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Exploring flaxseed's potential in enhancing bone health: Unveiling osteo-protective properties

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ABSTRACT

Flaxseed, also known as *Linum usitatissimum* L., is a valuable grain that can significantly prevent bone deterioration and promote bone health. Flaxseed plays a distinctive role in bone formation and growth. Flaxseed contains lignans, polyunsaturated fatty acids (PUFA), soluble and insoluble fibers, B vitamins, magnesium (Mg) and manganese. The recent nutritional characterization of flaxseed has underscored its importance as a crucial grain in diets aimed at enhancing bone mineral density (BMD) and reducing osteoporosis risk factors. Flaxseed plays a distinctive role in bone formation and growth evident in the elevation of bone production markers, increased BMD, enhanced biomechanical properties, structural changes in the femur, higher total and vertebral bone density and content, increased total bone surface area, raised osteocalcin levels and improved bone thickness. Omega-3 (ω-3) PUFAs are favorably connected with BMD and have beneficial effects on the control of bone metabolism. Alpha-linolenic acid (ALA), an essential fatty acid (FA), plays a crucial role in promoting bone health. In recent years certain experimental trials have supported the bone protective efficacy of flaxseed in different forms like oil, flour, extract and powder. These studies have conclusively shown that the inclusion of flaxseed in our diet can significantly prevent bone deterioration and promote bone health.

1. Introduction

Achieving peak bone mass is necessary to attain optimal bone health and hence, reducing the risk of osteoporosis in the future. Achieving peak bone mass will continue until late adolescence or early 20s, depending on interactions between hormones and growth factors, genetics, physical activity as well as nutrition, particularly Ca/Vit D. The role of other specific dietary compounds such as phytoestrogens, estrogens and anti-estrogenic agents remains unclear for achieving peak bone mass. Substances that are present in living organisms such as animals and plants and acting like estrogens are called peripheral estrogens. Some of these hormones called phytoestrogens are found in plants such as soy clover and flaxseed. The plants are becoming therapeutically popular [1] due to its anti-oxidants [2], essential oils [3–6], protein content [7], medicinal and pharmacological attributes [8,9]. Flaxseed, *Linum usitatissimum*, being a plant based product contains a high amount of phytoestrogens [10]. Flaxseed contains exceptionally high amounts of Ω-3 polyunsaturated fatty acids (PUFA), mainly Alpha-linolenic acid (ALA) (19–25 % by weight) (15–17) and lignans (0.165–0.375 % by weight) (18–21). ALA mainly undergoes beta-oxidation for the production of energy. However, it may also decrease the n6 PUFA production of pro-inflammatory compounds as a result of their competition for the same series of enzymes that are shared for n3 and n6 PUFA conversion. Secoisolariciresinol diglucoside (SDG) and other flax lignans

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(i.e. matairesinol, pinoresinol, lariciresinol) are metabolized by the action of colonic microflora into the mammalian lignans enterodiol and enterolactone that may regulate oestrogen signaling [11].

The flaxseed plant contains lignans, PUFA, soluble and insoluble fibers, B vitamins, magnesium (Mg) and manganese (Figueiredo et al., 2017). The nutritional advantages of its components, particularly lipids, proteins, lignans and fiber, coupled with its use in the production of several value-aided goods [12], have recently led to the recognition of flaxseed as a necessary grain to include in diets [13]. Flaxseed exhibited bone protective efficacy in different forms like oil, flour, extract and powder, which can be obtained through different processing methods such as cold press [14], grinding [15], solid-liquid extraction pressurised fluids and sub/supercritical fluids extraction [16]. Mineral are essential for the normal growth and development in living organism [17]. Calcium (Ca), Phosphorous (P), Mg and Sulfur (S) are microminerals needed by livestock to constructing body parts like bones and teeth [18]. Flaxseed contributes to maintaining bone strength and density while preventing bone loss [19] and the consumption of flaxseed oil (FO) has been shown to increase bone mineral density (BMD) and lower osteoporosis risk factor. According to a study, foods containing FO should be consumed by diabetic women to reduce their risk of osteoporosis [20].

The daily consumption of Long-chain omega-3 (ω-3) PUFA has been linked to improvements in overall health, particularly in terms of their effects on the cardiovascular, metabolic and inflammatory systems, making them potentially beneficial for women health [21]. According to studies, the ω-3 PUFAs found in flaxseed are favorably connected with BMD and have beneficial effects on the control of bone metabolism [22]. ALA, an essential fatty acid (FA) and its longer chain derivatives, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are key ω-3 PUFAs that play a crucial role in the regulation of osteoblastogenesis and the prevention of bone resorption [23]. The consumption of ω-3 PUFAs has been shown to successfully increase the stiffness of bone production and improve skeleton function [24]. Plant oils, such as flaxseed and soybean oil, are rich sources of PUFAs. High levels of PUFAs in these oils contribute to their potential health benefits, particularly in terms of bone health and overall well-being. According to important research, the essential FA, ALA is typically obtained through a variety of food sources, such as seeds and nuts (e.g., flaxseed, chia seed and walnuts) as well as vegetable oils like FO, canola oil and soybean oil [20,22]. The role of FO in bone homeostasis and its effects on alterations in osteogenic indicators have been studied. Researchers discovered that FO has positive effects on treatment of osteoporosis [25]. The current review aims to summarize all the studies conducted in the last ten years on the therapeutic effectiveness of flaxseed and its various forms in promoting bone density mineralization, thickness and prevention of bone diseases and decay. Therefore, achieving peak bone mass is vital for optimal health. Flaxseed, rich in phytoestrogens and (ω-3) PUFAs, has shown promising effect in bone density promotion and osteoporosis prevention. The benefits of flaxseed are described in Fig. 1.

2. Nutritional composition of flaxseed

Flaxseeds are rich in proteins (about 20–30 g/100 g), lipids (about 40 g/100 g) and carbohydrates (about 29 g/100 g). The composition and quantities of FA can vary and depend on growing conditions. ALA predominates (34–65 %), followed by oleic (16–24 %) and linoleic (12.5–17 %), while the quantities of saturated palmitic (3.0–6.0 %) and stearic acid (3.0–7.1 %) are lower [26]. FAs present in the flaxseed are crucial for bone health. Foods with high ALA (ALA, C18:3 n-3) concentration help to prevent bone loss, whereas high intake of linoleic acids (LA, C18:2 n-6) was associated with bone fragility [27]. Flaxseed produces a fixed oil known as linseed oil or FO. The oil contains unsaturated FAs like oleic acid (12–30 %), linoleic acid (8–29 %) and linolenic acid (35–67 %) [28].

