The effect of vitamin K insufficiency on histological and structural properties of knee joints in aging mice

M. Kyla Sheaa,*, Sarah L. Bootha, Stephanie G. Harshmana, Donald Smitha, Cathy S. Carlsons, Lindsey Harpers, Alexandra R. Armstrongs, Min Fangt, M. Leonor Cancelad, Márcio Simãoa, Richard F. Loesor

a USDA Human Nutrition Research Center on Aging at Tufts University, Boston MA, USA
b College of Veterinary Medicine, University of Minnesota, St. Paul MN, USA
c Small Animal Imaging Preclinical Testing Facility, Tufts University School of Medicine, Boston MA, USA
d Center of Marine Sciences University of Algarve, Faro Portugal
e Department of Biomedical Sciences and Medicine, University of Algarve, Faro Portugal
f Algarve Biomedical Centre and Centre for Biomedical Research, Universidade do Algarve, Faro, Portugal
g Thurston Arthritis Center, University of North Carolina School of Medicine, Chapel Hill, NC, USA

ARTICLE INFO

Keywords:
Vitamin K
Nutrition
Cartilage
Osteoarthritis
Aging
Rodent model

SUMMARY

Objective: While a role for vitamin K in maintaining joint tissue homeostasis has been proposed based on the presence of vitamin K dependent proteins in cartilage and bone, it is not clear if low vitamin K intake is causally linked to joint tissue degeneration. To address this gap, we manipulated vitamin K status in aging mice to test its effect on age-related changes in articular cartilage and sub-chondral bone.

Methods: Eleven-month old male C57BL6 mice were randomly assigned to a low vitamin K diet containing 120 mcg phylloquinone/kg diet (n = 32) or a control diet containing 1.5 mg phylloquinone/kg diet (n = 30) for 6 months. Knees were evaluated histologically using Safranin O and H&E staining, as well as using micro-CT.

Results: Eleven mice in the low vitamin K diet group and three mice in the control group died within the first 100 days of the experiment (p = 0.024). Mice fed the low vitamin K diet had higher Safranin-O scores, indicative of more proteoglycan loss, compared to mice fed the control diet (p = 0.026). The articular cartilage structure scores did not differ between the two groups (p = 0.190). The sub-chondral bone parameters measured using micro CT also did not differ between the two groups (all p ≥ 0.174).

Conclusion: Our findings suggest low vitamin K status can promote joint tissue proteoglycan loss in older male mice. Future studies are needed to confirm our findings and obtain a better understanding of the molecular mechanisms underlying the role of vitamin K in joint tissue homeostasis.

1. Introduction

Osteoarthritis (OA), a leading cause of joint pain and disability in older age, is characterized by pathophysiological changes in all joint tissues, including articular and meniscal cartilage and sub-chondral bone [1,2]. The molecular mechanisms underlying OA development and progression are not well-characterized and currently disease-modifying OA treatments are limited [3].

In observational studies, higher vitamin K status was associated with less articular cartilage and meniscus damage and a lower risk for incident OA [4,5], suggesting that vitamin K-dependent pathways are involved in cartilage homeostasis. Vitamin K is a fat-soluble nutrient that functions as an enzyme co-factor in the carboxylation of vitamin K-dependent proteins. To function, vitamin K-dependent proteins must be carboxylated, which requires vitamin K. Joint tissues contain several vitamin K-dependent proteins, including matrix gla protein (MGP) and gla-rich protein (GRP) (also known as unique cartilage matrix-associated protein (Ucma)), which are involved in inhibiting soft-tissue mineralization,
as well as growth arrest specific gene 6 (Gas6) which may contribute to chondrocyte survival [6–8].

Articular cartilage and meniscal mineralization have been linked to OA [9–11]. In cultured articular chondrocytes, loss of MGP expression was accompanied by an increase in expression of cartilage degrading enzymes [12]. In mice with surgically-induced OA, GRP deletion increased cartilage degradation [13]. Moreover, uncarboxylated MGP and GRP, which are non-functional, are abundant in human arthritic articular cartilage, while the carboxylated (functional) protein forms are more abundant in healthy articular cartilage [7,8]. While these proteins require vitamin K for carboxylation, the assumption inherent to the conclusions is that vitamin K is involved in the mechanism. This can only be empirically tested by manipulating vitamin K.

