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Review

RANKL inhibition: a new target of treating diabetes mellitus?

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***Abstract*:** Accumulating evidence demonstrates the link between glucose and bone metabolism. The receptor activator of nuclear factor-kB ligand (RANKL)/the receptor activator of NF-κB (RANK)/osteoprotegerin (OPG) axis is an essential signaling axis maintaining the balance between bone resorption and bone formation. In recent years, it has been found that RANKL and RANK are distributed not only in bone but also in the liver, muscle, adipose tissue, pancreas, and other tissues that may influence glucose metabolism. Some scholars have suggested that the blockage of the RANKL signaling may protect islet β-cell function and prevent diabetes; simultaneously, there also exist different views that RANKL can improve insulin resistance through inducing the beige adipocyte differentiation and increase energy expenditure. Currently, the results of the regulatory effect on glucose metabolism of RANKL remain conflicting. Denosumab (Dmab), a fully human monoclonal antibody that can bind to RANKL and prevent osteoclast formation, is a commonly used antiosteoporosis drug. Recent basic studies have found that Dmab seems to regulate glucose homeostasis and β-cell function in humanized mice or *in vitro* human β-cell models. Besides, some clinical data have also reported the glucometabolic effects of Dmab, however, with limited and inconsistent results. This review mainly describes the impact of the RANKL signaling pathway on glucose metabolism and summarizes clinical evidence that links Dmab and DM to seek a new therapeutic strategy for diabetes.

Keywords: denosumab, diabetes mellitus, glucose metabolism, insulin resistance, RANKL

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Introduction

With its increasing prevalence, diabetes mellitus (DM) has been a serious public health issue in contemporary society, which has already affected more than 537 million adults worldwide in 2021 and approximately 643 million by 2030.[1] A chronic hyperglycemia state caused by islet β-cell dysfunction (insufficient insulin secretion or insulin resistance) can lead to diabetic microvascular and macrovascular complications, which severely influence the patient's quality of life and expectancy. Therefore, it's necessary to look for effective approaches to maintain glucose homeostasis and improve β-cell function.

Osteoporosis is a common endocrine disease characterized by bone mass loss and bone microstructure destruction, which predisposes to fractures and contributes to significant disability and mortality. There is mounting evidence that supports a strong interaction between glucose and bone metabolism. DM patients are more likely to fracture than the overall population.[2–4] Patients with type 2 diabetes mellitus (T2DM) may experience detrimental effects on their bone health as a result of chronic hyperglycemia, oxidative stress, reduced sex hormones, and the use of some antidiabetic drugs such as thiazolidinedione.[5–7] In turn, some osteokines that are bioactive factors and peptides mainly secreted by bone tissue cells are also thought to have the potential to regulate glucose metabolism. Osteocalcin, secreted by osteoblast, can favor insulin secretion and improve glucose tolerance, which is regarded to be a protective factor against the onset of DM.[8,9] The receptor activator of nuclear factor-kB ligand (RANKL), the receptor activator of NF-κB (RANK), and osteoprotegerin (OPG) are also

crucial osteokines to modulate bone metabolism, forming a vital signaling axis that can maintain the balance between bone resorption and bone formation. Previous research has demonstrated that patients with T2DM had higher serum OPG levels, which are also strongly related to the onset of T2DM and its consequences.[10,11] However, several studies have presented opposite views: the RANKL/RANK signaling may be harmful to T2DM by inducing insulin resistance and reducing glucose uptake in peripheral tissues, while OPG, as a RANKL decoy receptor, can block the binding between RANKL and RANK, thereby enhancing insulin sensitivity.[12] Until now, research on the relationship between RANKL signaling and glucose metabolism has been limited and controversial. Similarly, the monoclonal RANKL antibody denosumab (Dmab), which functions in the same way as OPG, has been reported to promote β-cell proliferation and glucose metabolism in basic studies,[12,13] but the corresponding clinical evidence is insufficient. Therefore, this review investigates the effects of the RANKL signal pathway on glucose metabolism and summarizes clinical data related to Dmab to provide new insights into the treatment of diabetes.

