Does Dietary Polyunsaturated Fatty Acid Intake Associate With Bone Mineral Density and Limb Structural Changes in Early Rheumatoid Arthritis?

Nutrition and Metabolic Insights
Volume 16: 1–9

DOI: 10.1177/11786388231176169
Sage

Annika Valner[1,2], Raili Müller[1,2], Mart Kull[3], Kaja Põlluste[1], Margus Lember[1,2] and Riina Kallikorm[1,2]

[1]Institute of Clinical Medicine, University of Tartu, Tartu, Estonia. [2]Internal Medicine Clinic, Tartu University Hospital, Tartu, Estonia. [3]Viljandi County Hospital, Viljandi County, Estonia.

ABSTRACT

BACKGROUND: Rheumatoid arthritis (RA) is an inflammatory disease that can result in bone erosion, lean mass lowering, and increase of fat mass without changes in body weight. The dietary consumption of polyunsaturated fatty acids (PUFAs) has been assessed in many studies due to their potential anti-inflammatory effect.

AIM: The aim of this research was to identify if dietary intake of PUFAs associates with bone mineral density (BMD) and limb structural changes in early rheumatoid arthritis (ERA) compared to a population-based control group. The study was conducted because previous results have been insufficient.

METHODS: The study group consisted of 83 ERA patients and 321 control subjects. A dual-energy X-Ray absorptiometry (DXA) machine was used to measure hip, lumbar spine, and radius BMD, as well as arm and leg fat, lean, and bone mass. Dietary habits and inflammatory markers were assessed to evaluate the effects to BMD and limb structural changes.

RESULTS: In ERA subjects, higher dietary consumption of PUFAs was associated with a decrease in arm fat mass (b −28.17, $P = .02$) and possibly with higher lumbar BMD (b 0.008, $P = .058$). Limb bone and lean mass changes were not associated with dietary intake of PUFAs.

CONCLUSION: Balanced nutrition is essential. Consuming PUFAs could be beneficial in ERA preventing structural changes to hands, but additional research is needed.

KEYWORDS: Bone mineral density, early rheumatoid arthritis, polyunsaturated fatty acids, arm fat mass, inflammation

RECEIVED: February 24, 2023. **ACCEPTED:** April 28, 2023.

TYPE: Original Research

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The study was funded by European Regional Development Fund/Estonian Research Council (3.2.1002.11-0002) and Estonian Research Council (Institutional Research grant IUT 2-8).

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Annika Valner, Institute of Clinical Medicine, University of Tartu, L. Puusepa 8, Tartu 51014, Estonia. Email: annika.valner@kliinikum.ee

Introduction

Rheumatoid arthritis (RA) is an autoimmune inflammatory disorder of the synovial tissue of joints. It is characterized by immune cell infiltration and the hyperplasia of synovial fibroblasts, leading to articular cartilage destruction and bone erosion.[1] Osteoporosis (OP) has been classically considered a comorbidity of RA[2,3] and the prevalence of OP in RA is around 30%.[4,5] OP is a common systemic skeletal disorder outlined by low bone mass and microarchitectural deterioration of bone tissue, leading to bone fragility and susceptibility to fracture.[2] There are 3 types of bone loss in RA: local, juxta-articular and systemic, inducing periarticular osteopenia, bone erosions, generalized osteopenia, and/or OP.[2,6]

Furthermore, several studies of early RA (ERA) patients revealed that in addition to bone changes, lean mass loss and fat mass gain can be evident in the early stage of RA, although the involved factors are currently unknown.[7] Likewise, we have found in our previous work[8] that ERA patients had lower arm and leg lean mass, and higher arm fat mass. The decrease in limb lean mass in ERA was associated with the elevation of C-reactive protein (CRP),[8] indicating the effects of inflammation.

Current research shows that in addition to inflammation, oxidative stress (OS) also has a crucial role in the pathogenesis of RA.[9-11] The interaction between cellular immune system and a body's endogenous and/or exogenous antigens create reactive nitrogen species (RNS) and reactive oxygen species (ROS). ROS and RNS activate the signaling cascades of inflammatory cells to synthesize pro-inflammatory cytokines and chemokines.[9] ROS and RNS have distinct parts in the destructive, proliferative synovitis of RA.[10] Furthermore, OS distinctly contributes to the initiation and maintenance of systemic inflammation in RA.[11] Several randomized controlled trials (RCTs) into using fatty acids with antioxidative effects[12] in the treatment of RA patients have been published, but the results and interventions of these RCTs vary.[11]

A healthy diet is important in the prevention of several chronic conditions and adverse health effects such as obesity, OP.[13,14] The relationship between dietary consumption of polyunsaturated fatty acids (PUFAs) and RA has been evaluated

in a number of studies. PUFAs impact on immune system and inflammatory diseases is relevant. Omega-6 PUFAs exert mostly pro-inflammatory features, while omega-3 PUFAs have anti-inflammatory and pro-resolving effects.[15] Omega-3 PUFAs, mainly found in fish oils, present inverse correlations with Interleukin (IL)-6 and CRP levels.[7] Omega-3 PUFA supplementation has been found to reduce pain, morning stiffness in RA, the frequency of nonsteroidal anti-inflammatory drug (NSAID) consumption,[15,16] and has been associated with lower disease-activity-related markers.[17-19] In France, PUFA supplementation is already an official recommendation to RA patients for symptomatic relief.[20]

The main dietary sources of omega-6 PUFAs are vegetable oils and animal sources.[15] PUFA omega-6 arachidonic acid (AA) type is responsible for inhibiting osteoblastogenesis and inducing adipogenesis of human mesenchymal stem cells.[15] Omega-6 arachidonic AA also alters osteoblast differentiation process and advances the loss of bone mass.[15,21] Therefore, a higher ratio of omega-3/omega-6 fatty acid in diet is considered a protective element against decreases in bone mineral density (BMD),[15,22] and early manifestations of RA could be possibly delayed with dietary consuming of PUFAs.[7] For that reason, dietary intake of PUFAs could be beneficial in the prevention of RA-related BMD decreases.