To address this gap we evaluated the effect of vitamin K insufficiency on articular cartilage and subchondral bone morphology in aging mice. We hypothesized that mice aged on a low vitamin K diet would have more articular cartilage proteoglycan loss, articular cartilage structural damage, and sub-chondral bone thickening, characteristics of knee OA, than mice aged on a diet with sufficient vitamin K.

2. Methods

Sixty-two 11-month old retired breeder male C57BL6 mice obtained from Charles River Laboratories were randomized to a control diet (TD.120060, Envigo, with 5% tocopherol stripped corn oil containing 1.5 mg phylloquinone/kg diet; n = 30) or low vitamin K diet (a modification of TD.97053, described in Supplemental Table, n = 32). The low vitamin K diet was initially formulated to contain 80 mcg phylloquinone/kg diet. Within 3 weeks, due to the death of two animals on the low vitamin K diet, it was reformulated to contain 120 mcg phylloquinone/kg diet. This diet was used for the rest of the experiment. Mice on the low vitamin K diet were group pair-fed to the mice on the control diet. Mice were weighed weekly and observed daily for signs of distress or disease. Because of the early deaths that occurred disproportionately in the group fed the low vitamin K diet, seven mice in control group were sacrificed at 14.5 months, in an effort to balance follow-up time between the two groups. All remaining mice were sacrificed when they were 17-months old. Knee joints and tissues were harvested upon sacrifice.

Mice were maintained in AAALAC-accredited facilities with an environmentally controlled atmosphere (22 °C, 45% relative humidity, 15 air changes of 100% fresh hepa-filtered air per h and a 12/12-h light/dark cycle). All protocols were approved by the HNRCA Tufts University Animal Care and Use Committee.

2.1. Histology

The right hind limb from each mouse was fixed in formalin then transferred to 70% ethanol. The excess soft tissue was removed, after which the limbs were decalcified in 10% EDTA and stiffe joints embedded in paraffin [14]. The joints were sectioned along the coronal plane at 4 μm. Two adjacent midcoronal sections from each knee were stained with safranin-O and hematoxylin and eosin (H&E) and graded semi-quantitatively on a 0–12 scale as described [15]. The Safranin-O system scores depth and extent of loss of staining of the articular cartilage on a scale of 0–12 in each region (medial and lateral tibial plateau, medial and lateral femoral condyle), where 0 represents uniform staining of healthy cartilage and 12 represents complete, full thickness loss of staining of matrix and chondrocytes across more than two-thirds of the surface. Because the intensity of staining of articular cartilage with Safranin O is directly related to the proteoglycan content of the matrix, loss of staining is considered to be a reflection of the proteoglycan content of the tissue. The H&E sections were scored using the articular cartilage structure (ACS) score, which evaluates the integrity of the articular cartilage on a scale of 0–12 in each region, where 0 represents normal healthy cartilage and 12 represents full-thickness loss of the articular cartilage across more than two-thirds of the surface scored [14, 15]. Percentage of cartilage necrosis (area occupied by necrotic chondrocytes divided by total articular cartilage area X 100%) was evaluated in each region using the Nikon NIS Elements software package. In addition, osteophytes were graded on a 0–3 scale in each region: 0 = none, 1 = questionable osteophyte, 2 = small osteophyte, 3 = large osteophyte [14].

A single observer blinded to diet group performed all scoring. Only animals that survived until they were at least 15 months of age were analyzed for OA-related outcomes (n = 27 control diet, n = 22 low vitamin K diet).

2.2. Immunohistochemistry

Additional adjacent sections were immunostained using antibodies directed against MGP and GRP. GRP was detected using anti-GRP antibody (PA5-20768, ThermoFisher Scientific), at 1:50, followed by secondary antibody (anti HRP at 1:100). MGP was detected using anti-MGP

Fig. 1. Survival of male C57BL6 mice fed a low vitamin K diet (solid line) or control diet (dashed line) starting at 11 months of age (p = 0.024, based on log-rank test; + denotes censoring).
(Anti-MGP antibody (ab192396) Abcam) at 1:40 followed by secondary antibody (anti-HRP at 1:100). Both antibodies recognized the C terminal of each protein, encoded by the last exon of the corresponding gene.

