]. However, administering subcutaneous GLP-2 for 2 years to patients with short bowel syndrome does not improve bone mineral density in the spine, femoral neck, or forearm [93]. In postmenopausal women with osteoporosis, 4 months of subcutaneous GLP-2 administration significantly increases bone mineral density at the total hip, femoral trochanter, and femoral neck [94].

3.4. Glucagon

Glucagon (GCG) is produced by post-translational processing of the proglucagon in pancreatic α -cells [95]. It consists of 29 amino acids and plays a role in glucose, lipid and amino acid metabolisms, as well as energy homeostasis and haemodynamics. Glucagon function has been the topic of a recent comprehensive review and will not be detailed further here [95]. GCG exerts its effects by binding to its specific receptor, the GCGr which is found in various tissues including liver, kidney, heart, adipocytes, lymphoblasts, spleen, brain, adrenal glands, retina and the gastrointestinal tract [96]. However, little information regarding the presence of a functional GCGr in bone is available. A study by Mieczkowska et al. demonstrated the absence of GCGr in the murine osteoblast cell line MC3T3-E1, and administration of GCG in MC3T3-E1 cultures did not result in increased intracellular cAMP levels [77]. Furthermore, the expression of GCGr has not been investigated in human osteoblasts or rodent/human osteoclasts. However, in 1971, based on glucagon-mediated calcitonin secretion [97], Condon proposed the use of this hormone to treat the acute phase of Paget's disease of bone, a condition characterized by excessive bone destruction, abnormal bone formation, and bone pain [98]. Intravenous infusion of high concentrations of glucagon (1 mg infused at a rate of 0.2 – 0.8 mg/h) resulted in significant decreases in plasma alkaline phosphatase and urinary calcium excretion, and relief of bone pain. The mechanism by which glucagon can reduce bone resorption and formation remains unknown. However, with the development of dual/triple agonists for managing of T2DM and obesity, it is crucial to understand the role of glucagon in bone physiology.

4. Effects of gut hormone analogues on bone physiology

4.1. Single analogue molecules

4.1.1. GIP analogues

Circulating levels of intact bioactive GIP are extremely low due to the cleavage of the first two amino acids by dipeptidyl peptidase-4 (DPP-4), expressed in the mucosa of the small intestine by hematopoietic and endothelial cells [99]. To overcome this issue, several GIP analogues have been developed to be enzymatically resistant to degradation [100]. Preclinical data on the use of GIP mimetics in animal models are scarce. Nevertheless, administration of N-Acetyl-GIP (N-AcGIP) improved tissue material properties when administered for 28 days in healthy rodents [101]. Furthermore, in a rodent model of type 1 diabetes mellitus, in order to alleviate bone fragility, the administration of [D-Ala²]-GIP for 21 days resulted in bone turnover almost similar to that of non-diabetic animals, and a reduction in matrix collagen destruction [102]. These results are very interesting as insulin release was unchanged in [D-Ala²]-GIP-treated mice compared with saline-treated mice, suggesting that these beneficial skeletal effects of GIP mimetics are independent of insulin secretion. However, to better understand whether the skeletal expression of the GIPr is required for the bone strength effects, we developed the GIP-Tag analogue, which carries a tag composed of 6 aspartic acid residues at the C-terminal extremity, targeting only skeletal murine GIPr [103]. In diet-induced type 2 diabetes and obesity, GIP-Tag administration improved bone strength by exclusively targeting bone material properties and improving collagen crosslinking and tissue

mineralization [104]. This finding suggests that bone alterations observed in type 2 diabetes and obesity could be reversed by targeting skeletal GIPr. On the other hand, in the OVX-induced osteoporosis mouse model, GIP-Tag was ineffective in reversing bone fragility, suggesting that extraskelatal GIPr is important for bone strength in osteoporosis [103]. In healthy individuals, while the initial findings indicated that GIP had no effect on decreasing bone resorption [105], evidence suggests that administering exogenous GIP is effective in reducing circulating markers of bone resorption [59].