In established RA, up to two-thirds of patients have a phenotype characterized by muscle wasting and increase of fat mass without body weight variations.[7,23] Omega-3 fatty acids have been widely investigated for their potential health benefits, with evidence suggesting positive effects in sustaining muscle mass.[24] Recent meta-analysis also confirmed the finding that omega-3 long chain PUFA supplementation has a positive effect on overall body muscle mass and strength.[25,26] Therefore, consumption of dietary PUFAs could be advantageous in avoidance of RA-associated structural changes to limbs.

Dual-energy X-ray absorptiometry (DXA) is a commonly used low-price and sensitive method to evaluate BMD and bone mass.[27] Furthermore, DXA enables separately measuring arm and leg fat, lean, and bone mass. Hence, BMD lowering estimation together with evaluation of nutritional status (especially consumption of PUFAs) could be additionally helpful in RA patient osteoporosis risk assessment.

The aim of this study was to assess if intake of dietary PUFAs is associated with BMD and limb fat, lean, and bone mass changes in ERA compared with a population-based controls. To our knowledge, this is the first study in ERA that assesses the effect of consumption of dietary PUFAs to BMD and limb structural changes. On majority of RA patients develop arthritis specific bone structure changes in the first 3 years of disease. Furthermore, these bone changes may be accompanied with muscle wasting and increase of fat mass without body weight changes.[7,23] Therefore, as RA is a disease that can cause disability and affect quality of life, it is crucial to find out new factors that associate with these arthritis-related changes. By knowing these factors, we could possibly lessen or prevent the appearance of these alterations.

Methods

Current article is a sequence to our previous work and the same study group has been used in our earlier research.[8,28] The study group consisted of 83 patients with ERA (age: 19-79 years) and 321 subjects in a population-based control group (age: 20-79 years). One hundred consecutive patients referred to Tartu University Hospital with recently diagnosed RA were enrolled between January 2012 and May 2014 to form the ERA group. Their diagnosis was made according to American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2012 criteria for RA. RA patients with symptom duration up to 1 year (early arthritis) were invited to take part in the study. Nine patients were excluded due to missing outcome data, as well another 8 patients who did not fulfill ACR/EULAR 2012 criteria for RA on a follow-up visit 1 year after their first visit (n = 83) (Figure 1).[8,28]

To form the control group, 350 subjects adjusted for the age and gender composition of the Estonian population in 2013 were randomly selected from a primary healthcare center practice list (total number of subjects: 1854). Cross-sectional data were assembled from September 2014 to March 2015. Invitations containing introductory materials were sent to 350 people. The primary healthcare center was contacted by 332 subjects seeking further instructions, and 330 people were ultimately recruited to the study. Six patients with missing outcome data were excluded and 3 subjects missed their study appointment (n = 321) (Figure 1).

Study procedures were performed before noon. Height was measured with a stadiometer to the nearest 0.5 cm. An electronic scale was used to measure body weight in kilograms. Body mass index (BMI) was calculated according to the standard formula: weight in kilograms divided by height in meters squared.[8,28]

A 24 hours dietary recall method was used for nutritional assessment. The data were analyzed with NutriData software developed by the Estonian National Institute for Health Development.[8,28,29]

Smoking habits were stratified as ever smoked, including current and previous smoking anamnesis.[28]

A Lunar Prodigy dual-energy X-Ray absorptiometry (DXA) machine was used to separately measure femoral neck, trochanter, lumbar spine (1-4th lumbar vertebrae), proximal and ultradistal radius BMD. Additionally, arm and leg fat, lean, and bone mass were measured. An experienced and qualified technician performed all DXA measurements on all participating subjects.[8]

Blood samples were collected between 8 and 11 a.m. after an overnight fast. They included measuring CRP, calcium, vitamin D3. IL-6, tumor necrosis factor alpha (TNFa), and interleukin-1 beta (IL-1b) were evaluated using Luminex's xMAP technology. Anti-citrullinated protein antibodies (anti-CCP)

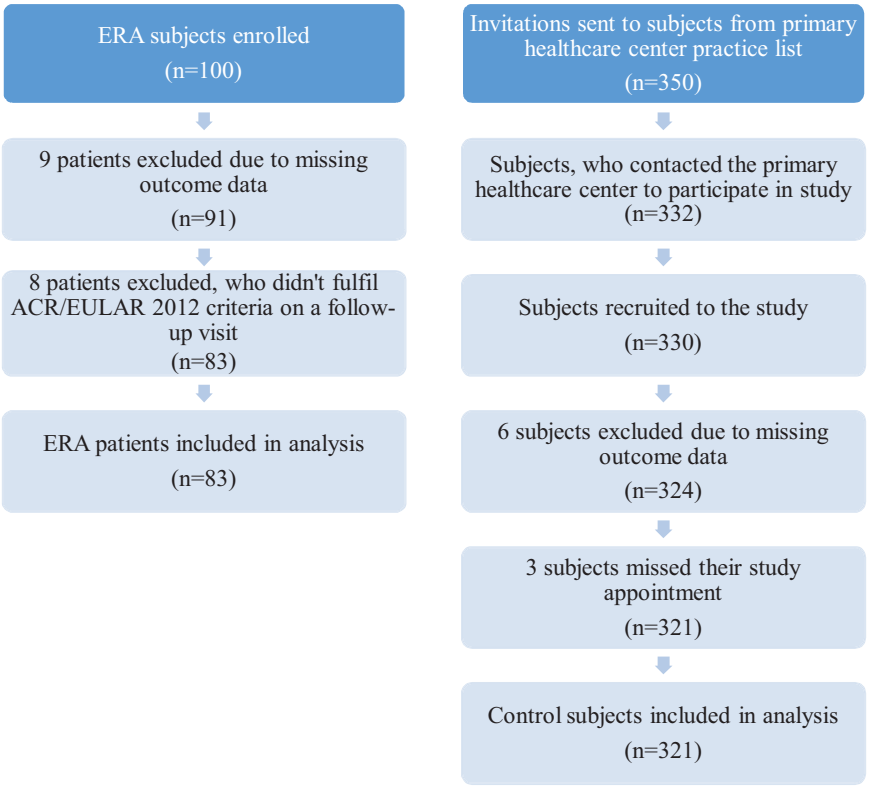

Figure 1. Study groups flow diagram. Abbreviation: ERA, early rheumatoid arthritis.

