Effects of Flaxseed Oil on Serum Bone Turnover Markers in Hemodialysis Patients

A Randomized Controlled Trial

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Introduction. Chronic kidney disease-mineral and bone disorder is a common complication in hemodialysis patients. The present study was designed to investigate the effects of flaxseed oil, a rich source of plant omega-3 fatty acid alpha-linolenic acid, on serum markers of bone formation and resorption in hemodialysis patients.
Materials and Methods. In this randomized controlled trial, 34 hemodialysis patients were randomly assigned to either the flaxseed oil or the control group. The patients in the flaxseed oil group received 6 g/d of flaxseed oil for 8 weeks, whereas the control group received 6 g/d of medium chain triglycerides oil. At baseline and the end of the 8th week, 7 mL of blood was obtained from each patient after a 12- to 14-hour fast and serum concentrations of osteocalcin, osteoprotegerin, N-telopeptide, and receptor activator of nuclear factor kappa B ligand were measured.
Results. Serum N-telopeptide concentration decreased significantly up to 17% in the flaxseed oil group at the end of week 8, as compared to baseline ($P < .01$), and the reduction was significant in comparison with the control group. There were no significant differences between the two groups in the mean changes of serum osteocalcin, osteoprotegerin, or receptor activator of nuclear factor kappa B ligand.
Conclusions. This study indicates that daily consumption of 6 g/d of flaxseed oil may reduce bone resorption in hemodialysis patients.

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INTRODUCTION

Chronic kidney disease-mineral and bone disorder is a common complication in hemodialysis (HD) patients and is associated with high fracture risk, vascular calcification, cardiovascular events, increased morbidity, and mortality, as well as a lower quality of life.[1-3] In addition, inflammation is prevalent in hemodialysis patients and has a negative impact on bone turnover.[4,5] It has been shown that omega-3 fatty acids can decrease inflammation in hemodialysis patients.[6,7] Furthermore, some animal and human studies indicated that marine omega-3 fatty acids could reduce bone resorption.[8-11] However, to our knowledge, only one study has shown that a diet high in plant omega-3 fatty acid alpha-linolenic acid has a protective effect on bone resorption.[12] Therefore, the present study was designed to investigate the effects of flaxseed oil, a rich source of alpha-linolenic acid,[13] on serum markers of

bone formation and resorption in hemodialysis patients.

MATERIALS AND METHODS

Trial Design and Ethics Aspects

This study was a parallel, randomized, double-blinded, clinical trial performed between December 2014 and March 2015. The study protocol was approved by the Ethics Committee of the National Nutrition and Food Technology Research Institute of Iran. The study was in adherence to the Declaration of Helsinki. Written informed consent was obtained from all patients before initiating the study. This study was part of the project on the effects of flaxseed oil in hemodialysis patients. This clinical trial was registered with the Iranian Registry of Clinical Trials (IRCT201412192716N3).

Sample Size Calculation

The minimum sample size estimated for each group was 16 at a power of 80% and an alpha of 0.05 for a 2-arm parallel study with 2-tailed testing to detect a difference of 8.5 ng/mL in serum osteocalcin concentration with a pooled standard deviation of 8.7 ng/mL, obtained from the study by Salari Sharif and coworkers.[8]

Participants

Thirty-eight hemodialysis patients were selected from the hemodialysis units at Taleghani and Modares Hospitals in Tehran, Iran. The inclusion criteria were: an age of18 years and greater and being on hemodialysis for at least 6 months. The exclusion criteria were having inflammatory and infectious diseases, receiving steroidal or nonsteroidal anti-inflammatory drugs, receiving omega-3 fatty acids supplements, receiving warfarin, and using flaxseed or flaxseed oil regularly.

In all cases, hemodialysis was performed with polysulfone capillary dialysis filters and bicarbonate dialysis solution, 3 times a week, 4 hours per session. During the study, the hemodialysis procedure and type of dialysis filters were not altered for any of the patients.