2.3. MicroCT

Prior to sectioning for histology, knees were scanned at 9 μm resolution on a Skyscan 1176 High Resolution Micro-CT Scanner. CTan software was used to evaluate bone surface density, bone volume, bone surface/volume ratio, trabecular pattern factor, trabecular thickness, trabecular number and trabecular separation of the trabecular bone of tibia. The region of interest was consistently defined by drawing manually with morphological and Boolean operations within Skyscan CTan using the growth plate as the anatomical reference. A single observer blinded to diet group evaluated the following parameters of the tibial plateau using the same threshold for each animal: bone volume, bone surface area, bone surface density, trabecular number, separation and thickness.

2.4. Vitamin K tissue concentrations

Phylloquinone (vitamin K1) and menaquinone-4 (a form of vitamin K2) concentrations in liver, kidney, brain, intestine, and serum were determined using reverse-phase HPLC, as described [16,17].

2.5. Statistical analyses

Sample size calculations were based on the differences in Safranin-O and ACS scores between 4.5 month and 17-month old male C57BL6 mice [18]. We determined 22 animals per group would provide 80–85% power to detect a 2.5 point difference in Safranin-O score and a 2.4 point difference in ACS score at the medial tibial plateau. Prior to this study, the only data available on survivorship of rodents on low vitamin K diets were in female rats [19]. Because male mice are more susceptible to vitamin K deficiency [16], and male C57BL6 mice develop knee OA naturally with age [20], we randomly assigned 62 male C57BL6 mice to one of the two diet groups (low vitamin K n = 32; control n = 30), to assure adequate sample sizes for our primary outcomes.

Changes in body weights over time were compared between the two groups using a one-way repeated measures ANOVA. The Wilcoxon two-sample test was used to compare the histological outcomes between the two groups. Safranin-O, ACS and osteophyte scores were analyzed at the medial tibial plateau (MTP; most severely affected site) and overall (for an assessment of the entire joint) by summing the scores across the four quadrants (medial and lateral tibial plateaus, medial and lateral femoral condyles). The percent cartilage necrosis area was compared at the MTP and overall using the average of the percentages across the four quadrants. The percent of cells stained positively for MGP and GRP in articular cartilage and meniscus were also compared using the Wilcoxon two-sample test. In an exploratory analysis, we evaluated the partial correlations of MGP and GRP expression (% positive cells) in articular cartilage and meniscus with one another and with Safranin-O and ACS scores using Spearman coefficients. The log-rank test was used to compare the survival between the low vitamin K group and the control group. Statistical analyses were conducted using SAS v.14.

3. Results

Within the first 100 days of the experiment, eleven mice on the low vitamin K diet and three mice in the control group died unexpectedly. The median (standard error) survival time (days) of the low vitamin K group was 128 (10) days, compared to 155 (6) days for the control group (p = 0.024, based on log-rank test) (Fig. 1). No necropsy evaluations were done to determine the cause of death in these animals; however, there were no overt signs of bleeding in any of the animals. Body weights increased similarly in both groups over time (group p = 0.63, group*t ime
$p = 0.772$, time $p < 0.001$) (Supplemental Fig. 1).

### 3.1. Histological outcomes

Mice fed the low vitamin K diet had higher Safranin-O scores (indicating greater loss of staining from the matrix) compared to mice fed the control diet, but the ACS scores did not differ between the two groups (Fig. 2). The osteophyte scores and percent area of cartilage necrosis also did not differ between the two groups ($p > 0.238$) (Table 1).

