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Characterization and preparation of food-derived peptides on improving osteoporosis: A review

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ABSTRACT

Osteoporosis is a systemic bone disease characterized by reduced bone mass and deterioration of the microstructure of bone tissue, leading to an increased risk of fragility fractures and affecting human health worldwide. Food-derived peptides are widely used in functional foods due to their low toxicity, ease of digestion and absorption, and potential to improve osteoporosis. This review summarized and discussed methods of diagnosing osteoporosis, treatment approaches, specific peptides as alternatives to conventional drugs, and the laboratory preparation and identification methods of peptides. It was found that peptides interacting with RGD (arginine-glycine-aspartic acid)-binding active sites in integrin could alleviate osteoporosis, analyzed the interaction sites between these osteogenic peptides and integrin, and further discussed their effects on improving osteoporosis. These may provide new insights for rapid screening of osteogenic peptides, and provide a theoretical basis for their application in bone materials and functional foods.

1. Osteoporosis

As the global population ages, the prevalence of diseases associated with aging, such as diabetes, arthritis, and dementia, also increases (Li et al., 2021; Lopes et al., 2022). Among these, the increasing incidence of chronic diseases such as osteoporosis have caused a significant societal burden (Su et al., 2020). Osteoporosis affects more than 200 million people worldwide (Shen et al., 2022). By 2030, the number of hip fractures among males are estimated to increase by 51.8% globally, and females are at even higher risk (Gasparik et al., 2023). The increasing incidence of osteoporosis may contribute to a higher risk of fractures and reduced life expectancy, necessitating investigations on the prevention and treatment of this condition.

In the human body, normal bone metabolism involves persistent bone tissue remodeling through osteoblast-dependent bone formation and osteoclast-dependent bone resorption. Enhanced bone resorption leads to reduced bone density, a primary factor in the development of osteoporosis (Malluche, Davenport, Lima, & Monier-Faugere, 2022). Osteoporosis is characterized by reduced bone density, deteriorating bone microstructure, and aggravated bone fragility, which increase the risk of fracture. These changes in bone structure are closely linked to the activities of osteoblasts and osteoclasts: osteoblasts secrete non-collagen proteins to regulate the mineralization of bone matrix, while osteoclasts are responsible for bone resorption (Kittivanichkul, Watanabe, Nagaoka, & Malaivijitnond, 2016). Osteocytes, the most abundant cells in mature bone (Fig. 1), interact with osteoblasts and osteoclasts to influence bone

Abbreviations: BLI, Biolayer Interferometry; CTX, C-Terminal peptide; DKK1, Dickkopf-related protein 1; DPD, deoxypyridoline; ICTP, Type I collagen cross-linked telopeptide; NTX, N-Terminal peptide; PICP, Procollagen Type I C-Terminal Propeptide; PINP, Procollagen Type I N-Terminal Propeptide; PYD, pyridine; RGD, Arg-Gly-Asp; TRACP5b, Tartrate-resistant acid phosphatase 5b..

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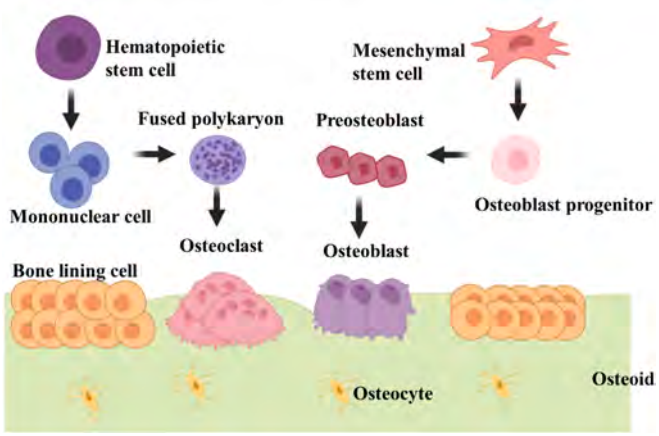

Fig. 1. The effects of bone cells in bone matrix on regulation of bone mass.

formation and resorption and are closely related to osteoporosis.

Bone formation and resorption occur through the activities of osteoblasts and osteoclasts, respectively, on osteoid and trabecular bone surfaces. Osteoid refers to the stage where osteoprogenitor cells multiply, differentiate into osteoblasts, and secrete bone matrix to form collagen fibers and matrix. Bone turnover, which includes bone formation and resorption, is higher in trabecular bone due to its greater surface area compared to cortical bone. Approximately 10% of the skeleton is remodeled annually to adapt to and repair mechanical stress-induced injury, indicating the importance of maintaining bone metabolism (Westendorf, 2007). The risk of osteoporosis increases in postmenopausal women due to estrogen deficiency, which could lead to bone loss. By 2025, hip fractures worldwide are expected to account for approximately 800,000 fractures per year, Additionally, approximately 13% of 50-year-old males and 40% of 50-year-old females will experience fragility fractures (Rinonapoli et al., 2021). Dual-energy X-ray absorptiometry, which measures bone mineral density (BMD), is a key technique for osteoporosis diagnosis. Generally, BMD with a T-score smaller than −2.5 indicates osteoporosis, a major risk factor for fractures. However, low BMD is not the only indicator for the high fracture risk (Bliuc, Alarkawi, Nguyen, Eisman, & Center, 2015; Chen, Ma, Liu, Cui, & Ma, 2020). Therefore, other diagnostic methods to evaluate the risk of osteoporosis fracture risk are necessary. Serum bone turnover markers, such as bone alkaline phosphatase and tartrate-resistant acid phosphatase 5b are effective indicators for this purpose since they provide the insights about bone remodeling and formation rates. These markers can be utilized to identify the fracture risk in certain populations, detect anabolic and anti-resorption processes, and predict osteoporosis risk in patients. The present review discusses bone turnover and resorption markers currently used for evaluating bone health.