DHA and EPA have larger health benefits against various diseases including cardiovascular, neurodegenerative, cancers and bone diseases [29]. Despite being a significant source of ALA, FO has only recently been used in humans due to its low conversion rate to DHA and EPA, posing a significant challenge to the scientific community today. The choice of flaxseed variety depends on the bioavailability of ALA. For instance, it is higher in FO than in the whole seed or milled form. Because flaxseed has a high concentration of unsaturated FA, it is vulnerable to oxidative damage, which needs to be addressed in all stages of processing to fully utilize the nutritional advantages of flaxseed [30].

Protein is an important building block for bones, muscles, cartilage and skin. Flaxseeds are a great source of high-quality, plant-based protein, constituting up to 23 % of the total seed mass. This percentage rises to 35–40 % when the oil is removed from the diet. Flaxseed has a high protein value (82 %), surpassing soybean and this is attributed to its balanced amino acid composition [31,32]. Protein makes up 50 % of bone volume and approximately one-third of its mass. It provides the structural matrix of bone, whereas calcium is the dominant mineral within that matrix. Collagen and a variety of noncollagenous proteins form the organic matrix of bone, so an adequate dietary protein intake would seem to be essential for optimal acquisition and maintenance of adult bone mass [33]. Cycloline peptides, also referred to as orbitides or linosurbs, is found in high amounts in flaxseed. More than 25 different varieties of these

Fig. 1. Benefits of flaxseed.

nutrients have been identified [16]. The orbitides typically consist of 8–10 amino acids. A well-known Cycloline peptide A has the following structural formula: Pro-Pro-Phe-Phe-Leu-Ile-Ile-Leu-Val. These compounds exhibit several advantageous qualities, including immunosuppression, anti-malarial, anti-cancer and protection against bone loss [14]. A krona chart displaying the proximate composition of flaxseed is shown in Fig. 2.

Flaxseed is comprised of 40 % fiber, with 25 % being soluble fiber and 75 % insoluble fiber [34]. Flaxseed is an attractive food to consume because it contains appreciable levels of essential nutrients (including n3 PUFA) and other healthful components such as fiber, lignans and plant sterols [11]. FO, fibers and flax lignans might aid in lessening heart disease risk, arosclerosis, diabetes, cancer, arthritis, osteoporosis, autoimmune diseases, & neurodegenerative problems [35]. Hence flaxseeds are nutrient dense food source, rich in proteins, (ω-3) FAs and fiber. Their potential health benefits include supporting bone health, reducing cardiovascular risk and contributing to overall well-being. Table 1 provides a breakdown of the composition of flaxseed.

3. Flaxseed association with bone

Flaxseed oil is a rich source of the essential FAs, linolenic acid (*ω*-6) and ALA (*ω*-3), which regulate prostaglandins synthesis and hence, induce the wound healing process. Deficiency in essential FAs results in phrynoderma or toad skin, horny eruptions on the limbs, poor wound healing [38]. The impact of FO on bone composition, mineral content, bone area, fracture toughness and maximum muscular strength is notable [39]. This effect is likely attributed to the presence of ALA and ω-3-FA in flaxseed. Flaxseed supplementation is beneficial for promoting bone formation in breastfeeding women [40]. Moreover, in hemodialysis patients with high bone disease, supplementing with FO has been shown to reduce bone resorption [41]. While supplementing with flaxseed does not significantly affect bone health in adults, it may have an impact during early postnatal development and stressful situations that can influence bone composition and strength [42]. In the context of arthritis, inflammatory chemicals and joint wear are common causes of painful joint condition [43]. Studies suggest that FO, with anti-inflammatory characteristics may be helpful in arthritis treatment [43]. Observational studies indicates that ω-3 long-chain FA can impede the progression and development of rheumatoid arthritis (RA) [44]. A meta-analysis of randomized controlled studies demonstrated that ω-3 long-chain FA (EPA and DHA) were associated with improvements in RA symptoms, reflected in reductions in morning stiffness, the number of tender joints and pain. These FA also showed potential in reducing increased pain, HAQ, ESR, leukotriene B4 concentration and grip strength [45]. The results suggest that flaxseed, being an excellent source of long-chain ω-3 FA, may also have a protective effect against RA symptoms. Hence FO is abundant in essential FAs like ALA and ω-3 that exhibits ameliorating roles in wound healing, bone health and arthritis symptoms. Its anti-inflammatory properties may contribute to enhanced bone formation and protective effect against RA. The various mechanisms proposed to account for the biological actions of flaxseed have been discussed in each section of the text above as they relate to specific biological activities of flaxseed (Fig. 2).

4. Osteo-protective impacts of flaxseed

Recent studies have provided evidence of the positive effects of flaxseeds and their various forms including oil, powder extract and flour against, bone deterioration and decay through animal modeling and human-based trials. In a study, early-weaned male rats treated with flaxseed and FO exhibited a positive impact on the shape of their femurs. The study involved early weaned pups on day 14, while control pups were weaned on day 21. After the initial 21 days, control group C60 received a standard diet. The EW group was then subdivided into three subgroups: the control diet (EWC60), the flax meal diet (EWFF60) and the FO diet (EWFO60) until 60 days of age. Various parameters, including BMD, surface area and biomechanical characteristics of the femur's structural components, were assessed. The results indicated EWFO60 group demonstrated increased stiffness compared to other groups, suggesting that flaxseed consumption reduced femoral fragility [46]. Another study evaluated the beneficial role of PUFAs obtained from fish oil, FO and soybean oil against GC osteoporosis. FA content in oils was measured using gas chromatography. Prednisolone control groups received a daily dose of prednisolone, along with groups supplemented with prednisolone plus soybean oil, FO, or fish oil. Male rats were divided into various groups, including a regular control group fed a balanced diet and a prednisolone control group. The study's findings revealed that the prednisolone control group had significantly lower BMD and femoral bone mass compared to the normal control group. Fish oil, in particular was superior to other oils in increasing BMD and achieving normal histological results. This study suggests that GC-induced osteoporosis could be prevented by consuming PUFA-rich dietary oils such as fish oil, soybean oil and FO [47]. It is a well-established fact that an individual's bone mass peaks around the age of 30, which in Wistar rats corresponds to approximately 180 days. The attainment of maximum bone mass is influenced by various factors such as physical activity, sex, genetics and nutrition. Recent research findings suggest that flaxseed flour (FF) possesses beneficial properties for bone formation. In a study conducted over 180 days, the impact of a flaxseed-rich diet on the bone health of adult male Wistar rats was investigated. Pups were randomly assigned to receive either a control diet or a diet containing FF during lactation and they continued on their respective diets after weaning. Dual-energy X-ray absorptiometry was used to measure body length, total bone architecture, femur and lumbar spine of rats on day 180. The result demonstrated that the FF diet significantly enhanced bone health compared to the control diet. The FF group exhibited improved femoral dimensions, BMD, peak strength, fracture strength and stiffness. Additionally, the FF group showed higher total and vertebral BMD and content, increased total bone surface area and elevated levels of osteocalcin indicating improved bone turnover. This experimental investigation suggested that consuming flax meal has beneficial effects on bone health [27].