RANKL/RANK/OPG pathway

RANKL, encoded by the tumor necrosis factor (TNF) ligand superfamily 11 gene, is a major activator stimulating osteoclast differentiation and maturation. RANK is the sole signaling receptor essential for RANKL-mediated osteoclastogenesis, which is a homotrimeric transmembrane protein with a 616 amino acid sequence.[14,15] RANKL and RANK are mainly expressed in bone, bone marrow, and lymphoid tissues.[16] OPG, belonging to a member of the TNF receptor superfamily, is a secretory protein that regulates bone density, first identified and named by Simonet's team in 1997.[17] It is produced in various organs and tissues, not only in bones where OPG levels are highest but also in the liver, kidneys, pancreas, heart, gastrointestinal tract, and vascular endothelium.[18]

RANKL plays a pivotal role in the development of osteoporosis. RANKL binds to RANK on the surface of osteoclast precursor cells, triggering a signaling switch called 'tumor necrosis factor receptor-associated factor 6 (TRAF6)', which then activates relevant signaling pathways, including NF-κB and mitogen-activated protein kinase (MAPK), to promote osteoclast differentiation and bone resorption. In turn, as a soluble decoy receptor for RANKL, OPG, can inhibit osteoclastogenesis and prevent bone loss by binding to RANKL and blocking the interaction between RANKL and RANK.[17,19] Therefore, the RANKL/RANK/OPG axis is the essential signaling pathway for modulating the balance of bone metabolism. And Dmab, as the specific RANKL inhibitor, has been approved for the treatment of osteoporosis to reduce bone resorption, increase bone mass, and improve bone density.

RANKL and glucose metabolism

The adverse effect of RANKL on glucose metabolism

Preceding studies have reported that RANKL and RANK can also be expressed in human liver tissues and pancreas β cells besides bones,[20] suggesting that it may participate in the regulation of glucose metabolism. The Bruneck Study, a prospective population survey on 844 Italian citizens with non-T2DM followed for 15 years, showed that RANKL concentration was associated with an increased risk of T2DM and was an independent predictor of the development of T2DM.[21] Bilger *et al.*[22] revealed that soluble RANKL (sRANKL) concentrations were higher in prediabetes groups than those in body mass index (BMI)- and age-matched control groups, and logistic regression analyses demonstrated that subjects with higher sRANKL levels have an increased risk of prediabetes. Similar findings were found in Karalazou *et al.*'s study,[23] where T1DM patients had higher serum RANKL levels and lower OPG levels compared with controls. As of now, the mechanism of the RANKL pathway impairing glucose metabolism has not been fully elucidated, which may mainly correlate with insulin resistance and islet β-cell proliferation (Figure 1).

RANKL induces insulin resistance. It is generally recognized that hepatic resistance is the key event in the etiology of T2DM, and abundant evidence supports that NF-κB pathway activation is a crucial step to cause hepatic insulin resistance and T2DM.[24–27] As a vital activator of NF-κB, RANKL may contribute to this process. Kiechl *et al.*[21] observed that blockage of RANKL signaling remarkably ameliorated liver insulin sensitivity