Intervention, Randomization, and Blinding

The patients were randomly allocated to either a flaxseed oil or control group by block randomization after stratification based on diabetes mellitus. For this block randomization, we chose a block size of 4 and possible balanced combinations with 2 C (control) and 2 F (flaxseed) subjects were calculated as 6 blocks (FFCC, FCFC, FCCF, CFFC, CFCF, CCFF). Then, blocks were randomly chosen, based on a simple random sampling method, to determine the assignment of all patients into the groups. The block randomization was performed by a trained dietician. Patients in the flaxseed oil group received 7 mL/d (6 g/d) of flaxseed oil, as one Iranian tablespoon of flaxseed oil, for a period of 8 weeks, whereas the control group received 7 mL/d (or 6 g/d) of medium chain triglycerides (MCT) oil. The participants consumed oils with salad at lunch or dinner. The flaxseed oil was provided by Barij Essence, Tehran, Iran, and MCT oil was provided by SHS International Ltd, Liverpool, UK. The flaxseed oil had 57.5% alpha-linolenic acid, 17.2% oleic acid, 15.2% linoleic acid, 5.1% palmitic acid, 4% stearic acid, and 1% other fatty acids, whereas MCT oil contained 59.4% caprylic acid, 39.6% capric acid, 0.7% caproic acid, 0.2% lauric acid, and 0.1% myristic acid.

The oils were provided in similar dark bottles without any indication of whether the bottle contained flaxseed oil or MCT oil. The taste of flaxseed oil was different from MCT oil, but none of hemodialysis patients had used flaxseed oil or MCT oil before the start of this study, and therefore, they did not have any experience of the taste of flaxseed oil or MCT oil.

Blinding was performed by a trained dietician, and the patients and researchers were kept blinded to the allocation. The participants were advised not to change their dietary habits, physical activities, and drug regimens. In addition, the study protocol did not change after the trial was commenced. At baseline and the end of the 8th week, 7 mL of blood was obtained from each patient after a 12- to 14-hour fast. Blood samples were kept at room temperature (20°C to 25°C) for 20 minutes. After clotting, the samples were centrifuged at 2000 rpm for 10 minutes. The samples of serum were separated into small aliquots and were frozen at -70°C, until they were used.

Measurements

Primary outcomes were osteocalcin, osteoprotegerin, N-telopeptide, and receptor activator of nuclear factor kappa B ligand (RANKL). Serum concentrations of osteocalcin, a

bone formation marker,[14] osteoprotegerin, a bone resorption inhibitor,[15] and N-telopeptide and RANKL as 2 bone resorption markers,[15,16] were determined by enzyme-linked immunosorbent assay kits (ZellBio GmbH, Ulm, Germany). Intra-assay coefficients of variation for serum osteocalcin, osteoprotegerin, N-telopeptide, and RANKL were 4.2%, 6.2%, 5.5%, and 4.1%, respectively. Serum intact parathyroid hormone (PTH) concentration was assessed by an enzyme-linked immunosorbent assay kit (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany), with an intra-assay coefficients of variation of 2.4%. Serum concentrations of phosphorus and calcium were assessed using commercial kits (Pars-Azmoon, Tehran, Iran) with the aid of a Selectra 2 Autoanalyzer (Vital Scientific, Spankeren, The Netherlands). Intra-assay coefficients of variation for these biochemical parameters were less than 3%.

Patients were weighed after hemodialysis, to determine dry body weight (or postdialysis weight), at baseline and at the end of weeks 4 and 8. In addition, the dietary intakes of the participants were assessed using a 2-day dietary recall (1 dialysis day and 1 nondialysis day) in weeks 1, 4, and 8. Patients' diets were analyzed by the Nutritionist IV software (N Squared Computing, San Bruno, CA, USA).

At baseline and the end of week 8, dialysis adequacy based on the dialysis adequacy Kt/V index was determined for each patient by a Kt/V calculator software using information recorded in patient files, including predialysis blood urea nitrogen concentration, postdialysis blood urea nitrogen, the dialysis session length, postdialysis weight, and ultrafiltration volume.[17]

Compliance

For the ascertainment of patients' compliance, we provided each patient with a fixed volume of oils and instructions to return the unused oils at the end of the study. The degree of compliance for each patient was determined according to the volume of returned oils.

Statistical Analysis

Statistical analysis of data was performed using the SPSS software (Statistical Package for the Social Sciences, version 21.0, SPSS Inc, Chicago, IL, USA). A chi-square test was used to compare qualitative variables between the two groups. Since all quantitative parameters according to the Kolmogorov-Smirnov test had normal distributions, we used a *t* test and paired *t* test to compare parameters between and within groups, respectively. In addition, because dietary and anthropometric parameters were measured 3 times during the study, analysis of variance for repeated measurements was used to compare data among these time points. The results are expressed as the mean ± standard deviation, and differences were considered significant at a *P* value less than .05.