and rheumatoid factor (RF) were measured only in the ERA group. An electrochemiluminescence assay with the cutoff value of 17 kU/L was used for anti-CCP positivity. RF was measured with the immunoturbidimetric method, and it was assessed positive if RF value was >14 IU/mL.[8,28]

The number of tender and swollen joints (28 joint scores) were assessed in the ERA group to evaluate disease activity. In accordance with the standard formula, DAS28 scores were calculated using CRP. According to the DAS28 score, ERA patients were grouped as having low disease activity (DAS28 score <3.2), moderate disease activity (≥3.2 to ≤5.1) or high disease activity (>5.1).[8,28,30]

Statistica version 13.3 for Windows and IBM SPSS Statistics version 27.0 for Windows were used to carry out statistical analyses. All data was evaluated in histograms to approximately normal distribution. The continuous data were presented as mean (standard deviation, SD) if distributed approximately normally, or by median with 25% and 75% percentiles if distributed non-normally. Unpaired two-tailed Student's *t*-test (for approximately normal distribution) and Mann–Whitney *U* test (for non-normally distributed variables) were used to make comparisons between ERA patients and controls.[8,28]

Multiple linear regression with binary exposure variables was carried out to assess femoral neck, trochanter, proximal and ultradistal radius BMD; with arm, leg and trunk bone mass mean difference between ERA (ERA = 1) and control group (control group = 0). Model was adjusted for age, gender, height, and weight as ERA patients differed from controls by age and gender.[8] Multiple linear regression analysis was done to assess in ERA the association of smoking, different inflammation markers and consuming of PUFAs to BMD and bone mass changes in various skeletal regions. The variables were chosen, as they can possibly associate with BMD changes.[7,15-19,21,22,28]

Multiple regression analysis (adjusted for age, gender, height, and weight) was carried out both in ERA and control subjects to evaluate if dietary intake of PUFAs was associated with arm and leg structural (fat, lean mass) changes. Additional analysis of the ERA and control group females (adjusted for age, height, and weight) was done to diminish gender related body structure differences. The variables were chosen since they can associate with hand and leg structural changes.[8,15-19,21,23,24] All tests were two-sided. *P*-values <.05 were considered significant.

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Research Ethics Committee of the University of Tartu (population group study approval number 238/M-15, date of approval 16 July 2014), ERA group study approval: 221/M-9 (date of approval 17 December 2012). All subjects participating in the study signed written informed consent forms.

Results

General characteristics of the study groups

The study group consisted of patients with ERA (n = 83) and control subjects (n = 321). Sixty-five percent (54) of ERA patients

Table 1. Characteristics of the study groups.

	ERA PATIENTS (N = 83)	CONTROLS (N = 321)	*P*-VALUE
General characteristics			
Age, years	52.7 (15.7)	47.9 (16.5)	.02
Female gender, n (%)	60 (72)	175 (54)	.004
Height, cm	166 (9)	171 (10)	<.0001
Weight, kg	74.7 (14.8)	80.2 (17.6)	.009
BMI, kg/m^2	27.2 (5.6)	27.2 (5.3)	.96
Smoking (ever), n (%)	28 (34)	66 (21)	.01
RA specific characteristics			
Time from first RA symptoms, days	88 (48-245)	-	-
Anti-CCP positivity, n (%)	59 (71)	-	-
RF positivity, n (%)	54 (65)	-	-
DAS28 score (CRP)	3.9 (1.3)	-	-
Inflammation and bone markers			
CRP, mg/L	4.0 (1.6-18.0)	0.9 (0.2-2.5)	<.0001
IL-6, pg/ml	2.9 (0-19.0)	0 (0-0)	<.0001
TNFa, pg/ml	2.2 (1.6-3.0)	1.8 (1.4-2.3)	<.0001
IL-1b, pg/ml	0.1 (0-1.0)	0 (0-0)	<.0001
Ca, mmol/L	2.4 (0.3)	2.3 (0.1)	.0005
25 (OH) Vitamin D, nmol/L	55 (22)	41 (18)	<.0001
Hand and leg structure			
Arm fat mass	2701 (1230)	2420 (1165)	.05
Arm lean mass	4893 (1307)	6018 (2042)	<.0001
Arm bone mass	355 (92)	393 (116)	.005
Leg fat mass	8913 (3850)	8235 (3864)	.16
Leg lean mas	14019 (2942)	16798 (4135)	<.0001
Leg bone mass	1042 (201)	1149 (262)	.0005

Abbreviations: Anti-CCP, anti-citrullinated protein antibodies. Anti-CCP positive, when ⩾17 kU/L; BMI, body mass index; Ca, calcium; CRP, C-reactive protein; DAS28 score, disease activity score calculated using 28 joints; ERA, early rheumatoid arthritis; IL-6, interleukin-6; IL-1b, interleukin-1 beta; RA, rheumatoid arthritis; RF, rheumatoid factor. RF positive, when RF > 14 IU/mL; TNFa, tumor necrosis factor alpha.
Values in table represent mean (standard deviation, SD) or median (with interquartile range). Hand and leg structure used units: mass (g).

were RF positive and 71% (59) anti-CCP positive. Thirty-one percent (26) of arthritis subjects had low disease activity, 52% (43) moderate and 17% (14) high disease activity respectively. Mean DAS28 score was 3.9, corresponding to moderate disease activity according to ACR recommendations.[31]

Fifty-eight percent of ERA patients were using disease-modifying anti-rheumatic drugs (DMARDs). The most frequently used DMARD (40%) was methotrexate (MTX). Furthermore, 71% (59) of ERA patients used NSAIDs, and 27% (22) glucocorticosteroids (GCS) (Table 1).

All the inflammatory markers were significantly ($P < .0001$) elevated in the ERA group (Table 1).