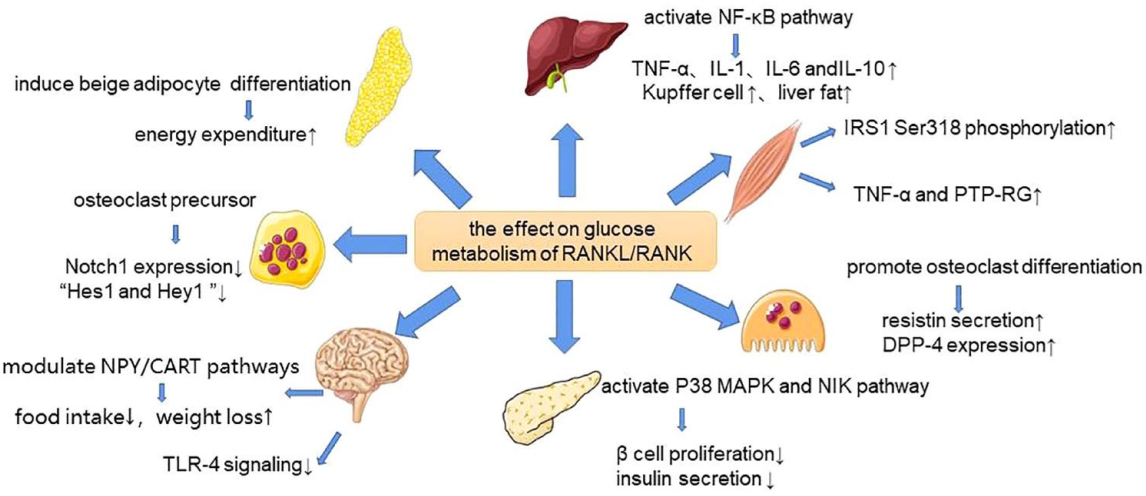

Figure 1. The effect on glucose metabolism of RANKL/RANK pathway.
CART, cocaine- and amphetamine-regulated transcript; DPP-4, dipeptidyl peptidase-4; IL-1, interleukin-1; IL-6, interleukin-6; IL-10, interleukin-10; IRS-1, insulin receptor substrate1; MAPK, mitogen-activated protein kinase; NIK, NF-κB-inducing kinase; NPY, neuropeptide Y; RANK, receptor activator of NF-κB; RANKL, receptor activator of nuclear factor-kB ligand; TLR-4, Toll-like receptor-4; TNF-α, tumor necrosis factor-α.

and lowered fasting glucose concentration in both high-fat-fed and leptin-deficiency (ob/ob) mouse models. A similar finding could be seen in a clinical study of 14 osteoporotic women from Italy that the administration of RANKL inhibitor (Dmab) for 4 weeks is helpful to reduce hepatic insulin resistance index (HIRI) (logHIRI: −0.2, $p = 0.01$).[28] Another study from Japan including 20 patients with osteoporosis also found that inhibiting RANKL can improve liver function, with the decline of alanine aminotransferase (ALT) (−1.6 U/L, $p = 0.004$) and aspartate transaminase (AST) (−1.8 U/L, $p = 0.014$).[29] Kiechl's team performed *in vitro* studies of cultured mouse hepatocytes and demonstrated that hepatocytes could steadily express RANK mRNA and the stimulation of hepatocytes with RANKL could promote upregulation of NF-κB-induced proinflammatory factors such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, and IL-10, and activate kupffer cells, which in turn caused excess accumulation of liver fat and insulin resistance.[21] Conversely, the role of RANKL disappeared after RANK genes in hepatocytes were knocked out.[21] It can be seen that the liver is a target organ of RANKL action. The combination of RANKL and RANK activates NF-KB signaling in hepatocytes and induces inflammation, which exerts an essential role in the pathogenesis of hepatic insulin resistance.

Reduced glucose uptake by peripheral tissues is another crucial reason to accelerate insulin resistance. Preceding studies have shown that RANKL mRNA and protein can be detected in the skeletal muscle of mice.[30,31] Recently, Bonnet *et al.*[12] observed that compared with wild-type (WT) mice, human RANKL transgenic (huRANKLTg$^+$) mice (5 times higher levels of human RANKL than WT mice) exhibited the attenuated muscle strength and decreased glucose uptake, accompanied by increased expression of inflammatory markers such as TNF-α and protein tyrosine phosphatase receptor gamma (PTP-RG). As confirmed previously, high TNF-α levels can attenuate the affinity of insulin receptor substrate1 (IRS1) to insulin and impede normal insulin signal transduction.[32,33] PTP-RG is also reported to cause inflammation-induced insulin resistance via binding to NF-kB.[34] Besides, Bonnet *et al.*[12] also found that prolonged RANKL exposure increased the phosphorylation of IRS1 ser318, which is known to downregulate the activity of insulin receptor 1, while OPG-Fc could reverse the deleterious effects of RANKL by blocking the NF-κB pathway, thereby improving insulin receptor activation. In addition, as expected, RANKL inhibitor Dmab, as with OPG, also improved muscle function, glucose entry, and insulin resistance in huRANKLTg$^+$ mice. Thus, RANKL signaling can reduce muscle glucose uptake possibly by

directly downregulating insulin receptors and indirectly increasing inflammation cytokines, while OPG and Dmab exert a protective effect on insulin sensitivity and glucose utilization by blocking the RANKL-mediated NF-κB pathway.