A diet rich in FO influenced the growth and bone production in healthy male Wistar rats. The pups and their mothers were divided into two groups: the experimental group received a meal containing 7 % FO while control group was fed a diet containing 7 % soybean oil. Pups were weaned on day 21 and fed separately. Various aspects of bone health and quality were examined at 67 days of age. Distinct differences were observed in the rats from FO group compared to the control group. The reduced body mass in the FO group indicated an initial sign that the diet might impact overall growth and development. Regarding bone measures, the FO group exhibited positive effects on bone health. Notably, the bone marrow area, representing the central cavity within the bone, was significantly lower in the FO group (10.1 %) than in the control group. This suggested that the bone remodeling and restructuring processes may have been influenced by the consumption of FO.

Furthermore, osteocalcin (+36.7 %) and OPG (+52.5 %) were both higher in the FO group. These indicators were found to be more prevalent, suggested that FO may have encouraged bone formation and reduced bone resorption, thereby boosting bone health and development. These results imply that the mineralization and structural integrity of the femur were positively impacted by the FO diet. The FO diet was associated with higher levels of indicators for bone production, better BMD, improved biomechanical characteristics and structural changes in the femur [40].

In another study bone characteristics in adult offspring were affected by maternal exposure to SDG, an estrogenic flaxseed lignan, during breastfeeding. The hypothesis was that maternal SDG supplementation would influence the offspring's bone metrics. After giving birth, dams were divided into various groups for the study. Other groups received different doses of SDG as a dietary supplement. 4 mg/100 g, 40 mg/100 g, or 400 mg/100 g. The control group consumed a typical diet. After

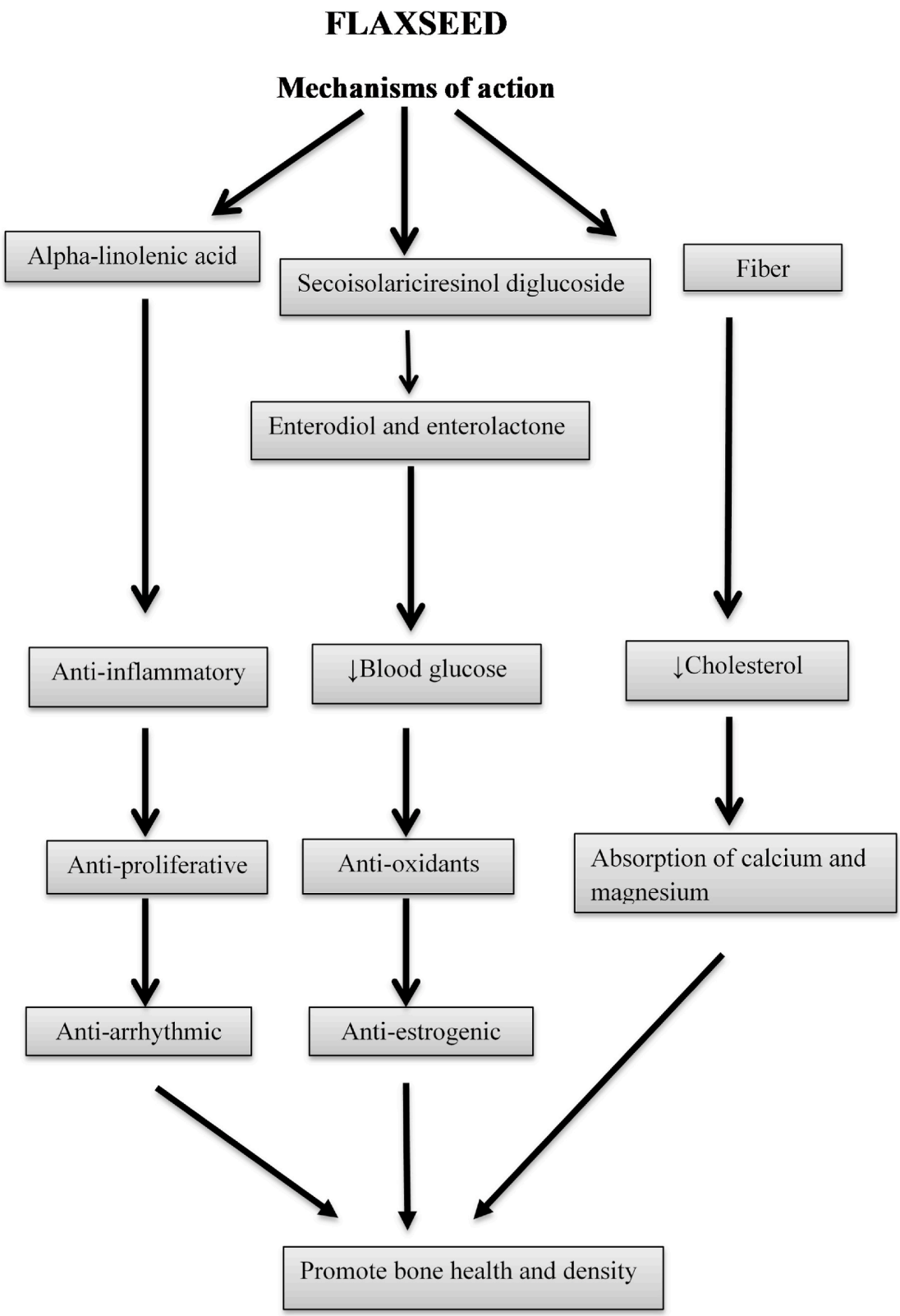

Fig. 2. Mechanism of action of dietary flaxseed.

Table 1
Flaxseed nutritional composition.

