Title

Glucagon-like peptide-1(GLP-1) receptor agonists: potential to reduce fracture risk in diabetic patients?

Journal

British journal of clinical pharmacology, 81(1)

ISSN

0306-5251

Authors

Luo, Guojing
Liu, Hong
Lu, Hongyun

Publication Date

2016

DOI

10.1111/bcp.12777

Peer reviewed

British Journal of Clinical Pharmacology

DOI:10.1111/bcp.12777

Glucagon-like peptide-1 (GLP-1) receptor agonists: potential to reduce fracture risk in diabetic patients?

Guojing Luo, Hong Liu & Hongyun Lu

Department of Endocrinology and Metabolism, The Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, Guangdong 519000, China

Correspondence
Dr H. Y. Lu, Department of Endocrinology and Metabolism, the Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, Guangdong 519000, China.
Tel.: +(86) 75 6252 8741
Fax: +(86) 75 6252 8300
E-mail: luhongyun2013@163.com

Keywords
bone mineral density, bone quality, glucagon-like peptide-1, osteoporosis

Received
21 June 2015

Accepted
1 September 2015

Accepted Article Published Online
7 September 2015

This review summarizes current knowledge about glucagon-like peptide 1 receptor agonists (GLP-1 RA) and their effects on bone metabolism and fracture risk. Recent *in vivo* and *in vitro* experiments indicated that GLP-1 RA could improve bone metabolism. GLP-1 could affect the fat-bone axis by promoting osteogenic differentiation and inhibiting adipogenic differentiation of bone mesenchymal precursor cells (BMSCs), which express the GLP-1 receptor. GLP-1 RA may also influence the balance between osteoclasts and osteoblasts, thus leading to more bone formation and less bone resorption. Wnt/β-catenin signalling is involved in this process. Mature osteocytes, which also express the GLP-1 receptor, produce sclerostin which inhibits Wnt/β-catenin signalling by binding to low density lipoprotein receptor-related protein (LRP) 5 and preventing the binding of Wnt. GLP-1 RA also decreases the expression of sclerostin (SOST) and circulating levels of SOST. In addition, GLP-1 receptors are expressed in thyroid C cells, where GLP-1 induces calcitonin release and thus indirectly inhibits bone resorption. Furthermore, GLP-1 RA influences the osteoprotegerin(OPG)/receptor activator of nuclear factor-κB ligand (RANKL)/receptor activator of nuclear factor-κB (RANK) system by increasing OPG gene expression, and thus reverses the decreased bone mass in rats models. However, a recent meta-analysis and a cohort study did not show a significant relationship between GLP-RA use and fracture risk. Future clinical trials will be necessary to investigate thoroughly the relationship between GLP-1 RA use and fracture risk in diabetic patients.

Introduction

The fracture risk of type 2 diabetic patients is increased compared with non-diabetic individuals [1, 2]. Even if the bone mineral density (BMD) in type 2 diabetic patients is not low, or even higher, the fracture risk of ankle, hip and upper extremity is still increasing [3–5]. The rising fracture risk cannot be simply explained by BMD. Bone strength reduced as a result of diabetes may not be completely reflected in the measurement of BMD. Poor bone quality may be another important factor that increases fracture risk in diabetic patients.

Previous studies have shown beneficial effect of glucagon-like peptide 2 (GLP-2) and glucose-dependent insulinotropic polypeptide (GIP) on bone formation or resorption [6–9], but the effects of GLP-1 on bone metabolism remains unclear. Recent *in vivo* and *in vitro* experiments showed that the glucagon-like peptide 1 receptor agonist (GLP-1 RA) can improve bone metabolism. The primary objective of this review was to focus on the current knowledge about GLP-1 RA and their effects on bone physiology and fracture risk and explore the therapeutic potential of GLP-1 RA in the treatment of osteoporosis in diabetic patients.

Overview of GLP-1 and GLP-RA

The discovery of the incretins (including GLP-1 and GIP) opens up a novel therapy in the treatment of diabetes. GLP-1, a 30/31-amino acid hormone, was found to be the second incretin hormone following the discovery of GIP. GLP-1 is a tissue-specific post-translational proteolytic product of the proglucagon gene which is secreted by intestinal L-cells in response to nutrient ingestion and stimulates insulin secretion from pancreatic β cells [10–12].

Various forms of GLP-1 are produced from their full length precursors by the action of prohormone convertase 1/3 [13] and appear to show similar insulinotropic effects in humans. GLP-1(7–37) and GLP-1(7–36) NH2 are thought to be biologically active and exert an insulinotropic effect on pancreatic β cells [14]. In humans, the majority of GLP-1 in the circulation is GLP-1(7–36) NH2 [15]. The half-life of bioactive GLP-1 in the circulation is approximately 2 min due to rapid inactivation by the widespread proteolytic enzyme dipeptidyl peptidase-4 (DPP-4) [16, 17]. DPP-4 is a serine protease that specifically cleaves dipeptides from the amino terminus of proteins or oligopeptides that contains an alanine or proline residue in position two, hence inhibiting their activity [18]. GLP-1, which contains a penultimate alanine residue, is metabolized rapidly to GLP-1(9–37) or GLP-1 (9–36) NH2, which have lost their insulinotropic effects [18, 19]. More remarkably, DPP-4 is also found on the position directly adjacent to the sites of GLP-1 secretion. Consequently, over half of GLP-1 has already been inactivated by DPP-4 in the portal circulation [10]. Ultimately, less than 5% of intact GLP-1 reaches the systemic circulation [20]. Glomerular filtration and tubular uptake and catabolism are the major route of GLP-1 elimination [21].

GLP-1 plays its biologic role primarily by binding to specific receptors, the GLP-1 receptor (GLP-1R) [22–24], which belongs to the G-protein coupled receptor family. It can activate adenylate cyclase and increase levels of intracellular cyclic adenosine monophosphate (cAMP) in pancreatic β cells which then stimulates glucose-dependent insulin secretion [20]. What is more, GLP-1 supresses appetite and lowers the rate of gastric emptying [25]. In addition to its well-known insulinotropic effects, GLP-1 exerts its critical effect on a variety of biological processes in diverse tissues and organs that express GLP-1R, including the bone tissue [20].

Different kinds of GLP-1 RA are being developed as anti-diabetic drugs, as GLP-1 increases post-prandial insulin secretion and suppresses post-prandial glucagon secretion [26]. In recent years, GLP-1 RA (e.g. exenatide and liraglutide) have been widely and successfully used in the clinic. Exenatide, a 39 amino acid peptide that was originally isolated from the venom of the *Heloderma suspectum* lizard, exhibits approximately 53% amino acid identity sequence with native GLP-1 and is a highly potent agonist for the mammalian GLP-1 receptor [27]. In contrast to native GLP-1, exenatide is not a substrate for DPP-4, as it contains a glycine at position two, and thus exhibits a longer circulating half-life. Liraglutide is a human GLP-1 analogue with 97% amino acid homology to native human GLP-1. Two amino acid modifications enable its non-covalent binding to albumin, thereby extending the half-life to 13 h. Liraglutide exhibits all the actions of native GLP-1[28].

