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Review Article

Magnesium in joint health and osteoarthritis

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ABSTRACT

Osteoarthritis (OA) is a prevalent debilitating age-related skeletal disease. The hallmark of OA is the degradation of articular cartilage that cushions the joint during movement. It is characterized by chronic pain and disability. Magnesium, a critical trace element in the human body, plays a pivotal role in metabolism homeostasis and the energy balance. Humans obtain magnesium mainly from the diet. However, inadequate magnesium intake is not uncommon. Moreover, the magnesium status deteriorates with ageing. There has been a growing body of clinical studies pointing to an intimate relationship between dietary magnesium and OA although the conclusion remains controversial. As reported, the magnesium ion concentration is essential to determine cell fate. Firstly, the low-concentration magnesium ions induced human fibroblasts senescence. Magnesium supplementation was also able to mitigate chondrocyte apoptosis, and to facilitate chondrocyte proliferation and differentiation. In this literature review, we will outline the existing evidence in animals and humans. We will also discuss the controversies on plasma or intracellular level of magnesium as the indicator of magnesium status. In addition, we put forward the interplay between dietary magnesium intake and intestinal microbiome to modulate the inflammatory milieu in the conjecture of OA pathogenesis. This leads to an emerging hypothesis that the synergistic effect of magnesium and probiotics may open a new avenue for the prevention and treatment of OA.

Abbreviations: OA, osteoarthritis; ROS, reactive oxygen species; ACL, anterior cruciate ligament; BMLs, bone marrow lesions; ACLT, anterior cruciate ligament transection; PA imaging, photoacoustic imaging; MRI, magnetic resonance imaging; Mg, magnesium; T2DM, type 2 diabetes mellitus; MetS, metabolic syndrome; MSCs, mesenchymal stem cells; SnCs, senescence cells; MMP, matrix metalloprotease; SaβGal, senescence-associated beta-galactosidase; $MgSO_4$, magnesium sulfate; K-L, Kellgren-Lawrence; FFQ, Food Frequency Questionnaire; JSN, joint space narrowing; hs-CRP, high-sensitivity C-reactive protein; RBCs, red blood cells; MBCs, mononuclear blood cells; MgRBC, Mg concentration in red blood cells; iMg^{2+}, ionized active form of Mg^{2+}; mTOR, mechanistic target of rapamycin; JNK, c-Jun N-terminal kinases; ATP, adenosine triphosphate.

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1. Introduction

Osteoarthritis (OA) is a serious disease characterized by articular cartilage degradation and damages to the other joint tissues [1]. OA is one of the most rising disability-associated conditions, leading to poor quality of life in older adults [2]. Dietary nutrition can be used as an important non-pharmacological treatment for OA. A diet supplemented with vitamin D has a positive effect on the thickness of the joint cartilage and joint lubrication [3]. Olive oil reduces the release of pro-inflammatory cytokines and increases lubricin synthesis, suggesting a positive protective effect on the joints [4,5]. Vitamin E supplementation can significantly increase the level of circulating antioxidant enzymes and relieve the pain of knee OA [6]. Fat-soluble vitamin K can affect the mineralization of bones and cartilage, which is associated with OA [7]. Obesity induced by a high-fat and high-sugar diet can cause inflammation and promote the development of OA. Supplementing prebiotic fiber can prevent the increase of serum endotoxin and microbial dysbiosis, so that it can improve knee joint damage [8,9]. Increasing intake of dietary fiber can reduce the risk of OA as well [10].

Magnesium (Mg) is an important trace element. Since the human body cannot produce this mineral by itself, humans need to obtain Mg from their diet. Mg is predominantly obtained from the diet by consuming green leafy vegetables, unprocessed beans and grains. As the modern diet has drifted away from these food sources in favor of fine dining or nutrient-poor foods, inadequate Mg intake is common in developed western countries such as the United States and France [11,12]. The suboptimal Mg level further deteriorates with age [13]. An estimated 10% of older adults have a low plasma Mg level and 20% of them have a low concentration of erythrocyte Mg [14]. There are a few possible reasons for Mg deficiency in the elderly. First, the intestinal absorption of Mg decreases with age [15]. Second, Mg deficiency is often observed in patients with type 2 diabetes mellitus (T2DM) or those taking diuretics, the anti-hypertension medication [16]. Two conditions often occurring in the elderly. Finally, the Mg deficit is further intensified by an increased intake of calcium which is advised for osteoporosis prevention [17]. Low Mg, together with excessive calcium, predisposes an individual to cardiovascular diseases. Not surprisingly, there is a growing body of evidence to indicate a link between a Mg deficiency and a plethora of age-related diseases, including OA [18,19], osteoporosis [20], metabolic syndrome (MetS) [21,22], stroke, cognitive impairment [23] as well as hypertension and T2DM [16].