4.1.2. GLP-1 receptor agonists (GLP-1RAs)

GLP-1RAs, such as liraglutide and exenatide/exendin-4, have also been used in several animal models of osteoporosis but also type 1 and type 2 diabetes mellitus. In rodent models of osteoporosis, liraglutide was administered at doses ranging from around 53 nmol/kg/day to around 530 nmol/kg/day. This resulted in a significant improvement in bone mineral density, trabecular and cortical bone microstructure at the appendicular and axial skeleton [106–108]. In the same animal models of osteoporosis, exenatide was administered at doses ranging from around 0.2 nmol/kg/day to around 5 nmol/kg/day. This led to higher trabecular bone mass, due to the preservation of trabecular number and thickness, as well as increases in circulating calcitonin and reduction in circulating sclerostin levels [108,109]. Interestingly, these positive effects on trabecular bone microstructure with exenatide were also associated with enhancement of cortical bone strength, without changes in cortical microstructure or mass [110], suggesting a possible improvement in bone material properties, although this was not investigated.

In rodent models of T1DM, a dose of 25 nmol/kg/day of liraglutide resulted in a slight improvement in tissue biomechanics and a reduction in collagen degradation [102], while a dose of around 160 nmol/kg/day improved both trabecular and cortical bone mineral density [111]. In rodent models of T2DM, liraglutide at doses of around 110–160 nmol/kg/day resulted in higher trabecular bone mineral density and better trabecular microarchitecture in the appendicular and axial skeleton [112–116]. At the lower dose of approximately 25 nmol/kg/day, only bone material properties were improved, with a significant restoration of the degree of mineralization, tissue water content, hydroxyapatite crystal size, and enzymatic collagen cross-linking [117]. In rodent models of T2DM, exenatide increases trabecular bone mass by reducing circulating sclerostin levels and by restoring limb blood flow [76,118,119]. The effects on bone strength are more controversial and seem to require a higher dose of exenatide, as used in osteoporosis, as 2.4 nmol/kg/day failed to evidence changes in bone biomechanics while observed at 25 nmol/kg/day [119,120], with significant changes in bone material properties, such as enzymatic collagen crosslinking acid phosphate content, carbonate-to-phosphate, and phosphate-to-CH₂ ratios [120,121]. In healthy young adults, administration of exenatide led to a reduction in circulating markers of bone resorption [122]. In individuals with T1DM, exenatide led to neutral effects on bone remodeling markers and bone mineral density, despite significant weight loss [123]. As several GLP-1 receptor agonists have been approved for the treatment of T2DM, it is important to ascertain their safety and whether GLP-1RAs could be beneficial for bone strength and fracture reduction. Several meta-analyses and retrospective case-control studies and clinical trials have been performed and concluded on the neutral effects of GLP-1RAs on fracture risk [124–128]. However, one has to bear in mind that weight loss is also a significant side effect of GLP-1RAs [129]. As weight loss has been shown to be a risk factor for bone fracture [130], it is plausible that the positive effects of GLP-1RAs on bone metabolism are mask by negative aspect of weight loss. Furthermore, bone fracture was not a specific outcome of clinical trials, and it is also plausible that the positive effects of GLP-1RAs were unnoted.

4.2. Double and triple analogue molecules

Recently, double and triple agonists of GIPr, GLP-1r, and the

glucagon receptor (GCGr) have been developed for the treatment of T2DM and obesity and are summarized in Table 1. Despite showing promising efficacy in improving glucose metabolism and mitigating the negative effects of obesity and diabetes, there have been no disclosed data regarding their impact on bone safety or their utility in reducing bone fractures. Currently, the only available data comes from Mansur et al., who conducted a study on bone strength in the db/db mouse model after administering [D-Ala²] GIP-Oxm, a triple agonist of GIPr, GLP-1r, and GCGr [131]. It appears that [D-Ala²] GIP-Oxm significantly enhanced bone strength at both the organ and tissue levels [131]. This improvement in bone strength was achieved through improvements in trabecular bone microarchitecture, collagen maturity, and reduced collagen glycation. Additionally, two sets of dual GIP/GLP-2 analogues have recently been developed for the prevention and treatment of bone fragility disorders based on the additive effects of GIP and GLP2 on BTMs and bone material properties [132,133].