### 3.2. MGP and GRP

MGP and GRP were expressed in articular and meniscal cartilage, but the expression did not differ significantly between the two groups (all $p \geq 0.241$) (Fig. 3). The expression of MGP (% positive stained cells) in articular cartilage was significantly positively correlated with MGP in the meniscus ($\text{Spearman } r = 0.46, p = 0.008$), but expression of GRP in the two tissues was not correlated ($\text{Spearman } r = 0.21, p = 0.242$). In the meniscus, MGP and GRP expression were significantly positively correlated with each other ($\text{Spearman } r = 0.46, p = 0.008$), but in articular cartilage, MGP expression was not correlated with GRP expression ($\text{Spearman } r = 0.21, p = 0.242$).

Fig. 3. Matrix gla protein (MGP) (A, C) and gla rich protein (GRP) (B, D) stained positive cells in articular cartilage (A, B) and menisci (C, D) of male C57BL6 mice fed a low vitamin K diet or control diet. Representative images of MGP (E,G) and GRP (F,H) stained knee joints of mice fed a low vitamin K diet (E,F) and control diet (G,H). (Adjacent separate sections were immunostained using antibodies directed against MGP and GRP. GRP was detected using anti-GRP antibody (PA5-20768, ThermoFisher Scientific), at 1:50, followed by secondary antibody (anti HRP at 1:100). MGP was detected using anti-MGP (Anti-MGP antibody (ab192396) Abcam) at 1:40 followed by secondary antibody (anti-HRP at 1:100). Both antibodies recognized the C terminal of each protein, encoded by the last exon of the corresponding gene.) MGP and GRP are stained in dark brown. Images are magnified 20x.
3.4. Vitamin K tissue concentrations

The vitamin K tissue concentrations were lower in the mice fed the low vitamin K diet compared to those fed the control diet (Fig. 4, all between group p < 0.001). Phylloquinone was not detected in the brain and menaquinone-4 was not detected in serum of either diet group. Serum phylloquinone was below the limit of detection in all mice fed the low vitamin K diet and in 13 mice fed the control diet.

4. Discussion

We evaluated the effect of low vitamin K intake on cartilage and subchondral bone morphology in aging male mice utilizing an aging mouse model of naturally-occurring OA to enhance translation, since age is a primary risk factor for OA development and progression in humans. We found the mice fed a low vitamin K diet for 6 months had higher (worse) articular cartilage Safranin-O scores, but not ACS scores, osteophyte scores, cartilage necrosis or bone changes evaluated using microCT compared to mice fed a diet with sufficient vitamin K. Loss of Safranin-O staining reflects proteoglycan loss, an early manifestation of cartilage degeneration [21]. Because proteoglycan loss occurs early in the disease process [22,23], the Safranin-O score is more sensitive than the ACS score, which is a measure of articular cartilage fibrillation and loss, at detecting the early stages of cartilage degeneration in OA. Although proteoglycan loss correlates with cartilage loss [24], we did not detect differences in cartilage necrosis between the two groups. Proteoglycan loss typically precedes loss of cartilage tissue [22], so our findings suggest that vitamin K may be more relevant to maintaining cartilage homeostasis before it begins to degenerate. However, future experiments are needed to clarify the importance of vitamin K to joint tissues at different stages of OA severity.

MGP and GRP are two vitamin K-dependent proteins expressed in joint tissues that inhibit soft-tissue mineralization when they are carboxylated, a process that requires vitamin K [25,26]. Articular cartilage and meniscal mineralization can be characteristic of OA [9,10,27], although whether mineralization contributes to OA development or progression directly is debatable [28–30]. Previous in vitro and ex vivo experiments using in-house antibodies found that uncarboxylated MGP and uncarboxylated GRP were more abundant in human OA cartilage, whereas the carboxylated (functional) protein forms were more abundant in healthy cartilage [7,8]. We detected MGP and GRP in the articular and meniscal cartilage of aged mice, the expression did not differ between the mice fed the low vitamin K diet and those fed the control diet. However, we evaluated MGP and GRP immunohistologically, regardless of the proteins’ carboxylation status. Because vitamin K is required for carboxylation, it is plausible the amount of carboxylated and/or uncarboxylated protein forms differed between the mice fed the low vitamin K and the control diet, while the total protein in the joint tissues remained similar in both groups. Unfortunately, antibodies that differentiate the carboxylated from uncarboxylated forms of these proteins in mice are not commercially available, so we were unable to measure the different forms, thereby limiting our ability to draw inferences about the effect of vitamin K intake on the carboxylation of vitamin K-dependent proteins in joint tissues. Our exploratory analyses indicated that MGP and GRP expression are positively correlated in meniscal cartilage. This may indicate that the two proteins function synergistically in some joint tissues, as has been reported in vascular tissue [31]. Since we did not detect a similar correlation in articular cartilage, additional experiments are needed to clarify the role of vitamin K in maintaining joint tissue homeostasis.