In this literature review, we aim to outline the existing evidence on the clinical and biological links between low dietary magnesium intake and OA and discuss potential interventions to address this challenge.

2. Searching strategy

The citations in this article were searched in PubMed and Google Scholar, using the search key words “Magnesium and Osteoarthritis,” “Magnesium and Mesenchymal Stem Cells," "Magnesium and Bone Cells," "Magnesium and chondrocyte," or "Magnesium and fibroblast." The search is not restricted by date, and all studies published before January 2020 are included. Total 2188 reference articles have been identified. After browsing, preliminary screening and re-screening, total 16 reference articles were finally selected for discussion.

2.1. *Magnesium storage and homeostasis*

Magnesium (Mg), a crucial micronutrient, plays a pivotal role in metabolism homeostasis and the energy balance in the human body [24]. The total amount of Mg in an adult human body is around 25 g, which is primarily used as an intracellular cofactor for the more than 300 ATP-dependent enzymatic activities. Moreover, the circadian rhythm of Mg^{2+} influx plays a role in controlling the daily metabolic activities at the cellular level [25]. In serum, around 30% Mg is bound to albumin and 55% is in the ionic form, Mg^{2+}. As a physiological calcium channel antagonist, Mg^{2+} is therefore essential for neuromuscular transmission and cardiovascular tone.

Mg plays an important role in bone health. The majority of Mg is distributed to bone (50%-60%) while the other 40% to 50% is found in muscles and other soft tissues and less than 2% is found in serum and red blood cells. One third of the Mg stored in bone can be used for exchange to maintain extracellular Mg levels [24,26].

2.2. *OA is a whole joint disease*

Traditionally, OA is considered a non-inflammatory joint disorder, in contrast to inflammatory arthritis such as rheumatoid arthritis. OA is simply regarded as a wear and tear problem of articular cartilage under abnormal mechanical loading [27]. Recently, the concept of OA is evolving with the advancement of multi-imaging modalities. It unveiled that OA is a whole joint disease involving not only the degradation of articular cartilage but also deformation of subchondral bone and low-grade inflammation of synovial tissues.

The hallmark of OA is the loss of articular cartilage, which cushions the joint during movement. Yet the homeostasis of articular cartilage relies on the underneath bone to provide mechanical support and nutritional supply [28]. Previous injuries, such as an anterior cruciate ligament (ACL) tear, form an important risk factor for knee OA; approximately 20% to 35% of knee OA patients are estimated to have had an incidental ACL injury [29,30]. In the situation of abnormal mechanical instability such as after ACL injury [31,32], subchondral bone exhibits edema-like changes under MRI, namely “bone marrow lesions” (BMLs) [33]. Cystic lesions develop in the regions of unresolved BMLs with cartilage loss in both human beings and animal models of posttraumatic OA induced by ACL transection (ACLT) [34,35].

Synovitis in arthritic joints is characterized by synovial effusion, angiogenesis, hypoxia and reactive oxygen species (ROS) generation [36]. Sixty-six percent of knee OA patients show synovial enhancement on gadolinium-Magnetic resonance imaging (MRI) [37]. MRI-detected effusion and synovitis correlate with pain and increased cartilage loss in knee OA patients [38]. Moreover, synovial hypoxia and ROS generation