As is known to all, resistin is an important adipocytokine that can induce insulin resistance by both adenosine 5′ monophosphate-activated protein kinase (AMPK)-dependent and AMPK-independent pathways. The latest data have indicated that the levels of resistin increase during RANK-induced osteoclast differentiation and osteoclast may decrease glucose uptake in C2C12 cells (a muscle cell) and further promote insulin resistance by secreting resistin.[34,35] Recently, another important regulator of glucose hemostasis, dipeptidyl peptidase-4 (DPP-4), is also identified to be an osteoclast-driving coupling factor in humans. In a novel finding, RANKL signaling in osteoclasts induced DPP-4 expression, high levels of which would accelerate the degradation of glucagon-like peptide-1 (GLP-1), leading to decreased insulin and increased glucagon secretion, thereby promoting hyperglycemia. Conversely, suppressing RANKL can decline the levels of circulating DPP-4 accompanied by the rising levels of GLP-1, and then improve insulin resistance and glucose metabolism.[36] Thus, RANKL-induced osteoclast differentiation drives the secretion of some crucial factors modulating glucose metabolism such as resistin and DPP-4, which may be an indirect mechanism of insulin resistance caused by RANKL.

RANKL induces islet β-cell dysfunction. Besides insulin resistance, RANKL/RANK/OPG may affect islet β-cell function. Through *in vivo* and *in vitro* experiments, Kondegowda *et al.*[13] verified that RANKL may be the breaker of human islet β-cell proliferation, while OPG can exert a protective effect by interfering with RANKL/RANK pathway. MAPK signaling is a vital signaling pathway that regulates cell proliferation, growth, differentiation, and apoptosis in mammal animals, for which P38 MAPK is a crucial component. Plentiful evidence highlights that activation of P38 MAPK is one of the key mechanisms inducing islet β-cell apoptosis.[37,38] It has been confirmed that RANKL can bind to RANK to activate the p38MAPK signaling pathway in osteoclasts, but whether this mechanism exists in the pancreas is unknown. As early as 2007, Schrader *et al.*[39] discovered that OPG could protect pancreatic beta cells from damage by inhibiting p38 MAPK phosphorylation in human islet cells. However, since no RANK mRNA was detected at that time, the effect of RANKL on MAPK and beta cells could not be evaluated. In 2018, Diedisheim's study described that human islets could also express RANKL and RANK mRNA. RANKL could phosphorylate p38 in a dose-dependent manner, while exogenous OPG prevented the phosphorylation of P38 by RANKL.[40] Thus, RANKL can activate the p38MAPK, leading to human islet β-cell dysfunction, while OPG can protect it by blunting RANKL signaling.

In addition, Malle's study demonstrated that the noncanonical NF-κB pathway mediated by NF-κB-inducing kinase (NIK) is a novel mechanism of damaging β-cell function. RANKL could induce the activation of NIK, further influencing islet β-cell mass and impairing glucose-stimulated insulin secretion in human islets.[41]

The beneficial effect of RANKL on glucose metabolism

Although much data show the deleterious effect of RANKL on glucose metabolism, there also exist contradictory results. In previous reports, high serum OPG level was regarded to increase the risk of obesity, insulin resistance, T2DM, and cardiovascular disease possibly due to the deleterious role on endothelial function.[42–44] Conversely, the latest study found that,[45] in the condition of the high-fat diet, compared with WT mice, OPG−/− mice exhibit white fat tissue (WAT) browning, lower weight gain, preserved insulin sensitivity, and improved glucose metabolism as well as higher serum RANKL levels. And RANKL treatment could increase energy expenditure by inducing beige adipocyte differentiation in preadipocytes and browning of WAT depots, which shed light on the possible beneficial aspect of RANKL from the perspective of adipocyte cells. However, the levels of serum adiponectin and osteocalcin, two important proteins that are considered to improve insulin sensitivity and glucose metabolism,[46,47] are also elevated in this OPG-deficiency mice model, which may interfere with the results.[45] In addition, besides RANKL, OPG also acts as a decoy ligand for TNF-related apoptosis-inducing ligand (TRAIL), a transmembrane belonging to the TNF

family number.[48] Previous studies have shown that TRAIL exhibited positive metabolic effects in mice by decreasing adipose tissue gene expression and inducing adipocyte apoptosis, as well as downregulating inflammatory factors TNF-α and monocyte chemotactic protein-1 (MCP-1) in adipose tissue.[49] Therefore, we speculated that the loss of OPG may enhance the action of TRAIL and play a protective effect on energy metabolism. Taken together, OPG has some biological effects independent of RANKL, which may limit the contribution of the RANKL activation on adipose tissue to some degree. Thus, the deeper mechanism of RANKL acting on adipocytes remains to be investigated.