Diabetes and osteoporosis

Although osteoporosis and T2DM are traditionally regarded as unrelated disease entities, emerging evidence indicates that they share many features including genetic predispositions, molecular mechanisms and pathophysiological mechanisms. ITGA1, a new candidate locus affecting both blood glucose and BMD, may partly explain the genetic contribution to the epidemiological observations linking T2DM and osteoporosis [29]. Besides, regulatory factors, including insulin, peroxisome proliferator activated receptor gamma (PPARγ), gut hormones such as GLP-1, and bone derived hormone osteocalcin are involved in the coordinated regulation of bone and energy homeostasis [30]. Wingless-related integration site (Wnt)/β-catenin signalling, which plays an important role in bone metabolism, is also thought to be a common element in the pathogenesis of osteoporosis and diabetes. The anomalies of this signalling pathway may lead to the occurrence of type 2 diabetes and osteoporosis. A single missense mutation in low density lipoprotein receptor-related protein 6 (LRP6), the co-receptor of the Wnt signalling pathway, was genetically associated with osteoporosis and diabetes [31]. Mice lacking osteocalcin show glucose intolerance [32, 33]. Contrary to predictions from mouse models, Schwartz *et al*. found that bisphopshonate-treated patients with low osteocalcin levels do not have an increase in the incidence of diabetes [34].

BMD is used as a strong predictor of fracture risk in clinical practice, but it does not encompass every aspect of bone strength. The fracture risk of T1DM increases as a consequence of decreased BMD that result from absolute deficiency of insulin, insulin-like growth factor-1 (IGF-1) and lower values of peak bone mass [35]. However in T2DM, increased load on bone because of obesity and hyperinsulinaemia as a result of insulin resistance may lead to increased bone formation. Several studies on osteoporosis and T2DM have demonstrated conflicting results, with BMD variously reported to be decreased [36, 37], increased [38–40] or unchanged [41, 42]. Despite the general increased BMD of individuals with T2DM [2], type 2 diabetic patients have a higher risk of fracture than non-diabetic patients with the same BMD measurement [43]. T2DM influences bone quality rather than BMD, which explains only 70 ~ 75% of the variance in bone strength, while other important factors, including the impaired bone micro-architecture, the accumulation of micro-fractures, the bone remodelling imbalance [44], or increased tendency to fall, may be ignored.

Besides, the increased bone fracture risk in diabetic patients could, in part, be due to the influence of anti-diabetic drugs. Previous research has shown that long term treatment with thiazolidinediones (TZDs) is

associated with an increased risk of fracture in women with T2DM compared with other glucose-lowering agents [45]. The effect of TZD on bone fractures could be due to a specific inhibition of osteoblast differentiation and activity [46]. In pharmacology, TZDs act as PPARγ activators [47]. In fact, PPARγ is expressed in bone and adipose tissue, and is able to switch the pluripotent mesenchymal stem cells (MSC) toward adipocyte at the expense of osteoblast formation [48]. As a result, adipogenesis exceeds osteogenesis.

GLP-1 and osteoporosis

GLP-1 and Wnt/β-catenin signalling pathway

GLP-1 exerts its influence by binding to its receptor. Its effect on bone metabolism can be speculated in some GLP-1R deletion animal experiments. Yamada *et al.* found that GLP-1R-deficient mice showed cortical osteopenia, bone fragility, increased osteoclastic numbers and bone resorption activity [49] (Table 1). In another similar study, Mabilleau *et al.* also demonstrated that GLP-1R knockout

Table 1

Published molecular and pre-clinical studies on the association between GLP-1 and bone metabolism

Author *et al.*	Study subject	Study method	Main result
Yamada [49]	GLP-1R knockout mice, bone marrow cells and osteoblasts	Exendin-4 and calcitonin treatment	GLP-1R knockout mice have cortical osteopenia and bone fragility as well as increased osteoclastic numbers and bone resorption activity and reduced levels of calcitonin mRNA transcripts in the thyroid. Exendin-4 increased calcitonin gene expression in the thyroid of mice
Mabilleau [50]	Male GLP-1R knockout mice	Analyze the presentation of GLP-1R knockout mice	GLP-1R knockout mice presented with a significant reduction in ultimate load, yield load, stiffness, cortical thickness, bone outer diameter and the maturity of the collagen matrix. But the mineral quantity and quality did not change significantly
Ma [51]	Old ovariectomy rats	Exendin-4 administration lasted for 16 weeks	Exendin-4 not only inhibited bone resorption by increasing the OPG : RANKL ratio, but also promoted bone formation by increasing the expression of OC and Runx2 in old ovariectomy rats
Nuche-Berenguer [52]	Streptozotocin-induced type 2 diabetic rats, fructose-induced insulin-resistant rats	Continuous infusion of GLP-1 for 3 days	GLP-1 increased OC and OPG in type 2 diabetic, insulin-resistant rats and RANKL in type 2 diabetic rats. GLP-1 induced an insulin- and PTH- independent bone anabolic action in insulin-resistant and type 2 diabetic rats
Nuche-Berenguer [53]	Hyperlipidic rats	Continuous infusion of GLP-1 and exendin-4 for 3 days	GLP-1 and exendin-4 similarly reversed the decreased femoral and vertebral bone mass by increasing OC gene expression and the OPG : RANKL ratio in hyperlipidic rats
Sanz [57]	hMSCs	Intervention with GLP-1 in cell proliferation and cell differentiation	GLP-1 significantly reduced the expression of PPARγ, C/EBPα, and LPL and prevented cell differentiation into adipocytes in hMSCs
Nuche-Berenguer [66]	Osteoblastic MC3T3-E1 cells	Analysis of GLP-1 binding and cross-linking studies	GLP-1 can directly and functionally interact with osteoblastic cells independent of the cAMP-linked GLP-1 receptor, possibly through a GPI/IPG-coupled receptor
Kim [78]	Type 2 diabetic OLETF rats, osteocyte-like MLO-Y4 cells and osteocytes of rat femurs	Investigated the presence of GLP-1 receptors and the effect of exendin-4 treatment through RT-PCR, Western blot and confocal microscopy	GLP-1 receptor was present on MLO-Y4 cells and osteocytes of rat femurs. Exendin-4 reduced the levels of SOST/sclerost in MLO-Y4 cells. Besides, exendin-4 reduced serum levels of SOST, increased serum levels of osteocalcin and femoral BMD in type 2 diabetic OLETF rats
Gier [88]	Thyroid tissue samples with medullary thyroid carcinoma, C cell hyperplasia, papillary thyroid carcinoma, and normal human thyroid	Immunofluorescence for expression of calcitonin and GLP-1 receptors	The neoplastic and hyperplastic lesions of thyroid C cells express the GLP-1 receptor and GLP-1 receptor expression is also detected in 18% of papillary thyroid carcinomas and in C cells in 33% of control thyroid lobes in humans
Hegedus [89]	T2DM or non-diabetic obese patients receiving liraglutide treatment	CT concentrations were measured at 3-month intervals for no more than 2 years	No significant change.
Bjerre [90]	The thyroid of mice, rats, cynomolgus monkeys and humans	The activation of the thyroid GLP-1 receptor with GLP-1 RA	GLP-1 RA stimulated calcitonin release, up-regulation of calcitonin gene expression and subsequently C-cell hyperplasia in rats. In contrast, humans and/or cynomolgus monkeys had low GLP-1 receptor expression in thyroid C-cells and GLP-1 RA did not activate adenylate cyclase or generate calcitonin release in primates