2. Osteoporosis evaluation

2.1. Bone formation and resorption biomarkers

Bone tissue proteins, fragments, and enzymes are biomarkers of bone turnover, with some indicating bone formation and others bone resorption (Table 1). Alkaline phosphatase (ALP), a cell surface enzyme, has tissue-specific and -nonspecific isoforms. For instance, bone-specific ALP is found in bones, liver, and kidney, while nonspecific isoforms detected as serum or total ALP are also expressed in the germline, intestine, and placenta. Some ALP genes exhibit both tissue-specific and -nonspecific expression, but the encoded isoenzymes feature distinct carbohydrate chain modifications depending on the tissue (Chen et al., 2017). Generally, liver and bone alterations can impact serum ALP content. Osteoblasts release ALP into blood circulation during bone mineralization. Over 50% of serum ALP in the normal adult human body originate from bones (Kulpa et al., 2024). Thus, serum ALP can be utilized as a bone turnover marker. However, it is not specific and sufficiently sensitive.

Table 1
Biomarkers of osteoporosis.

Type	Test sample	Tissue of origin
Bone formation marker		
Alkaline phosphatase	Serum	Bone, intestine, germ cell
Bone specific alkaline phosphatase	Serum	Bone (osteoblast), platelets
Osteocalcin (intact, total, carboxylated)	Serum	Bone (osteoblast), soft tissue
Procollagen I C-terminal extension peptide	Serum	Bone, soft tissue
Procollagen I N-terminal extension peptide	Serum	Bone, soft tissue
Bone resorption marker		
Osteocalcin	Urine	Bone
Tartrate resistant acid phosphatase 5b	Serum	Bone (osteoclast)
C-terminal cross-linking telopeptide of type I collagen	Serum/ urine	Bone, soft tissue
N-terminal cross-linking telopeptide of type I collagen	Serum/ urine	Bone, soft tissue
C-terminal cross-linking telopeptide of type I collagen, generated by metalloproteinases	Serum	Bone
Deoxypyridinoline	Urine	Bone, blood vessels
Pyridinoline	Urine	Bone

While bone-specific ALP offers enhanced sensitivity as a biomarker for bone turnover, there is a 15%–20% overlap between bone-specific ALP and liver ALP (Chew & Clarke, 2017), which may complicate its interpretation. Osteocalcin, the most abundant bone-derived non-collagen matrix protein, is a low-molecular-weight protein expressed by tissue-forming cells like osteoblasts, preosteoblasts, odontoblasts, and hypertrophic chondrocytes (Li, Zhang, Yang, Li, & Dai, 2016). This protein is characterized by three c-carboxyglutamate residues that interact with calcium ions (Ca^{2+}) in hydroxyapatite, the main inorganic component in bones. Osteocalcin is found both in the circulatory system and bone matrix. So, serum osteocalcin can serve as a marker for bone formation and osteoblast function. Osteocalcin released from bone matrix during bone resorption is quickly degraded followed by the entry into circulation (Kalia, Ansari, & Regmi, 2022). Osteocalcin fragments are also detected in the liver and kidney (Martiniakova et al., 2024). Urinary osteocalcin serves as a biomarker for bone resorption (Hurjui et al., 2020; Paldanius, Ivaska, Makitie, & Viljakainen, 2021), but not type I bone formation collagen synthesis. Type I collagen secreted by osteoblasts has amino- and carboxyl-terminal peptides, which are cleaved prior to fibril formation (Claeys et al., 2021). These peptides promote helical folding of collagen. Procollagen type I C-terminal propeptide and procollagen type I N-terminal propeptide derived from procollagen type I are present in circulation. While type I collagen is typically found in bones and soft tissues, the collagen regeneration rate is higher in bones than in other tissues. Therefore, procollagen type I C-terminal propeptide and procollagen type I N-terminal propeptide can serve as biomarkers for quantifying type I collagen in bone (Tridimas, Milan, & Marks, 2021; Claeys et al., 2021).

Bone resorption markers mainly include tartrate-resistant acid phosphatase (TRACP), C-/N-terminal cross-linked telopeptides of type I collagen generated *via* metalloprotease activity, osteocalcin, pyridinoline, and deoxypyridinoline. The catalytic enzyme TRACP5b, which targets phosphate esters under acidic conditions, is mostly expressed by osteoclasts, monocyte-derived macrophages, and alveolar cells. In blood, two TRACP5 isozymes can be detected: TRACP5a produced by macrophages, and TRACP5b by osteoclasts. Moreover, each isozyme functions optimally at different pH values. However, the function of TRACP5b in osteoclasts is unclear. TRACP endocytosis by cells promotes

matrix degradation through reactive oxygen species generation within endocytic vesicles. Thus, TRACP is an effective indicator of osteoclast quantity and bone resorption rate (Mira-Pascual et al., 2020). TRACP5b is widely used to access renal bone disease in chronic kidney disease (Vasikaran, Miura, Pikner, Bhattoa, & Cavalier, 2023). Additionally, due to the inter- and intramolecular interaction, TRACP5b shows high stability in serum (Lv et al., 2015). Deoxypyridinoline and pyridinoline, compounds composed of cross-linked collagen, are released during the degradation of collagen in bone and dentin (Pillalamarri, Manyam, Pasupuleti, Birajdar and Akula, 2022). These cross-linked compounds are excreted through the kidney and are detectable in urine and serum either as free form or bound to peptides. Type I collagen cross-linked telopeptide (ICTP) consists of cross-linked C- and N-terminal peptides (CTX and NTX, respectively). CTX exists in two forms: non-isomerized α-CTX and isomerized β-CTX. β-CTX is a marker of bone resorption (Monjardino et al., 2019). Additionally, different pathways of collagen breakdown yield various fragments. For instance, the process of cathepsin K-mediated type I collagen degradation could produce CTX and NTX, whereas that mediated by matrix metalloproteinases generates ICTP (Turco et al., 2018).