The Notch signaling pathway is a key pathway leading to islet β-cell impairment in diabetic patients, and there may be an interaction between the RANKL and the Notch pathway. *In vitro*, exogenous RANKL stimulation for mouse RAW264.7 cell can decrease Notch1 expression and suppress the Notch pathway downstream signal 'Hes1 and Hey1 molecules' to inhibit the Notch signal transduction,[50,51] which suggests that RANKL may prevent pancreatic β-cell damage by inhibiting the Notch 1 signal. However, the downregulation of Notch1 can increase the expression of RANK mRNA,[51] further promoting the combination between RANK and RANKL, which may be detrimental for islet β cells. The outcome of the reciprocity between RANKL and Notch1 signal on glucose metabolism is unclear; thus, it is necessary to investigate this mechanism in depth.

Moreover, Some studies have reported that RANKL and RANK are also expressed in the hypothalamus and septal regions of the brain.[31,52] Neuropeptide Y (NPY) and cocaine- and amphetamine-regulated transcript (CART) are two important neuropeptides in the hypothalamus that promote and inhibit appetite, respectively.[53] Zhu *et al.*[54] showed that NPY mRNA decreased and CART mRNA increased in RANKL-treated mice, suggesting that RANKL can reduce food intake and causes weight loss via modulating the hypothalamic NPY/CART pathways. Besides, it has been reported that RANKL/RANK signal exerted an anti-inflammatory influence by preventing Toll-like receptor (TLR)-4 signaling in the brain,[55] the activation of which can induce obesity and insulin resistance, thus leading to T2DM.[56] Nevertheless, it was conflicting with the results that RANKL/RANK can enhance the expression of proinflammatory factors.[21,12] To conclude, RANKL may play a beneficial role in glucose metabolism by regulating some pathways in the brain, but the specific mechanism remains to be well-studied.

Dmab and glucose metabolism

Dmab, a human monoclonal antibody of RANKL that can specifically bind to RANKL and prevent the combination of RANKL and RANK on osteoclasts, is currently applied to treat osteoporosis clinically due to the ability of anti-bone absorption. Multiple animal studies have demonstrated that Dmab may have the potential to improve glucose metabolism and β-cell function.[13,57] However, available clinical data on Dmab and glucose metabolism are inadequate and inconsistent (Table 1). Weivoda *et al.*[36] found that Dmab treatment could reduce circulating DPP-4 levels and increase GLP-1 levels in nondiabetic postmenopausal women. Based on this, he followed up a cohort including 345 T2DM patients treated with Dmab, *versus* bisphosphonate or calcium plus vitamin D (C/VitD) for 1 year (115 patients per group), the results indicating that Dmab was helpful to improve glucose homeostasis (HbA1c: −0.239%, +0.126%, and + 0.356% in Dmab, bisphosphonate, and C/VitD groups, respectively; $p < 0.05$) in T2DM patients compared with controls.[36] This not only reconfirms the interconnection between glucose and bone metabolism but also provides a new idea for the treatment of diabetes. Abe *et al.*[29] obtained the same findings from 20 patients with osteoporosis and T2DM: the levels of both fasting plasma glucose (FPG) and hemoglobin A1c (HbA1c) were decreased from baseline to end-points (52 weeks), simultaneously accompanied by the improvement of insulin resistance [the decrease of homeostasis model assessment for insulin resistance (HOMA-IR)] and liver enzymes [alanine aminotransferase (ALT) and aspartate transaminase (AST)]. Napoli performed a *post hoc* analysis from a large randomized controlled trial (RCT) on Dmab, and the results were slightly different from the above two studies.[58] He revealed that Dmab could only reduce FPG levels in diabetic patients who did not receive hypoglycemic drugs, whereas it did not affect FPG in the overall diabetic population. Moreover, he analyzed 1268 pre-diabetic postmenopausal women receiving

Table 1. Clinical studies focused on the effect of denosumab on glucose metabolism.

