

# The influence of zinc levels on osteoarthritis: A comprehensive review

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## Abstract

Osteoarthritis (OA), a disease with a multifactorial aetiology and an enigmatic root cause, affects the quality of life of many elderly patients. Even though there are certain medications utilised to reduce the symptomatic effects, a reliable treatment method to reverse the disease is yet to be discovered. Zinc is a cofactor of over 3000 proteins and is the only metal found in all six classes of enzymes. We explored zinc's effect on the immune system and the bones as OA affects both. We also discussed zinc-dependent enzymes, highlighting their significant role in the disease's pathogenesis. It is important to note that both excessive and deficient zinc levels can negatively affect bone health and immune function, thereby exacerbating OA. The purpose of this review is to offer a better understanding of zinc's impact on OA pathogenesis and to provide clarity regarding its beneficial and detrimental outcomes. We searched thoroughly systematic reviews, meta-analysis, review articles, research articles and randomised controlled trials to ensure a comprehensive review. In brief, using zinc supplementation in the treatment of OA may act as a doubled-edged sword, offering potential benefits but also posing risks.

**Key words:** bone; enzymes; immunity; osteoarthritis; pathophysiology; zinc

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## Introduction

Osteoarthritis (OA) is a chronic and debilitating condition that primarily impacts the articular cartilage and is more common in older adults, with increased risk associated with comorbidities or obesity<sup>(1,2)</sup>. It is of much importance to treat OA as it results in deteriorated quality of life and shortens lifespan, impacting the individual's socio-economic level highly<sup>(3)</sup>. OA is generally categorised into primary (idiopathic) and secondary types based on its aetiology or cause. It can also be categorised as generalised, which affects three or more joints, and localised, which affects one or two joints. Female sex, obesity, high-impact sports, weight lifting and, most importantly, aging are among the risk factors for OA<sup>(4)</sup>. The start and progression of this disease have been related to many hereditary and environmental factors. Even though many of them are hard to modify, certain risk factors, such as nutrition, may be more amenable to behavioural and medicinal interventions than others. When Zhuo *et al.* explored the correlation between OA and different blood levels

of five different minerals, they discovered that high zinc status, which was genetically predicted, was linked to OA<sup>(5)</sup>. The pathophysiology of OA is somewhat complex. Previously, scientists believed this disease develops from a solely mechanical 'wear and tear' condition. However, this postulation is refuted by modern literature owing to the overabundance of substantiated research demonstrating inflammation and immune responses as the causal factor. Additionally, scholars have introduced a condition known as 'inflammaging' which is characterised as the gradual inflammation state devoid of infection manifesting in the course of aging. Inflammaging entails innate and adaptive immune responses similar to what is observed in OA.

The human cartilage continuously undergoes remodelling throughout life, and some of the critical mechanisms involved in OA comprise pro-inflammatory (IL-6, IL-8, IL-1 $\beta$  and TNF- $\alpha$ ) cytokines and pro-catabolic pathways such as nuclear factor  $\kappa$ B (NF- $\kappa$ B) and mitogen-activated protein (MAP) kinase signalling

**Abbreviations:** ADAMTS, A disintegrin and metalloproteinase with thrombospondin motifs; BMP, Bone morphogenic protein; CIA, Collagen-induced arthritis; CMC, Carpometacarpal; DIP, Distal interphalangeal; Glis3, Gli-similar 3; GLUT, Glucose transporter; GPx1, Glutathione peroxidase 1; HIV, Human immunodeficiency virus; HK2, Hexokinase-2; IPFP, Infrapatellar fat pad; MAP, Mitogen-activated protein; MIA, Monosodium iodoacetate; MMP, Matrix metalloproteinase; MTF, Metal responsive transcription factor; NADPH, Nicotinamide adenine dinucleotide phosphate; NF- $\kappa$ B, Nuclear factor kappa B; Nrf2, Nuclear factor erythroid 2-related factor 2; OA, Osteoarthritis; Osr2, Odd-skipped related 2; PDH-E1 $\alpha$ , Pyruvate dehydrogenase- E1 $\alpha$ ; PHA, Phytohaemagglutinin; PKR, Protein kinase R; PMA, Phorbol myristate acetate; PMN, Polymorphonuclear neutrophil; PTOA, Post-traumatic osteoarthritis; ROS, Reactive oxygen species; Runx, Runt-related transcription factor; SLC, Solute carrier family; SOD, Superoxide dismutase; TLR, Toll-like receptor; TRAP, Tartrate-resistant acid phosphatase; Treg, Regulatory T cells; ZIP, Zrt-/Irt-like proteins; ZnT, Zn transporter.

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responses<sup>(6)</sup>. According to reports, the accumulation of reactive oxygen species (ROS) due to age-related oxidative stress and a disruption in energy metabolism as a consequence of impaired mitochondrial function are primary culprits in OA<sup>(7)</sup>. Each of these mechanisms promotes the apoptosis of chondrocytes, giving rise to articular cartilage degradation<sup>(8)</sup>. This can lead to high IL-1 levels, which results in inflammation in the area, provoking the degradation of the cartilage and eventually the bone underneath by attracting inflammatory cells such as macrophages and lymphocytes<sup>(9)</sup>. Elevated IL-1 induces the expression of a crucial enzyme in osteoarthritis progression matrix metalloproteinase (MMP)-13 in chondrocytes<sup>(10)</sup>. Two primary origins of ATP in a cell are cytosolic glycolysis and mitochondrial oxidative phosphorylation, and the trace element zinc up-regulates the expression of proteins involved in these processes<sup>(11)</sup>. Likewise, zinc exerts a substantial influence on most of the pathophysiological processes mentioned above.

The fact that obesity provokes OA in weight-bearing joints is reasonable, but the incidence also increases in non-load-bearing joints such as the carpometacarpal (CMC) and distal interphalangeal (DIP) joints<sup>(12)</sup>. Such manifestation prompts us to consider systemic factors that induce OA such as adipokines released by other cells<sup>(13)</sup>. Moreover, cellular cross-talk between local adipose tissue and other synovial tissue affects OA development<sup>(14)</sup>.