BMD, bone mineral density; cAMP, cyclic adenosine monophosphate; C/EBPα, CCAAT/enhancer-binding protein α; GLP-1RA, glucagon-like peptide-1 receptor agonists; hMSCs, human mesenchymal stem cells; LPL, lipoprotein lipase; OC, osteocalcin; OP, osteoporosis; OPG, osteoprotegerin; PPARγ, peroxisome proliferator-activated receptorγ; PTH, parathyroid hormone; RANKL, receptor activator of nuclear factor-kappaB ligand; Runx2, runt-related transcription factor 2; SOST, sclerostin.

mice presented with a significant reduction in ultimate load, yield load, stiffness, total absorbed, post-yield energies, cortical thickness and bone outer diameter, but the mineral quantity and quality were not significantly influenced [50]. What is more, the improvement of bone metabolism and anti-osteoporosis effects of GLP-1 are demonstrated in rodent experiments, mainly in the osteoblast lineage. Its significant role in the improvement of bone metabolism has been reported in aged ovariectomized rats models [51], streptozotocin-induced type 2 diabetic and fructose-induced insulin-resistant (IR) rat models [52] and hyperlipidic rat models [53]. This intrigues clinical doctors for its potential application in osteoporosis.

Bone formation is a series of events that include the differentiation of mesenchymal precursor cells into osteoblast precursors, the maturation of osteoblasts and the formation and the mineralization of matrix [54]. The fat-bone axis is involved in this important course. Osteoblasts and adipocytes differentiate from a common mesenchymal precursor cell, BMSCs [55], which can differentiate into osteoblasts, adipocytes as well as other cell types under the influence of local growth factors, hormonal regulators and transcriptional factors [56]. GLP-1 in humans can promote proliferation of BMSCs and inhibit adipocyte differentiation [57]. Consistent with these findings, Ma *et al*. also found that exenatide can promote the osteogenic differentiation and inhibit the adipogenic direction of differentiation of BMSCs [51]. Previous research has confirmed that the GLP-1 receptor is expressed in BMSCs and immature osteoblasts but not in mature osteoblasts [57–59]. Evidence from *in vitro* experiments suggests no direct effect of GLP-1 in either osteoblasts or osteoclasts [49]. This evidence suggests that BMSCs could be the key targets of GLP-1 [51] (Figure 1). The balance between osteoblast/adipocyte differentiation of BMSCs plays an important role in bone homeostasis. If too many BMSCs differentiate towards adipocytes, the number of osteoblasts will be reduced and bone absorption exceeds bone formation, resulting in the reduction of BMD and osteoporosis. Vermas *et al*. found that the number of adipocyte of bone marrow in patients with osteoporosis significantly increased [60]. This indicated that reduction of osteoblasts is often accompanied by an increase in adipocyte ratio. Similarly, in the ageing mouse model, when fat cells in bone marrow increased, bone formation decreased significantly [61]. Therefore, the direction of differentiation of BMSCs plays a crucial role in the pathogenesis of osteoporosis [62–64]. Examples of drugs that increase adiposity (especially visceral fat) at the expense of osteoblast differentiation are glucocorticoids [65]. Although it is widely believed that GLP-1 does not directly affect osteoblasts or osteoclasts, Nuche-Berenguer *et al*. found that GLP-1 can directly and functionally interact with osteoblastic cells, possibly through a

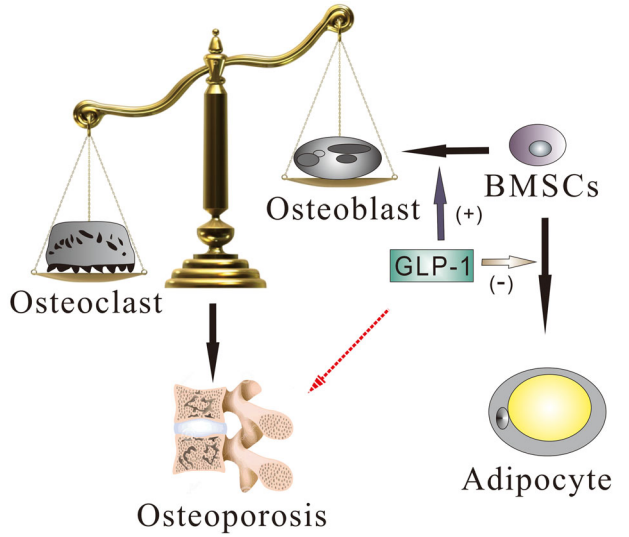

Figure 1

Model for the influence of GLP-1 on osteogenesis. Bone homeostasis is regulated by the balance between osteoblastic bone formation and osteoclastic bone resorption. An imbalance between these two factors will lead to osteoporosis. GLP-1 not only inhibits adipocyte differentiation from BMSCs, but also inhibits osteoblast differentiation, and thus improves bone metabolism. GLP-1 glucagon-like peptide-1; BMSCs bone mesenchymal precursor cells

glycosylphosphatidylinositols(GPI)/inositolphosphoglycans (IPG)-coupled receptor that is different from the cloned GLP-1 receptor in pancreas [66].

The canonical Wnt signalling pathway regulates the differentiation and maturation of the osteoblast. The signal pathway includes low density lipoprotein receptor related protein (LRP) 5/6, β-catenin, GSK-3β and other related regulative factors, such as Dkk1 and sclerostin [67]. LRP5 gene mutations can lead to different abnormal bone phenotypic abnormalities, including osteoporosis pseudoglioma syndrome (OPPG) and increased bone mass [68]. In animal experiments, LRP5 knockout mice are characterized by decreased bone mass, resulting from inactivating canonical Wnt signalling pathways [69]. Wnts are secreted glycoproteins, consisting of 350 ~ 400 amino acids and 23 ~ 24 residues of conserved cysteine [70], many of which are likely to participate in intramolecular disulfide bonds. In BMSCs, canonical Wnt signalling regulates a reciprocal relationship between adipogenesis and osteogenesis. In previous research, Wnt signalling is regarded as a key pathway for β cell growth and differentiation. Recent findings link GLP-1 as a direct activator of this pathway. GLP-1 could also stabilize β-catenin by binding to its receptor and increasing the level of intracellular cAMP [71]. In the inactive state of the Wnt/β-catenin pathway, Dkk1 forms a ternary complex with transmembrane proteins kremen and LRP5/6 and then induces rapid endocytosis and removal of the Wnt receptor LRP5/6 from the plasma membrane. Activation of the Wnt signalling pathway promotes bone

formation while inactivation of the pathway leads to reduced bone mass [72–74]. The activation of the GLP-1 receptor and the Wnt signalling pathway showed similar effect on adipocyte differentiation and bone metabolism. Specific gene expression of adipocyte differentiation including PPARγ, C/EBPα and LPL decreased [57] but bone formation-related gene including Runx2/cbfa1 [69, 74], OPG [51–53], and OC [51–53] increased (Figure 2).