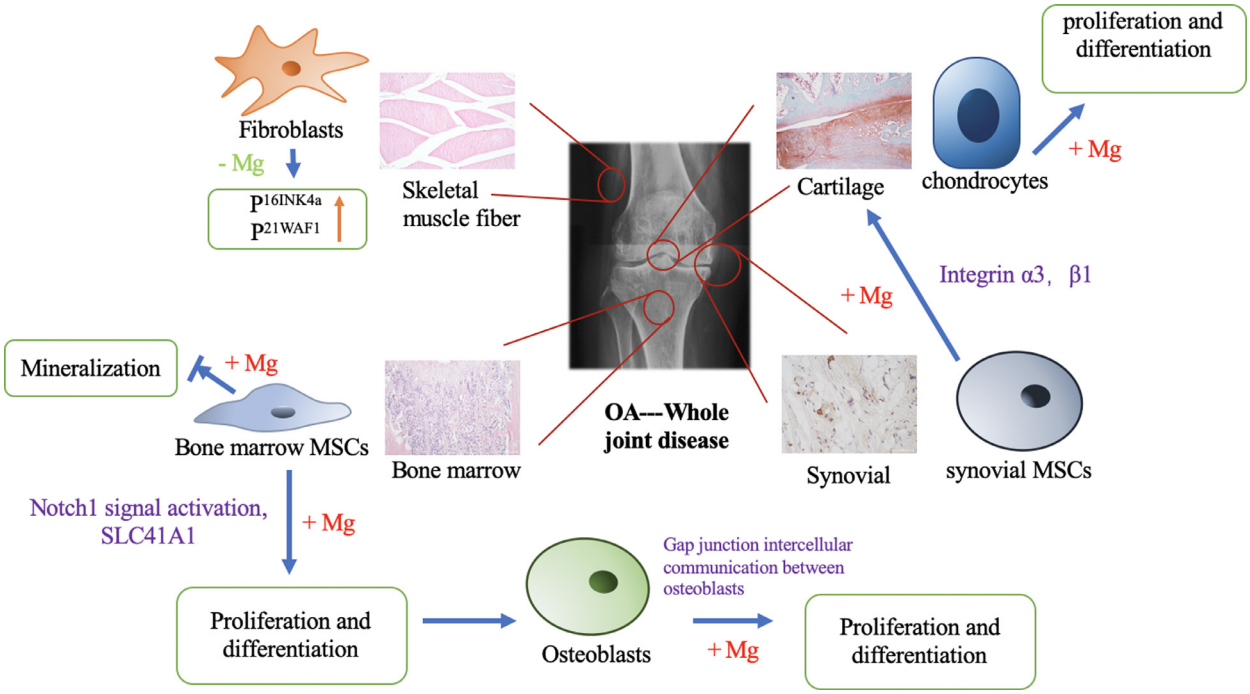

Fig. 1 – Proposed mechanism for magnesium in prevention and treatment of OA at molecular and cellular levels. OA is a whole joint disease. Mg enhances bone marrow MSCs proliferation through Notch 1 signal activation and induces differentiation of bone marrow MSCs through SLC41A1. High concentrations of extracellular Mg inhibit the mineralization process. High concentration of Mg extract promotes the proliferation and differentiation of osteoblasts and it induces osteoblast activity by enhancing gap junction intercellular communication to help bone formation. Mg enhances the proliferation and re-differentiation of chondrocytes. Mg promotes cartilaginous matrix assembly via enhancement of the adhesion of synovial MSCs, which is related to integrin α3 and β1. Mg deficiency causes cellular senescence of fibroblasts.

may induce oxidative damage to synovial tissue, mitochondrial mutagenesis and dysfunction.

In human OA cartilage lesions, senescence cells (SnCs) are detected near the cluster of chondrocytes [39] which exhibited the characteristics of progenitor cells with increased proliferation [40,41]. In response to altered mechanical loading [42,43] or oxidative stress [44], articular chondrocytes undergo premature senescence with shortening of telomeres, which provokes the onset of OA [45].

Overexpression of the senescence marker $p16^{Ink4a}$ was sufficient to induce two major cartilaginous-matrix remodeling enzymes: matrix metalloprotease (MMP)-1 and -13 [46]. In addition, the severity of OA was correlated with the senescence-associated beta-galactosidase (SAβGal) activity in articular chondrocytes close to the lesion while no staining of SAβGal was found in normal cartilage [47]. Very recent studies provided direct evidence that shows the involvement of SnCs in cartilage damage [45,48]. It was reported that the transplanting of SAβGal-positive SnCs into synovial joint led to an OA-like lesion in rodents [48]; and ablation of $p16^{Ink4a}$-positive SnCs using genetically modified mice model could mitigate OA [45]. Recently, $p16^{Ink4a}$-positive SnCs were identified in inflamed synovium [45] and aged bone microenvironment [49]. However, it is not yet fully understood how synovial or skeletal SnCs contribute to OA pathologies, further mechanistic studies to gain an overall picture of SnCs in the pathogenesis and management of OA.

3. Magnesium and OA: Evidence from cellular studies

3.1. *Magnesium and MSCs*

Mg is essential for MSCs interaction with extracellular matrix. Mesenchymal stem cells (MSCs) can divide multiple times, and their progeny can differentiate into skeletal tissues such as bone and cartilage [50]. As these tissues play a major role in OA, it is important to evaluate the effect of Mg on MSCs. Mg has been shown to enhance the adhesion of synovial MSCs and then promote cartilaginous matrix assembly (ref). The adhesion of human synovial MSCs to collagen-coated slides in the presence of magnesium showed that Mg can enhance the adhesion to collagen (ref). Moreover, this effect is inhibited by the neutralizing antibodies of integrin α3 and β1. This points to an important function of integrin in the adhesion process. Additionally, Mg promoted the synthesis of cartilage matrix during the chondrogenesis of the synovial MSCs *in vitro*, which was related to the neutralizing antibodies of integrin β1. Finally, an *in vitro* experiment revealed that Mg enhanced the adhesion of human synovial MSCs to osteochondral defects [51]. (Fig. 1, Table 1)

Mg also plays a role in MSCs proliferation and differentiation. For example,

It was found that high concentrations of extracellular Mg could inhibit the mineralization process during MSCs os-