Fig. 4. Vitamin K tissue concentrations of male C57BL6 mice fed a low vitamin K diet (♀) or control diet (♂) (All between group p < 0.001 based on Wilcoxon two sample test; error bars represent SEM.).
needed to better understand the presence and function of vitamin K-dependent proteins in other joint tissues. We confirmed that the mice fed a low vitamin K diet had significantly lower tissue vitamin K concentrations compared to the mice fed the control diet. Consistent with previous studies [16,32,33], we detected menaquinone-4, a metabolite of phylloquinone, in several tissues. Phylloquinone was the only form of vitamin K provided in the diet of both groups. Unfortunately, we were technically unable to extract vitamin K from articular cartilage and it is not known if joint tissues contain menaquinone-4 or phylloquinone. One potential mechanism relating vitamin K to OA that is independent of protein carboxylation may involve the SXR/PXR nuclear receptor, which menaquinone-4 can activate, but phylloquinone cannot [34,35]. Menaquinone-4 activation of the SXR in cultured human osteoblasts upregulated expression of extracellular matrix genes involved in collagen formation [36]. In mice, menaquinone-4 activation of PXR in chondrocytes was associated with less articular cartilage loss and age-related osteoarthritis [37], suggesting multiple mechanisms through which vitamin K may influence joint health.

Because vitamin K is required to maintain coagulation, a diet without any vitamin K can lead to hemorrhagic death. Currently the amount of vitamin K that is sufficient to support the carboxylation of coagulation proteins but not the carboxylation of extra-hepatic vitamin K-dependent proteins in C57BL6 mice is not established. Because we sought to maintain older male mice on a low vitamin K diet for 6 months, our diet initially contained 80 mcg phylloquinone/kg diet. However, two animals died unexpectedly within the first month on this diet. Out of caution, we increased the vitamin K to 120 mcg phylloquinone/kg diet. This corresponded to 8% of the amount of vitamin K in the control diet. Over the remainder of the study, there was still significantly higher mortality in the low vitamin K group compared to the control. The hepatic and extra-hepatic tissue vitamin K concentration in our low vitamin K group were significantly lower than the control. The hepatic and extra-

Contributions

MKS, SGH, DS performed the experiment; MKS, SGH, CSS, LH, AA, MF, MLC, and MS analyzed data; MKS, SLB, RFL drafted the manuscript; all authors contributed to manuscript revision and approved the final version. MKS had full access to the data and takes responsibility for data integrity.

Funding

Supported by the National Institute of Arthritis, Musculoskeletal and Skin Diseases (R01AR063167), a pilot grant from the USDA Human Nutrition Research Center on Aging at Tufts University, and the USDA ARS Cooperative Agreement (58-1950-7-707). Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the USDA.

Declarations of Competing Interest

The authors have no conflicts to declare.

Acknowledgements

The authors thank Katie McDermott from the University of Minnesota for histology assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jocarto.2020.100079.