One tablespoon (10 g) of whole flaxseed contains:					[36, 37]
Protein: 1.9 g	Carbohydrates: 3 g	Fats: 4.3 g	Sugar: 0.2 g	Fiber: 2.8 g	
B. % composition of flaxseed					
Protein: 18 %	Carbohydrates: 29 %	Water: 7 %	–	–	
42 g of fat, of which 73 % are polyunsaturated, 27 % are saturated and monounsaturated.					
20–40 % of the fiber is soluble and 60–80 % is insoluble.					

weaning, the offspring in each group were fed a standard diet. At postnatal day 180 (PN180), the researchers examined the adult offspring's bone parameters. According to the findings, male offspring from the 4 mg/100 g SDG group (4SDG) had more body fat mass than the control group. However, female progeny showed no discernible modifications. These results indicated that bone mineralization and density in male progeny were positively influenced by the intermediate dose of SDG. Comparatively, females in groups 4 and 40SDG had increased total and spine BMD compared to control groups. These results suggested that a nursing mother's exposure to SDG had a positive impact on mineral content and BMD in female offspring. Overall, this study suggested that exposure of newborns to low doses of SDG may result in denser bones in adults. These results draw attention to the potential advantages of dietary SDG in encouraging ideal bone growth [48]. Similarly, the effect of flaxseed extract on the BMD of Wistar rats was assessed through a digital radiography experiment. The trial included 25 male and 25 female Wistar rats randomly assigned to one of five groups: control, Ca and vitamin D (Ca/Vit D), 100 mg/kg flaxseed, 200 mg/kg flaxseed and 400 mg/kg flaxseed. The mice were then kept for a full month. Serum Ca, vitamin D and P levels were assessed at baseline and 30 days later, along with the maxillary and mandibular BMD. The highest improvement in alterations to maxillary and mandibular bone density was seen in a group receiving 200 mg/kg flaxseed, with mean differences of 24.56.1 for maxillary bone density and 26.53.1 for mandibular bone density. These findings confirm that flaxseed extract more efficiently raised bone density than the Ca/Vit D group. Compared with Ca/Vit D group, a group consuming 200 mg/kg flaxseed had higher maxillary and mandibular BMD ($p < 0.001$). These findings imply that flaxseed extract may have value as a natural supplement for enhancing bone health and possibly preventing or treating osteoporosis [10]. In a 22-week study, four groups of male Sprague-Dawley rats were fed various diets: Standard control mode, FO, high fat diet (HFD) and HFD mode with 10 % FO (HY). The findings showed that the mice in the HFD group had lower skeletal biomechanical characteristics than those of the normal control group, including peak load, peak fracture load, ultimate tensile strength, stiffness, energy absorption capacity and elastic modulus. Peak load, peak fracture load and ultimate tensile strength were among the bone biomechanical characteristics that HFD-induced attenuation of HY group that received HFD additional FO. The inclusion of FO in the HY group considerably enhanced these properties of cancellous bone compared to the HFD group. ALP and Procollagen type I N-terminal propertide (P1NP) serum levels were greater in the HY group compared to the HFD group, suggesting accelerated bone formation. These results imply that by encouraging bone formation and reducing bone resorption, FO supplementation reduced HFD-induced bone loss. Treatment with Palmitic acid (PA), a saturated FA, reduces ALP activity and expression of osteogenic genes and proteins in vitro studies using rat primary OBs. Treatment with ALA, however, dose-dependently reversed the inhibitory effects brought on by PA, suggested a potential role for this compound in enhancing osteoblast activity and bone formation. According to this study, FO, which is high in ALA, may have therapeutic potential for reducing bone loss caused by HFD. The potential of FO to encourage osteogenesis and enhance bone microstructure may be the cause of the positive benefits of FO supplementation on bone health [49].

Another investigation examined FO's ability to protect osteoporotic cavities and ALP alterations in the bones of adult male albino rats induced by ingesting caffeine-containing energy drinks. The experiment, conducted over 8 weeks at Shaikh Zayed Postgraduate Medical Institute's Department of Anatomy in Lahore involved four sets of 32 mature male albino rats weighing between 250 and 300 g, randomly assigned. The control group received a baseline diet for eight weeks along with a daily dose of 5 mL/kg of maize oil by body weight. In the experimental groups, members of group B received corn oil (5 mL/kg of body weight) and caffeinated energy drinks (15 mL/kg body weight) for 8 weeks. Groups C and D, also being evaluated, received a caffeinated energy drink (15 mL/kg body weight) daily for eight weeks along with 40 and 60 % FO, respectively. Blood samples were obtained before and 24 h before the final dose to test serum ALP levels and the rat's weights were recorded before and after the experiment. Right femora (thigh bones) were selected for histopathological examination. The study's findings revealed a highly significant difference in the mean osteoporotic femoral cavities between the experimental groups ($p = 0.000$) indicating that osteoporotic cavities in the bones were caused by the caffeinated energy drink alone. To counteract these osteoporotic alterations, FO was given along with the energy drink. The study found that FO had a protective effect against the osteoporotic alterations that were brought on by the consumption of caffeinated energy beverages [50]. Vitamin D plays a crucial role in various biological processes, including the regulation of bone metabolism, cell proliferation and differentiation. Imbalance in this endocrine system has been linked to prevalent disorders such as osteoporosis and diabetes. To investigate if changes in osteogenic indicators are influenced by variations in the vitamin D receptor gene (VDR), specifically the Bsm1 and Fok1 polymorphisms, a study was conducted using linseed oil. Researchers employed the restriction fragment length polymorphism (RFLP) technique to investigate these genetic alterations. The study involved five groups: a control group, diabetic rats, diabetic rats fed FO in the food, ovariectomized (OVX) rats and diabetic OVX rats fed linseed oil. The findings showed that the OVX group exhibited significantly higher levels of osteocalcin, urine deoxypyridinoline and insulin-like growth factor-1 (IGF-1) were considerably higher than those of other groups (1.470 ± 88.1), (160 ± 7.11) and (21.25 ± 2.33), respectively). There was a highly significant positive relationship between these markers ($p < 0.05$). Conversely, when FO was added to the diet of all groups, the lowest levels of these indicators were observed. According to the results, diabetes had a more profound effect on bone health than ovariectomy. The study also emphasized the impact of gene variants, particularly the Bsm1 and Fok1 polymorphisms in the VDR gene, on bone health. The outcomes suggested that FO is advantageous in preventing osteoporosis.

Vitamin D receptor gene variants play a role in bone health and illustrate how diabetes negatively impacts bone health, while FO may offer some protection. The results suggested that genetic variants in the VDR gene, along with dietary modifications such as FO intake, may significantly contribute to preventing osteoporosis and enhancing bone health [20].

In a study involving ovariectomized breast cancer mice (MCF-7), both SDG and FO, both individually and in combination, affected BMC, BMD and bone biomechanical strength. Tamoxifen (TAM), a medication known to increase BMC and BMD in breast cancer patients, was also tested. The mice were divided into four non-TAM and four TAM groups according to the factorial design. Each group received a unique diet, including a basal diet, SDG alone (1 g/kg), FO alone (38.5 g/kg) or a combination of SDG and FO. TAM implants were used for groups of 5 mg release TAMs over 60 days. Results showed that TAM significantly enhanced BMC, BMD and bone biomechanical strength in the lumbar and femur vertebrae of rats. However, SDG alone resulted in a significant decline in femur BMD (by 6 %), while FO alone, led to decreased vertebrae BMC (by 8 %) and BMD (by 6 %), without TAM therapy. These findings suggest that when used separately, SDG and FO may have