Author	Study design	Study Subjects	Dmab group sample	follow-up time (months)	Age (years)	Invention	Outcome
Weivoda *et al.*[36]	Randomized controlled trial	46 postmenopausal women with NGT	22	3	66.7 ± 4.9 69.5 ± 6.0	Dmab 60mg; placebo	Dmab increased GLP-1 levels and reduced DPP-4 levels, however, without the change of FPG and insulin levels
Weivoda *et al.*[36]	Case–control study	345 patients with osteoporosis and T2DM (or prediabetes)	115	6, 12	74.1 ± 10.0 71.4 ± 9.5; 70.3 ± 9.6	Dmab 60mg; calcium/vitamin D; bisphosphate	Compared with either bisphosphonates or calcium and vitamin D, Dmab treatment showed significant reductions in FPG levels at 6 months and HbA1c levels at 12 months in T2DM patients
Abe *et al.*[29]	Prospective observational study	20 patients with osteoporosis and T2DM	20	6,12	72.1 ± 7.2	Dmab 60 mg per 26 weeks	Dmab can significantly decrease the levels of FPG, HbA1c, and HOMA-IR as well as the liver enzymes at 12 months
Napoli *et al.*[58]	*Post hoc* analysis of FREEDOM study[a]	666 postmenopausal women with T2DM	342	36	73.5 ± 5.1; 73.4 ± 5.1	Dmab 60 mg per 6 months, for 36 months; placebo	DMAb did not improve FPG levels in postmenopausal osteoporotic women with diabetes, but the levels of FPG slightly lowered in those with diabetes who were not on antidiabetic drugs
		1268 postmenopausal women with prediabetes	628	36	72.6 ± 5.4; 72.4 ± 5.0	Dmab 60 mg per 6 months, for 36 months; placebo	Dmab had no impact on the levels of FPG in postmenopausal osteoporotic women with prediabetes
Passeri *et al.*[28]	Prospective observational study	14 postmenopausal osteoporotic women with NGT	14	1, 3	67.1 ± 11.6	Single 60 mg Dmab	Dmab did not affect the levels of FPG, insulin, and HOMA-IR, but the hepatic insulin resistance index was reduced at 1 month, accompanied by the decline of HbA1c
Lasco *et al.*[59]	Prospective observational study	48 postmenopausal women with NGT	48	1, 3, 6	57.89 ± 6.34	Single 60 mg Dmab	Dmab had no clinical effect on FPG and HOMA-IR in nondiabetic postmenopausal women
Rossini *et al.*[60]	Prospective observational study	14 postmenopausal Women with breast cancer and NGT	14	1, 5	68.1 ± 8.1	Single 60mg Dmab	Dmab had no influence on glucose metabolism in the population with NGT, but it can induce a short-term insulin sensitivity improvement in insulin-resistant patients

Dmab, denosumab; DPP-4, dipeptidyl peptidase-4; FPG, fasting plasma glucose; GLP-1, glucagon-like peptide-1; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment for insulin resistance; NGT, normal glucose tolerance; T2DM, type 2 diabetes mellitus.
[a]FREEDOM study: FREEDOM is a 3-year randomized, placebo-controlled trial on the efficacy of denosumab on vertebral, nonvertebral, and hip fractures in postmenopausal women with osteoporosis.

Dmab therapy and found the FPG levels were also not improved in these populations either.[58]

Other scholars had also similar conclusions that Dmab did not affect glucose homeostasis in individuals with normal glucose tolerance (NGT).[59] Notably, although some studies did not find the clinical effect of Dmab on glucose metabolism, they detected that it seems to improve hepatic insulin sensitivity.[28,60] In 2021, to determine whether Dmab can improve glycemic parameters, Pacheco-Soto *et al.*[61] did a meta-analysis involving 1203 participants and found that Dmab could reduce FPG levels (standardized mean difference, SMD = −0.388 mg/dl, $p = 0.017$) and ameliorate insulin resistance (HOMA-IR: −0.223, $p = 0.008$) in the overall population, but without the change of HbA1c (SMD = −0.075%, $p = 0.538$). Further subgroup analysis showed that this beneficial effect on FPG (SMD = −0.636 mg/dl, $p = 0.010$) and insulin resistance (HOMA-IR: −0.573, $p = 0.008$) was more prominent in individuals with prediabetes or diabetes than in those with NGT, and the significant improvement of HbA1c (SMD = −0.292%, $p < 0.005$) can also be seen.[61] However, there existed a relatively large heterogeneity across studies, which may affect the strength of the results. In brief, the current studies on Dmab and glucose metabolism are limited with relatively small sample sizes, variable study populations and follow-up times, and a lack of high-quality RCTs. But, undoubtedly, Dmab has shown some benefits in regulating glucose disorders, especially for patients with T2DM rather than NGT or prediabetes, the mechanism of which may be related to its ability to improve hepatic insulin resistance. In the future, more large-scale RCTs are still needed to evaluate the efficacy of Dmab in treating hyperglycemia.