Due to ethical constraints, conducting experiments on humans for OA research is challenging. Therefore, scientists have developed numerous successful models to facilitate research. OA models, both *in vitro* and *in vivo*, have been proposed for use in clinical trials to investigate primary and secondary OA. Examples of primary OA models include naturally occurring and genetically modified models, whereas post-traumatic OA (PTOA) is the most frequently investigated model of secondary OA<sup>(15)</sup>. PTOA can be further classified into invasive and non-invasive models. Invasive models include those created surgically or chemically, whereas non-invasive secondary OA models include those created by cyclic articular cartilage tibial compression and anterior cruciate ligament rupture via tibial compression overload<sup>(16)</sup>. For a more in-depth understanding, please refer to the comprehensive review by Samvelyan *et al.*<sup>(15)</sup>.

Intra-articular injections of monosodium iodoacetate (MIA), papain, quinolone and collagenase are examples of the relatively less invasive chemically induced OA model. Among their advantages are the avoidance of potential infectious problems and the elimination of the necessity for surgery<sup>(16)</sup>. To our knowledge, the majority of research examining the impact of zinc on OA has been conducted using MIA; nonetheless, investigations utilising alternative OA models are still needed for additional clarification.

Intra-articular MIA injection, the most widely reported chemically induced method<sup>(16)</sup>, is used to mimic articular cartilage changes seen in human OA<sup>(17)</sup>. MIA increases ROS, IL-1 $\beta$  and MMP-13 production, whose levels are reduced by zinc<sup>(18)</sup>. Moreover, MIA was shown to reduce ATP levels in cells by down-regulating the expression of proteins associated with glycolysis such as HK2, GLUT1, PDH-E1 $\alpha$  and mitochondrial complex I, II, IV and V subunits of the mitochondrial oxidative

phosphorylation system, and zinc supplementation reverses these changes. Recent studies have clearly indicated that zinc prevents MIA-induced alterations in the cartilage. Studies have also linked zinc with chondrocyte survival; meanwhile, zinc deficiency could restrict chondrocyte growth<sup>(19)</sup>. Additionally, zinc can inhibit the initiation of lipid oxidation by impeding the interactions with redox-active metals such as iron and copper<sup>(20)</sup>. Apart from its antioxidant properties, zinc also functions as an anti-inflammatory agent<sup>(21)</sup>. In contrast to all the beneficial effects of zinc mentioned above, many researchers have also evidenced deleterious outcomes of zinc that participate in articular cartilage degeneration. Zinc is a cofactor and a vital constituent for the function of many enzymes and proteins in the human body<sup>(22)</sup>. Research has provided evidence that zinc can up-regulate enzymes in charge of cartilage breakdown. In patients with OA, zinc-dependent matrix-degrading enzymes such as MMP and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) contribute to the degradation of the extracellular matrix cartilage<sup>(21)</sup>. Zinc relies on a transporter to facilitate its passage through the cell membrane, as it cannot freely traverse by itself. In response to high IL-1 $\beta$  levels seen in OA, among many other importers, the zinc carrier protein ZIP8 levels increase in chondrocytes<sup>(23,24)</sup>. This protein's expression is up-regulated in pro-inflammatory conditions and elevates intracellular zinc levels by carrying it from the extracellular environment or the organelles inside the cells to the cytoplasm<sup>(25)</sup>. As a result, zinc-dependent enzymes inside the cells such as MMPs which are related to OA are augmented<sup>(26)</sup>. Kim J. H. *et al.* revealed *in vivo* that, of the many importer-mediated zinc influx, ZIP-8-mediated zinc influx in particular stimulates a wide range of transcription factors, including NF- $\kappa$ B, MTF1, NRF1/NRF2, AP1, SP1 and p53. Their research revealed that MTF1 activation in chondrocytes is enough to trigger the expression of MMP and ADAMTS enzymes. To support their findings, they also showed that zinc influx, among other metals transported by ZIP8 such as iron, manganese and cadmium, is the crucial event that activates catabolic pathways in OA chondrocytes. In line with this, they demonstrated in mice that genetic ZIP8 deletion prevents OA pathogenesis while ZIP8 overexpression in cartilage tissue induces OA pathogenesis<sup>(24)</sup>.

We discussed the significance of zinc on bone health and the immune system in this review, outlining the critical pathways interconnected with OA pathology. We also elucidated the cardinal zinc-dependent enzymes contributing to cartilage degeneration. The purpose of this review is to offer a better understanding of zinc's impact on OA pathogenesis and to provide clarity regarding its beneficial and detrimental outcomes.

### The impact of zinc on bone health

Zinc is a key cofactor of numerous enzymes and proteins within the human body, and its level declines with age<sup>(27)</sup>. The vast majority of this element is bound to and is a potent inducer of metallothionein in the intracellular compartment and is bound to proteins such as albumin, transferrin and  $\alpha$ 2 macroglobulin in the plasma<sup>(28)</sup>. Zaichick and Zaichick claimed that the concentration of zinc was slightly higher in patients under the age of 35

compared with patients over the age of 55<sup>(29)</sup>. It is involved in many important pathways including DNA synthesis<sup>(30)</sup>, RNA transcription<sup>(31)</sup>, oxidative stress<sup>(32)</sup> and immune responses<sup>(33)</sup>. The bone accounts for about 30% of the body's overall zinc content<sup>(34)</sup>. Zinc improves the growth of the cartilage and has the ability to promote the differentiation of mesenchymal stem cells into chondrocytes<sup>(35)</sup>. An *in vitro* study conducted by Rodriguez and Rossilot revealed that low levels of zinc intake can augment chondrocyte proliferation by 40–50%<sup>(36)</sup>. Zinc is also accountable for the attachment of plenty of transcriptive factors to the DNA by forming molecules known as Zn-finger proteins<sup>(37)</sup>. It also protects the human body from ROS as the enzyme peroxide dismutase requires zinc<sup>(38)</sup>. Scientists agree that zinc is of high importance in the osteogenesis process and for the growth and quality of the bone. Ossein, the organic extracellular matrix (ECM) of the bone, requires zinc as a structural element<sup>(39)</sup>. Zinc deficiency can lead to significant bone issues, including reduced bone mass and increased brittleness. *In vivo* and *in vitro* studies have highlighted zinc's role in enhancing bone growth by promoting osteoblast proliferation, differentiation and collagen synthesis. Adequate zinc levels are also necessary for inhibiting osteoclast differentiation and preventing bone resorption. Despite its benefits, excessive zinc can harm osteoblastic and osteoclastic cells. Preserving bone health in OA is paramount, as deteriorating bone integrity can worsen the disease.