2.2. Computed tomography for osteoporosis diagnosis

Computed tomography is one of the most commonly used techniques in hospitals to assess bone health. This method can provide detailed information about several aspects of bone structure, including tissue and bone volumes, the ratio of bone volume to tissue volume, trabecular surface, pattern, number, separation, along with bone mineral density (BMD) (Yang, Yang, Pan, & Zhong, 2019). BMD, expressed as bone mineral content per unit volume of bone (g/cm^3)(Bristow, Gamble, Horne, & Reid, 2019), is a crucial indicator of bone strength. Due to variations in BMD measurement approaches, T-score is frequently used to clinically evaluate the bone density (Martineau, Silva, & Leslie, 2017). Normal T-scores range from −1 to +1 (Lee et al., 2017), while below −2.5 indicate low BMD (Leslie and Crandall, 2019). Bone density is a key indicator of osteoporosis and fracture risk. With development in measurement techniques and improved accuracy, BMD has been applied beyond osteoporosis diagnosis, including epidemiological investigations and clinical studies of drugs.

Currently, many methods to treat osteoporosis are evaluated by animal osteoporosis model combined with computed tomography measurement of BMD. Extensive osteoporosis models have been reported, including glucocorticoid intramuscular injection (Hofbauer et al., 2015), intragastric retinoic acid (Xiong, Hua, & Tu, 2017), and ovary and testis resection are the most common methods for model establishment (Min et al., 2018). Additionally, low-calcium diets (Wen-Ting, Wang, Liu, Kai, & Yang, 2015), and parathyroid gland modeling (Jung et al., 2016) have also been reported to study osteoporosis. Regardless of the osteoporosis model, BMD detected by computed tomography is an important basis for judging the effectiveness of osteoporosis treatment.

3. Treatment of osteoporosis

3.1. Agents on improving osteoporosis

Long-term chronic conditions that disrupt the homeostasis of bone formation and resorption in the skeletal system can lead to bone diseases, which are challenging to treat. Antiosteoporosis therapeutic agents can be classified into two main types: bone antiresorptive and bone anabolic agents (Riggs & Parfitt, 2005). Bone antiresorptive agents, such as bisphosphonates and denosumab, promote bone remodeling, enhance secondary mineralization, and stabilize skeletal structure stabilization, improving bone strength while decreasing fracture risk. However, these agents are not able to reverse bone or structural loss but only improve bone remodeling. In addition, selective estrogen receptor modulators, hormone replacement therapy, calcitonin, and divalent cation strontium ranelate are clinically used as antiresorptive agents (Fang & Zhu, 2019). Nitrogen-containing bisphosphonates, such as alendronate, ebandronate, minodronate, risedronate, and zoledronate, inhibit protein isoprenylation in osteoblasts, and diminish the reactivation of bone lining cells by parathyroid hormone (PTH). These bisphosphonates efficiently suppress the production of farnesyl pyrophosphate, which is involved in isoprenoid lipid production and has an essential role in osteoclast activity and survival. Thereby the treatment of bisphosphonates efficiently reduces bone resorption rate and risk of fractures (Ebetino et al., 2022). However, in severe osteoporosis cases with trabecular bone loss, antiresorptive agents cannot prevent subsequent fractures. Furthermore, antiresorptive agents are associated with the risk of chronic liver disease.

Bone anabolic agents, especially teriparatide, are frequently prescribed as an alternative treatment for osteoporosis. Teriparatide, an endogenous peptide with 34-amino acid of PTH (Rachner, Hofbauer, Göbel, & Tsourdi, 2019), interacts with PTH1R to promote anabolism through the cAMP-PKA pathway (Nishimori et al., 2019; Vilardaga, Lin, & Nissenson, 2001). In osteoblasts, activated PTH receptors transmit canonical Wnt signals, which promote their growth and differentiation. Additionally, drugs targeting Wnt antagonists inhibit sclerostin in bone cells, amplifying osteoblast signals, and PTH modulates Dickkopf-related protein 1 (DKK1), an antagonist of Wnt (Appelman--Dijkstra & Papapoulos, 2018; Pinet & McLaughlin, 2019). PTH 1–34 enhances DKK1 expression (Gatti et al., 2011). PTH's up and down-regulating effect indicates a steady-state response, which results in decreased levels of bone formation markers in patients with osteoporosis. PTH treatment triggers RUNX2 expression, promoting osteoblast differentiation (Gomathi, Akshaya, Srinaath, Moorthi, & Selvamurugan, 2020). Intermittent PTH treatment increases osteoblast population, promotes committed osteoblast precursor differentiation, and extends osteoblast survival.

Osteoclasts release growth factors, such as insulin-like growth factor, bone morphogenetic proteins, and transforming growth factor β, all of which are stored in the bone matrix. Transiently-activated osteoclasts produce molecules that enhance the anabolic activity of PTH. Intermittent treatment with rhPTH in combination with bisphosphonate can delay this anabolic response in bone (Wang et al., 2017). Such treatment with rhPTH not only increases bone mass and BMD, but also enhances trabecular bone connectivity and enhances bone microstructure. Additionally, intermittent treatment with rhPTH promotes the juxtaposition of cortical endometrium and periosteum, increasing cortical thickness and bone diameter and promoting intracortical porosity (Jilka et al., 2009). The resulting improved bone strength could further contribute to the mechanical strength that helps resist fracture. Additionally, teriparatide has been reported to decrease serum sclerostin content in women with postmenopausal osteoporosis and reduce bone fragility.

However, long-term use of anabolic agents is associated with severe side effects, including carcinogenesis, uterine bleeding, cardiovascular disease, jaw bone necrosis (McGreevy & Williams, 2011), and increased osteosarcoma risk (Taylor & Saag, 2019). Osteosarcoma, which has the highest morbidity rate among bone cancers, develops from mesenchymal cells. Synthesis of bone tissue or tumor bone-like tissue can lead to rapid tumor growth directly or indirectly. Therefore, food-derived active peptides that promote bone metabolism have received increasing attention. Currently, there are extensive studies of biologically active peptides like calcitonin gene-related peptides and osteogenic growth peptides, and their functions in enhancing bone metabolism. While the exact mechanisms by which certain food-derived peptides improve osteoporosis remain unclear, their notable osteogenic activity, effective absorption, and safety provide significant advantages for developing products with the potential to enhance osteoporosis treatment.

3.2. Food-derived peptides on improving osteoporosis

The scarcity of reported agents that enhance bone metabolism indicates the need to develop safe and effective new-generation therapeutics and alternatives to prevent osteoporosis. Consequently, there is growing interest in the development of novel dietary treatments to enhance bone formation and prevent osteoporosis.