Table 1 – Magnesium and OA in cellular studies

Authors	Year	Type of cells	Magnesium concentration	Measures	Key findings	remarks
Shimaya M, et al.	2010	Human synovial MSC	PBS with magnesium (0, 0.1, 0.8, 1, 5, and 10mM)	The number of cells attached to the collagen-coated glass slide and the defect.	Magnesium can enhance the adhesion of human synovial mesenchymal stem cells to collagen and it is inhibited by the neutralizing antibodies of integrin $\alpha 3$ and $\beta 1$. Magnesium can promote the synthesis of cartilage matrix in the process of chondrogenesis of synovial MSCs in vitro and enhance the adhesion of human synovial MSCs to osteochondral defects.	
Tsao Y T, et al.	2017	Mouse bone marrow-derived MSC, human MSC	Magnesium chloride (0.8mM, 5.8mM)	Osteogenic differentiation efficiency, cell morphology, and osteogenic marker gene expression	High concentration of extracellular magnesium can inhibit the mineralization during MSCs' osteogenic differentiation and the magnesium transporter SLC41A1 can regulate the interaction between magnesium and MSCs during osteogenic differentiation.	SiRNA transfection was used to knock down the magnesium transporter Slc41a1
Díaz-Tocados J M, et al.	2017	Rat bone marrow MSC	Magnesium chloride (0.8mM, 1.2nM, 1.8mM)	Alkaline phosphatase (ALP) activity, Matrix mineralization, osteogenic marker gene expression	Magnesium chloride can enhance MSC proliferation through Notch1 signal activation and induces osteogenic differentiation.	During differentiation, 2-APB was added to osteogenic medium containing 0.8 mM Mg 2+ to determine the effect of inhibiting the Mg 2+ channel TRPM7.
Wu L, et al.	2015	Human telomerase reverse transcriptase (hTERT) -transduced mesenchymal stem cells (SCP-1), peripheral blood mononuclear cells (PBMC)	Mg extract dilutions (0.93mM, 1.46mM, 3.50mM, 6.08mM, 10.13mM, 14.36mM and 26.67mM)	The mRNA expression of osteoblast and osteoclast specific genes, alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) activities	High concentration of magnesium extract can promote the proliferation and differentiation of osteoblasts, and monocytes co-cultured with osteoblasts show greater tolerance to higher Mg extract concentrations	
He L Y, et al.	2016	Human osteoblasts	Magnesium sulfate (1mM, 2mM and 3mM)	Osteoblast viability, function, osteocalcin levels, and alkali alkaline phosphate (ALP) activity and osteocalcin determinations, the ratios of fluorescence recovery (R)	Magnesium ions induce osteoblast activity by enhancing gap junction intercellular communication between osteoblasts, which can help bone formation. And this effect is proportional to the magnesium ion concentration and contact time.	Photobleached Fluorescence Recovery (FRAP) is used to quantitatively measure gap junction function in living cells.
Feyerabend F et al.	2006	Human articular chondrocytes (HACs)	Magnesium sulfate (0mM, 1mM, 2mM, 5mM, 10mM, 15mM, 20mM, 25mM and 30mM)	Cell number, chondrogenic markers, extracellular matrix (ECM) formation	The proliferation and redifferentiation of chondrocytes were enhanced in a dose-dependent manner, but excessive extracellular magnesium concentrations still inhibited. But cartilage formation is inhibited with increasing extracellular magnesium concentration.	Use three-phase system for cartilage tissue engineering: (1) proliferation in tissue culture flasks; (2) redifferentiation of chondrocytes in alginate, and (3) chondrogenesis in high-density pellets.
Killilea D W, et al.	2008	Fibroblasts IMR-90 cells	Magnesium-deficient DMEM with magnesium chloride (0.1mM, 0.4mM, 0.8mM)	Expression of senescence-associated biomarkers	Senescence-related β-galactosidase activity was increased in human fibroblasts cultured in magnesium deficiency conditions, and p16INK4a and p21WAF1 protein expressions also increased. Mg-deficient cell culture conditions also accelerate telomere attrition in human fibroblasts.	

teogenic differentiation. Moreover, this study revealed that the Mg transporter SLC41A1 could regulate osteogenic differentiation [52]. Magnesium chloride was used as a Mg supplement to treat rat bone marrow MSCs. It could enhance MSC proliferation through Notch1 signal activation and induces osteogenic differentiation [53]. In conclusion Mg has a significant effect on the proliferation and differentiation of MSCs. Moreover, high concentration of magnesium can promote the treatment effect of human synovial MSCs on osteochondral defects. (Fig. 1,Table 1)