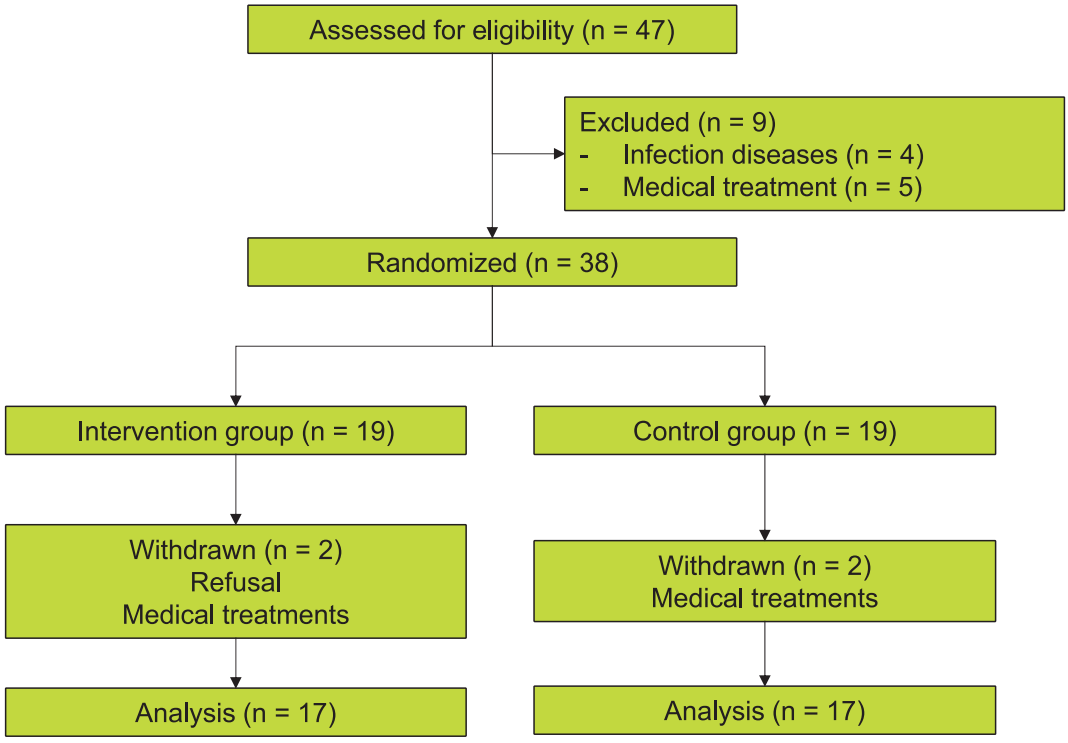
RESULTS

Of the 38 hemodialysis patients eligible for this trial, 2 in the flaxseed oil group and 2 in the MCT oil group were withdrawn because of lack of cooperation or medical treatments (Figure). The adherence rate of all of the patients was more than 90%, and no adverse events were reported.

The baseline characteristics of the patients did not differ significantly between the two groups. There was no significant difference in dialysis adequacy between the two groups at baseline and the end of week 8 (Table 1).

There were no significant differences in the mean dietary intake of calcium, phosphorus, protein, total fat, monounsaturated fatty acids, and omega 6-polyunsaturated fatty acids between the two groups on weeks 1, 4, and 8. In addition, these factors did not significantly change within each group during the study (Table 2). The mean dietary intake of saturated fatty acids was significantly higher in the MCT oil group compared with the flaxseed oil group on weeks 1 and 4 ($P < .05$; Table 2); whereas the mean dietary intake of omega 3-polyunsaturated fatty acids was significantly higher in the flaxseed oil group compared with the MCT oil group on weeks 1, 4, and 8 ($P < .01$; Table 2). There was no significant difference in the mean body weight and body mass index between the two groups on weeks 1, 4, and 8 (Table 2).

Serum N-telopeptide concentration reduced significantly in the flaxseed oil group at the end of week 8 compared to baseline ($P < .01$), whereas no significant change was observed in the MCT oil group. The reduction of serum N-telopeptide concentration in the flaxseed oil group was significant in comparison with the MCT oil group ($P < .05$; Table 3). No significant changes



Summary of participants' flow diagram.