Regulation of the Wnt/β-catenin signalling pathway is carried out mainly by proteins that either act as competitive binders of Wnts. Sclerostin (SOST), which might contribute to the pathogenesis of bone loss in T2DM, is the major one. SOST is released by mature osteocytes and inhibits Wnt/β-catenin signalling by binding to LRP5 and preventing the binding of Wnt protein to LRP5/6 [75]. Serum SOST levels of the diabetic patients were significantly higher than non-diabetic patients and were increased with age [76]. The high SOST level might indicate increased osteocyte activity in postmenopausal patients with T2DM [77]. Osteocytes could be another target of GLP-1 RA and GLP-1 receptor is expressed in osteocytes as showed in osteocyte-like MLO-Y4 cells and osteocytes of rat long bone [78]. In *in vitro* experiments, GLP-1 RA decreases the expression of SOST in MLO-Y4 cells whereas it decreases the circulating levels of SOST in T2DM rat models in *in vivo* experiments. These findings demonstrate that GLP-RA might increase the femoral BMD through decreasing the expression of SOST/sclerostin in osteocytes in T2DM rat models [78].

GLP-1, body weight and osteoporosis

DPP-4 inhibitors (DPP4-I) are used to prolong the action of GLP-1, so their effect on bone may be similar to GLP-1. To explore this possibility, a meta-analysis was performed to compare DPP4-I with placebo or other antidiabetic drugs in all randomized clinical trials with a

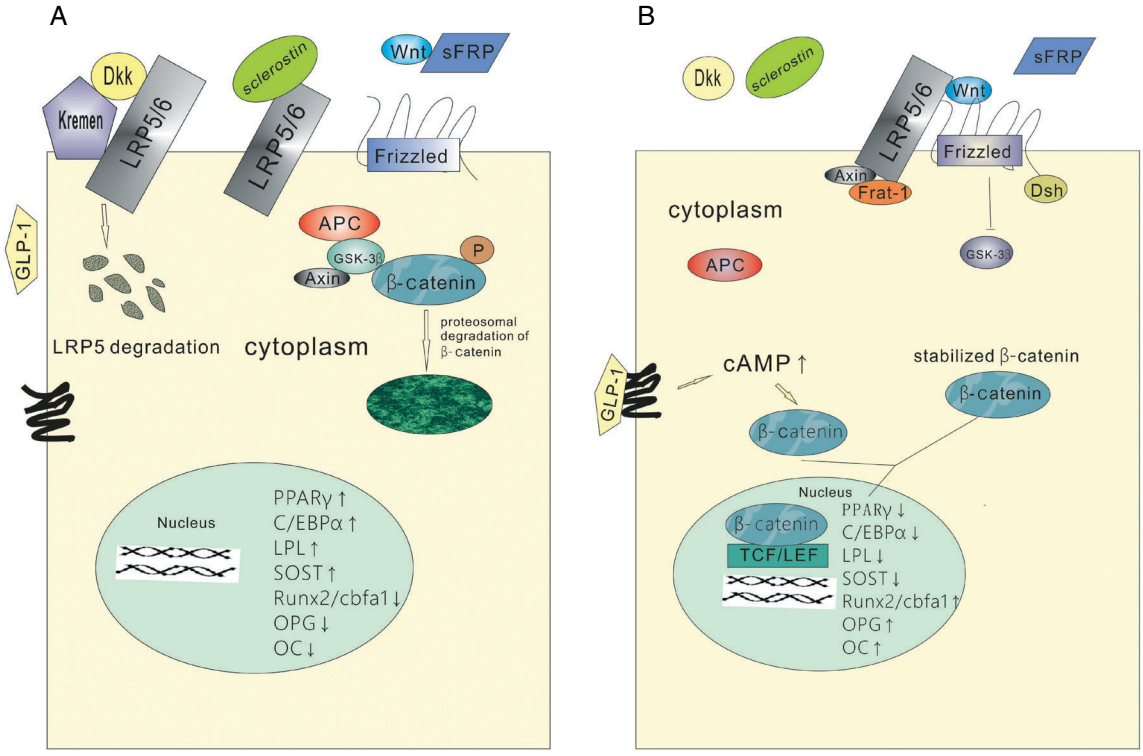

Figure 2

GLP-1 and Wnt signalling in the regulation of bone formation (A) In the inactive state of the Wnt/β-catenin pathway, Wnt is inhibited by a decoy receptor sFRP, where as its co-receptor LRP5/6 binds to inhibitory protein sclerostin or Dkk. Activated GSK-3β can result in proteosomal degradation of β-catenin, which then inhibits osteoblast differentiation. Specific gene expression of adipocyte differentiation including PPARγ, C/EBPα, and LPL and bone formation negative regulatory genes SOST increased but bone formation positive regulatory genes including Runx2/cbfa1, OPG, and OC decreased. (B) The Wnt/β-catenin pathway is activated by the binding of the Wnt to a co-receptor complex LRP5/6 and frizzled family member. Disruption of the GSK-3β inhibitory complex stabilizes the β-catenin, which is then translocated into the nucleus and activates transcription. GLP-1 could also stabilize β-catenin by binding to its receptor and increasing the level of intracellular cAMP. The changes in the expression of related genes are the opposite of Figure 2A. sFRP secreted frizzled related protein; Dsh dishevelled; LRP5/6 lipoprotein receptor related proteins 5 and 6; Dkk1 dickkopf1; APC adenomatous polyposis coli; GSK-3β glycogen synthesis kinase-3β; Frat1 frequently rearranged in advanced T-cell lymphomas-1; cAMP cyclic adenosine monophosphate; TCF/LEF T-cell factor/lymphoid enhancing factor; PPARγ peroxisome proliferator-activated receptorγ; C/EBPα CCAAT/enhancer-binding protein α; LPL lipoprotein lipase; SOST sclerostin; Runx2/cbfa1 runt-related transcription factor 2/core binding factor alpha1; OPG osteoprotegerin; OC osteocalcin

duration of at least 24 weeks. This meta-analysis showed a 40% reduction of fracture risk for DPP4-I users compared with other patients [79] (Table 2). However, a retrospective population based cohort study demonstrated no different risk of fracture comparing DPP4-I users with controls [80]. In another meta-analysis, Mabilleau *et al*. found that GLP-1 RA did not reduce bone fracture risk in T2DM compared with the use of other anti-diabetic medications [81]. Consistent with this study, Driessen *et al*. also found that GLP1-RA use (the median duration of actual GLP1-RA use was 1.2 years) was not associated with a decreased risk of bone fracture as compared with users of other anti-hyperglycaemic drugs in a population-based cohort study [82]. These clinical results seem to demonstrate that GLP-RA use is not related to bone fracture risk. However we should realize that the average duration of GLP1-RA and DPP-I use is relatively short which may make it fail to exert an effect on bone fracture risk. Besides, GLP-1 users often have a high BMI which may influence the final result. Careful assessment of the incidence of fractures in clinical trials will be important.

A primary difference between GLP-1 RA and DPP-4I lies in weight loss, which often accompanies treatment with GLP-1 RA. The weight loss effect of GLP-1 RA is beneficial to many overweight or obese type 2 diabetic patients who are advised to lose weight. The positive association between body weight and BMD has previously been established in some studies [83, 84]. Bone mobilization and loss could result from weight loss. According to Canadian osteoporosis guidelines, obvious weight loss is a risk factor for osteoporosis [85, 86]. A positive effect of GLP-1 RA on bone homeostasis could be weakened by weight loss-induced mechanical loading and bone mass decrease, thus causing neutrality of GLP-1 RA treatment on bone fracture risk in clinical trials. However from the perspective of the fat-bone axis, this positive effect could be independent of weight loss. Apart from the direct effect of soft tissue mass on bone through skeletal loading, GLP-1 can also act as a mediator of the fat and bone cells [87], but the exact mechanism remains unclear.

