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Emerging Issues in Vitamin K Research

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Abstract

Vitamin K is traditionally recognized for its role in blood clotting. More recently, new roles for vitamin K have emerged. The current evidence for the role of vitamin K in bone, cardiovascular, and reproductive health will be discussed. There will be a particular focus on populations who could be at risk for vitamin K deficiency.

Keywords

vitamin K, coagulation, calcification, phylloquinone, menaquinones, menadione

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Vitamin K is a fat-soluble vitamin that has long been recognized for its essential role in coagulation and, more recently, has been proposed as a key nutrient in the regulation of soft tissue calcification. It is found in 2 natural forms in the diet: phylloquinone and menaquinones. Phylloquinone (vitamin K_1) is present in green leafy vegetables, such as broccoli and spinach, and in certain plant oils. Menaquinones (vitamin K_2) are primarily of animal origin and/or from bacterial synthesis. Menadione (vitamin K_3), a naturally occurring metabolite of vitamin K, is also a synthetic form of vitamin K added to animal feed.

This review will focus on the different sources of vitamin K and their purported health benefits relative to recommended intakes, with an emphasis on subgroups at risk for vitamin K deficiency.

Biochemical Roles

The only known biochemical role of vitamin K is as a cofactor for the vitamin K–dependent carboxylase that catalyzes the amino acid glutamic acid (Glu) to γ-carboxyglutamic acid (Gla) (Figure 1).[1,2] This can be achieved by all forms of vitamin K, albeit with different enzyme affinities. This carboxylation reaction is critical to the calcium-binding function of vitamin K–dependent proteins. The degree to which a vitamin K–dependent protein is carboxylated has been used for the assessment of vitamin K nutritional status. As the vitamin K–dependent γ-carboxylation is a post-translational event, these carboxylated measures of vitamin K–dependent proteins are used as functional indicators of vitamin K status, whereas total concentrations of vitamin K–dependent proteins are influenced by other factors independent of vitamin K.

The hepatic vitamin K–dependent proteins involved in coagulation are factors II (prothrombin), VII, IX, and X and proteins C, S, and Z, all of which need vitamin K for physiologic activation.[1] Multiple vitamin K–dependent proteins have been identified in extrahepatic tissues; however, their biologic roles are still being elucidated. Of the extrahepatic proteins, osteocalcin and matrix γ-carboxyglutamic acid protein are perhaps the best studied for their role in regulation of calcium binding in bone and soft tissue.

All forms of vitamin K share a common naphthoquinone ring but differ in the position-3 side chain (Figure 2). Phylloquinone contains the phytyl group as its side chain, whereas the menaquinones contain a polyisoprenoid side chain of varying lengths at position-3 of the naphthoquinone ring. The phytyl side chain of phylloquinone is thought to be removed to form menaquinone-4. Even though menaquinone-4 is not abundant in the food supply, it is found in high concentrations in certain tissues. This has led to the hypothesis that menaquinone-4 can have unique roles in novel functions of vitamin K, independent of its role as an enzyme cofactor. These include prevention of oxidative injury to oligodendrocytes in the brain,[3] acting as a ligand for a xenobiotic receptor in bone cells,[4] playing a role in gene expression in osteoblasts,[5] and modulation of inflammatory responses.[6]

Sources and Recommendations

Green vegetables are the main source of phylloquinone in the diet (Table 1), and they contribute up to 60% of total phylloquinone intake.[7] Plant oils, such as soybean and canola oil, as well as margarine spreads are also an important dietary sources of phylloquinone in the US diet.[8] In the United States, poultry products are the primary dietary sources of menaquinone-4

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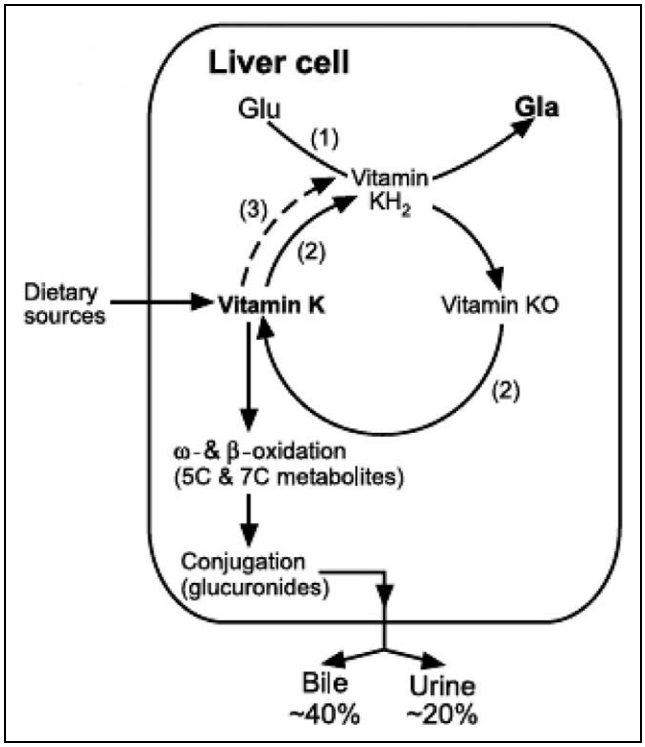