adverse effects on bone health. Furthermore, the study demonstrated that SDG and FO had no discernible effect on the biomechanical strength of bones, regardless of TAM use. This suggest that there was no significant difference between the tested flaxseed components and the overall mechanical strength of bones. The study also highlighted the beneficial effects of TAM on bone health in ovariectomized mice with breast cancer and these effects were not significantly influenced by flaxseed components, specifically SDG and FO. These results imply that, in this experimental setting, the use of flaxseed products in addition to TAM therapy may not have obvious negative consequences on bone health [51]. An animal trial investigated the impact of a flaxseed diet on the body obesity and bone health of rats over a 90-days period. Male Wistar rats were randomly divided into two groups: control group and experimental group (FF). Mothers in the FF group consumed a diet high in flaxseed during lactation, while those in the control group consumed a typical control diet. Puppies were weaned on day 21 and then fed either the control or test diet for 90 days. Measurements of variables such as food intake, body weight and height were made between 21 and 90 days. The researchers evaluated various aspects of body composition and bone health after 90 days, including using dual-energy X-ray absorptiometry for composition, analyzing serum hormone profiles, determining intra-abdominal fat levels and examining the lumbar spine and femur. Statistical significance was set at $p < 0.05$. The results revealed several important differences between the FF and control groups. The total fat-free mass was higher in the FF group (7 %), while the overall (−16 %) and intra-abdominal (−24 %) fat mass was lower. Additionally, the adipocyte area was 30 % lower in the FF group. The FF group exhibited better bone health in terms of the femur (5 %), BMD (5 %) and radiodensity (20 %). Moreover, the FF group demonstrated higher peak strength (10 %) and fracture toughness (11 %). In general, flaxseed meal possesses beneficial characteristics that support healthy body growth and reduce the risk of obesity, metabolic diseases and bone fragility. These results suggest a potential advantage of consuming flaxseed to promote healthy body composition and bone strength [52]. Flaxseed is considered to improve femur quality, prompting an investigative study on dam rats. Due to the bone mass modifications in women during pregnancy and post-delivery, there may be an increased risk of developing osteoporosis later in life, making this study particularly relevant. Two groups of mice were employed: the control group and the experimental group. After weaning, the rats were divided into two groups: experimental and control. During the lactation period, the experimental group received 25 g of FF and during the maintenance phase 15 g were provided. The control group was fed a standard diet without FF. 51 days after birth, several studies were conducted to determine the effect of flaxseed meal on bone quality. Analysis of the FA composition revealed that the experimental group had higher amounts of ALA and EPA, but lower levels of total PUFA and ARA. ARA is known for its pro-inflammatory qualities and the reduction in this level support an anti-inflammatory profile in the experimental group. Additionally, an increase in ω-3 FA, ALA and EPA is associated with better bone health. The research demonstrated that feeding lactating and post-weaning rats FF increased the quality of their femurs, as indicated Increased bone density, mineral content and a favorable FA composition. Due to its protein composition, Ca content and FA composition, flaxseed meal can potentially prevent future osteoporosis by creating an anti-inflammatory environment and encouraging the deposition of organic substrates in the bone [53].

ω-3 PUFAs are essential nutrients that the body cannot produce on its own and must be obtained from food. Studies on the transgenic Fat-1 mouse model have highlighted the significance of endogenous ω-3 PUFAs in bone quality and skeletal development. These mice possessed the gene for ω-3 desaturase, enabling their bodies to convert ω-6 PUFAs to ω-3 PUFAs. However, this model falls short of accurately simulating human dietary intake of ω-3 PUFAs. Therefore, additional studies using fish and FO-rich diets were conducted to better understand the role of ω-3 PUFAs in bone development. Significant discoveries emerged from this investigation regarding the effects of dietary ω-3 PUFAs. First, the addition of ω-3 PUFAs to the diet led to a reduction in the amount of fat stored in the liver and the quantity of circulating blood lipids. Furthermore, dietary ω-3 PUFA consumption altered the composition of FA in the liver and serum. In bone investigations, researchers employed the three-point bending test to evaluate the mechanical properties of bone. The results indicated that dietary ω-3 PUFAs improved these mechanical abilities. However, the effects of ω-3 PUFAs on osteogenesis varied and were more complicated depending on the source of the PUFA. Understanding the molecular mechanisms controlling bone remodeling advances our knowledge of the role of dietary ω-3 PUFAs in bone growth. This research sheds light on the different effects that dietary sources of ω-3 PUFAs, including linseed oil and fish oil, can have on bone structure and composition. These results contribute to our understanding of the potential role ω-3 PUFAs may play in improving skeletal health and influencing the development of targeted treatments for bone disease [54]. Another study examined the effect of a monounsaturated diet high in ω-9 FA on osteoporosis. The absence of tissue loss, lower mechanical strength and bone microarchitectural deterioration in ovariectomized (OVX) mice led the authors to speculate that ω-9 may be a useful dietary supplement for treating osteoporotic degeneration. Female C57BL/6J mice were divided into three groups: sham ovariectomy (control), Ovariectomy (OVX) and Ovariectomy with estradiol therapy. Each group was fed a high ω-9 diet for 12 weeks. Surprisingly, this diet altered the mechanical properties of bone, making it stiffer and more viscous. This study provide initial information on the potential benefits of a monounsaturated diet high in ω-9 for maintaining bone density and reducing fractures in Ovariectomized rats (Omer et al., 2023). Additionally, the impact of PUFAs on the bones of mice was investigated in another experiment using male and female C57BL/6J mice. The only dietary difference between the three mouse groups was the percentage of fat in each group's diet. One group was fed a diet high in ω-6 PUFA (soybean oil, soy), another DHA, a ω-3 PUFA and the third DHA and ARA in a 20:1 ratio, both of which are ω-6 PUFA. Using micro-computed tomography, the researchers examined the mice's femurs and evaluated the bone microstructure after nine weeks of nutritional therapy. They also measured bone strength using 3-point bending in mechanical testing. Blood samples were regularly collected to search for biomarkers connected to bone growth and resorption. Based on these findings, the study concluded that dietary PUFA intake did not appear to alter the bone structure or metabolism in this particular model of healthy-growing mice. Some minor alterations were seen at the microstructural level, although these were most likely the product of random factors and did not result in any discernible changes [55].