3.2. *Magnesium and bone cells*

Mg can promote the proliferation and differentiation of osteoblasts, as well as can induce the activity of osteoblasts. Two important cell types are involved in bone remodeling: osteoblasts for bone-formation and osteoclasts for bone-resorption [54]. There is a fine balance between osteoblasts and osteoclasts and when the balance is disturbed, the bone structure will be affected. High extracellular Mg ion concentrations have a positive effect on osteoblasts. The effects of different concentrations of Mg extract on a co-culture of osteoblasts and osteoclasts were investigated. High concentration of Mg extract promoted the proliferation and differentiation of osteoblasts. Monocytes co-cultured with osteoblasts showed greater tolerance to higher Mg extract concentrations [55]. Mg ions induce osteoblast activity by enhancing gap junction intercellular communication between osteoblasts, which can help bone formation. This effect is proportional to the magnesium ion concentration and contact time [56]. (Fig. 1,Table 1)

3.3. *Magnesium and chondrocytes*

Magnesium is beneficial to the proliferation and redifferentiation of chondrocytes. Chondrocytes are the only cells found in healthy cartilage [57]. A 3-phase tissue engineering model was been used to investigate the effects of high extracellular Mg concentrations, in the form of Mg sulfate, on chondrocytes. After supplementation with Mg sulfate, the proliferation and re-differentiation of chondrocytes were enhanced in a dose-dependent manner. However, this effect has an upper limit as excessive extracellular Mg lead to inhibition. Moreover, it was observed that cartilage formation is inhibited with increasing extracellular Mg concentrations [58]. (Fig. 1,Table 1)

3.4. *Magnesium and fibroblasts*

Mg can also affect human fibroblasts, more specifically a Mg deficiency causes cellular senescence of human fibroblasts. IMR-90 human fibroblasts were cultured long-term in moderate magnesium deficiency conditions. This increased senescence-related β-galactosidase activity and p16INK4a and p21WAF1 protein expressions compared with the culture in standard media conditions. Moreover, Mg-deficient cell culture conditions also accelerated telomere attrition in human fibroblasts [59]. (Fig. 1,Table 1)

4. Magnesium and OA: evidence from animal studies

In animal models, injecting a magnesium ion solution directly in the OA joint can relieve pain and slow down cartilage lesions. Moreover, in animals Mg ions also promote the formation of chondrocytes from synovial mesenchymal stem cells. In a study, a rat model of osteoarthritis was established by injecting collagenase into the knees of Wistar rats. Then the knee joints were injected with magnesium sulfate ($MgSO_4$) while a control group was injected with physiological saline. The results showed that the degree of cartilage degeneration in OA rats treated with intramuscular injection of magnesium sulfate was significantly lower than that of OA rats injected with saline. After treatment with magnesium sulfate, mechanical allodynia and thermal hyperalgesia in OA rats were alleviated. Moreover, these experiments also showed that intramuscular injection with magnesium sulfate could reduce the apoptosis of chondrocytes in OA rats [60]. In the *in vivo* study with rabbits, osteochondral defects were surgically created in the trochlear grooves of the knees of the rabbits and then filled with a synovial MSC suspension with or without 5mM magnesium. The *in vivo* study showed that magnesium promoted adhesion of the MSCs day 1 after administration and stimulated cartilage formation in synovial MSCs 2 weeks after treatment [51]. (Table 2)

5. Magnesium and OA: evidence from human studies

5.1. *Circulating magnesium and OA*

The serum magnesium concentration is inversely proportional to OA. A study showed that patients with severe osteoarthritis had significantly lower serum magnesium levels than patients with mild osteoarthritis, but there was no association between serum magnesium concentration and the two inflammatory biomarkers [61]. Multivariable logistic analysis was used in a study to illustrate the association between serum magnesium and radiographic knee OA in 2855 patients. It was concluded that the serum Mg concentration may have an inverse relationship with knee radiographic OA [62]. (Table 3)

5.2. *Dietary magnesium intake and OA*

There were some cohort studies showing a relationship between dietary magnesium and OA. Most of these studies defined knee radiographic OA as Kellgren-Lawrence (K-L) grade 2 in at least one knee and used the Food Frequency Questionnaire to assess Mg intake. It was showed that lower magnesium intake was associated with increased pain and worsened function in patients with knee OA. Patients with lower fiber and lower magnesium intake performed more significantly [19]. A population-based study in the United States found that the relationship between magnesium intake and radiographic knee OA was different between African Americans and Caucasians. In the Caucasian population, there was a moderate