References

[1] M.B. Goldring, S.R. Goldring, Osteoarthritis, J. Cell Physiol. 213 (2007) 626–634, https://doi.org/10.1002/jcp.21258.
[2] R.A. Terkelshab, What does cartilage calcification tell us about osteoarthritis? J. Rheumatol. 29 (2002) 411–415, http://www.jrheum.org/content/29/3/411.
[3] A. Ghouri, P.G. Conaghan, Prospects for therapies in osteoarthritis, Calcif. Tissue Int. (2020), https://doi.org/10.1007/s00223-020-00672-9.
[4] M.K. Shea, S.B. Kritchovsky, F.C. Hsu, M. Nevitt, S.L. Booth, C.K. Kwoh, et al., The association between vitamin K status and knee osteoarthritis features in older adults: the Health, Aging and Body Composition Study, Osteoarthritis Cartilage 23 (2015) 370–378, https://doi.org/10.1016/j.joca.2014.12.008.
[5] D. Misra, S.L. Booth, I. Tokshvky, D.T. Felson, M.C. Nevitt, C.E. Lewis, et al., Vitamin K deficiency is associated with incident knee osteoarthritis, Am. J. Med. 126 (2013) 243–248, https://doi.org/10.1016/j.amjmed.2012.10.011.
[6] R.P. Looser, B.C. Varum, C.S. Carlson, M.B. Goldring, E.T. Liu, S. Sadiev, et al., Human chondrocyte expression of growth-arrest-specific gene 6 and the tyrosine kinase receptor axl: potential role in autocrine signaling in cartilage, Arthritis Rheum. 40 (1997) 1455–1465, https://doi.org/10.1002/art.178040014.
[7] R. Wallin, L.J. Schurgers, R.P. Looser, Biosynthesis of the vitamin K-dependent matrix GlA protein (MGP) in chondrocytes: a fetuin-MGP protein complex is assembled in vesicles shed from normal but not from osteoarthritic chondrocytes, Osteoarthritis Cartilage 18 (2010) 1096–1103, https://doi.org/10.1016/j.joca.2010.03.019.
[8] M.S. Rafael, S. Cavaco, C.S. Viegas, S. Santos, A. Ramos, B.A. Willems, et al., Insights into the association of Gla-rich protein and osteoarthritis, novel splice variants and gamma-carboxylation status, Mol. Nutr. Food Res. 98 (2014) 1636–1646, https://doi.org/10.1002/mnfr.201300941.
[9] A. Abbishek, S. Doherty, R.A. Maciewicz, K. Muir, W. Zhang, M. Doherty, Does chondrocalcinosis associate with a distinct radiographic phenotype of osteoarthritis in knees and hips? A case-control study, Arthritis Care Res (Hoboken) 68 (2016) 211–216, https://doi.org/10.1002/acr.22852.
[10] M. Fuerst, O. Niggemeyer, L. Lammers, F. Scharer, C. Lohmann, W. Ruther, Articular cartilage mineralization in osteoarthritis of the hip, BMC Musculoskel. Disord. 10 (2009) 166, https://doi.org/10.1186/1471-2474-10-166.
[11] D. McDaniel, E. Tilton, K. Dominick, K. Floir, T. Ernest, J.C. Johnson, et al., Histological characteristics of knee menisci in patients with osteoarthritis, Clin. Anat. 30 (2017) 805–810, https://doi.org/10.1002/ca.22920.
[12] C. Shepherd, A.E. Reese, L.N. Reynard, J. Loughlin, Expression analysis of the osteoarthritis genetic susceptibility mapping to the matrix GlA protein gene MGP, Arthritis Res. Ther. 21 (2019) 149, https://doi.org/10.1186/s13075-019-1934-7.
[13] M. Stock, S. Menges, N. Eitzinger, M. Gesslein, R. Botschner, L. Wormser, et al., A dual role of upper zone of growth plate and cartilage matrix-associated protein in human and mouse osteoarthritic cartilage: inhibition of aggrecanases and promotion of bone turnover, Arthritis Rheum. 69 (2017) 1233–1245, https://doi.org/10.1002/art.40042.
[14] M.A. Rowe, L.R. Harper, M.A. McNulty, A.G. Lau, C.S. Carlson, L. Leng, et al., Reduced osteoarthritis severity in aged mice with deletion of macrophage.
migration inhibitory factor, Arthritis Rheum. 69 (2017) 352–361, https://doi.org/10.1002/art.39844.

[15] M.A. McNulty, R.F. Loeser, C. Davey, M.F. Callahan, C.S. Carlson, A comprehensive histological assessment of osteoarthritis lesions in mice, Cartilage 2 (2011) 354–363, https://doi.org/10.1177/1947603511402665.

