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Review on osteoporosis and its treatment using fish collagen peptides

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Abstract
Osteoporosis is a common skeletal disorder characterized by low bone mass and skeletal micro-architectural deterioration, increased risk of fracture and associated co-morbidities most prevalent in the elderly. Due to an increasingly aging population, osteoporosis has become a significant health issue requiring innovative disease management. Proteins are essential for healthy bone, protein intake plays a considerable role in both bone development and bone maintenance. The most abundant sources of collagen are land-based animals, such as cows and pigs. However, the extraction of collagen from non-mammalian sources such as fish has strongly influenced current society due to some religious and disease transmission issues. Many studies have dealt with collagen extraction and anti-osteoporotic properties from fish wastes. Collagen-derived products such as gelatin or collagen hydrolysates are well acknowledged for their safety from a nutritional point of view. In this manuscript, we critically review the evidence from the literature for the effect of fish collagen peptides on bone tissues to determine whether collage peptides may represent a relevant alternative in the design of future nutritional approaches to manage osteoporosis prevention.

Keywords: Fish, collagen peptides, anti-osteoporotic, bone

Introduction
Collagen is the most abundant protein in vertebrates and constitutes about 25% of total proteins (Harnedy and FitzGerald, 2012) [12]. Collagen and gelatin from animal sources are excellent raw materials to obtain hydrolysates and peptides with important biological activities, such as antioxidant, antimicrobial, antihypertensive or anticancer properties (John, 2003) [13]. There are more than 27 different types of collagen, such as type I collagen, type II collagen, type III collagen, etc.; among these, type I collagen is the most common. Type I collagen plays a significant role in the maturation and mineralization of bone cells and in supporting organic bone growth (Sun *et al*., 2004) [32]. Studies confirm that eating collagen can promote the absorption of minerals, increase bone density and decrease the risk of osteoporosis (Aleman *et al*., 2011) [1].
Fish scales, mainly composed of type I collagen and hydroxyapatite, and are also discarded as waste in the fish processing industry (Nomura *et al*., 1996) [27]. Scales from different fish species, such as lizardfish, sardine or deep-sea redfish, serve as raw materials for collagen and gelatin extraction (Sun *et al*., 2004) [32].

Fish collagen peptides
Collagen peptide is a hydrolyzed form of collagen. After hydrolysis, the product loses its gelling ability and becomes soluble in water (Kumar *et al*., 2019) [21]. Collagen peptide differs from other proteins, as it contains amino acids such as glycine, proline and hydroxyproline in an accessible form at around 10-20 times higher (Nomura *et al*., 1996) [27]. Collagen peptide contains all essential amino acids except tryptophan (Murray and Baker, 1952) [25].

Osteoporosis
In the 1830s, Lobstein, a French pathologist and surgeon, observed that some patient's bones were more prominent than typical porous structures and named the disorder "osteoporosis" ("osteo" means bone, "porosis" means porous) (Gallagher *et al*., 1994) [9]. Initially, it was considered an unavoidable consequence of aging. Fuller Albright, an endocrinologist, associated osteoporosis with the postmenopausal state in women (Orsini *et al*., 2005) [28].

It is estimated that worldwide, every third women, and one in five men over the age of 50, sustain an osteoporotic-induced bone fracture (Klibanski *et al*., 2001) [19]. Osteoporosis results from the imbalance in bone turnover associated with excessive resorption and exiguous remodeling (Orsini *et al*., 2005) [28]. Osteoporosis is defined as a chronic and asymptomatic disease characterized by low bone mineral density (BMD) and skeletal microarchitectural deterioration, leading to increased risk of fracture and associated comorbidities, such as lumbago and arthralgia (Turner *et al*., 2009) [37].

Osteoporosis is often diagnosed when an unsubstantial fall causes a fracture. The spine, hip, and wrist are the common osteoporotic fracture sites since they contain high percentages of trabecular bone (Mazziotti *et al*., 2006) [24]. Trabecular bone, called cancellous bone, is a porous bone composed of trabeculated bone tissue. It can be found at the end of long bones like the femur, where the bone is not solid but is full of holes connected by thin rods and plates of bone tissue. Also, the trabecular bone is more assailable to turnover rate (Zylberberg *et al*., 1992) [42].