Table 2 – Magnesium and OA in animal studies

Authors	Year	Animal model	Dosage of magnesium	Treament approach of magnesium	Key findings	Remarks
Lee CH, et al.	2009	Wistar rats	Magnesium sulfate (500 μg) twice a week for 5 consecutive weeks	Intra-articular injection	Intramuscular injection of magnesium sulfate can significantly reduce the degree of cartilage degeneration in osteoarthritis rats, as well as alleviate mechanical allodynia and thermal hyperalgesia, and reduce chondrocyte apoptosis.	Knee OA was induced by injecting collagenase.
Shimaya M, et al.	2010	Rabbits	PBS with 5 mM magnesium (unspecified)	Surgical filling of magnesium-containing cell suspensions	Magnesium promoted adhesion at 1 day and promoted cartilage formation in synovial MSCs at 2 weeks.	Osteochondral defects were formed in the trough of the rabbit knee

inverse threshold correlation between dietary magnesium intake and knee OA. Mg intake above the threshold did not have more benefit for OA. In the African American population, no statistically significant association was found between Mg intake and radiographic knee OA. The relationship between dietary Mg intake and knee OA might vary by race [63]. A study conducted in the Chinese population reported a negative correlation between magnesium intake and radiographic knee OA and joint space narrowing. The result suggests that Mg has a potential role in preventing knee osteoarthritis [64]. In 2017, a study analyzed the data of 936 early radiographic knee OA patients in China, and then proposed that the dietary Mg and serum Mg of early radiographic knee OA patient were inversely associated with serum high-sensitivity C-reactive protein (hs-CRP) level [65]. However, It was found that although Mg intake was inversely associated with serum hs-CRP level in Finland in 2019, the result could not explain how low Mg intake could be beneficial for the development of knee osteoarthritis [66]. (Table 3)

6. Knowledge gaps to be filled

6.1. *Lack of enough information for a normative range of magnesium level*

An estimated 50% of Americans have inadequate Mg intake (What we eat in America, NHANES 2005-2006), with approximately 19.2% to 37% of the adults, age 45 or above, having radiographic knee OA [67]. Clinically, hypomagnesemia or hypermagnesemia is diagnosed based on the serum Mg level. Due to the important physiological function of Mg, the serum Mg level is tightly controlled by balancing intestinal absorption and urinary excretion. Therefore, the serum Mg level cannot reflect the Mg intake level. Furthermore, 99% of Mg locates intracellularly for a variety of biochemical reactions. It is doubtful that measuring the serum Mg, which is less than 1% of total Mg, will give an indication of the total body Mg status. This forms the base for the long-time debate on the necessity to monitor the Mg level of the elderly patients in routine clinical practice [68]. Currently, Mg is not included in the routine electrolyte examination unless on special request.

To our knowledge, a well-received normative range of serum Mg for healthy adults at the age of 18-74 years old (0.75-0.96 mmol/L, equivalent to 1.8-2.3 mg/dL) was based on a United States nationwide nutrition survey conducted in the 1970s [69]. A reference range of serum ionized Mg^{2+} (0.54~0.67 mmol/L) has also been documented [70]. Compared with the United States data, the serum Mg level was relatively higher in the Chinese population [0.92 $\pm$ 0.07 mmol/L from a study in Shanghai (n = 1170)], particularly for the northern Chinese [3.5 $\pm$ 0.5 mg/dL from a study in Jinlin province (n = 50)]. Such large ethnic variation warrants a large-scale community-based study on a normative range of circulating Mg level for southern Chinese.

6.2. *Challenges in measuring magnesium status*

An alternative way to quantify Mg is to measure the concentration in tissue (bones and muscles) and in liquid biopsies, which is assumed to better reflect the total body Mg status [71,72]. Bone and muscle tissue biopsies are invasive and traumatic; liquid biopsies on red blood cells (RBCs) and mononuclear blood cells (MBCs) are much more favorable. Mg concentration in red blood cells (MgRBC) was 2.34±0.27 mmol/L in randomly selected elderly Americans between the age of 65 and 74 (n = 381) [14]. By contrast, a relatively lower erythrocyte Mg content was found in the non-hypertensive and non-diabetic northern Chinese at the age of 64.0±4.8 years old (2.0±0.7 mmol/L, n = 142) [73]. Moreover, the ionized active form of Mg^{2+} (iMg^{2+}) would be an ideal biomarker to reflect functional Mg deficiency [13], and to better correlate the Mg concentration with the clinical outcome [16]. This prompts