GLP-1 and calcitonin

Calcitonin may contribute to the improvement of bone metabolism with GLP-1 treatment. GLP-1 receptors are expressed in thyroid C cells, where GLP-1 induces postprandial calcitonin release and thus bone resorption is indirectly inhibited [49]. However, these previous findings reported in rodents may not apply to humans. Recent studies have found that only 33% of the normal thyroid C cells express GLP-1 receptors [88]. Clinical experimental data do not support an effect of GLP-1 receptor activation on serum calcitonin levels in humans in a clinical trial with over 5000 subjects receiving 2 years liraglutide treatment [89]. As a result, considering the differences between rodents and humans, it is plausible that the observed bone protective effects of GLP-1 in rodent models may be the result of direct effects on bone cells and indirect effects mediated via calcitonin. In contrast, non-human primate and human thyroid glands and the human TT cell line, which is derived from human medullary thyroid carcinoma, express very low levels of GLP-1 receptors. The human TT cell line, unlike rat thyroid C-cell lines, does not respond to GLP-1 RA with an acute release of calcitonin [90]. Collectively, it seems that the influence of GLP-1 RA on the human thyroid is limited.

GLP-1 and OPG/RANKL/RANK

The discovery of the osteoprotegerin (OPG)/receptor activator of nuclear factor-κB ligand (RANKL)/receptor activator of nuclear factor-κB (RANK) system in the past decade provides us with a better understanding of bone metabolism, especially bone resorption. OPG, RANK and its ligand RANKL, which all belong to the family of

Table 2

Clinical studies of the relationship between GLP-1 RA and DPP-4 inhibitors use and bone fracture

Author *et al*.	Study subject	Study method	Main result
Monami [79]	Type 2 diabetic patients treated with DPP-4 inhibitors with a duration of at least 24 weeks	Meta-analysis of bone fracture risk	DPP-4 inhibitors were associated with a reduced risk of fractures even after the exclusion of comparisons with thiazolidinediones or sulfonylureas
Driessen [80]	Type 2 diabetic patients treated with DPP-4 inhibitors from the CPRD database	retrospective population based cohort study of bone fracture risk	DPP4-I use was not associated with fracture risk compared with controls and with other non-insulin anti-diabetic drug users
Mabilleau [81]	Type 2 diabetic patients treated with GLP-1 RA in randomized clinical trials	Meta-analysis of bone fracture risk	The use of GLP-1RA does not reduce the risk of bone fracture in T2DM compared with the use of other antidiabetic medications
Driessen [82]	Type 2 diabetes patients treated with GLP-1RA from the CPRD database.	Population-based cohort analysis of bone fracture risk	There was no decreased risk of fracture with current use of GLP-1RA compared to never-GLP-1RA use. Osteoporotic fracture risk was also not decreased by current GLP-1RA use

CPRD, Clinical Practice Research Datalink; GLP-1RA, glucagon-like peptide-1 receptor agonists.

tumour necrosis factor receptors (TNFR), mediate osteoclastogenesis [91]. In the skeleton, RANKL stimulates the differentiation, activation and survival of osteoclasts and thus enhances bone resorption [5, 8]. OPG acts as a decoy receptor by binding to RANKL therefore preventing the interaction with its receptor RANK that is necessary for the activation of RANKL [92]. Consequently, OPG acts as a negative regulator of osteoclast differentiation, activation and survival and therefore inhibits bone resorption [93]. High RANKL and low OPG levels are associated with high bone turnover and bone loss, whereas low serum RANKL may relate to accumulation of microdamage and reduced bone quality [94]. An imbalance of OPG/RANKL/RANK expression associated with diabetes may contribute to the delay of fracture repair during the course of diabetes [95]. Elevated OPG in patients with T1DM may be the body's response to increased bone resorption [96]. New drugs targeting the OPG/RANKL/RANK system proved to be efficient in reducing bone resorption and preventing bone loss in post-menopausal osteoporosis [97]. Recently, Nuche-Berenguer *et al*. observed that exenatide administration exerts osteogenic effects in streptozocin-induced type 2 diabetes and fructose-induced insulin-resistant rats. They found that GLP-1 RA administration increased OPG gene expression and the OPG/RANKL ratio and reversed the decreased femoral and vertebral bone mass in these rats [52]. Their subsequent studies demonstrated that GLP-1 and exendin-4 are similarly efficient in reversing the bone alterations in the hyperlipidic-related rat model [53].

Conclusion

Reduced bone strength in type 2 diabetic patients involves many complex factors. Some oral anti-diabetic drugs even aggravate the impaired bone turnover and increase bone fracture risk. The recently reported bone-related effects of GLP-1, together with their known glucose-lowering action, make them candidates for reducing fracture risk in diabetic conditions. Results from *in vivo* and *in vitro* experiments are quite encouraging. However, the clinical data about the relationship between GLP-1RA and fracture risk are disappointing. A meta-analysis and cohort study did not find any relationship between GLP-RA use and fracture risk. Also, the influence of GLP-1 RA on human thyroid is limited. However, we should not jump to a conclusion prematurely. Weight loss may weaken the protective effect of GLP-1 RA on bone. The short duration of GLP1-RA use and high BMI of GLP-RA users are two main shortcomings of the clinical trials. Careful assessment of the fracture risk in future clinical trials with GLP-1RA will be necessary.

Competing Interests

All the authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

REFERENCES

1 Chau DL, Edelman SV, Chandran M. Osteoporosis and diabetes. Curr Diab Rep 2003; 3: 37–42.

2 Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes–a meta-analysis. Osteoporos Int 2007; 18: 427–44.

3 Luetters CM, Keegan TH, Sidney S, Quesenberry CP, Prill M, Sternfeld B, Kelsey J. Risk factors for foot fracture among individuals aged 45 years and older. Osteoporos Int 2004; 15: 957–63.

4 Lipscombe LL, Jamal SA, Booth GL, Hawker GA. The risk of hip fractures in older individuals with diabetes: a population-based study. Diabetes Care 2007; 30: 835–41.

5 Ivers RQ, Cumming RG, Mitchell P, Peduto AJ. Diabetes and risk of fracture: The Blue Mountains Eye Study. Diabetes Care 2001; 24: 1198–203.

6 Henriksen DB, Alexandersen P, Bjarnason NH, Vilsboll T, Hartmann B, Henriksen EE, Byrjalsen I, Krarup T, Holst JJ, Christiansen C. Role of gastrointestinal hormones in postprandial reduction of bone resorption. J Bone Miner Res 2003; 18: 2180–9.

7 Gottschalck IB, Jeppesen PB, Holst JJ, Henriksen DB. Reduction in bone resorption by exogenous glucagon-like peptide-2 administration requires an intact gastrointestinal tract. Scand J Gastroenterol 2008; 43: 929–37.

8 Tsukiyama K, Yamada Y, Yamada C, Harada N, Kawasaki Y, Ogura M, Bessho K, Li M, Amizuka N, Sato M, Udagawa N, Takahashi N, Tanaka K, Oiso Y, Seino Y. Gastric inhibitory polypeptide as an endogenous factor promoting new bone formation after food ingestion. Mol Endocrinol 2006; 20: 1644–51.