ERA patients had higher fat mass of arm, as well as lower arm and leg lean mass. Furthermore, ERA subjects also had lower arm and leg bone mass (Table 1).

Characteristics of study groups daily diet

ERA patients consumed less calories, proteins, fats (including polyunsaturated fatty acids), $P < .005$ (Table 2).

Table 2. Daily diet (last 24 hours) characteristics of the study groups.

	ERA PATIENTS (N = 83)	CONTROLS (N = 321)	*P*-VALUE
General characteristics of diet			
Energy (kcal)	1507 (625)	1832 (786)	.0005
Carbohydrates (g)	196 (97)	210 (91)	.22
Carbohydrates (% E)	50.4 (10.6)	46.1 (10.5)	.0009
Fats (g)	53 (40-72)	66 (46-97)	.002
Fats (% E)	34.7 (8.7)	34.1 (8.3)	.84
Proteins (g)	53 (38-69)	73 (53-99)	<.0001
Proteins (% E)	14.8 (4.4)	17.5 (5.4)	.00004
Saturated fatty acids (% E)	14.2 (4.6)	13.8 (4.5)	.42
Polyunsaturated fatty acids (g)	8.9 (4.9)	12.2 (8.6)	.0008
Polyunsaturated fatty acids (% E)	5.3 (2.2)	5.8 (2.4)	.11

Values in table represent mean (standard deviation, SD) or median (with interquartile range).

Table 3. Characteristics of study group bone mineral density.

	ERA PATIENTS (N = 83)	CONTROLS (N = 321)	*P*-VALUE
General BMD characteristics			
Femoral neck BMD, g/cm^2	0.97 (0.21)	1.02 (0.16)	.03
Trochanter BMD, g/cm^2	0.84 (0.17)	0.89 (0.16)	.01
Total hip BMD, g/cm^2	1.02 (0.17)	1.06 (0.16)	.04
Ultradistal radius BMD, g/cm^2	0.48 (0.10)	0.50 (0.10)	.14
Proximal radius BMD, g/cm^2	0.88 (0.14)	0.91 (0.13)	.07
Total radius BMD, g/cm^2	0.39 (0.10)	0.39 (0.09)	.82
Lumbar L1- L4 BMD, g/cm^2	1.17 (0.18)	-	-
T and Z-scores			
Femoral neck T-score	−0.4 (−1.4 to 0.6)	-	-
Femoral neck Z-score	0.5 (−0.3 to 1.2)	-	-
Total hip T-score	−0.2 (−1.0 to 0.9)	-	-
Total hip Z-score	0.6 (−0.3 to 1.4)	-	-
Lumbar L1-L4 T-score	−0.4 (−1.2 to 0.8)	-	-
Lumbar L1-L4 Z-score	0.3 (−0.6 to 1.2)	.	-

Abbreviation: BMD, bone mineral density.
T-score, a comparison of a person's bone density with that of a healthy 30-year-old of the same gender. Z-score, a comparison of a person's bone density with that of an average person of the same age and gender. Values in table represent mean (standard deviation, SD) or median (with interquartile range).

Characteristics of study group bone mineral density

In the ERA group, BMD was significantly lower in the femoral neck and trochanter regions, but not in the radius area compared with the controls. According to the BMD classification,[32] the median T-score of ERA subjects indicated normal bone mineral density both in the lumbar spine and femoral neck (Table 3).

Characteristics of body structure changes associated with ERA

We examined how the presence of ERA alternates body BMD in several skeletal regions compared with control subjects. The multiple regression model adjusted for age, gender, height, and weight indicated that the presence of ERA was not associated

Table 4. Mean estimated difference of body structure in ERA compared to controls.*

DEPENDENT VARIABLE	REGRESSION COEFFICIENT (B)	STANDARD ERROR	*P*-VALUE
Femoral neck BMD	0.01	0.02	0.73
Trochanter BMD	−0.004	0.02	0.80
Ultradistal radius BMD	0.01	0.01	0.15
Proximal radius BMD	0.02	0.01	0.09
Arm fat mass	220.0	62.0	0.0005
Arm lean mass	−304.6	98.8	0.002
Arm bone mass	9.6	6.5	0.14
Leg fat mass	417.5	241.0	0.08
Leg lean mass	−943.4	184.9	<0.0001
Leg bone mass	7.5	14.0	0.62

Abbreviation: BMD, bone mineral density
*Multiple regression models adjusted for age, gender, height, and weight.

Table 5. Summary of regression analysis for variables predicting ERA-specific lumbar BMD changes.*

DEPENDENT VARIABLE	PREDICTOR VARIABLE	REGRESSION COEFFICIENT (B)	STANDARD ERROR	*P*-VALUE
L1-L4 BMD	Smoking	−0.042	0.043	.34
	CRP	−0.001	0.001	.47
	IL-6	−0.001	0.001	.18
	TNFa	0.012	0.009	.15
	IL-1b	−0.005	0.009	.58
	RF positivity	−0.078	0.042	.07
	Proteins (g)	0.001	0.001	.28
	PUFAs (g)	0.008	0.004	.058

Abbreviations: BMD, bone mineral density; CRP, C-reactive protein; IL-6, interleukin-6; IL-1b, interleukin-1 beta; PUFAs, polyunsaturated fatty acids; RF, rheumatoid factor. RF positive, when RF > 14 IU/mL; TNFa, tumor necrosis factor alpha.
*Different multiple regression models adjusted for age, gender.

with BMD changes in femoral neck, trochanter, or radius area compared with control subjects (Table 4).

Additionally, the multiple regression models revealed that the presence of ERA was associated with higher arm fat mass (b 220.0, $P = .0005$) compared with control group subjects. Furthermore, the lean mass of arm (b −304.6, $P = .002$) and leg (b −943.4, $P < .0001$) were lower in ERA subjects compared with control group subjects (Table 4). The arm and leg bone mass changes due to ERA itself were not significant (Table 4).