Table 3 – Serum/dietary Magnesium and OA in humans

Authors	Year	Study design	No. of subjects	Population	Type of OA	Magnesium level	Outcomes	remarks
Zeng C et al.	2015	Cross-sectional study	2855	Chinese	Radiographic knee OA	Serum level (chemiluminescence method)	Serum Mg concentration may have an inverse relationship with radiographic OA of the knee.	
Ike Coşkun Benlidayı et al.	2017	Retrospective study	75	Turk	Radiographic knee OA	Serum level (chemiluminescence method)	Patients with severe osteoarthritis had significantly lower serum magnesium levels than patients with mild osteoarthritis, but there was no association between serum magnesium concentration and the two inflammatory biomarkers, CRP and ESR.	
Shmagel A, et al.	2018	Cohort study	2548	existing data from the Osteoarthritis Initiative	Radiographic knee OA	Dietary level (Block Brief 2000 food frequency questionnaire)	Low magnesium intake can aggravate knee OA pain and worsen function, especially in people with lower fiber intake.	Outcomes included self-reported annual WOMAC, and its pain and function subscales, as well as KOOS
Qin B et al.	2013	Cohort study	2112	African American and Caucasian men and women	Radiographic knee OA	Dietary level (the National Cancer Institute block food frequency questionnaire)	The relationship between magnesium intake and radiographic knee OA varies by race. In Caucasians, there is a moderate inverse threshold association between magnesium intake and knee OA risk, but this association has not occurred in African Americans.	The multivariate logistic regression model using standard energy adjustment methods was used to estimate the relationship between magnesium intake and radiographic knee osteoarthritis.
Zeng C et al.	2015	Cross-sectional study	1626	Chinese	Radiographic knee OA	Dietary level (semi-quantitative food frequency questionnaire)	Magnesium has a potential role in preventing osteoarthritis of the knee, because the magnesium intake is inversely related to radiographic knee OA and JSN.	use multivariate logistic analysis models to test various associations.
Li H et al.	2017	Cross-sectional study	936	Chinese	Radiographic knee OA	Dietary level (block food frequency questionnaire)	In early radiographic knee OA patients, both dietary and serum Mg were inversely associated with serum hsCRP.	multivariable logistic regression was used to test the associations of dietary and serum Mg with the serum hsCRP in early radiographic knee OA patients.
Konstari S et al.	2019	Cohort study	4953	Finns	Diagnosis of knee OA in hospital	Dietary level (a validated self-administered food frequency questionnaire)	The results showed that magnesium intake is inversely associated with serum hs-CRP levels, but it couldn't prove that low magnesium intake was helpful for the development of clinical knee OA.	Cox proportional hazards model was used to estimate the strength of the association between dietary magnesium intake and the incidence of knee OA after adjusting for the potential influencing factors.

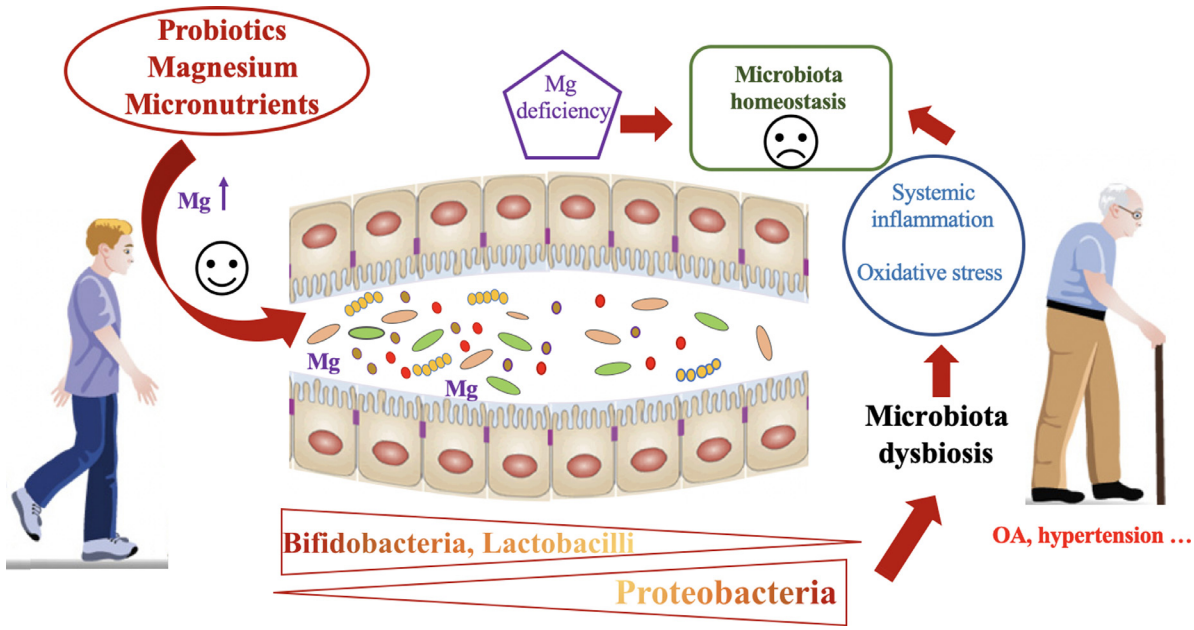

Fig. 2 – Proposed mechanisms of prebiotic Mg micronutrients on the interaction between gut microbes and the host in ageing and age-related disorders such as OA. With aging, the abundance of beneficial bacteria decreases which leads to microbiota dysbiosis. The inflammatory milieu in OA might interplay with the gut microbiome. Mg deficiency also affects the gut microbiota homeostasis. Probiotics Mg micronutrients can not only regulate the gut microbiome, but also supplement Mg. Mg supplementation is beneficial to regulate the gut microbiome and restore the absorption and deposition of dietary Mg, potentially prevent and treat OA.