9 Yamada Y, Miyawaki K, Tsukiyama K, Harada N, Yamada C, Seino Y. Pancreatic and extrapancreatic effects of gastric inhibitory polypeptide. Diabetes 2006; 55: S86–91.

10 Hansen L, Deacon CF, Orskov C, Holst JJ. Glucagon-like peptide-1-(7–36)amide is transformed to glucagon-like peptide-1-(9–36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. Endocrinology 1999; 140: 5356–63.

11 Kreymann B, Williams G, Ghatei MA, Bloom SR. Glucagon-like peptide-1 7–36: a physiological incretin in man. Lancet 1987; 2: 1300–4.

12 Schmidt WE, Siegel EG, Creutzfeldt W. Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets. Diabetologia 1985; 28: 704–7.

13 Dhanvantari S, Seidah NG, Brubaker PL. Role of prohormone convertases in the tissue-specific processing of proglucagon. Mol Endocrinol 1996; 10: 342–55.

14 Orskov C, Wettergren A, Holst JJ. Biological effects and metabolic rates of glucagonlike peptide-1 7–36 amide and glucagonlike peptide-1 7–37 in healthy subjects are indistinguishable. Diabetes 1993; 42: 658–61.

15 Orskov C, Rabenhoj L, Wettergren A, Kofod H, Holst JJ. Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. Diabetes 1994; 43: 535–9.

16 Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ. Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. Diabetes 1995; 44: 1126–31.

17 Deacon CF, Johnsen AH, Holst JJ. Degradation of glucagon-like peptide-1 by human plasma *in vitro* yields an N-terminally truncated peptide that is a major endogenous metabolite *in vivo*. J Clin Endocrinol Metab 1995; 80: 952–7.

18 Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum. Eur J Biochem 1993; 214: 829–35.

19 Kieffer TJ, McIntosh CH, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 *in vitro* and *in vivo* by dipeptidyl peptidase IV. Endocrinology 1995; 136: 3585–96.

20 Seino Y, Fukushima M, Yabe D. GIP and GLP-1, the two incretin hormones: Similarities and differences. J Diabetes Investig 2010; 1: 8–23.

21 Ruiz-Grande C, Alarcon C, Alcantara A, Castilla C, López Novoa JM, Villanueva-Penacarrillo ML, Valverde I. Renal catabolism of truncated glucagon-like peptide 1. Horm Metab Res 1993; 25: 612–6.

22 Dillon JS, Tanizawa Y, Wheeler MB, Leng XH, Ligon BB, Rabin DU, Yoo-Warren H, Permutt MA, Boyd AR. Cloning and functional expression of the human glucagon-like peptide-1 (GLP-1) receptor. Endocrinology 1993; 133: 1907–10.

23 Stoffel M, Espinosa RR, Le Beau MM, Bell GI. Human glucagon-like peptide-1 receptor gene. Localization to chromosome band 6p21 by fluorescence in situ hybridization and linkage of a highly polymorphic simple tandem repeat DNA polymorphism to other markers on chromosome 6. Diabetes 1993; 42: 1215–8.

24 Thorens B Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc Natl Acad Sci USA 1992; 89: 8641–5.

25 van Bloemendaal L, Ten KJ, la Fleur SE, Ijzerman RG, Diamant M. Effects of glucagon-like peptide 1 on appetite and body weight: focus on the CNS. J Endocrinol 2014; 221: T1–16.

26 Cho YM, Wideman RD, Kieffer TJ. Clinical application of glucagon-like Peptide 1 receptor agonists for the treatment of type 2 diabetes mellitus. Endocrinol Metab (Seoul) 2013; 28: 262–74.

27 Eng J, Kleinman WA, Singh L, Singh G, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. J Biol Chem 1992; 267: 7402–5.

28 Degn KB, Juhl CB, Sturis J, Jakobsen G, Brock B, Chandramouli V, Rungby J, Landau BR, Schmitz O. One week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycemia and alpha- and beta-cell function and reduces endogenous glucose release in patients with type 2 diabetes. Diabetes 2004; 53: 1187–94.

29 Billings LK, Hsu YH, Ackerman RJ, Dupuis J, Voight BF, Rasmussen-Torvik LJ, Hercberg S, Lathrop M, Barnes D, Langenberg C, Hui J, Fu M, Bouatia-Naji N, Lecoeur C, An P, Magnusson PK, Surakka I, Ripatti S, Christiansen L, Dalgard C, Folkersen L, Grundberg E, Eriksson P, Kaprio J, Ohm KK, Pedersen NL, Borecki IB, Province MA, Balkau B, Froguel P, Shuldiner AR, Palmer LJ, Wareham N, Meneton P, Johnson T, Pankow JS, Karasik D, Meigs JB, Kiel DP, Florez JC. Impact of common variation in bone-related genes on type 2 diabetes and related traits. Diabetes 2012; 61: 2176–86.

30 Lecka-Czernik B Safety of Anti-Diabetic Therapies on Bone. Clin Rev Bone Miner Metab 2013; 11: 49–58.

31 Mani A, Radhakrishnan J, Wang H, Mani A, Mani MA, Nelson-Williams C, Carew KS, Mane S, Najmabadi H, Wu D, Lifton RP. LRP6 mutation in a family with early coronary disease and metabolic risk factors. Science 2007; 315: 1278–82.

32 Ferron M, Wei J, Yoshizawa T, Del FA, DePinho RA, Teti A, Ducy P, Karsenty G. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. Cell 2010; 142: 296–308.

33 Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G. Endocrine regulation of energy metabolism by the skeleton. Cell 2007; 130: 456–69.

34 Schwartz AV, Schafer AL, Grey A, Vittinghoff E, Palermo L, Lui LY, Wallace RB, Cummings SR, Black DM, Bauer DC, Reid IR. Effects of antiresorptive therapies on glucose metabolism: results from the FIT, HORIZON-PFT, and FREEDOM trials. J Bone Miner Res 2013; 28: 1348–54.

35 Jackuliak P, Payer J. Osteoporosis, fractures, and diabetes. Int J Endocrinol 2014; 2014: : 820615.

36 Levin ME, Boisseau VC, Avioli LV. Effects of diabetes mellitus on bone mass in juvenile and adult-onset diabetes. N Engl J Med 1976; 294: 241–5.

37 De Leeuw I, Abs R. Bone mass and bone density in maturity-type diabetics measured by the 125I photon-absorption technique. Diabetes 1977; 26: 1130–5.

38 Wakasugi M, Wakao R, Tawata M, Gan N, Koizumi K, Onaya T. Bone mineral density measured by dual energy x-ray absorptiometry in patients with non-insulin-dependent diabetes mellitus. Bone 1993; 14: 29–33.

39 van Daele PL, Stolk RP, Burger H, Algra D, Grobbee DE, Hofman A, Birkenhager JC, Pols HA. Bone density in non-insulin-dependent diabetes mellitus. The Rotterdam Study. Ann Intern Med 1995; 122: 409–14.

40 Dobnig H, Piswanger-Solkner JC, Roth M, Obermayer-Pietsch B, Tiran A, Strele A, Maier E, Maritschnegg P, Sieberer C, Fahrleitner-Pammer A. Type 2 diabetes mellitus in nursing home patients: effects on bone turnover, bone mass, and fracture risk. J Clin Endocrinol Metab 2006; 91: 3355–63.