Table 1 summarises pre-clinical studies demonstrating the effects of different concentrations of zinc on bone tissue and bone cells in various study methods.

#### *The effects of zinc deficiency on bone health*

Zinc deficiency augments bone resorption and can prompt thin and brittle bones due to the gradual reduction in bone density<sup>(40)</sup>. About 30% of zinc is found in bones, meaning that most of the body's zinc is stored in the skeleton. Zinc-deficient bones are prone to abnormal development.

#### *In vivo studies showing the effects of zinc deficiency on bone health*

Research on male Sprague-Dawley rats showed that, in comparison with the control group (60 mg zinc/kg), rats fed with a low-zinc diet (0.76 mg zinc/kg) for 42 d showed a decrease in bone mass, reduced growth and decreased body weight<sup>(41)</sup>. One of the reasons zinc deficiencies lead to these results is that zinc stimulates the release and enhances the functionality of IGF-1 and growth hormone in bony tissue<sup>(42)</sup>. This suggestion confirms the symptoms including agenesis of long bones, micrognathia, ossification abnormalities, bending of long bones, and abnormal rib and vertebra development observed in patients with low zinc concentration. Studies have also indicated a correlation between zinc deficiency and dwarfism<sup>(43)</sup>.

Zinc activates aminoacyl-tRNA synthetase in osteoblasts and affects osteoblast differentiation by Zn-finger transcription factors, Gli-similar 3 (Glis3) and Odd-skipped related 2 (Osr2)<sup>(44)</sup>. This metal also enhances bone growth by stimulating osteoblast proliferation and differentiation, and through activating the enzyme alkaline phosphatase and stimulating collagen synthesis, zinc ameliorates osteoblastic bone mineralisation<sup>(45)</sup>.

Dietary zinc deficiency has negative impacts on skeletal metabolism. Rossi *et al.* conducted a study where Sprague-Dawley rats were given either a zinc-deficient diet (1 mg zinc/kg) or a normal diet (50 mg zinc/kg) for 28 d. The zinc-deficient rats exhibited a significantly slower weight growth rate ( $0.9 \pm 0.3$  g/d) compared with the rats on the normal diet ( $8.0 \pm 0.5$  g/d) and showed noticeably greater humerus elasticity. Furthermore, IGF-1 levels were notably lower in zinc-deficient rats. The bone trabeculae and bone volume were also markedly reduced in the zinc-deficient rats in comparison with the normal group<sup>(46)</sup>.

In another animal study involving female Wistar/ST rats, the number of osteoclasts in the distal femur's growth plate decreased by 50% after 3 weeks of a zinc-free diet. Osteoblast counts also decreased by 70%. Alkaline phosphatase (ALP) activity in the distal femur and serum osteocalcin levels were significantly reduced in the zinc-deficient group, reaching approximately 50% of the control values. Additionally, tartrate-resistant acid phosphatase (TRAP) and cathepsin K activities, along with serum levels of CTx-1, dropped to approximately 65%, 50% and 60% of the control levels, respectively. These results indicate that both osteoclastogenesis and osteoblastogenesis are impaired during zinc deficiency<sup>(47)</sup>.

*In vitro studies showing the effects of zinc deficiency on bone health.* Zinc deficiency ( $1 \mu\text{M}$  of  $\text{ZnCl}_2$ ) inhibited the expression of BMP-2 and its downstream regulator Smad-1 in an *in vitro* study using MC3T3-E1 cells. This down-regulation decreased Runx2 and osterix, two crucial bone-specific transcription factors, which in turn reduced expression and synthesis of bone marker proteins such as ALP, osteopontin, osteocalcin and COL-1. The results indicate that osteoblast development may be impeded by low zinc levels<sup>(48)</sup>.

Another study demonstrated that the apoptosis rate of the MC3T3-E1 cells was 7% in zinc-adequate medium ( $5 \mu\text{M}$  tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN) +  $15 \mu\text{M}$  zinc), 80% in zinc-deficient ( $5 \mu\text{M}$  TPEN +  $1 \mu\text{M}$  zinc) medium and 90% in zinc-absent ( $5 \mu\text{M}$  TPEN only) media<sup>(49)</sup>. Scientists assumed that the reason for the increase in apoptosis rates was the increased level of cytochrome C in the cytoplasm of the osteoblasts which consequently activates the mitochondrial (intrinsic) apoptotic pathway.

#### *The effects of excessive zinc on bone health*

Nevertheless, even though zinc enhances osteoblastic proliferation, intracellular zinc overload can also have deleterious effects on the osteoblastic cells by inhibiting their osteogenic activity and mineralisation, which will eventually damage the tissue<sup>(50)</sup>. Likewise, zinc affects osteoclast differentiation.

*In vivo studies showing the effects of excessive zinc on bone health.* Some researchers also indicate that zinc suppresses osteoclast differentiation and therefore inhibits bone resorption. Hie and Tsukamoto observed that giving zinc-supplemented water containing 75 mg Zn per litre of zinc acetate to female Wistar/ST strain rats for 1 week reduced osteoclastogenesis. This reduction occurred by suppressing ROS production, extracellular signal-regulated kinase activation and receptor activator of NF- $\kappa$ B