the need to redefine subclinical Mg deficiency using iMg^{2+} in MBCs and/or RBCs (iMg^{2+}MBCs, iMg^{2+}RBCs)

6.3. *Poor understanding of magnesium homeostasis in the gut microbiota-host interaction*

6.3.1. *Effects of low magnesium on the gut microbiota*

The gut microbiome is emerging as a major determinant of the wellbeing of its host, affecting both ageing and diseases [74,75]. It has been known that the loss of microbial diversity is associated with increased frailty in the elderly [76]. The latest studies have shown that alterations in gut microbiota can elicit the onset of hypertension [77], obesity and OA [78], and alter neurobehavioral functions in animals [79]. Gut microbiota affects ageing through several established longevity pathways such as mechanistic target of rapamycin (mTOR), c-Jun N-terminal kinases (JNK), and insulin/IGF signaling as well as through caloric restriction. Very recently, researchers have identified a novel mitochondrial pathway independent of all the above pathways. Gut microbes send signals to the host mitochondria by suppressing the production of polysaccharide colonic acid that promotes mitochondrial fission and enhances the mitochondrial unfolded protein response to stress, through which the microbes can regulate the lifespan of their host [80]. In short, factors that regulate the host-gut microbiota interaction in mitochondrial dynamics would be candidate therapeutic targets to extend human lifespan and health span.

Aging, gender, obesity and nutrition are risk factors of osteoarthritis and enteric malnutrition. Changes in gut microbiome may also be a trigger for the onset of osteoarthritis [81]. Environmental factors, nutrition and lifestyle have a significant impact on gut microbiota [82]. A low Mg diet results in a reduction of intestinal *Bifidobacteria* [83], which are believed to contribute to systemic inflammation in obesity and the onset of OA [78]. Yet effects of Mg supplementation on the host-gut microbiota interactions in ageing and age-related pathologies remain largely unknown.

6.3.2. *Effects of low magnesium on the host*

Intracellular Mg^{2+}, mainly located in mitochondria - energy-producing organelles-, is responsible for oxidative phosphorylation, for adenosine triphosphate (ATP) production and activation [84]. Disruption of the Mg homeostasis increases oxidative stress and induces mitochondrial dysregulation [85], and ultimately triggers cellular senescence [59]. It is known that a low Mg concentration accelerates senescence of human endothelial cells and fibroblasts in culture. Moreover, a low Mg diet aggravates elevated blood pressure and increases the risk of cardiovascular events *in vivo* [59,86]. In addition to endothelial cells and fibroblasts, it remains unknown whether low Mg will accelerate articular chondrocytes senescence and trigger the development of OA; on the other hand, Mg supplementation can remove ageing chondrocytes and mitigate OA.

7. Perspectives

The concentration of the extracellular Mg ion affects cells that are related to articular joints such as mesenchymal stem cells, osteoblasts, chondrocytes and human fibroblasts. However, the association of dietary intake and serum levels of Mg

with the risk of knee osteoarthritis remains controversial. Intake of high level of Mg has been associated with low risk of osteoporotic fracture [87], yet it is not associated with low risk of radiographic knee OA in the older adults [88]. We postulate that the inflammatory milieu in arthritic condition might interplay with the gut microbiome. Among gut microbiota, the relative abundance of the beneficial bacteria, such as *Bifidobacterium* species from the Actinobacteria phylum and *Lactobacillus* species from the Firmicutes phylum, has been shown to decrease in frail older individuals. The commercial products containing *Lactobacillus* species are quite substantial but those containing the obligate anaerobe *Bifidobacterium* species are very limited as they are difficult to stay alive in the products. Mg deficiency appears to reduce the abundance of *Bifidobacterium* species, which contributes to low-grade systemic inflammation, obesity and OA [78,83]. It is reported that the Mg requirements of Gram-positive bacteria are much higher than those of Gram-negative bacteria. Maintaining a healthy intestinal flora may be a potential prevention and treatment method for OA. We propose the hypothesis that a low-Mg diet may affect the composition of intestinal microbes, as well as that the probiotics or prebiotics could be a potential therapeutic strategy to restore the absorption and deposition of dietary Mg and potentially reduce cellular oxidative stress and senescence in joint degeneration and OA. Therefore, the combined effect of appropriate dietary Mg intake and healthy intestinal environment may be beneficial to the prevention and treatment of OA. (Fig. 2) The above conjecture needs further study to confirm.

Author contributions

KXQ, KL, JC and CYW conceived this review. KXQ and CYW conducted literature search, systemic review and analyses. KXQ and CYW prepared the draft of the manuscript, which was revised by KL and JC. All authors have read and approved the final version of the manuscript.

Declaration of Competing Interest

None to declare.

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