Flaxseed has gained widespread use in food due to its health benefits. Investigating whether altering the type and amount of FA consumed can improve bone metabolism, especially during childhood, is crucial, as changes in dietary FAs can affect bone health. While the benefits of whole flaxseed or its pure lignans on bone formation have been studied, no research has explored the effects of ALA-rich FO on bone development. This study aimed to examined how a 10 % FO diet affect key markers of bone health, such as bone mass and DHA levels. Experimental results demonstrated that mice fed on a 10 % FO diet had significantly higher serum EPA and DHA levels, indicating an increase in these beneficial FA. Additionally, other FAs in the serum were examined, revealing that animals on the FO diet exhibited significantly lower levels of LA and ARA as compared to mice on other diets. This suggested that the FO diet influenced the overall FA composition of mice. Importantly, the study found that the amount of ALA in the 10 % FO diet did not affect bone mass, bone strength, or bone density in growing mice, indicating that the ALA levels in this diet are safe for bone growth. Furthermore, mice on the 10 % FO diet showed no significant positive or negative impacts on bone formation. While the FO diet increased EPA and DHA levels, which are beneficial for overall health, it did not affect serum cytokines or bone health markers. Consequently, providing FO as an ALA source to mice at the investigated amount poses no harm to bone

Table 2
Effect of consuming flaxseed on bone health.

Study object	Induction	Duration	Methodology	Results	Conclusion	Citation
Male rats	Femoral dimension, BMD, BMC and biomechanical properties were determined	60 days	After 14 days (early weaning) or 21 days (control), puppies were divided. A control diet was given to the C60 control group. According to control (EWC60), FF (EWFF60) and FO diet (EWFO60), the EW group was divided.	EW FO 60 showed a lower ($P < 0.05$) femur mass. BMC was decreased ($P < 0.05$) in EWC60, although stiffness was increased ($P < 0.05$) in EWFF60.	The early weaning-related femoral fragility was reduced by FF.	(Pessanha et al., 2016)
Male rats	Using gas chromatography, the concentration of FA oil was measured.	3 weeks	5 groups, each with 8 rats. G1: Standard control (healthy diet) G2: Control with prednisolone (10 mg/kg/daily) G3: Soybean oil (10 mg/kg of prednisolone plus 7 % soybean oil by weight) G4: FO (10 mg/kg prednisolone plus 7 % FO by weight). Fish oil (from cod liver; 10 mg/kg prednisolone plus 7 % fish oil by weight).	Improvements in plasma Ca concentrations and inhibition of oxidative stress and inflammatory markers were achieved with the use of fish oil, soybean oil or FO supplements. Significant increases in plasma Ca concentrations and suppression of oxidative stress and inflammatory markers were achieved through supplemental use of fish oil, soybean oil or FO. Fish oil was substantially more effective than the other two oils with a significant increase in BMD.	Particularly fish oil, which protects against GC-induced osteoporosis, ω-3 PUFAs such as soybean oil, FO or fish oil help stop bone loss	[47]
Adult Wistar male rats	Femur resistance, bone structure and BMD were examined by DEXA.	180 days	2 groups of rats Group under control (C, n = 12) Group using FF (FF, n = 12)	The FF group generated more total BMD (+3.4 %), spine (+5.9 %) BMD, total bone area (+8.7 %), BMD (+5.2 %), maximum force (+10.6 %) and stiffness (+23 %).	At 180 days, FF improved BMD and femur resistance.	[27]
Male Wistar rats	Osteocalcin, OPG levels, head radiodensity, BMD and biochemical properties of the right femur were evaluated.	67 days	Six mother dogs and six puppies from each group the control and flaxseed groups were given diets containing either 7 % soy oil (C) or 7 % FO.	In the FO group, body weight decreased (3.7 %), osteocalcin increased (+36.7 %), OPG (+52.5 %), femoral width (+3.8 %), femoral BMD (+3.6 %) and head A partial X-ray density (+6.1 %) was observed. Compared to the control group.	In healthy male Wistar rats, the FO diet improved the quality of their femurs.	[40]
Three-month-old Wistar rats	Body mass, BMC, food intake, body composition, and BMD were evaluated by DEXA.	180 days	Standard food (n = 10) was given to 4 male and 4 female pups. SDG-supplemented diet: 4 mg/100 g, 40 mg/100 g, or 400 mg/100 g groups.	Male 4SDG displays greater body fat mass at PN180. In PN180, 40SDG men had higher total spine BMC and BMD. At PN180, only 40SDG women demonstrated greater total BMC, although 4SDG and 40SDG women demonstrated higher spinal BMD.	Adults with SDG have denser bones, indicating that eating SDG may be beneficial for bone formation.	[48]
Wistar rats 25 male and 25 females	Serum levels of Ca, vitamin D and P were measured in the maxilla and mandibular at baseline.	30 days	Five groups of male and female rats were created: 1: Control 2: Vitamin D & Ca 3: 100 mg/kg of flaxseed 4: 200 mg/kg of flaxseed 5: 400 mg/kg of flaxseed	The Ca/vit D group had increased serum vitamin D concentrations. The highest change in maxillary and mandibular BMD was seen in the group that received flaxseed at a dose of 200 mg/kg, with a mean difference of 24, 56.1 and 26, 53.1.	The 200 mg/kg flaxseed group showed an increase in the maxillary and mandibular BMD.	[10]
Male Sprague-Dawley rats	Micro CT and three-point bend test performed.	22 weeks	HFD with 10 % FO (HY, 60 % fat), HFD with 10 % FO + Control (NC, 10 % fat) and FO diet (NY, 10 % fat).	The HFD group displayed weaker biomechanical features as compared to the NC group ($p < 0.05$).	By encouraging osteogenesis, FO is a possible treatment drug for HFD-induced bone loss.	[49]
32 adult male albino rats	Blood samples were taken & right femora was used for histological purposes.	8 weeks	4 group G1: (Control) gets corn oil 5 mL/kg BW in addition to basal diet G2: (Experimental) gets CED (15 mL/kg BW) and corn oil (5 mL/kg BW). G3: (Experimental) gets CED (15 mL/kg BW) and 40 % FO (5 mL/kg BW). G4: (Experimental) gets CED	Significant difference in mean osteoporotic cavities of femora among experimental groups ($p = 0.000$) An insignificant difference was found in mean serum ALP ($p = 0.072$).	In mature male albino rats, a caffeinated energy drink caused osteoporotic alterations in the femora, which could have been reduced with FO.	[50]

(continued on next page)

Table 2 (*continued*)