Recently, there has been a growing interest among consumers and researchers in functional foods with therapeutic properties and biological activities, particularly those that help prevent osteoporosis. Proteins are the most widely used components in pharmaceuticals, foods, and nutritional supplements that promote human health. Bioactive peptides enhance protein diversification and exhibit properties such as high solubility, low toxicity, and favorable pharmacokinetics (Marcone, Belton, & Fitzgerald, 2017; Singh, Aluko, Hati, & Solanki, 2022; Zvereva, Dudko, & Dikunets, 2018). Hence, bioactive peptides are extensively used as therapeutic agents. Globally, peptide therapeutics are expected to grow at a compound annual rate of nearly 9% from 2016 to 2024, the sales of metabolic disease peptide drugs such as liraglutide and glucagon-like peptide 1 have reached at least 2 billion US dollars (Anand, Bandyopadhyay, Jha, Pérez de la Lastra, & Dey, 2023).

Table 2 lists some example peptides with the ability to prevent

Table 2
Osteogenic peptides.

No.	Amino acid sequence	Origin	Model
1	SVSEIQLMHNLGKHLNSMERVGWLRKKLQDVHNF	PTH 1–34	*In vitro*
2	IAGVGGEKSGGP	Collagen type III	*In vitro* and *In vivo*
3	ALKRQGRTLYGFGG/YGFGG/GGFGY	Histone4	*In vitro*
4	YQPPSTNKNTKSQRRKGSTFEEHK	Insulin-like growth factor I	*In vitro*
5	GLRSKSKKFRRPDIQYPDATDEDITSHM	Osteopontin	*In vitro* and *In vivo*
6	NGVFKYRPRYYLYKHAYFYPHLKRFPVQ	Bone sialoprotein	*In vitro* and *In vivo*
7	RKKNPNCRRH	Bone morphogenetic protein-4	*In vitro*
8	NSVNSKIPKACCVPTELSAI	Bone morphogenetic protein-2	*In vivo*
9	KIPKASSVPTELSAISTLYL	Bone morphogenetic protein-2	*In vitro*
10	SKIPKASSVPTELSAISTLDDD	Bone morphogenetic protein-2	*In vivo*
11	ALKRQGRTLYGFGG	Histone H4	*In vivo*
12	VEIQLLHQXALWLHD	PTH(1–17)	*In vivo*
13	HWAWFK	Hexarelin	*In vivo*
14	GFOGER	Collagen	*In vivo*
15	KPSSAPTQLN	Bone morphogenetic protein-7	*In vitro*
16	CGGKVGKACCVPTKLSPISVLYK	pBone morphogenetic protein-9	*In vitro*
17	VEHDKEFFHPRYHHR	Bone morphogenetic protein-7	*In vitro*
18	DVSTSQAVLPDDFPRYPVGKFFKFDTWRQSAGRL	Preptin	*In vitro* and *In vivo*
19	PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC	C-Type Natriuretic Peptide	*In vivo*
20	GVVPPQVLSQPNEEAGAALSPLPEVPPWTGEVSPAQR	C-Type Natriuretic Peptide	*In vitro*
21	HSDGIPTDSYSRYRKQMAVKKYLAAVLGKQRVKNK	Pituitary Adenylate Cyclase-Activating Polypeptide	*In vitro*
22	GTPGPQGIAGQRGVV	Collagen type I	*In vitro*
23	NGLPGPIGP	Human collagen type I	*In vitro*
24	IAGVGGEKSGGF	Collagen IIIa	*In vitro* and *In vivo*
25	NAVPITPTL	Buffalo casein	*In vitro*
26	LDLNLDLSKFRLPQPSSGRESPRH	Protein from rat stomach	*In vitro*
27	CPP	Casein	*In vivo*
28	RQLKIWFQNRRMKWKKIPVGESLKDLIDQ	Casein kinase 2	*In vitro*
29	GETNPADSKPGSIR	Protein from *Gadus morhua*	*In vitro* and *In vivo*
30	SVSEIQLMHNLGKHLNSMERVGWLRKKLQDV	rhPTH(1−31)	*In vitro* and *In vivo*
31	KCNTATVATERLANPLVHSSNNPGAILSSTNVGSNYY	Amylin	*In vitro*
32	VTHRLAGLLSRSGGVVKNNFVPTNVGSKAF	Calcitonin	*In vitro* and *In vivo*
33	KRQWAQFKIQWNQRWGRR	Human calcitonin receptor	*In vitro* and *In vivo*
34	IPP, VPP, LKP	Chemical synthesis	*In vitro*
35	TRSAWLDSGVTGSGLEGDHLSDTSTTSLELDSR	PTH-related protein (PTHrP)	*In vitro*
36	CGRP	Calcitonin	*In vitro*
37	SVVYGLR	Chemical synthesis	*In vitro*
38	VLPVPQK	Buffalo milk casein	*In vivo*
39	DGEA	Collagen type I	*In vitro*
40	RGD	Fibronectin	*In vitro*
41	TDLQERGDNDISPFSGDGQPFKD	Matrix extracellular phosphoglycoprotein	*In vitro*
42	LVQPRGDTNGPGPWQGGRRKFRRQRPRLSHKGPMPF	Apelin	*In vitro*
43	EEEEEEEPRGDT	Chemical synthesis	*In vitro*
44	DVDVPDGRGDSLAYG	Chemical synthesis	*In vitro*
45	AGYKPDEGKRGDACEGDSGGPFV	Thrombin	*In vivo*
46	IERGDVVVQDSPSD	Transmembrane protein 182 OS = *Larimichthys crocea*	*In vitro*
47	RGDLGIEIPTEK	Pyruvate kinase OS = *Larimichthys crocea*	*In vitro*
48	YRGDVVPK	Tubulin Alpha-1C chain in *Crassostrea gigas*	*In vitro and In vivo*
49	YPRKDETGAERT	Chitinase-like protein-3 in *Mytilus edulis*	*In vitro*
50	IEELEEELEAER	Pedal retractor muscle myosin heavy chain in *Mytilus edulis*	*In vitro* and *In vivo*
51	RVYFFKGKQYWE	Human vitronectin	*In vitro and In vivo*