In the series proposed by Gabe and colleagues, although the hit molecules were balanced at the human GIPr and GLP2r, they exhibited a weaker activity at the murine GIPr, with no significant changes at the murine GLP2r [132]. Furthermore, to date, no bone data have been reported with these molecules. Another series of dual GIP/GLP2 analogues was developed by Gobron and colleagues at the University of Angers [133]. The strategy employed by Gobron et al. was different and reposed on modulating amino acids at key positions in the structure. The hit molecule, GL-0001, presented with balanced dual agonism at the human and murine receptors. The selection of the best candidate was based on the capacity of dual GIP/GLP2 analogues to enhance enzymatic collagen crosslinking in vitro in murine and human osteoblasts rather than solely its capacity to induce intracellular signalling and cAMP. In opposition to the dual GIP/GLP2 analogues developed by Gabe and colleagues, no bias towards the murine GIPr or GLP2r has been reported in vitro with this series. GL-0001 was further validated in vivo in the ovariectomized mouse model of osteoporosis, where it led to improvement in bone toughness and strength through enhancement of enzymatic collagen cross-linking. The effects of GL-0001 were also compared to those of the bisphosphonate zoledronic acid, one of the most effective treatments for osteoporosis [133]. GL-0001 was more potent than zoledronic acid in improving bone strength in the murine OVX model of osteoporosis. If the positive effects of GL-0001 on bone material properties observed in rodent models are transferable to humans, it would represent a new and interesting molecule to be added in the therapeutic arsenal against bone fragility.

5. Conclusions

Gut hormones, including GIP, GLP-1, and GLP-2, are closely related

Table 1
Summary of the dual and triple analogues targeting the GIPr, GLP-1r or GCGr approved or currently in development.

| Target | Name | Company | Status | |
|-------------|----------------------|---------------------------|------------------------|-------------|
| GLP-1r/GIPr | Tirzepatide | Eli Lilly | Approved 13th May 2022 | |
| | CT-388 | Carmot Therapeutics | Phase 1 | |
| | VK2735 | Viking Therapeutics | Phase 2 | |
| | AMG133 | Amgen | Phase 1 | |
| | GLP-1r/GCGr | AZD9550 | Astra Zeneca | Preclinical |
| GLP-1r/GCGr | Survodutide | Boehringer Ingelheim | Phase 3 | |
| | Pemvidutide | Altimmune | Phase 2 | |
| | Pegapamodutide | Eli Lilly | Phase 2 | |
| | Efinopegdutide | Merck | Phase 2 | |
| | Mazdutide | Innovent Biologicals, Inc | Phase 3 | |
| | GLP-1r/GIPr/ GCGr | Retatrutide | Eli Lilly | Phase 2 |
| | | Efocipegtrutide | Hanmi Pharmaceutical | Phase 2 |

to bone physiology. At low concentrations, they improve bone material properties and bone strength in preclinical rodent models, suggesting that the use of gut hormone analogues might be an option for the treatment of bone fragility. However, preliminary data gathered with GLP-1RAs does not fully reflect the potential of these molecules to preserve bone strength. However, the major challenge in this field for the future will be to determine the mechanisms by which gut hormones exert their control on skeletal physiology. As the field has evolved in recent years, leading to the development of dual or triple analogues, it will also be necessary to further investigate the effects of such molecules on bone strength, particularly in the case of diabetes and obesity.

CRedit authorship contribution statement

Guillaume Mabileau: Writing – review & editing, Writing – original draft, Conceptualization. **Béatrice Bouvard:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of Competing Interest

None of the authors has a conflict of interest to declare

Data Availability

No data was used for the research described in the article.

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Authors' contribution

GM and BB actively wrote the manuscript. All authors have approved the final version of this manuscript.

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