Study object	Induction	Duration	Methodology	Results	Conclusion	Citation
			(15 mL/kg per body weight) and 60 % FO (5 mL/kg per body weight)			
Ovariectomis-ed diabetic rats	Urine collection, blood collection, tissue collection, Biochemical parameters, BMC, BMD and DEXA analysis for bones were evaluated.	8 weeks	5 groups 1. Regular control rats were given a simple diet 2. Rats with diabetes provided a basic diet. 3. Rats with diabetes are fed flaxseed rather than maize oil. 4. Non-diabetic ovariecto-mized (OVX) rats were fed a regular diet. 5. FO-fed OVX diabetic rats.	Urinary levels of osteocalcin, deoxypyridinoline (DPD) and insulin growth factor 1 (IGF-1) were all elevated (1.470 ± 88.1), (160 ± 7.11) and (21.25 ± 2.33), in OVX respectively. The FO group experienced an increase in BMC and BMD, with a ratio of 13.90 ± 0.01:21.10 ± 0.40.	FO is helpful in preventing osteoporosis, and diabetes has an impact on bone health that is greater than that of ovariectomy.	[20]
Ovariectomized athymic mice	The femur and lumbar vertebrae's BMC, BMD and biomechanical strength were all measured.	60 days	There are four TAM groups and one non-TAM group. Mice were fed a basal diet (BD), SDG (1 g/kg), FO (38.5 g/kg), or SDG + FO diet in each group. TAM is available in 5 mg doses in the TAM group.	Without TAM therapy, SDG decreased femoral BMD (6 %), whereas FO reduced vertebral BMC by 8 % and BMD by 6 %. FO and SDG had no effect on femoral or vertebral BMC and BMD under TAM therapy.	TAM's beneficial effects on bone were not diminished by FS components, indicating that there were no negative consequences on bone health.	(Chen et al., 2011)
Lactating rats	Body weight and length of food intake were assessed by DEXA	90 days	Flaxseed meal (FF, n = 12), mothers fed a diet comprising 25 g of FF/100 g, and controls (C, n = 12), mothers fed a diet containing 20 g of casein/ 100 g.	The FF group had a larger intra-abdominal fat mass (24 %), higher femur mass (+5 %), higher BMD (+5 %) and high radioactivity (+20 %).	FF diet reduces the risk of obesity, metabolic changes and bone fragility.	(Da Costa et al., 2016)
Post-Partum Female Rats	Postnatal studies of FA composition, bone compartment, serum hormones, computed tomography and biomechanics were performed.	51 days	During breastfeeding, the experimental (F, n = 7) and control (C, n = 7) groups were fed a diet containing 25 g of FF and 15 g during maintenance.	Total PUFA (−17 %, $p < 0.0001$) and ARA (−44 %, $p < 0.0001$) were lower in Group F, but ALA (+695 %, $p < 0.0001$) and EPA (+160 %, $p < 0.05$) were greater. However, the observed fracture strength (+25 %, $p < 0.05$), stiffness (+17 %, $p < 0.0001$) and femoral head radiographic density (+15 % $p < 0.05$) were higher.	The lower level of ARA and a higher level of EPA increased the deposition of organic matrix and resulted in the prevention of osteoporosis.	[53]
48 female C57BL6 mice	Micro-CT analysis, BMD and a three-point bending test were evaluated.	9 weeks	Six groups of eight mice each, including a control group, an experimental group, a group using FO, and a group using fish oil	In a micro-CT study, a FO diet enhances trabecular bone micro-architecture. A fish oil diet encourages increased BMD. N-3 PUFAs improve mechanical characteristics, according to examinations of bone.	Diets that support bone health should include ω-3 as a key component.	(Rozner et al., 2020)
Female C57BL/6J mice	DMA, 3-point bending, his-tomorphometry, and micro-CT were used to assess tibiae.	12 weeks	Anesthesia and analgesia were administered to the control group. Mice with ovariectomies (OVX) were fed a high ω-9 unsaturated diet.	Significant reductions in lean mass ($p = 0.05$) and tibial area ($p = 0.009$) were observed. OVX bone displayed increased ductility, elastic modulus, storage modulus, and loss modulus.	This study gives evidence that ω-9 contributes to bone health and postmenopausal osteoporosis	[59]
3 weeks oil male and female mice C57BL/6J	Femurs were collected for three-point mechanical bending tests and micro-computed tomography	9 weeks	3 groups G1: ω-6 PUFA-rich soybean oil (SOY). G2: DHA, a PUFA-three. G3: ARA and ω-6 PUFA and DHA in a 20:1 ratio.	In various dietary groups, male mice's trabecular bone metrics showed statistically significant differences.	In healthy mice, PUFA intake does not appear to affect bone structure or metabolism.	*(Anez-B*ustillos et al., 2019)
Male and female mice	Evaluation of serum LA, ARA, EPA, and DHA levels	91 days	Male and female mice were given meals that contained 10 % FO or 10 % corn oil, respectively. ALA was converted to EPA and DHA in mice given FO, both male and female.	Serum LA and ARA levels were significantly lower in mice given FO.	Regarding osteogenesis in developing mice, the ALA content in the 10 % FO diet is safe.	[56]
82 patients with hand OA	The Visual Analogue Scale (VAS) and the Auskan OA Hand Index were assessed using data acquisition equipment.	15 days	Three flaxseeds compress trial groups, intervention G1 (n = 33), control group (n = 20) and intervention G2 (warm compress) group (n = 29)	Patients in group 1 had VAS scores of 6.03 ± 0.25 on day 0, 2.2 ± 0.30 on day 8 and 3.39 ± 0.32 on day 15. AUSCAN scores for patients in intervention G1 ranged from 40.84 ± 1.76 on day 0, 14.03	Patients receiving flaxseed poultice compresses have improved hand function	[57]

(*continued on next page*)

Table 2 (*continued*)

Study object	Induction	Duration	Methodology	Results	Conclusion	Citation
				± 1.66 on day 8, and 15.78 ± 1.66 on day 15.		
34 hemodialytic patients	serum concentrations of osteocalcin, N-telopeptide, were measured by ELISA kit.	8 weeks	FO group 6 g/d for 8 weeks. Control group 6 g/d of MCT oil. At baseline 7 mL of blood was obtained	Significant decreases in serum N-telopeptide content of up to 17 % occurred in the FO group.	Patients on hemodialysis who consume 6 g/d of FO may experience less bone loss.	(Mirfatahi et al., 2018)
120 patients with RA	The Disease Activity Score 28 (DAS28) and the Health Assessment Questionnaire (HAQ) were used to evaluate disability and pain.	12 weeks	Three groups were divided. G1: An anti-inflammatory diet and flaxseed (30 g every day). G2: Regular diet and 30 g of flaxseed/day (RF group) G3: Regular diet (RW group) with toasted wheat (30 g/day).	Flaxseed reduced DAS28 in the RF group compared to the RW group (-0.87 ± 1.11 vs. - 0.24 ± 0.78; P = 0.014). The AIF and RF groups had considerably lower levels of pain ($P \leq 0.001$), morning stiffness ($P < 0.05$) and exhaustion ($P < 0.01$).	Flaxseed can be used as an effective adjunctive therapy in people with rheumatoid arthritis.	(Ghaseminasab-Parizi et al., 2022)

ELISA: enzyme-linked immunosorbent assay, BMD: bone mineral density, HFD: high-fat diet, FO: flaxseed oil, MCT: medium chain triglycerides, SDG: secoisolariciresinol diglucoside, PUFA: polyunsaturated fatty acids, ARA: arachidonic acid, ALA: alpha-linolenic acid, OPG: osteoprotegerin, AUSCAN: Australian–Canadian, RA: rheumatoid arthritis, BW: body weight, CED: caffeinated energy drink, TAM: tamoxifen, CO: camelina oil, DHA: docosahexaenoic acid, FA: fatty acid.