**Table 1.** Pre-clinical research

	Study method	Treatment	Effect	Reference
<b>Zinc deficiency on bone health</b>	<i>In vivo</i> (male Sprague-Dawley rats)	Rats consumed a diet containing 0.76 mg/kg of zinc for 42 d.	A decrease in bone mass, reduced growth, and decreased body weight was observed.	(41)
	<i>In vivo</i> (male Sprague-Dawley rats)	Rats either fed a zinc-deficient diet (1 mg zinc/kg) or a regular diet (50 mg zinc/kg) for 28 d.	The weight growth rate, IGF-1 levels and bone trabeculae volume were significantly lower. In contrast, the elasticity of the humeri bone was grater.	(46)
	<i>In vivo</i> (female rats of the Wistar/ST strain)	The rats were subjected to a zinc-free diet for a period of 3 weeks.	The activity of ALP, TRAP and cathepsin K significantly decreased. The number of osteoclasts and osteoblasts in the growth plate of the distal femur were reduced by 50% and 70%, respectively.	(45)
	<i>In vitro</i> (MC3T3-E1 cells)	ZnCl <sub>2</sub> at a concentration of 1 µM was added to the culture medium.	Reduced BMP-2 expression resulting in poor osteoblast differentiation.	(48)
	<i>In vitro</i> (MC3T3-E1 cells)	Zinc-deficient group was incubated in a medium containing 5 µM TPEN + 1 µM zinc.	Apoptosis was effectively induced in osteoblastic cells.	(49)
<b>Excessive zinc on bone health</b>	<i>In vivo</i> (female rats of the Wistar/ST strain)	Water containing 75 mg Zn per litre of zinc acetate was given to the rats for 1 week.	In rats given zinc supplementation, osteoclast numbers decreased to approximately 64% of the control, while osteoblast numbers showed no change.	(51)
	<i>In vitro</i> (mouse marrow cells)	10 <sup>-6</sup> to 10 <sup>-4</sup> M of zinc sulphate was included in a culture with factors that stimulate osteoclast formation.	The formation of osteoclast-like cells induced by RANKL was markedly suppressed.	(52)
	<i>In vitro</i> (SaOS-2 human osteoblast-like cells)	Cells in the culture medium were exposed to different concentrations of zinc (0, 1, 10, 25 and 50 µM).	ALP activity was significantly enhanced with 1 µM or 10 µM zinc treatment, but notably reduced with 50 µM zinc compared with zinc-free cells.	(53)

IGF-1, insulin-like growth factor; ALP, alkaline phosphatase; TRAP, tartrate-resistant acid phosphatase; BMP-2, bone morphogenic protein-2; TPEN, tetrakis-(2-pyridylmethyl) ethylenediamine; RANKL, receptor activator of nuclear factor kappa B ligand.

expression. Approximately 64% of the control value of osteoclasts was observed in the zinc-administered rats. However, zinc administration did not alter the number of osteoblasts<sup>(51)</sup>. To shed light on this complexity with previous results regarding osteoclastogenesis, O'Conner J. P. *et al.* suggested in a thorough review that zinc restrains osteoclastogenesis at low (<0.2 µM) and high (>10 µM) concentrations<sup>(50)</sup>.

***In vitro studies showing the effects of excessive zinc on bone health.*** Zinc has been shown to have inhibitory effects on osteoclastogenesis *in vitro* as well. In a study by Yamaguchi and Uchiyama, the marrow cells of the mouse were cultured for 3 d with osteoclastogenesis-stimulating factors such as lipopolysaccharide, TNF-α and receptor activator of nuclear factor kappa B ligand (RANKL). The findings revealed that the formation of RANKL-induced osteoclast-like cells was significantly inhibited in the presence of 10<sup>-6</sup> to 10<sup>-4</sup> zinc sulphate, which suppressed the signalling pathway related to RANKL stimulation<sup>(52)</sup>.

Using ALP activity as an early indicator of osteoblast differentiation, Cerovic *et al.* examined the impact of zinc on the differentiation of SaOS-2 human osteoblast-like cells. The results indicated that ALP activity significantly increases with 1 µM or 10 µM of zinc treatment, whereas exposure to 50 µM zinc significantly reduces ALP activity compared with zinc-free cells<sup>(53)</sup>.

In short, zinc plays a role in both osteoclast and osteoblast differentiation and survival, which are critical for bone remodelling and overall bone health. Thus, it is not a surprise that zinc is commonly recommended for bone diseases such as OA. However, it is also important to consider zinc's impact on the

immune system, as current literature suggests that immunity plays a role in the pathogenesis of the disease.

### Zinc's impact on inflammation and immunity

As discussed previously, the belief that OA originates purely mechanically is rejected by numerous scientists, and the immune system contribution credence has been accepted lately. The human body's second most prevalent trace element, zinc, is essential for both immunity and inflammation. Thus, zinc homeostasis necessitates tight regulation as it can be detrimental in both high and low concentrations.

Low zinc concentrations in blood plasma may cause the body to be susceptible to some mortal infectious diseases<sup>(54)</sup>. Immunosenescent is also related to zinc deficiency, which is a drop in immune response with increasing age<sup>(55)</sup>. Long-term zinc supplementation could lessen the infection rate in adults<sup>(56)</sup>. In addition, zinc supplementation may improve infections such as pneumonia, diarrhoea and viral infections<sup>(57)</sup>. During inflammation, zinc is concentrated at the site of the incident<sup>(58)</sup>. When the toll-like receptors (TLR) 3, 4 and 7 are stimulated, the expression of various zinc carriers is induced, accumulating zinc in the vesicles of macrophages<sup>(59)</sup>. Zinc is then transported from the extracellular environment to the cytoplasm of macrophages by SLC39A family members also known as ZIP proteins<sup>(60)</sup>. Subsequently, macrophages accumulate zinc in phagosomes by zinc exporters, ZnT or SLC30A family members, which transport zinc into pathogen-encapsulated vesicles<sup>(61)</sup>. Neutrophils were observed to accumulate zinc in lysosomes and azurophilic