41 Sosa M, Dominguez M, Navarro MC, Segarra MC, Hernandez D, de Pablos P, Betancor P. Bone mineral metabolism is normal in non-insulin-dependent diabetes mellitus. J Diabetes Complications 1996; 10: 201–5.

42 Isaia GC, Ardissone P, Di Stefano M, Ferrari D, Martina V, Porta M, Tagliabue M, Molinatti GM. Bone metabolism in type 2 diabetes mellitus. Acta Diabetol 1999; 36: 35–8.

43 Schwartz AV, Vittinghoff E, Bauer DC, Hillier TA, Strotmeyer ES, Ensrud KE, Donaldson MG, Cauley JA, Harris TB, Koster A, Womack CR, Palermo L, Black DM. Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes. JAMA 2011; 305: 2184–92.

44 Sanyal A, Gupta A, Bayraktar HH, Kwon RY, Keaveny TM. Shear strength behavior of human trabecular bone. J Biomech 2012; 45: 2513–9.

45 Grey A, Bolland M, Gamble G, Wattie D, Horne A, Davidson J, Reid IR. The peroxisome proliferator-activated receptor-gamma agonist rosiglitazone decreases bone formation and bone mineral density in healthy postmenopausal women: a randomized, controlled trial. J Clin Endocrinol Metab 2007; 92: 1305–10.

46 Benvenuti S, Cellai I, Luciani P, Deledda C, Baglioni S, Giuliani C, Saccardi R, Mazzanti B, Dal Pozzo S, Mannucci E, Peri A, Serio M. Rosiglitazone stimulates adipogenesis and decreases osteoblastogenesis in human mesenchymal stem cells. J Endocrinol Invest 2007; 30: C26–30.

47 Lecka-Czernik B, Gubrij I, Moerman EJ, Kajkenova O, Lipschitz DA, Manolagas SC, Jilka RL. Inhibition of Osf2/Cbfa1 expression and terminal osteoblast differentiation by PPARgamma2. J Cell Biochem 1999; 74: 357–71.

48 Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002; 418: 41–9.

49 Yamada C, Yamada Y, Tsukiyama K, Yamada K, Udagawa N, Takahashi N, Tanaka K, Drucker DJ, Seino Y, Inagaki N. The murine glucagon-like peptide-1 receptor is essential for control of bone resorption. Endocrinology 2008; 149: 574–9.

50 Mabilleau G, Mieczkowska A, Irwin N, Flatt PR, Chappard D. Optimal bone mechanical and material properties require a functional glucagon-like peptide-1 receptor. J Endocrinol 2013; 219: 59–68.

51 Ma X, Meng J, Jia M, Bi L, Zhou Y, Wang Y, Hu J, He G, Luo X. Exendin-4, a glucagon-like peptide-1 receptor agonist, prevents osteopenia by promoting bone formation and suppressing bone resorption in aged ovariectomized rats. J Bone Miner Res 2013; 28: 1641–52.

52 Nuche-Berenguer B, Moreno P, Esbrit P, Dapia S, Caeiro JR, Cancelas J, Haro-Mora JJ, Villanueva-Penacarrillo ML. Effect of GLP-1 treatment on bone turnover in normal, type 2 diabetic, and insulin-resistant states. Calcif Tissue Int 2009; 84: 453–61.

53 Nuche-Berenguer B, Lozano D, Gutierrez-Rojas I, Moreno P, Marinoso ML, Esbrit P, Villanueva-Penacarrillo ML. GLP-1 and exendin-4 can reverse hyperlipidic-related osteopenia. J Endocrinol 2011; 209: 203–10.

54 Martin TJ, Sims NA, Ng KW. Regulatory pathways revealing new approaches to the development of anabolic drugs for osteoporosis. Osteoporos Int 2008; 19: 1125–38.

55 Gharibi B, Abraham AA, Ham J, Evans BA. Adenosine receptor subtype expression and activation influence the differentiation of mesenchymal stem cells to osteoblasts and adipocytes. J Bone Miner Res 2011; 26: 2112–24.

56 Karsenty G Minireview: transcriptional control of osteoblast differentiation. Endocrinology 2001; 142: 2731–3.

57 Sanz C, Vazquez P, Blazquez C, Barrio PA, Alvarez MM, Blazquez E. Signaling and biological effects of glucagon-like peptide 1 on the differentiation of mesenchymal stem cells from human bone marrow. Am J Physiol Endocrinol Metab 2010; 298: E634–43.

58 Bollag RJ, Zhong Q, Phillips P, Min L, Zhong L, Cameron R, Mulloy AL, Rasmussen H, Qin F, Ding KH, Isales CM. Osteoblast-derived cells express functional glucose-dependent insulinotropic peptide receptors. Endocrinology 2000; 141: 1228–35.

59 Pacheco-Pantoja EL, Ranganath LR, Gallagher JA, Wilson PJ, Fraser WD. Receptors and effects of gut hormones in three osteoblastic cell lines. BMC Physiol 2011; 11: 12.

60 Verma S, Rajaratnam JH, Denton J, Hoyland JA, Byers RJ. Adipocytic proportion of bone marrow is inversely related to bone formation in osteoporosis. J Clin Pathol 2002; 55: 693–8.

61 Kodama Y, Takeuchi Y, Suzawa M, Fukumoto S, Murayama H, Yamato H, Fujita T, Kurokawa T, Matsumoto T. Reduced expression of interleukin-11 in bone marrow stromal cells of senescence-accelerated mice (SAMP6): relationship to osteopenia with enhanced adipogenesis. J Bone Miner Res 1998; 13: 1370–7.

62 Akune T, Ohba S, Kamekura S, Yamaguchi M, Chung UI, Kubota N, Terauchi Y, Harada Y, Azuma Y, Nakamura K, Kadowaki T, Kawaguchi H. PPARgamma insufficiency enhances osteogenesis through osteoblast formation from bone marrow progenitors. J Clin Invest 2004; 113: 846–55.

63 Bennett JH, Joyner CJ, Triffitt JT, Owen ME. Adipocytic cells cultured from marrow have osteogenic potential. J Cell Sci 1991; 99: 131–9.

64 Beresford JN, Bennett JH, Devlin C, Leboy PS, Owen ME. Evidence for an inverse relationship between the differentiation of adipocytic and osteogenic cells in rat marrow stromal cell cultures. J Cell Sci 1992; 102: 341–51.

65 Shapses SA, Sukumar D. Bone metabolism in obesity and weight loss. Annu Rev Nutr 2012; 32: 287–309.

66 Nuche-Berenguer B, Portal-Núñez S, Moreno P, González N, Acitores A, López-Herradón A, Esbrit P, Valverde I, Villanueva-Peñacarrillo ML. Presence of a functional receptor for GLP-1 in osteoblastic cells, independent of the cAMP-linked GLP-1 receptor. J Cell Physiol 2010; 225: 585–92.

67 Shi YC, Worton L, Esteban L, Baldock P, Fong C, Eisman JA, Gardiner EM. Effects of continuous activation of vitamin D and Wnt response pathways on osteoblastic proliferation and differentiation. Bone 2007; 41: 87–96.

68 Johnson ML, Harnish K, Nusse R, Van Hul W. LRP5 and Wnt signaling: a union made for bone. J Bone Miner Res 2004; 19: 1749–57.