growth in developing mice [56]. In a recent human-based trial utilizing a randomized controlled intervention research design, the effects of flaxseed poultice application on pain and hand function in patients with primary hand interphalangeal osteoarthritis (OA). The study included 82 participants from a university hospital's rheumatology clinic, divided into three groups: control group, intervention group I (hot compress) and intervention group II (linseed compress). Result showed that patients treated with a flaxseed poultice compress (intervention group I) had significantly better hand function efficiency and pain relief than those treated with a hot compress (intervention group II) and the control group. This suggested that the flaxseed poultice intervention is a beneficial nursing intervention to reduce pain and improve hand function in patients with hand OA, in addition to pharmacological treatment [57]. Hemodialytic patients suffering from chronic kidney-mineral-bone disease (CKD-MBD) were the focus of another randomized controlled experiment. The study aimed to explore the potential benefits of FO, a rich source of plant-based ω-3 FA and ALA. 34 hemodialysis patients were randomized to either the FO group or the control group, with the FO group receiving a daily dose of 6 g of FO and a control group receiving 6 g of medium-chain triglyceride oil daily. At the start of the research and after the eighth week, blood samples were obtained from all patients following a 12- to 14-h fast. Result showed a substantial drop in serum N-telopeptide content in the FO group compared to the baseline, indicating a reduction in bone loss. Additionally, this decrease was noticeably more pronounced than that seen in the control group. These findings back up the study's claim that hemodialysis patients who consumed 6 g of FO daily may experience a reduction in bone loss. In comparison to the baseline and the control group, the N-telopeptide level, a measure of bone resorption, was considerably lower in the FO group. This randomized controlled research provide evidence that adding FO to hemodialysis patients' diets may improve their bone health by lowering bone resorption. The results raised the possibility that FO is helpful in treating CKD-MBD in hemodialysis patients [41]. A recent study explored the effects of flaxseed and an anti-inflammatory diet on R patients. Previous studies have suggested that long-chain ω-3 FAs, such as those found in flaxseed, can reduce RA symptoms. The experiment's goal was to find out whether including flaxseed and an anti-inflammatory diet in the treatment regimen would reduce symptoms, disability, quality of life and inflammatory indicators. The 12-week intervention involved 120 RA patients divided into three groups: roasted wheat plus normal diet (RW), flaxseed plus anti-inflammatory diet (AIF) and flaxseed plus regular diet (RF). Results indicated that flaxseed supplementation may be an effective adjuvant therapy for RA patients, improving pain, morning stiffness and disease perception reducing disease activity. Combining an anti-inflammatory diet with flaxseed supplements led to even more significant improvements in disease severity, pain, impairment level and the quality of life. The study emphasized the potential of a supplemental anti-inflammatory diet for treating RA symptoms [58]. Hence recent studies highlight the positive impact of flaxseed, FO and flaxseed lignans on bone health, demonstrating effects such as increased femoral strength, preventing GC induced osteoporosis and enhanced bone formation in various experimental models. Flaxseed derived components, especially (ω-3) FAs, exhibits potential in promoting optimal bone development and countering bone related disorders. These findings suggest the promising role of flaxseed-based interventions in enhancing bone health. Table 2 discusses the effects and roles of flaxseed as described in different studies.

5. Conclusion

The peak bone mass achievement is crucial for optimal bone health and the prevention of osteoporosis later in life. The process of reaching peak bone mass is influenced by various factors, including hormones, genetics, physical activity and nutrition, particularly the intake of Ca, vitamin D and ω-3 FAs. Flaxseed, derived from *Linum usitatissimum*, is rich in phytoestrogens, ω-3 PUFAs, proteins, fibers and essential nutrients. The presence of phytoestrogens, including SDG, makes flaxseed a unique dietary component that may influence estrogen signaling and contribute to bone health. Additionally, the high content of ω-3 PUFAs, particularly ALA in flaxseed has been associated with positive effects on bone density and the regulation of bone metabolism. The nutritional composition of flaxseed, including proteins, lipids and fibers further supports its potential benefits for bone health. Flaxseed has been studied in various forms, such as oil, flour, extract and powder, with different processing methods and demonstrated bone protective efficacy. The consumption of flaxseed, particularly its long chain ω-3 PUFAs, has been linked to improvements in overall health, cardiovascular health and inflammatory responses, making it potentially beneficial for women's health and bone density. Consuming FO has shown to enhance BMD and reduce osteoporosis risk factors. FO, comprising 53 % LA, 19 % oleic acid, 17 % ALA, 5 % palmitic acid and 3 % stearic acid, exhibits promising effects in osteoporosis prevention. This study aimed to assess the impact of flaxseed and fish oil treatment on the femur shape of early-weaned male rats. The EWFO60 group exhibited lower femoral mass compared to the other groups. Fish oil supplements compared to the normal control group, notably increased the BMD and achieved normal histological results. This suggests that incorporating flaxseed into the diet might enhance bone health during the initial stages of weaning. Another study involving adult male Wistar rats revealed BMD and

femoral resistance significantly improved after a 180-day flax meal diet. Notably maximal bone mass in humans is typically attained around age 30 and is influenced by factors such as physical activity, sex, genetics and nutrition. In this context, the FF group displayed improved femoral dimensions, increased total and vertebral BMD and content, as well as total bone surface area and osteocalcin levels. This investigation specially explored how a diet rich in FO influenced bone growth and production in healthy male Wistar rats. Osteocalcin and OPG levels were higher in the FO group which also exhibited lower body mass. The FO diet correlated with elevated indicators of bone production, improved BMD, enhanced biomechanical characteristics and structural alterations in the femur. Moreover, the bone density and mineralization of adult offspring were positively impacted by maternal exposure to SDG during lactation. These results suggest the potential contribution of FO as a source of ALA in promoting bone health.

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CRediT authorship contribution statement

Itrat Batool: Writing – original draft, Methodology, Data curation, Conceptualization. **Ammar B. Altemimi:** Writing – review & editing, Software. **Seemal Munir:** Writing – review & editing, Data curation, Conceptualization. **Shifa Imran:** Writing – review & editing, Methodology, Data curation. **Naymal Khalid:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Muhammad Asif Khan:** Writing – review & editing, Conceptualization. **Gholamreza Abdi:** Writing – review & editing, Resources, Investigation, Conceptualization. **Muhammad Saeeduddin:** Writing – review & editing. **Muhammad Abid:** Writing – review & editing, Conceptualization. **Rana Muhammad Aadil:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization.

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

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

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