**Table 2.** Pre-clinical research

	Study method	Treatment	Effect	Reference
<b>Zinc deficiency on immunity</b>	<i>In vivo</i> (female BALB/c mice)	<i>H. polygyrus</i> infected mice were provided with either a zinc-deficient (0.75 mg zinc/kg diet) or a zinc-sufficient (60 mg zinc/kg) diet for 3 weeks.	Zinc-deficient mice showed reduced spleen size. The production of cytokines by T cells and APCs was also impaired.	(86)
	<i>In vivo</i> (male Sprague-Dawley rats)	Rats were fed with a zinc-deficient (<1 mg Zn/kg) or control diet (30 mg Zn/kg) for 3 weeks.	The numbers of thymic pre-T cell and splenic helper and cytolytic T cells were reduced in zinc-deficient rats. Serum cortisone levels were elevated.	(89)
	<i>In vitro</i> (isolated neutrophil granulocytes)	Cells in the culture medium were stimulated with LPS followed by TPEN treatment.	Cells showed a decreased capacity to secrete IL-8 and IL-1ra following TPEN treatment.	(92)
	<i>In vitro</i> (HUT-78 (Th0) and D1.1 (Th1) cell lines)	PMA/PHA stimulated cells were cultured in a medium containing less than 0.9 μM of zinc.	Both IL-2 mRNA levels and cytokine production were significantly decreased compared with zinc-sufficient medium.	(81)
<b>Excessive zinc on immunity</b>	<i>In vivo</i> (C57BL/6 mice)	Mice injected with type-II collagen intradermally received water with 3000 ppm of zinc for 30 d.	Zn suppressed CIA development, lowered serum levels of IL-17A and decreased the number of Th17 cells.	(99)
	<i>In vitro</i> (THP-1 cells)	Cells stimulated with LPS were pre-exposed to 20 μM and 40 μM of ZnCl <sub>2</sub>	Both groups showed significant reduction in monocyte adherence, ROS formation and IL-1β mRNA expression.	(100)
	<i>In vitro</i> (human T cell lymphoma cell line Jurkat E6.1)	PMA + PHA stimulated cells were incubated in a medium supplemented with 50 μM zinc acetate.	Zinc levels at or above 50 μM effectively reduced IFN-γ expression.	(101)
	<i>In vitro</i> (PBMC)	Pokeweed mitogen stimulated PBMCs were incubated with different concentrations of zinc.	Zinc had a concentration-dependent impact on DNA synthesis and cytokine production. Both functions were strongly suppressed at 0.2 mM zinc. Concentrations higher than 0.5 mM were toxic to immune cells.	(102)

APC, antigen-presenting cell; TPEN, tetrakis-(2-pyridylmethyl)ethylenediamine; LPS, lipopolysaccharides; PMA, phorbol myristate acetate; PHA, phytohemagglutinin; CIA, collagen-induced arthritis; ROS, reactive oxygen species.

granules, implicating zinc delivery to a phagocytosed pathogen such as *Streptococcus pyogenes*<sup>(62)</sup>. These findings imply that innate immune cells utilise zinc as a poison to kill pathogens, as the zinc-rich environment is toxic to pathogens through various mechanisms<sup>(57)</sup>. Some bacteria express several transport systems that export zinc out of the cytoplasm for their preservation<sup>(63)</sup>. It is predicted that zinc binds to proteins in lieu of other first-row transition metal ions leading to mismetallation of essential proteins<sup>(64)</sup>. For instance, *Streptococcus pneumoniae*, *Bacillus anthracis* and *Staphylococcus pseudintermedius* are examples of bacteria that entail manganese acquisition proteins. The irreversible binding of zinc to these proteins prompts manganese depletion in the bacteria, which leads to increased sensitivity to oxidative stress<sup>(65)</sup>.

Zinc was also shown to decrease transplant rejections as it acts as a pro-antioxidant agent and can induce regulatory T cells (Treg)<sup>(66)</sup> since the frequency of intra-graft Treg cells within the graft appears to be linked with graft acceptance, function and survival<sup>(67)</sup>. Maintaining zinc homeostasis is crucial for modulating immune responses and ensuring bone health, particularly in preventing and managing OA.

Table 2 summarises the pre-clinical research on the impact of varying zinc concentrations on immune responses across different study methods.

### The effects of zinc deficiency on immune function

Zinc deficiency alters the immune system in a significant way. Lymphocytes, especially CD4<sup>+</sup>, decrease in number, lowering

the CD4<sup>+</sup>/CD8<sup>+</sup> ratio<sup>(68)</sup>. Chemotaxis and phagocytosis are also adversely affected in conditions of zinc deficiency<sup>(69)</sup>. In the course of an immune response, zinc is relocated to the affected tissue, resulting in a transient hypozincaemia and is rebalanced after the resolution of the incident<sup>(70)</sup>. This transient hypozincaemia acts as a warning sign for the immune system<sup>(71)</sup>. Additionally, IL-6 enhances hypozincaemia by up-regulating the expression of zinc-binding proteins such as metallothionein and α2 macroglobulin<sup>(72)</sup>. Zinc ions are crucial in regulating signalling pathways through reversible binding to intracellular signalling proteins, and they are also vital for the proper function of several hormones<sup>(73)</sup>. For example, T cell development is sensitive to altered zinc signals as this metal is a key co-factor for thymulin. Thymulin, a hormone secreted by the thymus, induces immature T cell differentiation<sup>(74)</sup> and is significant for T cell function. Several reports show that zinc deficiency leads to thymic atrophy in children<sup>(75)</sup>. Premature immune cell apoptosis rates also increase in zinc deficiency owing to the activation of the hypothalamic-pituitary-adrenal axis resulting in increased circulating glucocorticoid levels, which is a strong apoptogen for immature lymphoid cells<sup>(76)</sup>. Endogenous glucocorticoid signalling causes damage in osteoblasts and chondrocytes in OA<sup>(77)</sup>. Yet another reason for the increased apoptosis in a zinc-deficient state is the induction of CDK2<sup>(78)</sup>, a member of the serine-threonine protein kinase family which is also attributed to cell survival depending on the pathway involved, by caspase-3-dependent cleavage of the cell cycle regulator p21. Thymulin also triggers IL-2 production and CD8<sup>+</sup> cell proliferation<sup>(79)</sup>. Mild zinc deficiency can impact thymulin levels highly. A study in

human volunteers showed that cytokines secreted by T helper 1 (TH1) cells such as IFN- $\gamma$  and IL-2 are diminished when a low-zinc diet (3–5 mg/d) is applied for 20 weeks<sup>(80)</sup>, consequently resulting in impaired natural killer (NK) cells and monocyte behaviour as these cytokines are important for their activity and function<sup>(81)</sup>. However, in primary human T cells, even high zinc concentrations such as 100  $\mu$ M can inhibit IFN- $\gamma$  production that are stimulated by IL-1 $\beta$  by reducing interleukin-1 receptor-associated kinase activity, which is important in the signalling pathway when the IL-1 receptor is activated<sup>(70)</sup>. IFN- $\gamma$  strongly activates the protein kinase R (PKR), a key regulator involved in transcription, translation, apoptosis, growth, differentiation and metabolism in chondrocytes. This activation subsequently enhances the production of IL-6 and TNF- $\alpha$ , promoting cartilage breakdown. Moreover, PKR is further stimulated by pro-inflammatory cytokines. It was demonstrated *in vitro* that the activation of PKR also amplifies the synthesis of matrix-degrading enzymes and contributes to proteoglycan degradation<sup>(82)</sup>. This perplexity was elucidated in a study conducted on T cell line Hut-78 demonstrating that extremely low concentrations (1  $\mu$ M) of zinc significantly lower IFN- $\gamma$ , IL-2 and TNF- $\alpha$  secretion, and high (50–100  $\mu$ M) zinc concentrations leads to mild decreases in these cytokines compared with moderate (15  $\mu$ M) zinc concentrations<sup>(83)</sup>. Nevertheless, cytokine levels produced by TH2 cells such as IL-4, IL-6 and IL-10 are unaffected. This shifts the TH1/T helper 2 (TH2) balance in favour of TH2 dominance generating altered immune function<sup>(84)</sup>.