Figure 1. Scheme showing the hepatic metabolism of vitamin K
Adapted with permission from Shearer.[2] The conversion of glutamic acid (Glu) to γ-carboxyglutamic acid (Gla) in vitamin K–dependent proteins is linked to an enzyme cycle called the vitamin K–epoxide cycle, which carries out both γ-glutamyl carboxylation and serves as a salvage pathway to recover vitamin K from its epoxide (KO) for reuse in carboxylation. Enzyme activities shown are (1) γ-glutamyl carboxylase, (2) vitamin K epoxide reductase (VKOR), and (3) NAD(P)H-dependent quinine reductase(s). The active form of vitamin K needed by the γ-glutamyl carboxylase is the reduced-form vitamin K quinol (KH_2). An obligatory metabolic consequence of γ-carboxylation is that KH_2 is oxidized to KO, which in turn undergoes reductive recycling, first to the quinine and then to KH_2. Under usual physiological conditions, vitamin K is probably mainly recycled by VKOR. The liver is also the site of a catabolic pathway, common to phylloquinone and menaquinones, whereby their respective side chains undergo ω-oxidation followed by β-oxidation, leading to 2 major aglycone metabolites with side chain lengths of 5 and 7 carbon atoms, respectively (5C and 7C metabolites). After conjugation (mainly with glucuronic acid) these metabolites are excreted in the bile and urine. Phylloquinone, the major dietary form, is rapidly and extensively catabolized in humans, with about 40% of the daily physiological dose being excreted via the bile and 20% via the urine. There are no equivalent excretion data for the menaquinones.

because poultry feed is a rich source of menadione, which is subsequently converted to menaquinone-4 in certain tissues.[9,10] There are also small amounts of menaquinone-4 in dairy products. In Japan, a unique source of menaquinone-7 is found in the traditional food *natto*, a fermented soybean product. Menaquinone-7 has recently emerged on the market as a dietary supplement. Certain cheeses also contain some bacterially produced menaquinones, although menaquinone concentrations appear to be dependent on the processing techniques used.[11]

There is no current recommended dietary allowance for vitamin K. The adequate intake of a nutrient is the median level of usual phylloquinone intake that is assumed to be adequate based on observation of groups of apparently healthy people. The adequate intake in the United States for vitamin K is based on representative dietary phylloquinone intake data from healthy individuals from the Third Nutrition and Health Examination Survey and is currently set at 120 and 90 μg/d for men and women, respectively.[12] The adequacy of these intakes for promotion of health has not been determined because there are currently no physiological outcomes available that can be reliably used to assess nutritional adequacy. Although there is growing interest in the role of menaquinones in human health, the food composition data for the various menaquinones are incomplete. At the time of this writing, it appears that the menaquinones are limited to certain cheeses and a few fermented products, the majority of which are not regularly consumed in North America.

Vitamin K is considered safe at the recommended adequate intake dosages discussed. There is no tolerable upper limit set because there are no known cases of toxicity with vitamin K. A common misconception is that excessive vitamin K will result in overcoagulation. However, the vitamin K–dependent proteins have a limited number of glutamic acid residues capable of γ-carboxylation per molecule, beyond which there can be no further γ-carboxylation or excessive coagulation.