[16] S.G. Harshman, X. Fu, J.P. Karl, K. Barger, S. Lamon-Fava, A. Kulinopulos, et al., Tissue concentrations of vitamin K and expression of key enzymes of vitamin K metabolism are influenced by sex and diet but not housing in C57Bl/6 mice, J. Nutr. 146 (2016) 1521–1527, https://doi.org/10.3945/jn.116.233130.

[17] K.W. Davidson, J.A. Sadowski, Determination of vitamin K compounds in plasma or serum by high-performance liquid chromatography using postcolumn chemical reduction and fluorometric detection, Methods Enzymol. 282 (1997) 408–421, https://doi.org/10.1016/s0076-6879(97)82124-6.

[18] M.A. McNulty, R.F. Loeser, C. Davey, M.F. Callahan, C.S. Carlson, Histopathology of naturally occurring and surgically induced osteoarthritis in mice, Osteoarthritis Cartilage 20 (2012) 949–956, https://doi.org/10.1016/j.oa.2012.05.001.

[19] I. Carrie, E. Belanger, J. Portoukalian, J. Rochford, G. Ferland, Lifelong low-phylloquinone intake is associated with cognitive impairments in old rats, J. Nutr. 141 (2011) 1495–1501, https://doi.org/10.3945/jn.110.137638.

[20] G.J. Van Osch, P.M. van der Kraan, E.L. Vitters, L. Blankevoort, W.B. Van Den Berg, G.J. Van Osch, P.M. van der Kraan, E.L. Vitters, L. Blankevoort, W.B. Van Den Berg, T1rho magnetic resonance: basic physics principles and applications in knee and intervertebral disc imaging, Ther. Adv. Musculoskelet. Dis. 4 (2012) 77, https://doi.org/10.1177/1759720X11431005.

[21] A.H. Mokdad, K. Ballestros, M. Echko, S. Glenn, H.E. Olsen, E. Mullany, et al., The state of US health, 1990-2016: burden of diseases, injuries, and risk factors among jama.2018.0158.

[22] M.A. McNulty, R.F. Loeser, C. Davey, M.F. Callahan, C.M. Ferguson, C.S. Carlson, Determination of vitamin K compounds in plasma or serum by high-performance liquid chromatography using postcolumn chemical reduction and fluorometric detection, Methods Enzymol. 282 (1997) 408–421, https://doi.org/10.1016/s0076-6879(97)82124-6.

[23] K. Azuma, S.C. Casey, T. Urano, K. Horie-Inoue, Y. Ouchi, B. Blumberg, et al., Steroid and xenobiotic receptor SXR mediates vitamin K2-activated transcription of extracellular matrix-related genes and collagen accumulation in osteoblastic cells, J. Biol. Chem. 281 (2006) 16927–16934, https://doi.org/10.1074/jbc.M600896200.

[24] T. Ichikawa, K. Horie-Inoue, K. Ikeda, B. Blumberg, S. Inoue, Steroid and xenobiotic receptor SXR regulates vitamin K2-activated transcription of extracellular matrix-related genes and collagen accumulation in osteoblastic cells, J. Biol. Chem. 281 (2006) 16927–16934, https://doi.org/10.1074/jbc.M600896200.

[25] K. Azuma, S.C. Casey, T. Urano, K. Horie-Inoue, Y. Ouchi, B. Blumberg, et al., Pregnane X receptor knockout mice display aging-dependent wearing of articular cartilage, PLoS One 10 (2015), e0119177, https://doi.org/10.1371/journal.pone.0119177.

[26] S. Bapat, D. Hubbard, A. Munjal, M. Hunter, S. Pulzle, Pros and cons of mouse models for studying osteoarthritis, Clin. Transl. Med. 7 (2018) 36, https://doi.org/10.1186/s40169-018-0215-4.

[27] R.F. Loeser, A.L. Olex, M.A. McNulty, C.S. Carlson, M.F. Callahan, C.M. Ferguson, et al., Microarray analysis reveals age-related differences in gene expression during the development of osteoarthritis in mice, Arthritis Rheum. 64 (2012) 705–717, https://doi.org/10.1002/art.33386.