osteoporosis. Osteoporosis primarily results from a higher bone resorption rate relative to bone formation. Thus, enhancing growth and differentiation of osteoblasts while suppressing those of osteoclasts may prevent osteoporosis. In Table 2, polypeptides with serial numbers 1–29 enhance osteoblast growth and differentiation and trigger osteogenesis, while those with serial numbers 30–38 inhibit osteoclast growth and differentiation and reduce bone resorption. Integrins are proteins expressed on osteoblasts and osteoclasts. Peptides bound to integrins mediate osteoblast and osteoclast adhesion, enhancing or suppressing their growth and differentiation. RGD and DEGA are examples of peptides that bind to integrins. Peptides 39–48 in Table 2 contain DEGA and RGD structures and can interact with integrins. Furthermore, peptides 46–51 promote bone formation and inhibit bone resorption by interacting with the RGD active binding site in integrin. The interaction of a peptide with the RGD-binding active site on integrins may be a crucial criterion for evaluating its performance to improve osteoporosis. Therefore, the peptides listed in Table 2 have been recognized as effective resources for osteoporosis improvement. The identification and preparation of the bioactive peptides for osteoporosis treatment will be reviewed in the following sections.

4. Preparation and identification of peptides on improving osteoporosis

4.1. Preparation of protein hydrolysates

Protein hydrolysates are produced by acid, base, thermal, or enzymatic hydrolysis. However, acid, base, and thermal hydrolysis may cause amino acid loss and lower the nutrient value, decreasing consumer satisfaction. Enzymatic hydrolysis is a suitable and commonly used alternative method. Although food-grade enzymes cannot effectively cleave peptides at the same sites as digestive enzymes, they provide greater control over food processing (Cheng, Tu, Liu, Zhao, & Du, 2019; Tu, Cheng, Lu, & Du, 2018). Commercial enzymes, sourced from plants, animals, and microorganisms, are extensively used in natural protein processing. There are a variety of commercial enzymes currently available, such as Bromelain, protamex, flavourzyme, alkaline protease, pancreatin, trypsin, pepsin, α-pancreas, proteinase K, chymotrypsin, papain, and neutral protease, ect. (Yathisha, Bhat, Karunasagar, & Mamatha, 2019).

Enzymatic hydrolysis efficiency is crucial for protein processing. To optimize yield, identifying the ideal pH and temperature for the hydrolysis of each protein is necessary. Neutral proteases exhibit optimal activity at a pH of around 7 and a temperature of 45 °C (Xu et al., 2019). Pepsin, an acidic protease, is most effective at pH = 2 (Chen et al., 2019). In contrast, the basic protease trypsin is effective at a pH of 8. Enzymes related to digestion in the gastrointestinal tract function optimally at a temperature of around 37 °C. Under ideal conditions, hydrolysis level, material-to-liquid ratio, and hydrolysis duration impact polypeptide composition and osteogenic activity (Xu et al., 2019).

4.2. Identification of peptides on improving osteoporosis

Protein hydrolysates generated by enzymatic hydrolysis can be classfied into peptides of diverse molecular weights, properties, and amino acid sequences by centrifugation. Low-molecular-weight peptides may exhibit better osteogenic activity (Jiang et al., 2022). Peptides with osteogenic activity can be separated using techniques like membrane filtration, column chromatography, and membrane ultrafiltration. To maintain the quality, activity, and storage longevity of peptides, quick freeze-drying is essential after each step of peptide collection and washing (Du et al., 2022). Peptides containing three amino acids, such as peptides 27, 34, and 40 in Table 2, are among the shortest that can improve osteogenesis (Huttunen, Pekkinen, Ahlstrom, & Lamberg-Allardt, 2008; Song et al., 2013). While the longest peptide, peptide 1 in Table 2, contains 34 amino acids (Montagnani, 2014). Determining osteogenic peptide content and sequence is essential for understanding osteogenesis-promoting mechanisms. Bacterial fermentation and enzymatic hydrolysis produce bioactive peptides along with other byproducts. However, to investigate the mechanism of action of a peptide, purification methods such as membrane filtration, alcohol precipitation, and gas/ion exchange chromatography are necessary. Moreover, target peptides can be identified using techniques like ultra-performance liquid chromatography-mass spectrometry (MS), high-performance liquid chromatography-MS, matrix-assisted laser desorption ionization-time of flight (TOF)-MS, ultra-performance liquid chromatography-TOF-MS (Li, Fan, & Xu, 2022), and capillary electrophoresis-TOF-MS (Chen et al., 2018), *etc.*

Fig. 2 summarizes the synthesis, purification, identification, characterization, and absorption of osteogenic peptides. Osteogenic peptides have crucial effects on modulating bone turnover, and obtaining their precise amino acid sequences is key to evaluating activities and exploring mechanisms (Xu et al., 2020, 2021). In this review, methods for the preparation, purification, and identification of peptides have been summarized. It was found that peptides 39–50 in Table 2 can interact with domains in integrins. Therefore, we will describe methods for predicting and verifying interactions between food-derived peptides and integrins, and it was determined the osteogenic peptide based on the interaction with the RGD-binding active site in integrin may be used as a new method. Additionally, the interactions between osteogenic peptides and integrins, their effects on the adhesion of osteoblasts, and the potential application of osteogenic peptides in promoting cell adhesion to bone materials have also been discussed in the following sections.