69 Kato M, Patel MS, Levasseur R, Lobov I, Chang BH, Glass DN, Hartmann C, Li L, Hwang TH, Brayton CF, Lang RA, Karsenty G, Chan L. Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor. J Cell Biol 2002; 157: 303–14.

70 Uusitalo M, Heikkila M, Vainio S. Molecular genetic studies of Wnt signaling in the mouse. Exp Cell Res 1999; 253: 336–48.

71 Gustafson B, Smith U. WNT signalling is both an inducer and effector of glucagon-like peptide-1. Diabetologia 2008; 51: 1768–70.

72 Milat F, Ng KW. Is Wnt signalling the final common pathway leading to bone formation? Mol Cell Endocrinol 2009; 310: 52–62.

73 Shahnazari M, Yao W, Corr M, Lane NE. Targeting the Wnt signaling pathway to augment bone formation. Curr Osteoporos Rep 2008; 6: 142–8.

74 Gaur T, Lengner CJ, Hovhannisyan H, Bhat RA, Bodine PV, Komm BS, Javed A, van Wijnen AJ, Stein JL, Stein GS, Lian JB. Canonical WNT signaling promotes osteogenesis by directly stimulating Runx2 gene expression. J Biol Chem 2005; 280: 33132–40.

75 Moester MJ, Papapoulos SE, Lowik CW, van Bezooijen RL. Sclerostin: current knowledge and future perspectives. Calcif Tissue Int 2010; 87: 99–107.

76 Gaudio A, Privitera F, Battaglia K, Torrisi V, Sidoti MH, Pulvirenti I, Canzonieri E, Tringali G, Fiore CE. Sclerostin levels associated with inhibition of the Wnt/beta-catenin signaling and reduced bone turnover in type 2 diabetes mellitus. J Clin Endocrinol Metab 2012; 97: 3744–50.

77 Zhou YJ, Li A, Song YL, Zhou H, Li Y, Tang YS. Role of sclerostin in the bone loss of postmenopausal Chinese women with type 2 diabetes. Chin Med Sci J 2013; 28: 135–9.

78 Kim JY, Lee SK, Jo KJ, Song DY, Lim DM, Park KY, Bonewald LF, Kim BJ. Exendin-4 increases bone mineral density in type 2 diabetic OLETF rats potentially through the down-regulation of SOST/sclerostin in osteocytes. Life Sci 2013; 92: 533–40.

79 Monami M, Dicembrini I, Antenore A, Mannucci E. Dipeptidyl peptidase-4 inhibitors and bone fractures: a meta-analysis of randomized clinical trials. Diabetes Care 2011; 34: 2474–6.

80 Driessen JH, van Onzenoort HA, Henry RM, Lalmohamed A, van den Bergh JP, Neef C, Leufkens HG, de Vries F. Use of dipeptidyl peptidase-4 inhibitors for type 2 diabetes mellitus and risk of fracture. Bone 2014; 68: 124–30.

81 Mabilleau G, Mieczkowska A, Chappard D. Use of glucagon-like peptide-1 receptor agonists and bone fractures: a meta-analysis of randomized clinical trials. J Diabetes 2014; 6: 260–6.

82 Driessen JH, Henry RM, van Onzenoort HA, Lalmohamed A, Burden AM, Prieto-Alhambra D, Neef C, Leufkens HG, de Vries F. Bone fracture risk is not associated with the use of glucagon-like peptide-1 receptor agonists: a population-based cohort analysis. Calcif Tissue Int 2015; 97: 104–12.

83 Felson DT, Zhang Y, Hannan MT, Anderson JJ. Effects of weight and body mass index on bone mineral density in men and women: the Framingham study. J Bone Miner Res 1993; 8: 567–73.

84 Coin A, Sergi G, Beninca P, Lupoli L, Cinti G, Ferrara L, Benedetti G, Tomasi G, Pisent C, Enzi G. Bone mineral density and body composition in underweight and normal elderly subjects. Osteoporos Int 2000; 11: 1043–50.

85 Shapses SA, Riedt CS. Bone, body weight, and weight reduction: what are the concerns? J Nutr 2006; 136: 1453–6.

86 Papaioannou A, Morin S, Cheung AM, Atkinson S, Brown JP, Feldman S, Hanley DA, Hodsman A, Jamal SA, Kaiser SM, Kvern B, Siminoski K, Leslie WD. 2010 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada: summary. CMAJ 2010; 182: 1864–73.

87 Naot D, Cornish J. Cytokines and Hormones That Contribute to the Positive Association between Fat and Bone. Front Endocrinol (Lausanne) 2014; 5: 70.

88 Gier B, Butler PC, Lai CK, Kirakossian D, DeNicola MM, Yeh MW. Glucagon like peptide-1 receptor expression in the human thyroid gland. J Clin Endocrinol Metab 2012; 97: 121–31.

89 Hegedus L, Moses AC, Zdravkovic M, Le Thi T, Daniels GH. GLP-1 and calcitonin concentration in humans: lack of evidence of calcitonin release from sequential screening in over 5000 subjects with type 2 diabetes or nondiabetic obese subjects treated with the human GLP-1 analog, liraglutide. J Clin Endocrinol Metab 2011; 96: 853–60.

90 Bjerre KL, Madsen LW, Andersen S, Almholt K, de Boer AS, Drucker DJ, Gotfredsen C, Egerod FL, Hegelund AC, Jacobsen H, Jacobsen SD, Moses AC, Molck AM, Nielsen HS,

Nowak J, Solberg H, Thi TD, Zdravkovic M, Moerch U. Glucagon-like peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. Endocrinology 2010; 151: 1473–86.

91 Ta HM, Nguyen GT, Jin HM, Choi J, Park H, Kim N, Hwang HY, Kim KK. Structure-based development of a receptor activator of nuclear factor-kappaB ligand (RANKL) inhibitor peptide and molecular basis for osteopetrosis. Proc Natl Acad Sci U S A 2010; 107: 20281–6.

92 Wright HL, McCarthy HS, Middleton J, Marshall MJ. RANK, RANKL and osteoprotegerin in bone biology and disease. Curr Rev Musculoskelet Med 2009; 2: 56–64.

93 Kong YY, Boyle WJ, Penninger JM. Osteoprotegerin ligand: a regulator of immune responses and bone physiology. Immunol Today 2000; 21: 495–502.

94 Wagner D, Fahrleitner-Pammer A. Levels of osteoprotegerin (OPG) and receptor activator for nuclear factor kappa B ligand (RANKL) in serum: are they of any help? Wien Med Wochenschr 2010; 160: 452–7.

95 de Amorim FP, Ornelas SS, Diniz SF, Batista AC, Da ST. Imbalance of RANK, RANKL and OPG expression during tibial fracture repair in diabetic rats. J Mol Histol 2008; 39: 401–8.

96 Rasmussen LM, Tarnow L, Hansen TK, Parving HH, Flyvbjerg A. Plasma osteoprotegerin levels are associated with glycaemic status, systolic blood pressure, kidney function and cardiovascular morbidity in type 1 diabetic patients. Eur J Endocrinol 2006; 154: 75–81.

97 Marie P, Halbout P. OPG/RANKL: role and therapeutic target in osteoporosis. Med Sci (Paris) 2008; 24: 105–10.