Novel hypoglycemic drug: GLP-1RA and RANKL

Diabetic patients are prone to bone damage and have more risk of bone fractures, so the safety of hypoglycemic drugs on bone has always been a research hotspot. Some traditional hypoglycemic drugs that have been identified to affect bone metabolism, such as metformin and sulfonylureas, may be beneficial to the bone, and thiazolidinediones are thought to increase the risk of fracture.[62,63] Glucagon-like peptide-1 receptor agonist (GLP-1RA) is a new class of hypoglycemic drugs, which can enhance the effect of GLP-1, an incretin secreted by intestinal L-cell, to potentiate glucose-stimulated insulin secretion. Recent studies have demonstrated that GLP-1RA can also improve bone metabolism, in addition to weight reduction and hypoglycemic effects as well as significant cardioprotection.

In some views, GLP-1RA mainly exerts a pro-osteoblast effect, promoting the differentiation of bone mesenchymal precursor cells (BMSCs) and the proliferation of osteoblast cells through acting on the Wnt/β-catenin signaling pathway, which in turn facilitates bone formation,[64–66] while other scholars also reveal that it can influence osteoclast function. The previous report showed that GLP1 could decrease bone resorption through the calcitonin-dependent pathway,[67] but the latest research works find that it can also regulate the RANKL pathway. In ovariectomized (OVX) mice, exendin-4, a GLP-1RA, can decrease telopeptides of type I collagen (CTX-I) (a bone resorption marker) and RANKL/OPG ratio.[68] In another experiment, liraglutide manifested the same effect not only in OVX mice but also in the streptozotocin (STZ) + OVX model,[69] which implied that it may help hinder osteoclast function in diabetic patients. Furthermore, Li *et al.*[70] also discovered that liraglutide could reduce RANKL-induced osteoclast differentiation by inhibiting NF-κB and MAPK-nuclear factor of activated T cell (NFATc1) signaling pathways. The above animal experiments indicated the potential benefits of GLP-1RA on bone health by inhibiting RANKL. As for clinical studies, there exist some inconsistent results. In some studies, neither exenatide nor liraglutide treatment showed any effect on bone mineral density (BMD) in T2DM patients compared with other hypoglycemic drugs;[71,72] besides, exenatide did not change the level of CTX-1.[71] However, two latest large meta-analyses, including 54 RCTs and 38 RCTs, respectively, indicate that GLP-1R therapy can increase BMD and lower the risk of bone fracture in T2DM individuals.[73,74] Despite different results, possibly related to GLP-RA dose, type, and treatment duration, overall, GLP-1RA seems to be a beneficial hypoglycemic agent for bone health. The effect of GLP-1RA on osteoclast, especially on RANKL, deserve to be deeply explored.

Conclusion and future perspective

In summary, in addition to being a classical regulator of bone metabolism, RANKL can also play

a role in glucose metabolism. Most of the evidence supports that RANKL signaling is detrimental to glucose homeostasis. Experimental studies demonstrate that RANKL not only increases hepatic and muscle insulin resistance by activating the NF-κB pathway and promoting inflammatory response, but also induces pancreatic β-cell dysfunction and upregulates negative glycemic regulators such as resistin and DPP-4. In the clinical aspect, the RANKL inhibitor, Dmab, shows a potential beneficial effect on glucose profile in patients with T2DM, although it seems to have no impact on the normal glucose tolerance population. Besides, GLP-1RA, as a new antidiabetic agent, also manifests a protective power on bone, whose mechanism may be related to the inhibition of osteoclast differentiation induced by RANKL, which also indicates that RANKL bridges the link between glucose and bone. Nevertheless, there exist counterarguments. Some scholars find that RANKL may be beneficial to improve glucose metabolism by promoting energy consumption in adipose tissue and feeding signaling and inflammatory pathways in the brain. Although the correlation between RANKL and glucose metabolism has not been fully elucidated at present, indubitably, RANKL signaling will be a promising research target in the field of diabetes treatment. Therefore, it is necessary to investigate the pathophysiological mechanism behind its involvement in glucose metabolism in the future.

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Not applicable.

Consent for publication
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Author contributions
Baodi Xing: Conceptualization, Data curation, Writing – original draft, Writing – review & editing.

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