***In vivo studies showing the effects of zinc deficiency on immune function.*** *In vivo* studies show that functions of polymorphonuclear neutrophils (PMNs) were impaired in a zinc-deficient state owing to reduced activity and phagocytosis<sup>(85)</sup>.

Female BALB/c mice infected with *Heligmosomoides polygyrus* were provided free access to either a zinc-sufficient (60 mg zinc/kg) or a zinc-deficient diet (0.75 mg zinc/kg). Three weeks post infection, the zinc-deficient group exhibited increased worm numbers and reduced spleen size. Analysis of T cells and APCs from zinc-deficient mice showed diminished cytokine production such as IFN- $\gamma$ , IL-4 and IL-5 by T cells and suppressed functions of APCs<sup>(86)</sup>.

The stimulation of macrophages through the adaptive immune system is also impaired due to reduced secretion of IFN- $\gamma$  by TH1 cells<sup>(80)</sup>. Furthermore, zinc deficiency alters IL-12 generation<sup>(87)</sup> by monocytes and macrophages, which is a key regulator of TH1 cell differentiation<sup>(88)</sup>.

Sprague-Dawley rats given either a zinc-deficient (<1 mg Zn/kg) diet or a control diet (30 mg Zn/kg) for 3 weeks displayed higher serum cortisone levels and fewer cytolytic T cells and thymic pre-T cells in the spleen than the controls<sup>(89)</sup>.

While B cells are less reliant on zinc compared with T cells, zinc deficiency still affects pre-B cell counts owing to increased glucocorticoid levels. Research conducted in mice indicates that B cells in the bone marrow are less affected by zinc deficiency than T cells<sup>(33)</sup>. However, zinc deficiency can alter antibody production by B cells<sup>(90)</sup>. Zinc-deficient states *in vivo* report reduced response to vaccination, suggesting that zinc treatment in advance of vaccination may improve antibody response<sup>(91)</sup>.

***In vitro studies showing the effects of zinc deficiency on immune function.*** Neutrophil granulocytes, prepared at a concentration of  $1 \times 10^6$  cells/ml in RPMI-1640 medium, were subjected to stimulation with lipopolysaccharides (LPS, 250 ng/ml) for 4 h at 37 °C. This triggered significant IL-8 release, a response significantly attenuated by the zinc chelator TPEN. Concurrently, TPEN administration also decreased the capacity to release IL-1ra in response to LPS stimulation<sup>(92)</sup>.

Furthermore, reduced levels of free cellular zinc were found to promote monocyte differentiation of HL-60 cells by relieving zinc-mediated inhibition of adenylate cyclase<sup>(93)</sup>. This alteration, however, contrasts with findings that macrophage stimulation through the adaptive immune system is hindered due to reduced IFN- $\gamma$  secretion by TH-1 cells<sup>(80)</sup>.

Meanwhile, a separate investigation by Bao B. *et al.* delved into zinc's influence on IL-2 production, pivotal for T cell activation and immune function. They observed that zinc deficiency, with levels below 0.9  $\mu$ M, markedly reduced both IL-2 mRNA levels and cytokine production in phytohaemagglutinin + phorbol myristate acetate (PHA + PMA)-stimulated HUT-78 (Th0) and D1.1 (Th1) cell lines after 6 h, highlighting zinc's critical role in immune response modulation<sup>(81)</sup>.

### *The effects of excessive zinc on immune function*

Elevated zinc concentrations can disrupt the balance of other trace elements. For example, zinc excess can cause copper depletion<sup>(94)</sup> but raise iron concentration in bacteria<sup>(95)</sup>. In addition, excess zinc can disrupt metabolic and growth pathways and glycolysis in bacteria<sup>(96)</sup>. These mechanisms arising from high zinc concentrations therefore exert antimicrobial effects on microorganisms. Duncan *et al.* noted that the potential of developing copper deficiency in patients prescribed high zinc doses is frequently overlooked<sup>(97)</sup>. Copper is crucial for promoting the regeneration of articular cartilage and subchondral bone by enhancing the transformation of macrophages into the M2 phenotype. This transformation increases anti-inflammatory cytokine secretion, thereby reducing cartilage tissue damage<sup>(98)</sup>. However, copper deficiency disrupts lysyl oxidase function, impairing the cross-linking of collagen and elastin. This weakening of the bone matrix and cartilage structure makes the cartilage vulnerable to fragmentation and weakens its integrity, potentially leading to OA<sup>(98)</sup>. As a result, by decreasing copper levels, zinc can indirectly adversely affect the osteoarthritic cartilage.

***An in vivo study showing the effects of excessive zinc on immune function.*** Kitabayashi *et al.* investigated the impact of zinc on TH17 cells using a collagen-induced arthritis (CIA) mouse model. CIA, which depends on cytokine produced by Th17 cells such as IL-6 and IL-17A, was induced in C57BL/6 mice by intradermal injection of type II collagen. The researchers observed significant suppression of CIA development when animals were provided drinking-water containing 3000 ppm of zinc for 30 d. Zinc administration led to reduced serum levels of IL-17A and decreased numbers of Th17 cells. The study concluded that high zinc concentration directly inhibits STAT3 activation, a critical process in Th17 cell development<sup>(99)</sup>.

*In vitro* studies showing the effects of excessive zinc on immune function. The induction of monocyte activation by LPS was investigated following pre-incubation with THP-1 cells, a non-adherent human monocytic cell line. The results showed that ZnCl<sub>2</sub> concentrations of 20 and 40 μM resulted in reduced LPS-induced monocyte activation, including adherence, ROS formation and the expression of IL-1β mRNA<sup>(100)</sup>.