As potential new roles for vitamin K emerge, supplemental vitamin K is becoming more widely available in the forms of topical creams, capsules, tablets, and softgels. Vitamin K creams are marketed for reduction of skin redness and varicose veins. Many multivitamin formulations contain vitamin K in the form of phytonadione, a synthetic water-soluble form of phylloquinone, ranging from 40 to 100 μg, which is well within normal dietary intakes in the US adult population. Supplements containing vitamin K alone are also available in the forms of phytonadione or menaquinones, in daily doses of up to 4050 μg. Many of these supplements are marketed with the claim that they support bone strength and cardiovascular health. Unpublished data from our laboratory indicate that the actual vitamin K content of many supplements is inconsistent with the amount stated on the label. This is of particular concern for individuals on coumarin-based drugs (eg, warfarin) given that variability in vitamin K intake is often cited as a risk factor for instability of oral anticoagulant therapy.[13]

Vitamin K supplementation is currently recommended in certain subgroup populations. Vitamin K deficiency bleeding is a significant public health issue that is of concern in healthy appearing neonates.[14] The low vitamin K content of breast milk, low placental transfer of vitamin K, low levels of clotting factors at birth, and a sterile gut are all contributing factors to vitamin K deficiency bleeding. Vitamin K deficiency bleeding classically presents as bleeding from the nose, gut, and umbilical cord and in the most severe cases can result in intracranial bleeding. Premature infants are at greater risk of intracranial hemorrhage secondary to vitamin K deficiency bleeding.

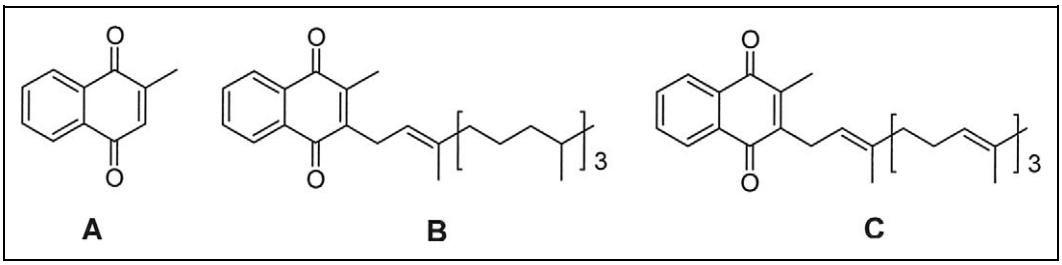

Figure 2. Chemical structures of vitamin K. A, Menadione; B, Phylloquinone; C, Menaquinone-4

Table 1. Food Sources of Vitamin K[53]

Food	Vitamin K (μg per Serving)
Foods high in vitamin K	
Kale, frozen (½ cup cooked)	573
Collards, frozen (½ cup cooked)	530
Spinach, frozen (½ cup cooked)	514
Turnip greens, cooked (½ cup)	265
Foods moderately high in vitamin K	
Brussels sprouts, cooked (½ cup)	110
Broccoli, cooked (½ cup)	110
Cabbage, cooked (½ cup)	82
Bibb lettuce, raw (1 cup)	56
Foods low in vitamin K	
Potato, baked (1 medium, flesh and skin)	4
Raisins, golden seedless (½ cup)	3
Corn muffin (1 small, commercially prepared)	2
Orange, fresh (1 navel orange without skin)	0

Prevention of vitamin K deficiency bleeding by oral or intramuscular administration of vitamin K at birth is standard practice in many countries. A systematic review assessing the effects of vitamin K administered to women at risk of very preterm birth to prevent intracranial hemorrhage found no beneficial effect in neonatal outcomes, supporting the need for prophylaxis in the newborn to prevent vitamin K deficiency bleeding.[15]

Vitamin K supplementation is also standard practice for patients with cystic fibrosis in doses ranging from 300 to 500 μg/d.[16] Vitamin K status is low in patients with cystic fibrosis due to low dietary intake, malabsorption, and the use of antibiotics that decrease production of vitamin K by gut microorganisms. Despite routine supplementation of vitamin K in these patients, vitamin K status often remains suboptimal or deficient, and a recent study found that only patients taking high-dose vitamin K supplements ($\geq$1000 μg/d) achieved a vitamin K status similar to healthy subjects.[17]

Other subgroups of the population also have been shown to have low intakes of vitamin K, which can result in suboptimal vitamin K status. Nursing home residents have been reported to consume lower amounts of phylloquinone than free-living elderly.[18] In the United States, adults aged 70 years and older do not on average meet the adequate intake.[12] Thus, these subgroups could benefit from supplementation. However, this is not standard practice, and the risks and benefits of supplementation in these subgroups have not been systematically studied.