Characteristics of factors associated with body BMD and bone structure changes

Regression analysis carried out for the ERA group indicated that higher lumbar BMD was associated with higher consumption of dietary PUFAs (b 0.008, $P = .058$). Still, smoking and serum inflammatory markers did not seem to have a relevant effect on lumbar BMD (Table 5). The same variables also didn't seem to have significant effect on femoral neck, proximal and ultradistal radius BMD. Interestingly, anti-CCP positivity was associated with lower ultradistal radius BMD (b −0.047, $P = .04$).

To eliminate gender differences, further multiple regression analysis of only ERA females was done. After being adjusted for age, height, and weight, the analysis indicated that lower arm bone mass was associated with higher level of CRP (b −0.82, $P = .04$). However, no such association was observed in leg bone mass.

Characteristics of dietary PUFAs intake associated with hand and leg structure changes in ERA

Multiple regression analysis of the ERA group (adjusted for age, gender, height, and weight) showed that a reduction in

Table 6. Summary of regression analysis for variables predicting ERA-specific body structural changes.*

DEPENDENT VARIABLE	PREDICTOR VARIABLE	REGRESSION COEFFICIENT (B)	STANDARD ERROR	*P*-VALUE
ERA arm fat mass	PUFAs (g)	–28.17	11.88	.02
Control arm fat mass	PUFAs (g)	–2.23	3.31	.50

Abbreviation: PUFAs, polyunsaturated fatty acids.
*Different multiple regression models adjusted for age, gender, height, and weight.

arm fat mass was associated with increased dietary consumption of PUFAs (b –28.17, $P = .02$). At the same time, in the control group, PUFA intake showed no relevant effect on arm fat mass (Table 6). Further analysis of the ERA and control group females (adjusted for age, height, and weight) confirmed the same results - only in ERA females was a decrease in arm fat mass associated with higher consumption of PUFAs (b –35.50, $P = .02$).

In contrast, dietary consumption of PUFAs showed no association with leg fat mass changes both in the ERA and control groups, including females. This indicates that in ERA, the lowering of arm fat mass was associated with higher dietary intake of PUFAs, and the finding was not influenced by some other factor, as the statistical models were adjusted.

Arm, leg lean, and bone mass changes both in ERA and controls, as in female groups, were not associated with dietary consumption of PUFAs or with proteins.

Discussion

The aim of this study was to evaluate if diet rich in PUFAs is associated with BMD, hand and leg structural changes in ERA compared with the control group. As far as we know, this is the first study to analyze ERA BMD and hand as well as leg structural change associated with the consumption of PUFAs.

The beneficial health effects of the Mediterranean diet have been often assigned to antioxidants.[33] A recent study also indicated that omega-3 PUFAs intake had antioxidative effects.[12] Furthermore, it has been assumed that dietary antioxidants protect cells and tissues from oxidation and prevent or delay the development of several diseases.[7,15,22,33,34] Both OP[35] and RA[36] are associated with increased inflammatory burden. PUFAs are characterized with a decrease in inflammation[37] and intake of PUFAs could relieve the symptoms of arthritis through lowering pro-inflammatory cytokine Interleukin 17 (IL-17) production. For that reason, dietary supplementation of PUFAs could have a therapeutic potential in the treatment of RA[38] by ameliorating the disease course in RA.

Our ERA patients ate significantly fewer calories, proteins, and fats (including PUFAs) compared with control group subjects. The analysis of dietary habits of our study subjects showed a strong tendency in the ERA group, where higher dietary intake of PUFAs was associated with higher lumbar BMD. This result is in line with a previous finding demonstrating PUFAs to have a positive effect on BMD.[15-19,21,22] As oxidative damage has been found to be significantly noticeable in RA patients,[39] diet rich in PUFAs could be beneficial in ERA for preventing a reduction of BMD. Still, in our ERA subjects, the association may have been weaker, as the ERA subjects consumed less fats compared with controls.

Our research confirmed that ERA patient BMD was remarkably decreased in the trochanter and femoral neck areas, but not in the radius region, compared with the control subjects. This finding is in accordance with previous data showing that in ERA, femoral neck BMD can already be reduced at the time of diagnosis.[40] Considering the pathogenesis of RA, we expected the radius BMD also to be lower, similar to a previous study.[41] However, it was not, possibly due to the short duration of RA. Also, in our study the BMD changes due to ERA itself were not significant, again possibly due to the early stage of arthritis. Therefore, the association between higher consumption of dietary PUFAs and higher BMD were also possibly not so evident.

As expected, the inflammatory markers were considerably higher in arthritis subjects compared to controls. Nevertheless, the inflammatory activity didn't seem to have an impact on BMD changes in all the observed skeletal areas.

Anti-CCP positivity can associate with BMD changes, as anti-CCP positive RA has been a more erosive disease subtype.[2] Our research showed that anti-CCP positivity was only associated with decreased ultradistal radius BMD. This finding may suggest the pathophysiological effects of RA, as the disease mostly affects small joints.

Various studies of ERA subjects (including our previous study) indicated that lean mass lowering and fat mass increase can be observable in ERA.[7,8] The results of our current research indicated that in ERA subjects, higher dietary intake of PUFAs was associated with lower arm fat mass. The finding may be because consuming increased amounts of long chain n-3 PUFAs is potentially beneficial due to an anti-inflammatory effect (they result in the suppressed production of pro-inflammatory cytokines).[42,43] High intake of PUFAs has been associated with changes in body composition: reductions in central adiposity and increases in lean body mass. Furthermore, higher consuming of plant-based essential fatty acids (ePUFAs), n-6 linoleic acid (LA), and n-3 α-linolenic acid (ALA), have a greater impact on body composition (fat and lean mass).[43] Hence, we can speculate that balanced nutrition containing PUFAs could be favorable in lessening ERA-related arm fat mass changes. Still, in our study dietary consumption of PUFAs and proteins revealed no association with arm or leg lean mass changes, possibly due to the short duration of arthritis.

Our research showed that in ERA female subjects, a fall in arm bone mass was associated with the elevation of CRP, indicating the effects of inflammation. Still, arm and leg bone mass changes in ERA were not associated with dietary intake of PUFAs, probably due to the early stage of RA studied.