Table 1. Baseline Characteristics of Patients in the Flaxseed Oil and the Medium Chain Triglycerides (MCT) Oil Groups*

Characteristics	Flaxseed Oil Group (n = 17)	MCT Oil Group (n = 17)	*P*
Sex			
Male	12 (71.0)	10 (59.0)	
Female	5 (29.0)	7 (41.0)	> .05
Age, y	68.0 ± 3.0	59.0 ± 4.0	> .05
Intake of drugs			
Calcium carbonate	6 (35.0)	8 (47.0)	> .05
Calcitriol	7 (41.0)	6 (35.0)	> .05
Cinacalcent	0	0	> .05
Sevelamer	0	0	> .05
Duration of dialysis, y	4.4 ± 1.0	4.6 ± 1.0	> .05
Dialysis adequacy (Kt/V)			
Baseline	1.20 ± 0.05	1.30 ± 0.05	> .05
Week 8	1.30 ± 0.10	1.20 ± 0.10	> .05

*Values are mean ± standard deviation or frequency (percentage).

were observed in serum RANKL, osteocalcin, osteoprotegerin, PTH, calcium, and phosphorus within each group during the study (Table 3).

DISCUSSION

Chronic kidney disease-mineral and bone disorder is a serious complication in hemodialysis patients,[1,2] leading to a substantial increase in the fracture risk, vascular calcification, cardiovascular events, morbidity, and mortality, and to decreased quality of life.[2,3] Bone is formed by osteoblasts while osteoclasts induce bone resorption.[18] N- telopeptide, or N-terminal telopeptide, is mobilized from bone by osteoclasts.[19] In our study, daily consumption of 6 g of flaxseed oil, a rich source of plant omega-3 fatty acid alpha-linolenic acid, significantly reduced serum N-telopeptide concentration up to 17% during 8 weeks. In addition, there were no significant differences between the two groups in mean changes of serum calcium, phosphorus, and PTH. Therefore, the reduction of serum N-telopeptide in hemodialysis patients receiving flaxseed oil was not due to the changes in serum calcium, phosphorus, and PTH during the study period. To our knowledge, no studies to date have investigated the effects of flaxseed oil consumption on serum N-telopeptide concentration, in hemodialysis patients, to compare with the results of our study. However, in agreement with our study, Griel and colleagues showed that the consumption of a diet high in alpha-linolenic acid (including 38% total fat, 8% saturated fatty acids, 12% monounsaturated fatty acids, 10.5% linoleic acid, and 6.5% alpha-linolenic acid), for 6 weeks,

Table 2. Changes in Dietary and Anthropometric Factors in the Flaxseed Oil and the Medium Chain Triglycerides (MCT) Oil Groups*

Factors	Week 1	Week 4	Week 8
Calcium, mg/d			
Flaxseed oil	468.0 ± 55.0	422.0 ± 40.0	582.0 ± 156.0
MCT oil	415.0 ± 45.0	516.0 ± 51.0	466.0 ± 53.0
Phosphorus, mg/d			
Flaxseed oil	836.0 ± 69.0	786.0 ± 52.0	1039.0 ± 207.0
MCT oil	800.0 ± 69.0	941.0 ± 72.0	836.0 ± 92.0
Protein, g/d			
Flaxseed oil	54.0 ± 5.0	56.0 ± 5.0	63.0 ± 4.0
MCT oil	55 ± 4.0	59.0 ± 5.0	58.0 ± 8.0
Fat, g/d			
Flaxseed oil	48.0 ± 2.0	49.0 ± 4.0	56.0 ± 8.0
MCT oil	49.0 ± 4.0	50.0 ± 5.0	49.5 ± 6.0
Saturated fatty acids, g/d			
Flaxseed oil	14.0 ± 1.0	15.5 ± 2	20.0 ± 4.0
MCT oil	20.0 ± 1.5†	23.0 ± 2.0†	23.0 ± 3.0
Monounsaturated fatty acids, g/d			
Flaxseed oil	17.0 ± 1.0	17.0 ± 1.5	21.0 ± 3.5
MCT oil	16.5 ± 1.5	16.5 ± 2.0	16.0 ± 2.0
Omega 6- polyunsaturated fatty acids, g/d			
Flaxseed oil	8.6 ± 0.6	8.5 ± 0.9	8.5 ± 1.4
MCT oil	9.0 ± 0.9	7.6 ± 0.9	7.3 ± 1.0
Omega 3- polyunsaturated fatty acids, g/d			
Flaxseed oil	4.00 ± 0.05‡	4.20 ± 0.09‡	4.00 ± 0.09‡
MCT oil	0.60 ± 0.09	0.70 ± 0.12	0.40 ± 0.06
Weight, kg			
Flaxseed oil	70.0 ± 3.0	71.0 ± 3.0	71.0 ± 3.0
MCT oil	63.0 ± 3.0	63.0 ± 3.0	63.0 ± 3.0
Body mass index, kg/m^2			
Flaxseed oil	26.0 ± 1.0	26.5 ± 1.0	26.5 ± 1.0
MCT oil	25.0 ± 1.5	25.0 ± 1.5	25.0 ± 1.5