5. Effect of peptides on improving osteoporosis by regulating integrins

5.1. Prediction of peptides and integrins interaction

Integrin is a heterodimeric adhesion glycoprotein comprising two distinct subunits, α and β. Integrin is a signal receptor protein in cells and has specific binding sites for extracellular matrix proteins which modulate cell survival and invasion. There are 18 α and 8 β subunits (De Marco, Tolomelli, Juaristi, & Gentilucci, 2016), forming 24 integrins (Dhanesha, Nayak, Doddapattar, & Chauhan, 2019; Morshed, Abbas, Hu, & Xu, 2019). Depending on their ligand, integrins are mainly classified into two types: RGD and non-RGD binding receptors (Finney, Stokes, Pattillo, & Orr, 2017; Perez, Leyton, & Valdivia, 2022). A bioactive peptide is a characteristic protein fragment that interacts with a specific receptor. It is generated through protein proteolysis, and exhibits activity to modulate physiological functions (Shi et al., 2018). Integrin α5β1 can not only upregulate phosphorylated RUNX2 but also activate the FAK-ERK pathway, promoting the activation of the ERK1, ERK2, and MAPK pathways. Moreover, it triggers osteoblast differentiation (Oh et al., 2017). Integrin αvβ3 contributes to the activation of the Shc and FAK pathways. Integrin-RGD complexes participate in and promote growth factor-induced cell cycle processes (Ambesi & McKeown-Longo, 2014; Ashe, 2016). The interaction between integrin β1 and the peptide RVYFFKGKQYWE critically impacts osteoblast growth promotion (Lemieux, Horowitz, & Kacena, 2010; Min, Kang, Jung, Jang, & Min, 2018; Zhou, Zu, Zhuang, & Yang, 2015). Integrin α5β1 promotes osteoblast growth, adhesion, and differentiation (Lawson et al., 2022; Taubenberger, Woodruff, Bai, Muller, & Hutmacher, 2010). Bioactive peptides interfering with integrin αvβ3 can suppress osteoclast growth by inhibiting its cytoskeleton (Park et al., 2016). Thus, interactions between bioactive peptides and integrins α5β1 and αvβ3 in the extracellular matrix are important for osteoblast and osteoclast proliferation and differentiation. The interactions of peptides with integrins α5β1 and αvβ3 and their potential osteogenic effects promote osteoblasts and inhibit osteoclasts (Fig. 3A).

The interaction between receptor proteins and bioactive peptides has been characterized by many methods, such as circular dichroism,

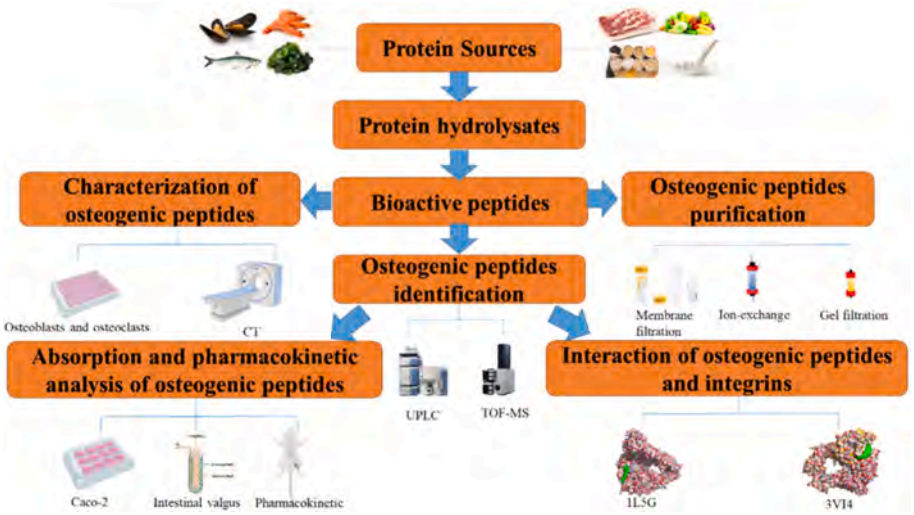

Fig. 2. Preparation, purification, identification, characterization, absorption, and structure-activity of osteogenic peptides.

nuclear magnetic resonance, X-ray crystal diffraction and fourier transform infrared spectroscopy. These methods, however, are time-consuming and costly. For initial interaction site prediction, computer simulation dynamics techniques are more suitable (Geng, Chen, Ye, & Jiang, 2019). There are several peptide structure-predicting online tools, including Pep-Fold (Shen, Maupetit, Derreumaux, & Tuffery, 2014), PEPstrMOD (Singh et al., 2015), Protinfo, Hmmstr/Rosetta (Hashemi et al., 2021), and I-Tasser (Zhou et al., 2022). Discovery Studio, Sybyl, and ArgusLab are able to predict peptide structure and perform molecular dynamics simulation through energy minimization. Discovery Studio 2017 predicts molecular structure based on the force field theory (Dasetty, Meza-Morales, Getman, & Sarupria, 2019), a set of parameters and equation positions used in molecular mechanics simulations. Molecular docking aims to predict small molecule affinity and receptor protein binding site (Ahmed, Mam, & Sowdhamini, 2021; Chen et al., 2019; Talluri, 2021), which influence the selection of osteogenic peptides. Docking between peptide IEELEEELEAER (PIE) and integrins 3VI4 and the 1L5G 3D structure are shown in Fig. 3B.

CDOCKER energy utilizes CHARMm force field. The interaction energy between RGDN and integrin α5β1 (PDB: 3VI4) is 116.249 kcal/mol, while that between PIE and integrin α5β1 is 191.364 kcal/mol. Fig. 3B shows the amino acids of integrin α5β1 that interact with PIE and RGDN. The binding sites of integrin α5β1 with PIE are similar to those of integrin α5β1 with RGDN (Xu et al., 2019). The osteogenic peptides YGRDVVPK and YPRKDETGAERT show strong affinity to integrin, indicated by more binding sites compared with RGD does (Chen, Xu, et al., 2019; Chen et al., 2020; Xu et al., 2019). The interaction energy between c(RGDf-MVA) and integrin αvβ3 (PDB: 1L5G) is 85.245 kcal/mol, while that between PIE and integrin αvβ3 is 209.321 kcal/mol. The binding sites of integrin αvβ3 with c(RGDf-MVA) are similar to those of integrin αvβ3 with PIE and include LYS253, GLU220, ALA218, ALA215, TYR178, TYR122, and SER121 (Xu, Chen, Fan, et al., 2019). A novel small-molecule PPI and the osteogenic peptide YPRKDETGAERT have shown a strong affinity to integrin (Min, Kang, Jung, Jang, & Min, 2018; Xu, Chen, Wang, et al., 2019). PIE promotes the growth and differentiation of osteoblasts and inhibits those of osteoclasts (Xu, Chen, Fan, et al., 2019). RVYFFKGKQYWE, the novel bioactive core vitronectin-derived peptide, has been reported to inhibit bone resorption by suppressing resorption mediated by integrin αvβ3-c-Src-PYK2. It could also activate FAK through direct interaction with integrin β1 to accelerate osteoblast differentiation and activity, promoting bone formation (Min et al., 2018).