As a matter of fact, inflammation has a crucial role in other conditions too, including gastrointestinal disorders,[44] autoimmune conditions,[45] obesity,[46] type 2 diabetes mellitus (DM),[47] and metabolic syndrome.[48] Previous studies have shown that abundance of PUFAs in diet, especially omega-3 PUFAs can have positive effect on inflammatory bowel disease (IBD) by reverting the gut microbiota to a healthier composition.[49-52] Furthermore, the interplay between gut microbiota, omega-3 fatty acids, and immunity helps to maintain the intestinal wall integrity[50] and via it possibly lower inflammation.[51] Intake of omega-3 PUFAs may also possibly decrease risk of IBD.[53]

An imbalanced intake of omega-3/omega-6 PUFAs may lead to gut microbe dysbiosis, which ultimately leads to overweight and obesity.[51,54] Obese patients have impaired intestinal immunity, which can be alleviated by supplementation with omega-3 PUFAs.[51,55] Omega-3 PUFAs may prevent the development of obesity by modulating the gut microbiota and influencing the function of white adipose tissue.[56] In addition, obese patients usually present low levels of inflammation, which is often associated with metabolic syndrome.[51]

Omega-3 PUFAs alleviate alcoholic steatosis and alcohol-induced liver injury, suggesting that omega-3 PUFAs may be promising treatments in the management of alcoholic liver disease (ALD).[51,57] Moreover, many clinical studies have indicated that dietery supplementation with omega-3 PUFAs could also lessen hepatic steatosis in nonalcoholic fatty liver disease (NAFLD).[51,58]

The diet rich in omega-3 PUFAs could decrease LDL-cholesterol,[59] prevent myocardial infarction, and reduce the morbidity and mortality of cardiovascular disease.[51] Accumulating evidences revealed that omega-3 PUFAs has an advantageous effect on a variety of inflammation-related diseases, including cancer,[60] asthma[51,61] and it may even possibly prevent allergic diseases.[51] Still, a recent metaanalysis indicated that increased intake of omega-3, omega-6, or total PUFAs had little or no effect on prevention and treatment of type 2 DM.[62]

Diet rich in PUFAs can have an ameliorative effect on inflammation in many conditions. Therefore the dietary intake of PUFAs could be relevant in preventing arthritis related changes and also lessening the symptoms of several conditions.

Our study was limited by small study groups, which was in turn partially due to the small total size of the Estonian population.[63] This study's short period of nutritional assessment was a further limitation. Taking into consideration the low mean amount of calories in the study group, it is possible that some subjects underreported their nutrition in the 24-hour dietary recall interview. Nevertheless, the statistical analysis confirmed a significant association between the consumption of dietary PUFAs and a reduction in arm fat mass in ERA subjects. The finding was relevant and not influenced by some other factor, as the statistical models were adjusted accordingly.

Conclusion

The results of the conducted study indicated that in ERA subjects, higher amount of PUFAs in diet was associated with a reduction in arm fat mass and presumably with higher lumbar BMD. Hand and leg lean, bone mass changes were not associated with consumption of PUFAs. The findings suggest that the nutritional assessment should be a part of the everyday clinical practice in the treatment of rheumatoid arthritis. A healthy eating is extremely important. The diet rich in PUFAs could be advantageous in preventing arthritis related limb structural changes and osteoporosis for those with ERA. Still, further research remains needed.

Author Contributions

Conceptualization: Annika Valner, Margus Lember and Riina Kallikorm. Methodology: Annika Valner, Raili Müller, Margus Lember and Riina Kallikorm. Formal analysis: Annika Valner. Investigation: Annika Valner, Mart Kull and Raili Müller. Writing—original draft preparation: Annika Valner. Writing—review and editing: Annika Valner, Raili Müller, Kaja Põlluste, Mart Kull, Margus Lember and Riina Kallikorm. Funding acquisition: Kaja Põlluste, Margus Lember and Riina Kallikorm. Resources: Kaja Põlluste. Supervision: Margus Lember and Riina Kallikorm. All authors read and approved the final version of the manuscript.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Research Ethics Committee of the University of Tartu (population group study approval number 238/M-15, date of approval 16 July 2014), ERA group study approval: 221/M-9 (date of approval 17 December 2012).

Informed Consent Statement

A written informed consent was obtained from all subjects involved in the study prior to participating.

REFERENCES

1. Patidar V, Shah S, Kumar R, Singh PK, Singh SB, Khatri DK. A molecular insight of inflammatory cascades in rheumatoid arthritis and anti-arthritic potential of phytoconstituents. *Mol Biol Rep*. 2022;49(3):2375-2391.
2. Llorente I, García-Castañeda N, Valero C, González-álvaro I, Castañeda S. Osteoporosis in rheumatoid arthritis: Dangerous Liaisons. *Front Med*. 2020;7:601618.
3. Wade SW, Strader C, Fitzpatrick LA, Anthony MS, O'Malley CD. Estimating prevalence of osteoporosis: examples from industrialized countries. *Arch Osteoporos*. 2014;9:182.
4. Haugeberg G, Uhlig T, Falch JA, Halse JI, Kvien TK. Bone mineral density and frequency of osteoporosis in female patients with rheumatoid arthritis: results from 394 patients in the Oslo County Rheumatoid Arthritis register. *Arthritis Rheum*. 2000;43(3):522-530.
5. Hauser B, Riches PL, Wilson JF, Horne AE, Ralston SH. Prevalence and clinical prediction of osteoporosis in a contemporary cohort of patients with rheumatoid arthritis. *Rheumatology*. 2014;53(10):1759-1766.
6. Komatsu N, Takayanagi H. Immune-bone interplay in the structural damage in rheumatoid arthritis. *Clin Exp Immunol*. 2018;194(1):1-8.