*Values are mean ± standard deviation.
†$P < .05$ compared with the flaxseed oil group
‡$P < .01$ compared with the MCT oil group

significantly reduced serum N-telopeptide in comparison with the American diet (including 34% total fat, 13% saturated fatty acids, 13% monounsaturated fatty acids, 7.7% linoleic acid, and 0.8% alpha-linolenic acid) in human adults.[12] Also, Zwart and colleagues indicated that a higher intake of omega-3 fatty acids was associated with less N-telopeptide excretion during bed rest.[9] In contrast, in Appleton and colleagues' study, daily administration of 1.48 g of omega-3 long-chain fatty acids to mild-moderately depressed individuals for 12 weeks had no effect on serum C-telopeptide (C-terminal telopeptide).[20] The disagreement of Appleton and colleagues' finding with that of our study may be due to the administration of low dose of omega-3 fatty acids.

Inflammation is a common complication in hemodialysis patients that induces osteoclast differentiation and activation [4,18]; therefore, one possible mechanism on the effect of omega-3 fatty acids on serum N-telopeptide is that these fatty acids can lower the osteoclastic activity by reducing the production of inflammatory cytokines.[15] It has also been shown that serum N-telopeptide positively correlated with serum concentration of tumor necrosis factor, an inflammatory cytokine.[12] In agreement with the mentioned mechanism, we previously showed serum C-reactive protein, an inflammatory marker, reduced up to 24% in the flaxseed oil group.[21] However, it is unclear whether alpha-linolenic acid itself exerts these effects or whether they are the result of its conversion to eicosapentaenoic acid and docosahexaenoic acid. Another mechanism by which omega-3 fatty acids can affect bone resorption is a decrease in the production of prostaglandin E2.[15] It has been shown

Table 3. Serum Concentrations of Bone Turnover Markers, Parathyroid Hormone, Calcium, and Phosphorus in the Flaxseed Oil and the Medium Chain Triglycerides (MCT) Oil Groups*

Parameters	Baseline	Week 8	Change
N-telopeptide, nmol/L			
Flaxseed oil	80 ± 19	66 ± 17†	-14 ± 4‡
MCT oil	71 ± 23	70 ± 23	-1 ± 4
RANKL, pg/mL			
Flaxseed oil	267 ± 69	290 ± 74	23 ± 23
MCT oil	212 ± 67	217 ± 66	5 ± 18
Osteocalcin, ng/mL			
Flaxseed oil	23 ± 7	23 ± 6	0 ± 2
MCT oil	36 ± 14	36 ± 14	0 ± 4
Osteoprotegerin, ng/mL			
Flaxseed oil	6.0 ± 0.5	6.0 ± 0.5	0.0 ± 0.5
MCT oil	6.0 ± 0.5	6.0 ± 0.5	0.0 ± 0.3
Parathyroid hormone, pg/mL			
Flaxseed oil	57 ± 19	71 ± 20	13 ± 6
MCT oil	45 ± 9	44 ± 13	-1 ± 10
Calcium, mg/dL			
Flaxseed oil	10.0 ± 0.2	10.3 ± 0.2	0.3 ± 0.3
MCT oil	9.7 ± 0.2	9.9 ± 0.3	0.2 ± 0.3
Phosphorus, mg/dL			
Flaxseed oil	5.5 ± 0.3	5.8 ± 0.3	0.3 ± 0.3
MCT oil	6.0 ± 0.3	6.0 ± 0.4	0.0 ± 0.3
Calcium-phosphorus product, mg^2/dL^2			
Flaxseed oil	55 ± 3	61 ± 4	6 ± 5
MCT oil	59 ± 3	59 ± 5	0 ± 4