Vitamin K and Bone Health

Osteoporosis is a significant public health issue, particularly as the population ages. Considerable interest in vitamin K and prevention of osteoporosis has arisen as osteocalcin, a vitamin K–dependent protein, is present in high concentrations in bone. The exact role of osteocalcin in bone metabolism is unknown, but it is thought to be involved in the regulation of bone mineral maturation.[19] Osteocalcin is produced by mature osteoblasts and thus elevated plasma concentration has commonly been used as a marker for increased bone formation and turnover. Osteocalcin synthesis is upregulated by 1,25-dihydroxyvitamin D. However, the mineral-binding capacity of osteocalcin depends on the vitamin K–dependent γ-carboxylation of its 3 glutamate residues, such that partially carboxylated osteocalcin could have reduced binding to the mineral in bone. Fully carboxylated osteocalcin is often assumed to be required for skeletal health. However, there is no clear evidence that supports an association between increased concentrations of plasma undercarboxylated osteocalcin and decreased mineralization in human bone.

As vitamin K is required for carboxylation of osteocalcin, it has been assumed that insufficient concentrations of plasma phylloquinone can lead to insufficient bone mineralization. Several large epidemiologic studies have shown that low phylloquinone intake is associated with increased hip, spine, or femoral neck fracture risk.[20-23] In addition, an association between lower serum phylloquinone and menaquinone-7 concentrations and decreased bone mineral density has been seen in observational studies.[24,25] However, these positive associations found between increased vitamin K status and skeletal health do not necessarily imply causation as observational studies are limited by the potential confounding effect of overall poor diet and unhealthy lifestyle since vitamin K is primarily found in foods associated with a healthy diet.

In Japan, a common therapy for osteoporosis is vitamin K supplementation in the form of menaquinone-4. The standard dose of menaquinone-4 that is employed is 45 mg/d, and it is used as a drug therapy rather than as a nutritional supplement.

This dosage is 450-fold higher than the current US dietary requirements for vitamin K and cannot be achieved by diet alone. In a systematic review and meta-analysis of randomized controlled trials, it was concluded that supplementation with menaquinone-4 is associated with a reduction in hip and vertebral fracture and an increase in bone mineral density.[26] However, it was noted by these authors that several of the studies included in the meta-analysis employed concurrent use of calcium and vitamin D supplementation, lack of a placebo arm, lack of blinding, and insufficient sample size. Subsequently, the results of a postsurveillance marketing study indicated a lack of beneficial effect of 3 years of menaquinone-4 supplementation in preventing fractures in more than 3000 elderly Japanese subjects with or without a history of fracture.[27] Inclusion of the results of this study could have altered the results of the meta-analysis and supports the need for a new meta-analysis, particularly in view of recent published findings on the effects of phylloquinone supplementation on bone loss.

Several randomized controlled trials have assessed the effect of phylloquinone supplementation on bone loss.[28-31] Supplementation over a 3-year period using a high dose of phylloquinone (1000 μg/d) in combination with a calcium, zinc, magnesium, and vitamin D supplement was shown to reduce bone loss at the femoral neck but not at the lumbar spine in postmenopausal women.[28] Another study of postmenopausal women compared the effect of placebo, 200 μg/d of phylloquinone, a calcium and vitamin D supplement, and the calcium and vitamin D supplement plus phylloquinone.[29] In this study, there was no significant difference in bone mineral density between any of the intervention groups at the end of 2 years. However, a modest increase was seen in bone mineral density of the ultradistal radius when compared with baseline values in the calcium and vitamin D plus phylloquinone group. A 3-year randomized controlled trial in healthy 60- to 80-year-old men and women assessed the effect of a multivitamin preparation containing calcium and vitamin D to the same preparation with 500 μg/d phylloquinone added.[30] This study found no differences in the change in bone mineral density at the femoral neck or lumbar spine. Binkley and colleagues conducted a 1-year study of healthy postmenopausal women that compared the effect of a calcium and vitamin D supplement alone, or this supplement combined with 1000 μg/d phylloquinone or 45 mg of menaquinone-4 on bone mineral density.[31] There was no effect seen on bone mineral density at the lumbar spine or proximal femur. Thus, the preponderance of evidence does not support the efficacy of phylloquinone supplementation on reducing bone loss. The one trial that did report a beneficial effect of phylloquinone plus calcium and vitamin D on reducing bone loss relative to calcium and vitamin D supplementation alone was unique in that the study participants were perimenopausal and apparently unresponsive to calcium and/or vitamin D supplementation alone. Therefore, it is plausible that phylloquinone is effective for subgroups that do not otherwise respond to calcium or vitamin D. As discussed later, early postmenopausal women appear to be less efficient in utilization of vitamin K[32] and as such could be more responsive to phylloquinone supplementation. Similarly, patient groups with known vitamin K deficiency, such as patients with cystic fibrosis,[17] could also benefit from phylloquinone supplementation on bone health. However, these findings would need to be replicated in different populations before any recommendations for phylloquinone supplementation to support bone health are made.