5.2. Evaluation of peptide-integrin interactions

Molecular docking is extensively used to predict the structures of biologically active substances for investigations and analyses, but there are challenges in computational models and underlying theory limit the accuracy of the results. As for the evaluation of molecular docking results, there are two ways to verify the interactions of peptides with receptor proteins. One is the indirect method that measures the function of peptides in RNA and receptor proteins (Min et al., 2018). The RNA level of the receptor protein could be determined through reverse transcription quantitative polymerase chain reaction (Wang et al., 2019), and receptor protein level could be measured by Western blotting (Xu et al., 2020). The other method is more intuitive and directly measures the interaction energy between the peptide and the receptor protein or the interactions were further analyzed by electron microscopy based on crystal culture of peptide and protein. Isothermal titration calorimetry is used to titrate peptides and proteins (Liu et al., 2020), determining the binding constant, stoichiometry, enthalpy, and entropy change of the entire reaction system. Biolayer interferometry is a rapid technique for marker detection and molecular dynamics analysis of biological interference signals. This technique has been applied to evaluate the interactions of bioactive proteins. The affinity constant of biolayer interferometry shows the biotin-labeled target protein and peptide (Li et al., 2020). In addition, crystal culture is performed using peptides and receptor proteins. The obtained crystals are analyzed by crystal diffraction (Juvvadi et al., 2019), revealing the interaction sites between peptides and proteins. Finally, these results are compared with those of molecular docking to determine the mechanism of action of the osteogenic peptide and integrin.

Some osteogenic peptides are used as factors for repairing bone defects after determining their interactions with integrins. Integrins belong to the cell adhesion receptor family, which regulates the location and activities of proteases as well as cell adhesion and proliferation (Hou, Wang, Li, Hao, & Hang, 2021). Bioactive peptides, such as osteogenic peptides and integrins, can bind with each other to promote various cell functions, including growth, differentiation, and adhesion (Lara-Ochoa, Ortega-Lara, & Guerrero-Beltrán, 2021; Xu et al., 2022, 2023). As shown in Fig. 4, the abovementioned interactions of integrins and osteogenic peptides can accelerate the healing of repaired bone defects. These evaluation methods, once verified, can be quickly applied, thereby reducing costs and increasing efficiency.