*Values are mean ± standard deviation. RANKL indicates receptor activator of nuclear factor kappa B ligand.
†$P < .01$ compared with baseline
‡$P < .05$ compared with the MCT oil group

that prostaglandin E2 promotes osteoclastogenesis.[15]

Receptor activator of nuclear factor kappa B ligand is expressed in different cells including osteoblasts. It is a ligand for the receptor activator of nuclear factor kappa-B (RANK) on the surface of osteoclasts and functions as a key factor for osteoclast differentiation and activation.[15] Osteoprotegerin is produced by different cells including osteoblasts and functions as a decoy receptor for the RANKL.[15] Osteoprotegerin prevents RANK activation by binding RANKL; therefore, it reduces osteoclast differentiation and activation.[15] In our study, daily consumption of 6 g of flaxseed oil had no effects on serum concentrations of RANKL and osteoprotegerin. We found no human study on the effects of flaxseed oil consumption or omega-3 fatty acids on serum concentrations of RANKL and osteoprotegerin to compare with the results of our study. However, some in vitro and animal studies showed that omega-3 fatty acids inhibited osteoclastogenesis through decreasing RANKL expression or increasing osteoprotegerin expression.[11, 22-24] The disagreement of findings from in vitro and animal studies with those of our study may be due to assessing the expression of RANKL and osteoprotegerin instead of measuring their serum concentrations.

Osteocalcin is produced exclusively by osteoblasts.[25] In the present study, flaxseed oil had no effect on serum osteocalcin concentration. To date, no research has investigated the effects of flaxseed oil consumption on serum osteocalcin concentration, in hemodialysis patients, to compare with the results of our study. However, in agreement with our study, some animal and human studies indicated that omega-3 fatty acids had no effect on serum osteocalcin concentration.[8,26-28]

The most important strength of our study was its design as a randomized controlled trial. Our study had 2 limitations; we did not measure bone density, and the sample size of this study was small.