Although osteoporosis is not a life-threatening disease, it drastically deteriorates the quality of life, resulting in immobilization and other complications, ultimately leading to hospitalization. Trabecular bone loss is more profound and starts earlier than cortical bone loss. In women, trabecular bone connectivity is drastically reduced and resorbed compared to men, where trabeculi become thinner (Currey, 2003) [3]. Post-menopause bone mineral density (BMD) at the hip and in the lumber spine is reduced at the annual rate of 2% and 1.6%, respectively (Turner *et al*., 2009) [37].

Aging increases the prevalence of osteoporosis dramatically in all geographic areas. It progresses from 5% among women of 50 years of age to 50% at the age of 85 years, whereas among men, the comparable numbers are 2.4% and 20%, respectively (Klibanski *et al*., 2001) [19]. Furthermore, around 9 million osteoporotic fractures occur annually, of which 1.7 million occur at the forearm, 1.6 million at the hip, and 1.4 million at the vertebra (Currey, 2003) [3]. As life expectancy increases, osteoporosis has become a growing public health concern. The incidence of hip fractures is expected to rise from 1.6 million to over 6 million by the year 2050. Particularly among Asian countries, the prevalence of this skeletal disorder is strikingly increasing because of the development of socio-economic status and aging (WHO, 1994) [39].

Risk Factors

Gender and age are the best predictors of osteoporosis, but also other factors like genetic history of maternal hip fracture, early menopause, low calcium intake, smoking, low body weight, fracture after four years of age, and various other primary diseases or their treatment enhances the chance of fractures. In addition, poor vision, balance or substandard muscle strength enhance the probability of osteoporosis and therefore increase the fracture risk (Lane, 2006) [22]. Other conditions associated with osteoporosis are glucocorticoid therapy resulting in decreased bone mass (Klibanski *et al*., 2001) [19], immobilization and inflammatory bowel disease (IBD), rheumatic disease, renal disease, chronic liver, osteolytic bone metastasis and organ transplantation. Among these factors, primary diseases, immobilization, and medication induced bone alterations are known as secondary osteoporosis, whereas estrogen and androgen deficit induced bone loss due to aging is known as primary osteoporosis (Tan *et al*., 2013).

Hypertension and Osteoporosis

High blood pressure is associated with abnormalities of calcium metabolism, leading to an increase in calcium loss, secondary activation of the parathyroid gland, and increased movement of calcium from bone, thereby increasing the risk of osteoporosis (Varenna *et al*., 2013) [38]. Angiotensin I - converting enzyme (ACE), plays an important physiological role in regulating blood pressure (Mazziotti *et al*., 2006) [24]. The ACE inactivates the vasodilator bradykinin in the Kinin - Kallikrein system (Erdos, 1975) [6] and produces potent vasopressor angiotensin II, an octapeptide from angiotensin I, by the hydrolysis of C-terminal histidyl-leucine (Erdos, 1975) [6]. Angiotensin II is involved in the release of the sodium-retaining steroid aldosterone. Angiotensin also increases the blood pressure by raising vascular resistance and fluid volume via vasopressor and aldosterone, respectively.

World Health Organization (WHO) has defined three stages of bone loss depending on the severity of an individual's bone loss based on bone mineral density (BMD) as T-score. Osteopenia is a condition of bone loss diagnosed when the T-score fall between 1 and 2.5 times below the standard deviation (SD) of the mean value of young adult women's peak bone mass, whereas osteoporosis is diagnosed when the T-score is 2.5 below the mean value. In addition to the osteoporotic T-score, when the patient has a fragility fracture present, severe osteoporosis is diagnosed (WHO, 1994) [39].

Osteoporosis treatment

Osteoporosis is still a challenge to medicine. With a better understanding of the molecular level regulation of bone remodeling, novel treatments are accessible for prevention and treatment. Traditional target sites for the treatment of osteoporosis include a target that promotes osteoblast function (anabolic therapies) and inhibits osteoclast function (anti-resorptive or anti-catabolic therapies). Of the two dimensions of osteoporosis therapies, anti-resorptive-based medicines are most commonly prescribed (Gallagher *et al*., 1994) [9].