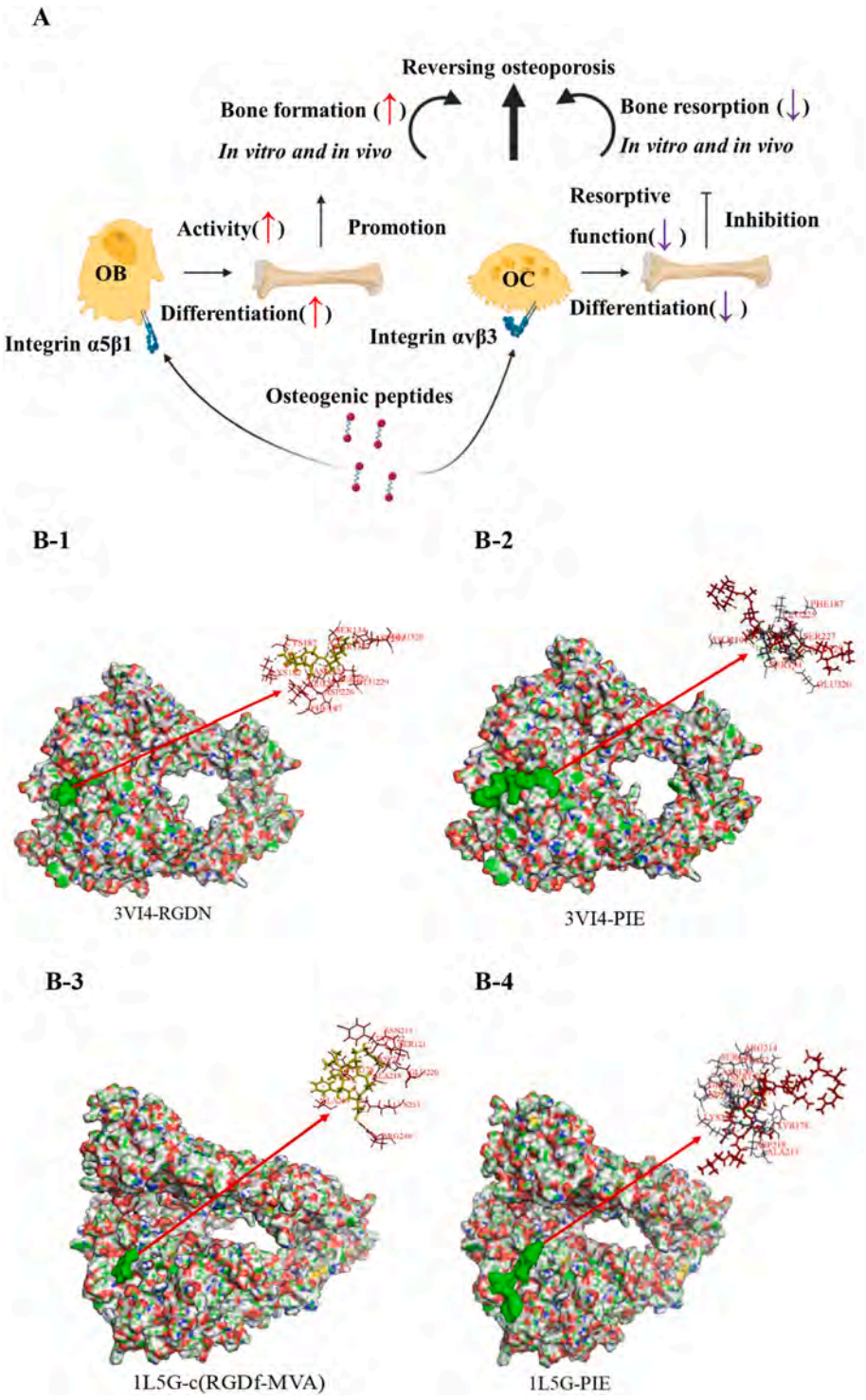

Fig. 3. The interactions of peptide IEELEEELEAER (PIE) with integrins 3VI4 and 1L5G. (A) Proposed pathways for osteogenic peptide-mediated reversal of estrogen deficiency-induced bone loss. OB, osteoblasts; OC, osteoclasts. (B) Docking for the interactions of PIE with integrins 3VI4 and 1L5G, 3D structures of integrins complex as a surface image, generated by the surface menu of Discovery Studio 2017 software based on the PDB database, peptide PIE, and original RGD after docking with integrins.

6. Perspectives

The value of food protein digests and bioactive peptides as functional foods in the future cannot be estimated. A healthy diet should contain osteogenic peptides that have been investigated through preclinical research and clinical trials. Prediction of osteogenic peptides directly from enzymatic hydrolysis products has significantly reduced the cost of osteogenic peptides compared to multi-step purification, isolation, screening and identification. Moreover, advanced and powerful technologies, such as mass spectrometry, protein purification, bioinformatics, crystallography, micro-computed tomography, and biofilm interference, are widely utilized to study molecular structure and provide a theoretical basis for understanding the relationship between structure and activity at the molecular level. In this review, the

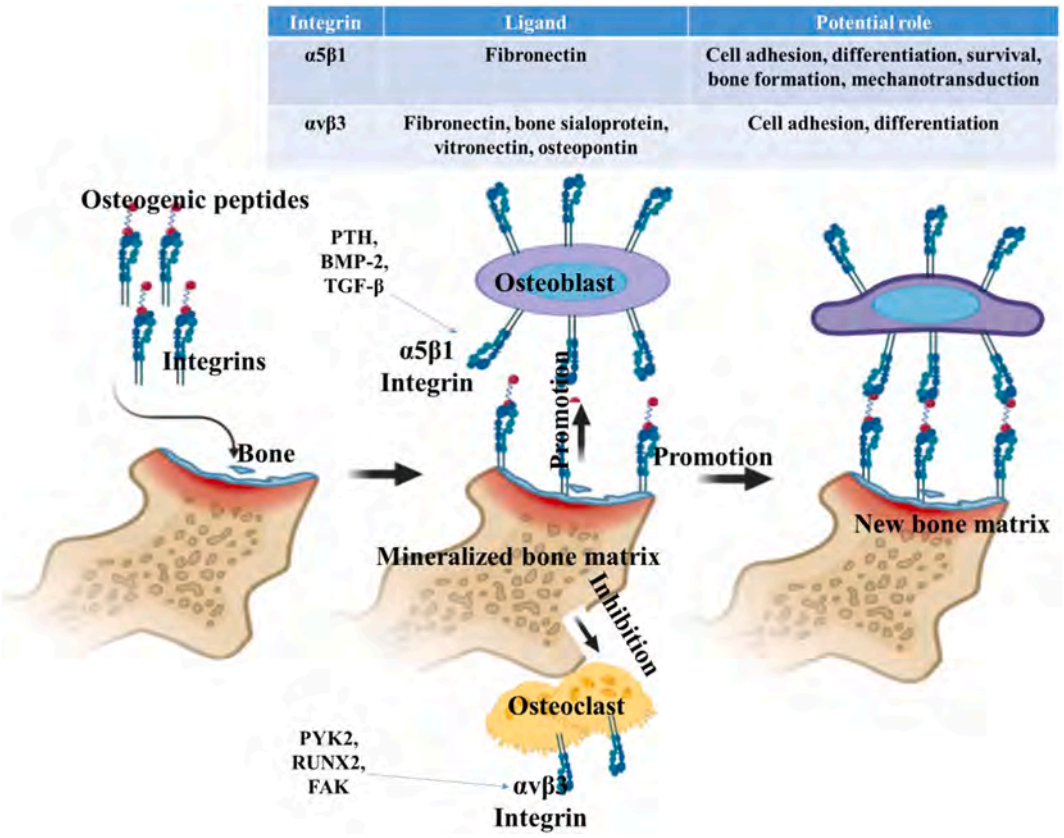

Fig. 4. A scheme for the usage of osteogenic peptides as self-adhesive integrin mimics.

evaluation methods and treatment methods of osteoporosis are reviewed, and it is found that osteopoietic peptides from food sources are a potential source to replace drugs with side effects for osteoporosis treatment. Peptides can improve osteoporosis by interacting with RGD binding sites within integrins, which may be used in future computer simulations to rapidly predict and screen new methods of osteogenic peptides. Taken together, these not only improve the safety of osteoporosis treatment but also reduce the cost of screening osteogenic peptides. This review aims to encourage studies on the structure-activity relationships between osteogenic peptides and integrins that regulate cell adhesion, promoting osteoblast and inhibiting osteoclast proliferation and differentiation. Osteogenic peptides also have the potential for application in the development of bioadhesives for bone materials. Therefore, research ideas for predicting and screening bone-forming active peptides have been summarized, these ideas may lead to the development of bone materials, active drugs that promote bone formation and functional foods.

CRediT authorship contribution statement

Zhe Xu: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis. **Rui Zhang:** Writing – original draft, Methodology, Formal analysis. **Hongrui Chen:** Methodology, Data curation. **Lijuan Zhang:** Writing – review & editing, Methodology. **Xu Yan:** Writing – original draft, Formal analysis. **Zijin Qin:** Writing – review & editing, Formal analysis. **Shuang Cong:** Writing – review & editing. **Zhijian Tan:** Writing – review & editing, Project administration. **Tingting Li:** Writing – review & editing, Resources. **Ming Du:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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