7. Gioia C, Lucchino B, Tarsitano MG, Iannuccelli C, Di Franco M. Dietary habits and nutrition in rheumatoid arthritis: can diet influence disease development and clinical manifestations? *Nutrients*. 2020;12(5):1456.
8. Valner A, Kirsimägi Ü, Müller R, et al. Structural and functional changes of hands and legs in early rheumatoid arthritis. *Medicina*. 2021;57(4):317.
9. Bala A, Mondal C, Haldar PK, Khandelwal B. Oxidative stress in inflammatory cells of patient with rheumatoid arthritis: clinical efficacy of dietary antioxidants. *Inflammopharmacology*. 2017;25(6):595-607.
10. Khojah HM, Ahmed S, Abdel-Rahman MS, Hamza AB. Reactive oxygen and nitrogen species in patients with rheumatoid arthritis as potential biomarkers for disease activity and the role of antioxidants. *Free Radic Biol Med*. 2016;97:285-291.
11. Zeng L, Yu G, Yang K, Li J, Hao W, Chen H. The efficacy of antioxidative stress therapy on oxidative stress levels in rheumatoid arthritis: a systematic review and meta-analysis of randomized controlled trials. *Oxid Med Cell Longev*. 2021;2021:33028861-33028930.
12. Richard D, Kefi K, Barbe U, Bausero P, Visioli F. Polyunsaturated fatty acids as antioxidants. *Pharmacol Res*. 2008;57(6):451-455.
13. World Health Organization. Nutrition in universal health coverage. Accessed October 16, 2021. https://www.who.int/publications/i/item/WHO-NMH-NHD-19.24
14. World Health Organization. Obesity and overweight. Accessed October 16, 2021. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
15. Navarini L, Afeltra A, Gallo Afflitto G, Margiotta DPE. Polyunsaturated fatty acids: any role in rheumatoid arthritis? *Lipids Health Dis*. 2017;16(1):197.
16. Skoczyńska M, Świerkot J. The role of diet in rheumatoid arthritis. *Reumatologia*. 2018;56(4):259-267.
17. Gioxari A, Kaliora AC, Marantidou F, Panagiotakos DP. Intake of ω-3 polyunsaturated fatty acids in patients with rheumatoid arthritis: a systematic review and meta-analysis. *Nutrition*. 2018;45:114-124.e4.
18. Philippou E, Petersson SD, Rodomar C, Nikiphorou E. Rheumatoid arthritis and dietary interventions: systematic review of clinical trials. *Nutr Rev*. 2021;79(4):410-428.
19. Sigaux J, Bellicha A, Buscail C, et al. Serum fatty acid profiles are associated with disease activity in early rheumatoid arthritis: results from the ESPOIR cohort. *Nutrients*. 2022;14(14):2947.
20. Daien C, Czernichow S, Letarouilly JG, et al. Dietary recommendations of the French Society for Rheumatology for patients with chronic inflammatory rheumatic diseases. *Joint Bone Spine*. 2022;89(2):105319.
21. Casado-Díaz A, Santiago-Mora R, Dorado G, Quesada-Gómez JM. The omega-6 arachidonic fatty acid, but not the omega-3 fatty acids, inhibits osteoblastogenesis and induces adipogenesis of human mesenchymal stem cells: potential implication in osteoporosis. *Osteoporos Int*. 2013;24(5):1647-1661.
22. Albertazzi P, Coupland K. Polyunsaturated fatty acids. Is there a role in postmenopausal osteoporosis prevention? *Maturitas*. 2002;42(1):13-22.
23. Ferguson LD, Siebert S, McInnes IB, Sattar N. Cardiometabolic comorbidities in RA and PsA: lessons learned and future directions. *Nat Rev Rheumatol*. 2019;15(8):461-474.
24. Cena H, Calder PC. Defining a healthy diet: evidence for the role of contemporary dietary patterns in health and disease. *Nutrients*. 2020;12(2):334.
25. Bird JK, Troesch B, Warnke I, Calder PC. The effect of long chain omega-3 polyunsaturated fatty acids on muscle mass and function in sarcopenia: A scoping systematic review and meta-analysis. *Clin Nutr ESPEN*. 2021;46:73-86.
26. Ma WJ, Li H, Zhang W, et al. Effect of n-3 polyunsaturated fatty acid supplementation on muscle mass and function with aging: a meta-analysis of randomized controlled trials☆. *Prostaglandins Leukot Essent Fatty Acids*. 2021;165:102249.
27. Kilic G, Ozgocmen S. Hand bone mass in rheumatoid arthritis: a review of the literature. *World J Orthop*. 2015;6(1):106-116.
28. Valner A, Kirsimägi Ü, Müller R, et al. Factors associated with hand bone changes in early rheumatoid arthritis. *Musculoskeletal Care*. 2023;21:108-116.
29. NutriData Software Homepage. Accessed January 30, 2018. http://tap.nutridata.ee/
30. Anderson J, Caplan L, Yazdany J, et al. Rheumatoid arthritis disease activity measures: American College of Rheumatology recommendations for use in clinical practice. *Arthritis Care Res*. 2012;64(5):640-647.
31. Fleischmann R. 2019 update of the American College of rheumatology- recommended rheumatoid arthritis disease activity measures: comment on the article by England et al. *Arthritis Care Res*. 2020;72(5):736.
32. Lu Y, Genant HK, Shepherd J, et al. Classification of osteoporosis based on bone mineral densities. *J Bone Miner Res*. 2001;16(5):901-910.
33. Barbouti A, Goulas V. Dietary antioxidants in the Mediterranean diet. *Antioxidants*. 2021;10(8):1213.
34. Sies H. Oxidative stress: concept and some practical aspects. *Antioxidants*. 2020;9(9):852.
35. Pietschmann P, Mechtcheriakova D, Meshcheryakova A, Föger-Samwald U, Ellinger I. Immunology of osteoporosis: A mini-review. *Gerontology*. 2016;62(2):128-137.