Three types of agents are currently used for the treatment of osteoporosis: 1) Anti-resorptive drugs that decrease bone loss, e.g., bisphosphonates, calcitonin, selective receptor modulators (SERMs), and calcium. 2) Anabolic agents that increase bone formation, e.g., teriparatide and 3) Strontium ranelate has both actions (Chiu *et al*., 2014) [2].

Most of these treatments showed a reduction in vertebral and non-vertebral fracture risks. However, current therapies only slow down the progress of the disease but are not potent enough to recover the lost bone mass back to normal. In addition, current therapies have more of adverse effects, which can sometimes be even of greater magnitude than the reported beneficial effects on the bone (Zhang *et al*., 2018) [41].

Osteoporotic drugs have some side effects also, which include gastrointestinal disturbances, including peptic ulcers, nausea, vomiting, facial flushing, tingling sensation in the hands and unpleasant taste in the mouth, raised blood pressure, oesophagitis, bone pain, osteonecrosis of the jaw, thrombophlebitis and arterial thromboembolism (Tan *et al*., 2013). Collagen peptides can form a suitable alternative in the treatment of osteoporosis without exhibiting any side effects.

Bioactive peptides and osteo-metabolism

Few scientific reports have demonstrated the beneficial effect of bioactive peptides on bone metabolism studied under in vitro and in vivo conditions. Bone regeneration is a complex process that involves the early stages of cell proliferation and later stages of cell differentiation, and other functions of osteoblast cells. Therefore, it is essential to improve the osteo-anabolic functions in terms of osteoblast proliferation and differentiation. Various milk-derived bioactive peptides promoted bone health in an anabolic manner. Milk-derived casein phosphor peptide (CPP) formed in the gastrointestinal tract or during fermentation enhances calcium absorption by preventing the formation of insoluble calcium salts in the intestine (Kitts and Nakamura, 1992).

Bovine casein-derived peptides have a stimulatory effect on MC3T3-El osteoblast cells (Tulipano *et al*., 2010) [36]. Also, bovine casein derived peptide enhanced the proliferation and differentiation of osteoblastic cells under in vitro oxidative stress model (Mada *et al*., 2017) [23]. These bioactive peptides reduced lipid peroxidation, superoxide dismutase (SOD), and catalase level, thereby protecting osteoblasts against oxidative stress (Mada *et al*., 2017) [23]. The trans-epithelial transport of milk-derived bioactive peptide using Caco-2 monolayer cells was reported to be 1% (Raisz, 2005) [29].

Collagen peptides and osteo-metabolism

In the last decade, researchers are mainly focusing on studies to improve bone mineral density (BMD) in osteoporosis patients. Collagen peptide metabolism in bone might be a new therapeutic approach to improve skeleton health. The capacity of bone to resist fractures and mechanical forces depends not only on the quantity of bone minerals, but also on the collagen framework (Tan *et al*., 2013). Few studies have reported the effect of collagen hydrolysates on improving osteoporosis (Kim *et al*., 2013) [17]. In an in vitro study, the collagen hydrolysate-derived Asp-Gly-Glu-Ala peptide could trigger osteoblast metabolism in bone marrow cells. Moreover, Hyp-containing peptides foster osteoblast activity, resulting in increased bone mineralization and synthesis of organic bone components (Gao *et al*., 2013) [10]. An in vivo study showed that fish-bone peptides could increase calcium retention and inhibit bone loss in ovariectomised (OVX) rats (Jung *et al*., 2006) [14]. Besides, collagen hydrolysates from pigs or deer also exerted significant effects in preventing bone loss, improving BMD, and increasing histo-morphometric parameters and mechanical indicators in OVX animal models (Zhang *et al*., 2014). Clinical trials further demonstrated that particular collagen peptides improved BMD and bone markers in postmenopausal women (Konig *et al*., 2018) [20].