In another study, it was demonstrated *in vitro* that zinc acetate at elevated concentrations, specifically 50 μM, attenuated IFN-γ expression in the human T cell lymphoma cell line Jurkat E6.1. This suppression occurred via inhibition of the calcium-independent PKC-AP-1 pathway. The effect of zinc on IFN-γ expression was assessed 6 h after PHA + PMA stimulation<sup>(101)</sup>.

Additionally, stimulating peripheral blood mononuclear cells (PBMC) with pokeweed mitogen (PWM) showed that zinc influences DNA synthesis and cytokine production (IL-2, IL-6 and IL-10) in a concentration-dependent manner. A zinc concentration of 0.2 mM strongly suppressed both functions, and concentrations above 0.5 mM, corresponding to approximately 45 mg of zinc salt daily, were observed to be toxic to immune cells<sup>(102)</sup>.

In brief, altered zinc homeostasis can influence lymphocyte activity directly by altering their formation and cytokine production, or indirectly by affecting their interaction with innate immune cells<sup>(33)</sup>. It is also important to keep immune conditions stable, as the expression of ZIP8, which is an important importer of zinc into cells, can be affected under different immune conditions. Therefore, altering zinc concentrations locally on OA sites could have an impact on disease progression.

### Zinc-dependent enzymes on osteoarthritis pathogenesis

Zinc is an element present in many enzymes and is the only metal found in all six classes of enzymes<sup>(103)</sup>. It is a cofactor of many metalloproteins as well, and these proteins make up the largest category of metalloproteins<sup>(104)</sup> which are engaged in numerous significant biological functions such as RNA and DNA synthesis, cell differentiation and proliferation, cell structure, cell membrane stabilisation, apoptosis and redox reactions<sup>(105)</sup>. Zinc serves as a cofactor for the enzyme superoxide dismutase (SOD)<sup>(106)</sup>, which is an important antioxidant agent, and restrains ROS-forming enzyme NADPH oxidase activity<sup>(107)</sup>. To stay within the boundaries of this article, two principal zinc-dependent enzyme families involved in OA will be discussed.

#### Matrix metalloprotein family

The first enzyme family under discussion is MMPs, which belong to the family of calcium-dependent zinc-containing endopeptidases responsible for breaking down ECM proteins<sup>(108)</sup>. These enzymes, particularly MMP-13, are strongly linked with articular cartilage destruction in OA<sup>(109)</sup>. Type II collagen is a substrate of MMP-13 which is among the most abundant components of the articular cartilage. Its overexpression in mice has been shown to manifest OA-like phenotypes<sup>(110)</sup>. The conditional knockout of MMP-13 in mouse chondrocytes decelerated OA progression post-meniscal-ligamentous injury surgery model<sup>(111)</sup>. *N*-O-isopropyl

sulphonamide-based hydroxamate, a zinc-chelating inhibitor exclusive to MMP-13, substantiated effectiveness in an *in vitro* cartilage degradation model<sup>(112)</sup>. Similarly, curcumin<sup>(113)</sup> and resveratrol<sup>(114)</sup> are natural compounds that were reported to restrain MMP-13 expression.

#### A disintegrin and metalloproteinase with thrombospondin motifs

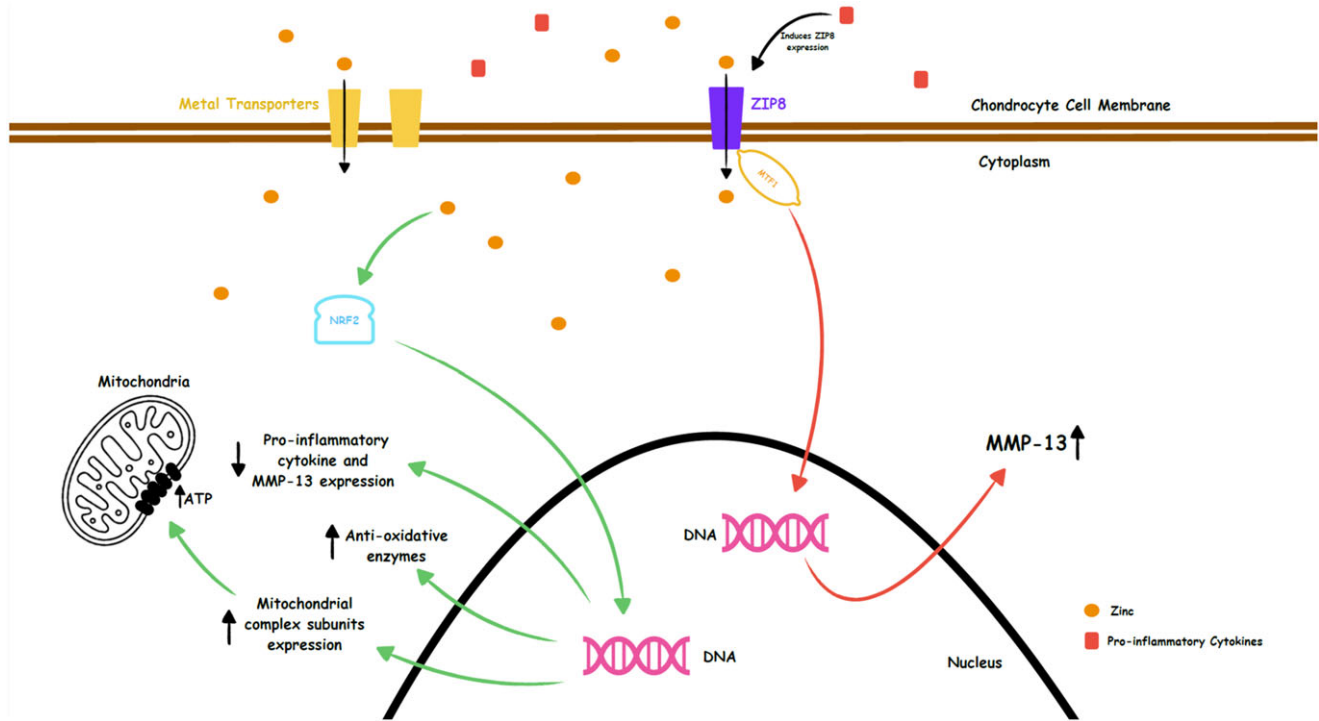
The second significant enzymatic family involved is a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS). They are membrane-bound zinc-dependent proteins that mediate ECM degradation<sup>(115)</sup>. ADAMTS 4/5, which are elevated within articular chondrocytes even at the outset of OA, are the primary proteases that break down the key constituent of the cartilage ECM, aggrecan, in human and animal osteoarthritic cartilage<sup>(116)</sup>. The elimination of ADAMTS 5 secured joints from damage in a surgically induced murine OA model<sup>(117)</sup>.