CONCLUSIONS

This study indicates that daily consumption of

6 g of flaxseed oil may reduce bone resorption in hemodialysis patients.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Delmez JA, Kaye M. Bone disease. In: Daugirdas JT, Blake PG, Ing TS, editors. Handbook of Dialysis. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 530-47.
2. Heaf JG. Chronic kidney disease-mineral bone disorder in the elderly peritoneal dialysis patient. Perit Dial Int. 2015;35:640-4.
3. Cozzolino M, Ureña-Torres P, Vervloet MG, et al. Is chronic kidney disease-mineral bone disorder (CKD-MBD) really a syndrome? Nephrol Dial Transplant. 2014;29:1815-20.
4. Stenvinkel P. Inflammation in end-stage renal failure: could it be treated? Nephrol Dial Transplant. 2002;17:33-8.
5. Eleftheriadis T, Kartsios C, Antoniadi G, et al. The impact of chronic inflammation on bone turnover in hemodialysis patients. Ren Fail. 2008;30:431-7.
6. Saifullah A, Watkins BA, Saha C, Li Y, Moe SM, Friedman AN. Oral fish oil supplementation raises blood omega-3 levels and lowers C-reactive protein in haemodialysis patients: a pilot study. Nephrol Dial Transplant. 2007;22:3561-7.
7. Rasic-Milutinovic Z, Perunicic G, Pljesa S, et al. Effects of N-3 PUFAs supplementation on insulin resistance and inflammatory biomarkers in hemodialysis patients. Ren Fail. 2007;29:321-9.
8. Salari Sharif P, Asalforoush M, Ameri F, Larijani B, Abdollahi M. The effect of n-3 fatty acids on bone biomarkers in Iranian postmenopausal osteoporotic women: a randomized clinical trial. Age (Dordr). 2010;32:179-86.
9. Zwart SR, Pierson D, Mehta S, Gonda S, Smith SM. Capacity of omega-3 fatty acids or eicosapentaenoic acid to counteract weightlessness-induced bone loss by inhibiting NF-kappaB activation: from cells to bed rest to astronauts. J Bone Miner Res. 2010;25:1049-57.
10. Sun D, Krishnan A, Zaman K, Lawrence R, Bhattacharya A, Fernandes G. Dietary n-3 fatty acids decrease osteoclastogenesis and loss of bone mass in ovariectomized mice. J Bone Miner Res. 2003;18:1206-16.
11. Bhattacharya A, Rahman M, Banu J, et al. Inhibition of osteoporosis in autoimmune disease prone MRL/Mpj-Fas(lpr) mice by N-3 fatty acids. J Am Coll Nutr. 2005;24:200-9.
12. Griel AE, Kris-Etherton PM, Hilpert KF, Zhao G, West SG, Corwin RL. An increase in dietary n-3 fatty acids decreases a marker of bone resorption in humans. Nutr J. 2007;6:2.
13. Bloedon LT, Szapary PO. Flaxseed and cardiovascular risk. Nutr Rev. 2004;62:18-27.
14. Bandeira F, Costa AG, Soares Filho MA, Pimentel L, Lima L, Bilezikian JP. Bone markers and osteoporosis therapy. Arq Bras Endocrinol Metabol. 2014;58:504-13.
15. Kajarabille N, Díaz-Castro J, Hijano S, López-Frías M, López-Aliaga I, Ochoa JJ. A new insight to bone turnover: role of ω-3 polyunsaturated fatty acids. Sci World J. 2013;2013:589641.
16. Nakashima A, Yorioka N, Mizutani T, Yamagata Z, Ueno T, Takasugi N. Serum cross-linked N-terminal telopeptide of type I collagen for evaluation of renal osteodystrophy in hemodialysis patients. Nephron Clin Pract. 2005;99:c78-85.
17. Daugirdas JT, Stone JCV. Physiologic principles and urea kinetic modeling. In: Daugirdas JT, Blake PG, Ing TS, editors. Handbook of Dialysis. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 15-45.
18. Alves CH, Farrell E, Vis M, Colin EM, Lubberts E. Animal models of bone loss in inflammatory arthritis: from cytokines in the bench to novel treatments for bone loss in the bedside - a comprehensive review. Clin Rev Allergy Immunol. 2016;51:27-47.
19. Wheater G, Elshahaly M, Tuck SP, Datta HK, van Laar JM. The clinical utility of bone marker measurements in osteoporosis. J Transl Med. 2013;11:201.
20. Appleton KM, Fraser WD, Rogers PJ, Ness AR, Tobias JH. Supplementation with a low-moderate dose of n-3 long-chain PUFA has no short-term effect on bone resorption in human adults. Br J Nutr. 2011;105:1145-9.
21. Mirfatahi M, Tabibi H, Nasrollahi A, Hedayati M, Taghizadeh M. Effect of flaxseed oil on serum systemic and vascular inflammation markers and oxidative stress in hemodialysis patients: a randomized controlled trial. Int Urol Nephrol. 2016;48:1335-41.
22. Rahman MM, Bhattacharya A, Fernandes G. Docosahexaenoic acid is more potent inhibitor of osteoclast differentiation in RAW 264.7 cells than eicosapentaenoic acid. J Cell Physiol. 2008;214:201-9.
23. Boeyens JC, Deepak V, Chua WH, Kruger MC, Joubert AM, Coetzee M. Effects of ω3- and ω6-polyunsaturated fatty acids on RANKL-induced osteoclast differentiation of RAW264.7 cells: a comparative in vitro study. Nutrients. 2014;6:2584-601.
24. Nakanishi A, Iitsuka N, Tsukamoto I. Fish oil suppresses bone resorption by inhibiting osteoclastogenesis through decreased expression of M-CSF, PU.1, MITF and RANK in ovariectomized rats. Mol Med Rep. 2013;7:1896-903.
25. Zoch ML, Clemens TL, Riddle RC. New insights into the

biology of osteocalcin. Bone. 2016;82:42-9.

26. Damsgaard CT, Mølgaard C, Matthiessen J, Gyldenløve SN, Lauritzen L. The effects of n-3 long-chain polyunsaturated fatty acids on bone formation and growth factors in adolescent boys. Pediatr Res. 2012;71:713-9.
27. Dong H, Hutchins-Wiese H, Kleppinger A, et al. Effects of omega-3 polyunsaturated fatty acid supplementation on bone turnover in older women. Int J Vitam Nutr Res. 2014;84:124-32.
28. Banu J, Bhattacharya A, Rahman M, Kang JX, Fernandes G. Endogenously produced n-3 fatty acids protect against ovariectomy induced bone loss in fat-1 transgenic mice. J Bone Miner Metab. 2010;28:617-26.

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