Guillerminet *et al*. (2012) [11] have shown that diets including porcine collagen hydrolysates significantly increased the BMD of OVX mice compared to the standard AIN-93N diet. An in vitro study demonstrated that collagen hydrolysates could stimulate the expression of type I collagen mRNA and its protein production (Yamada *et al*., 2013) [40], and the ALP activity increased in a dose-dependent manner. The TGF-β1/S mad signaling pathway plays an important role in regulating osteoblast collagen synthesis and mineralization (Sowa *et al*., 2002) [31]. Zhang *et al*. (2014) concluded that collagen hydrolysates regulate bone remodeling via the important role of S mad3 pathway in osteoblastic bone formation.

Preclinical in vitro studies with rodents have shown that administration of collagen peptides increased the organic component of bones, improved bone metabolism as well as bone microarchitecture and enhanced the biomechanical resistance of vertebrae. Moreover, supplementation with collagen peptides in combination with calcitonin has shown positive effects in postmenopausal women (Fouchereau-Peron *et al*., 1999) [7]. In another study, supplementation with collagen peptides significantly decreased the excretion of bone collagen breakdown products compared to placebo treatment. Moreover, the effect of therapy with collagen peptides was persistent over a period of at least three months after the last administration, suggesting an anabolic effect of collagen peptide treatment (Zhang *et al*., 2014).

Fish collagen peptides and osteo-metabolism

Hydrolyzed collagen from both fish and shrimp was described to contain both, a biologically related calcitonin and/or calcitonin-gene-related peptide (Fouchereau- Peron *et al*., 1999) [7]. Jung *et al*. (2006) [14] isolated fish-bone peptides with a high affinity to calcium and a high content of phosphopeptides. Using OVX rats, they observed higher calcium retention and preservation of both bone mineral density and bone strength when rodents were supplemented with those peptides. Hydrolyzed collagen was found to affect lipid absorption and metabolism in rats resulting in a lower transient increase of plasma triglycerides and associated inflammation (Saito *et al*., 2009) [30].

Fu and Zhao (2013) [8] observed that salmon skin hydrolysates induced cell proliferation, accelerated cell cycle progression and inhibited cell apoptosis in human hFOB1.19 cells, mainly when skin collagen was hydrolyzed with papain compared to other proteases. The dose-dependent effect of fish collagen hydrolysate on human osteoblast proliferation was observed (Kim *et al*., 2013) [17]. Incubation of human osteoblasts with 0.1% fish collagen hydrolysate increased osteocalcin, osteopontin, BMP-2 and integrin β3 mRNA expression and accelerated matrix mineralization compared to untreated cells (Yamada *et al*., 2013) [40]. In vitro and in vivo studies have shown that specific peptide fractions in fish protein hydrolysates may stimulate the nonspecific immune defense system (Khora, 2013) [16].

Collagen peptides (1.4, 2, and 5 kDa) from bovine, porcine, and fish had up-regulated the proliferation and ALP activities of human osteoblastic MG-63 and MC3T3-E1 cells (Guillerminet *et al*., 2012 [11]; Kim *et al*., 2013) [17]. Recently, Chiu *et al*. (2014) [2] reported that type-II collagen treatment increased the level of integrin α2β1 complex (VLA-2) expression in BMMS cells surface and mammalian collagen-treated cells accrued more calcium than control in the previous study Chiu *et al*., (2014) [2]. Daneault *et al*. (2017) [4] reported that hydrolyzed collagen rich in hydroxyproline had increased the mineralization of osteoblasts in vitro. Elango *et al*. (2019) [5] also found that the addition of collagen peptide of Mahi mahi fish (Coryphaena hippurus) bones had improved the proliferation and differentiation in bone marrow mesenchymal stem (BMMS) cells.

Conclusion

A growing body of evidence demonstrates that collagen peptides obtained from fishery sources own bioactive properties beneficial for bone tissue, including stimulation of bone-forming cells, improvement of calcium absorption, and anti-inflammatory and antioxidant capacities. Those properties render collagen peptides a new and innovative

candidate for putative dietary intervention in the prevention of osteoporosis. In the light of the increasing prevalence of osteoporosis-related to the worldwide increasing longevity, good candidates for dietary prevention are of particular relevance. As such, collagen peptides could offer additional values to calcium and vitamin D, thus responding to the growing demand for primary prevention.

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