36. Cakır L, Aktas G, Mercimek OB, et al. Are red cell distribution width and mean platelet volume associated with rheumatoid arthritis? *Biomed Res*. 2016;27:292-294.
37. Yates CM, Calder PC, Ed Rainger G. Pharmacology and therapeutics of omega-3 polyunsaturated fatty acids in chronic inflammatory disease. *Pharmacol Ther*. 2014;141(3):272-282.
38. Kim JY, Lim K, Kim KH, Kim JH, Choi JS, Shim SC. N-3 polyunsaturated fatty acids restore Th17 and treg balance in collagen antibody-induced arthritis. *PLoS One*. 2018;13(3):e0194331.
39. Ferreira HB, Melo T, Paiva A, Domingues MDR. Insights in the role of lipids, oxidative stress and inflammation in rheumatoid arthritis unveiled by new trends in lipidomic investigations. *Antioxidants*. 2021;10(1):45.
40. Theander L, Willim M, Nilsson JÄ, et al. Changes in bone mineral density over 10 years in patients with early rheumatoid arthritis. *RMD*. 2020;6(1):e001142.
41. Jin S, Li M, Wang Q, et al. Bone mineral density and microarchitecture among Chinese patients with rheumatoid arthritis: a cross-sectional study with HRpQCT. *Arthritis Res Ther*. 2021;23(1):127.
42. Calder PC. Polyunsaturated fatty acids and inflammation. *Biochem Soc Trans*. 2005;33(Pt 2):423-427.
43. Monnard CR, Dulloo AG. Polyunsaturated fatty acids as modulators of fat mass and lean mass in human body composition regulation and cardiometabolic health. *Obes Rev*. 2021;22 Suppl 2(Suppl 2):e13197.
44. Balci SB, Aktas G. A comprehensive review of the role of hemogram-derived inflammatory markers in gastrointestinal conditions. *Iran J Colorectal Res*. 2022;10:2-13.
45. Ustaoglu M, Aktas G, Avcioglu U, Bas B, Bahceci BK. Elevated platelet distribution width and red cell distribution width are associated with autoimmune liver diseases. *Eur J Gastroenterol Hepatol*. 2021;33(1S Suppl 1):e905-e908.
46. Aktas G, Kocak MZ, Taslamacioglu Duman T, et al. Mean platelet volume (MPV) as an inflammatory marker in type 2 diabetes mellitus and obesity. *Bali Med J*. 2018;7(3):650-653. doi:10.15562/bmj.v7i3.806
47. Aktas G, Kocak MZ, Bilgin S, Atak BM, Duman TT, Kurtkulagi O. Uric acid to HDL cholesterol ratio is a strong predictor of diabetic control in men with type 2 diabetes mellitus. *Aging Male*. 2020;23(5):1098-1102.
48. Kocak MZ, Aktas G, Erkus E, Sincer I, Atak B, Duman T. Serum uric acid to HDL-cholesterol ratio is a strong predictor of metabolic syndrome in type 2 diabetes mellitus. *Rev Assoc Med Bras*. 2019;65(1):9-15.
49. Patterson E, O' Doherty RM, Murphy EF, et al. Impact of dietary fatty acids on metabolic activity and host intestinal microbiota composition in C57BL/6J mice. *BR J Nutr*. 2014;111(11):1905-1917.
50. Costantini L, Molinari R, Farinon B, Merendino N. Impact of Omega-3 fatty acids on the gut Microbiota. *Int J Mol Sci*. 2017;18(12):2645.
51. Fu Y, Wang Y, Gao H, et al. Associations among dietary Omega-3 polyunsaturated fatty acids, the gut Microbiota, and intestinal immunity. *Mediators Inflamm*. 2021;2021:8879227.
52. Belluzzi A. Polyunsaturated fatty acids (n-3 PUFAs) and inflammatory bowel disease (IBD): pathogenesis and treatment. *Eur Rev Med Pharmacol Sci*. 2004;8(5):225-229.
53. Marion-Letellier R, Savoye G, Ghosh S. Polyunsaturated fatty acids and inflammation. *IUBMB Life*. 2015;67(9):659-667.
54. Liu HQ, Qiu Y, Mu Y, et al. A high ratio of dietary n-3/n-6 polyunsaturated fatty acids improves obesity-linked inflammation and insulin resistance through suppressing activation of TLR4 in SD rats. *Nutr Res*. 2013;33(10):849-858.
55. Tilg H. Obesity, metabolic syndrome, and microbiota: multiple interactions. *J Clin Gastroenterol*. 2010;44 Suppl 1:S16-S18.
56. Kalupahana NS, Goonapienuwala BL, Moustaid-Moussa N. Omega-3 fatty acids and adipose tissue: inflammation and browning. *Annu Rev Nutr*. 2020;40:25-49.
57. Wang M, Ma LJ, Yang Y, Xiao Z, Wan JB. N-3 Polyunsaturated fatty acids for the management of alcoholic liver disease: a critical review. *Crit Rev Food Sci Nutr*. 2019;59:S116-S129.
58. Araya J, Rodrigo R, Videla LA, et al. Increase in long-chain polyunsaturated fatty acid n - 6/n - 3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease. *Clin Sci*. 2004;106(6):635-643.
59. Yue H, Qiu B, Jia M, et al. Effects of α-linolenic acid intake on blood lipid profiles: a systematic review and meta-analysis of randomized controlled trials. *Crit Rev Food Sci Nutr*. 2021;61(17):2894-2910.
60. Jing K, Wu T, Lim K. Omega-3 polyunsaturated fatty acids and cancer. *Anticancer Agents Med Chem*. 2013;13(8):1162-1177.
61. Liu J, Li X, Hou J, Sun J, Guo N, Wang Z. Dietary intake of N-3 and N-6 polyunsaturated fatty acids and risk of cancer: meta-analysis of data from 32 studies. *Nutr Cancer*. 2021;73(6):901-913.
62. Brown TJ, Brainard J, Song F, Wang X, Abdelhamid A, Hooper L. Omega-3, omega-6, and total dietary polyunsaturated fat for prevention and treatment of type 2 diabetes mellitus: systematic review and meta-analysis of randomised controlled trials. *BMJ*. 2019;366:l4697.
63. Estonian population statistics database. Statistics Estonia Homepage. Accessed October 18, 2021. https://www.stat.ee/en/avasta-statistikat/valdkonnad/rahvastik/population-figure