Concerning these data, suppression of these enzymes might be a therapeutic target to retard OA progression.

### The impact of zinc on osteoarthritis

As mentioned above, zinc has multiple positive effects on bone health and immunity. Zinc by promoting osteoblast and chondrocyte differentiation, being toxic to pathogens and regulating the innate and adaptive immune system fosters optimistic thoughts in treating OA. To our knowledge, an inhibitor of glyceraldehyde-3-phosphate, MIA, is the most widely reported method to study osteoarthritis<sup>(16,118)</sup>. A recent study carried out by Huang *et al.* showed that the addition of MIA to chondrosarcoma cell line SW1353 *in vitro* and to the Wistar rat model *in vivo* reduces antioxidative glutathione peroxidase 1 (GPx1) and Mn-SOD enzyme expression, induces oxidative stress, increases pro-inflammatory cytokine levels and increases MMP-13 levels mimicking human osteoarthritis closely<sup>(18)</sup>. The introduction of zinc to these cells blocks these changes as it augments NRF2 translocation to the nucleus, leading to increased gene expression of antioxidants and decreased pro-inflammatory cytokine and MMP-13 expression. The author indicated that the supplementation of 1.6 mg/kg/d and 8 mg/kg/d zinc is equally effective in preventing MIA-induced OA progress<sup>(18)</sup>. This study clearly indicates that zinc protects against cartilage degradation brought on by MIA. Huang L. *et al.* showed that MIA also decreases ATP production in SW1353 cells by down-regulating the expression of glycolysis-associated proteins such as HK2, GLUT1 and PDH-E1α and mitochondrial complex I, II, IV and V subunits of the oxidative phosphorylation pathway. The addition of zinc to these cells reverses these changes and results in elevated ATP levels, thus indicating that it is likely chondroprotective<sup>(11)</sup>.

Nevertheless, a cross-sectional study carried out by Yang W. *et al.* suggested that a higher incidence of OA is linked to zinc intake<sup>(21)</sup>. The authors recommended that the daily intake of zinc should be reduced in individuals at high risk of OA. However, the dietary data relied on participants' recall, which could lead to inaccuracies, and the study had a relatively small sample size. Further large-scale, longitudinal studies are required to validate



**Fig. 1.** A schematic representation of the possible effects of zinc on osteoarthritic cartilage. Cellular zinc that has been transported through various metal transporters augments NRF2 translocation to the nucleus, boosting antioxidative gene expression and lowering pro-inflammatory cytokine and MMP-13 levels. During inflammatory conditions, ZIP8-mediated zinc influx increases, translocating MTF1 to the nucleus, leading to higher MMP-13 expression. ATP, adenosine triphosphate; MMP-13, matrix metalloproteinase-13; MTF1, metal responsive transcription factor 1; NRF2, nuclear factor erythroid 2-related factor 2; ZIP8, Zrt/Irt-like proteins 8. ↑ indicates increase; ↓ indicates decrease.

these findings. A study carried out by Kim *et al.* supports this suggestion, showing that zinc negatively impacts cartilage integrity. They indicated *in vitro* that IL-1 $\beta$ , which is a key mediator for OA, induces the zinc transporter ZIP8 expression in chondrocytes. This leads to increased influx of zinc to the cytoplasm. Zinc influx mediated by other importers, particularly ZIP8, translocates MTF1 to the nucleus. This translocation induces the expression of zinc-dependent MMP enzymes which are pivotal in the pathophysiology of OA, contrary to the findings of Huang *et al.* The authors propose that the local depletion of zinc in the cartilage could be a promising therapeutic approach for OA treatment<sup>(24)</sup>. In addition, J. Zhou and colleagues investigated the impact of various elements in the blood on OA, and their findings concluded that genetically predicted elevated zinc levels showed a positive correlation with OA<sup>(5)</sup>. However, the data for this work were sourced from the UK Biobank, which predominantly includes older individuals of European ancestry. Therefore, the results might not be applicable to younger populations or individuals of diverse ethnic backgrounds<sup>(5)</sup>. Figure 1 provides a schematic representation of the possible mechanisms by which zinc influences OA pathogenesis.

### Conclusion

Due to its numerous beneficial effects on bone health and immunity, zinc is often thought to have favourable impacts on

patients with OA. Evidence suggests that zinc can inhibit the progression of certain OA models, such as those induced by MIA. However, several studies also suggest potential negative effects of zinc on OA. Altered zinc levels can influence various physiological processes including the innate and adaptive immune system, osteoblast and osteoclast differentiation, enzyme activities and numerous intracellular signalling pathways. We propose that the contradictory results may be explained by the fact that, under normal physiological conditions without risk factors, zinc can have beneficial and protective effects on bone against OA. However, when risk factors are present and a pro-inflammatory environment develops in the joint, ZIP8 levels rise, causing zinc to pass through this transporter and become more harmful than beneficial for osteoarthritic bone. The concern in this matter is that even though OA models mimic certain pathological pathways observed in the disease, these models do not yet replicate human OA completely. To our knowledge, there is a lack of sufficient studies involving various OA models and human subjects that explore the association between zinc and OA. Further research is necessary to thoroughly understand the link between zinc and OA. Health professionals should be cautious when prescribing medications containing zinc to patients with OA, being mindful that both excessive and insufficient zinc levels could be factors in the disease. Therefore, utilising zinc supplementation in managing patients with OA could pose a double-edged sword. Additional studies with a variety of OA models and large sample sizes are required



to more accurately investigate the influence of zinc on OA and to delineate the range of zinc consumption by patients with OA that is considered innocuous.

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The author(s) declare none.

### Authorship

Conceptualisation and design: İ.T. Literature search, data collection, drafting and editing: M.Z.Ş. Analysis and interpretation of data: A.K. and K.N. All authors have reviewed and approved the final manuscript.

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