Vitamin K and Cardiovascular Health

An increased risk of cardiovascular disease is associated with atherosclerosis, which is caused in part by coronary artery calcification. The vitamin K–dependent protein, matrix γ-carboxyglutamic acid protein, is expressed in vascular smooth muscle cells, which are involved with the calcification process in coronary arteries.[33,34] In the matrix γ-carboxyglutamic acid protein knockout mouse model, the animals develop rapid calcification of the elastic lamellae of the arterial media, which is fatal within 2 months of age.[35] In rats, vitamin K antagonism with warfarin inhibits the vitamin K–dependent carboxylation of matrix γ-carboxyglutamic acid protein, which leads to arterial calcification.[36] In addition, diets high in vitamin K have been shown to reverse aortic calcification and improve elasticity in warfarin-treated rats.[37]

In humans, the potential role of vitamin K intake in protecting against vascular calcification is limited. Several cohort studies have examined the association between dietary phylloquinone intake and cardiovascular disease risk and have concluded that high phylloquinone intake is not an independent risk factor for cardiovascular disease. Instead, it has been suggested that higher phylloquinone intakes are a marker of an overall heart-healthy dietary and lifestyle pattern as they track green vegetable consumption.[38-40] However, authors of a large epidemiologic study in the Netherlands reported an inverse relationship between dietary menaquinone and aortic calcification.[42] Interpretation of these findings is made difficult because phylloquinone and menaquinone intakes in this population were high relative to current recommendations (~275 μg/d) and intakes of menaquinones represented only about 10% of the total. Of note, phylloquinone intake was not associated with arterial calcification in this study.[41] Currently, there is no obvious biological explanation for the potency of the menaquinones in reducing cardiovascular disease risk when there was no concomitant protective effect of phylloquinone, even though the latter was consumed in higher amounts, and both forms of vitamin K support carboxylation of matrix γ-carboxyglutamic acid protein. There is speculation that menaquinone-7 is not as readily absorbed and metabolized compared with phylloquinone, which renders it more available to extrahepatic tissues.[42] However, more data are still required on the relative absorption of different vitamin K forms in humans.

A 3-year randomized controlled trial in postmenopausal women that assessed the effect of supplementation phylloquinone, calcium, and vitamin D found improved carotid artery elasticity and compliance compared with supplementation without phylloquinone.[43] It was proposed by the authors that this improvement was a result of an increase in vitamin

K–dependent carboxylation of matrix γ-carboxyglutamic acid protein, which led to a decrease in vascular deposition, but neither matrix γ-carboxyglutamic acid protein nor vascular calcification were measured.[43] To further explore this theory, a 3-year prospective randomized controlled trial studied the effects of 500 μg/d phylloquinone supplementation on age-related bone loss and progression of vascular calcification was performed.[44] Daily supplementation of vitamin K in amounts achievable by high dietary intake of green, leafy vegetables resulted in less progression of coronary calcification in older men and women compared with those who did not take vitamin K.[44] This effect was found to be independent of changes in serum matrix γ-carboxyglutamic acid protein.[44] At the time of the study, there was no available assay for measurement of the carboxylation status of matrix γ-carboxyglutamic acid protein. There is also uncertainty regarding the ability of serum matrix γ-carboxyglutamic acid protein concentrations, total or uncarboxylated, to reflect matrix γ-carboxyglutamic acid protein function at the level of the vascular tissue.[45]

Mechanisms underlying how vitamin K supplementation plays a role in reducing cardiovascular disease still need to be elucidated, and further study is needed to determine if and how different forms of vitamin K have variable efficacy in reducing cardiovascular disease risk.

Vitamin K and Reproductive Endocrinology

It has been reported in rodent studies that the vitamin K concentrations in ovaries are higher than in other organs.[46] Furthermore, the most abundant form of vitamin K measured in reproductive organs is menaquinone-4, suggestive of a role for vitamin K in female reproduction that is independent of γ-carboxylation. However, to date there are no reports of vitamin K linked to reproductive functions.

In China, vitamin K acupuncture point injection has been a standard treatment for dysmenorrhea since the 1980s.[47] Dysmenorrhea is a common gynecologic complaint and has been reported to be one of the most common causes of periodic absenteeism from school or work in young women.[48] Common pharmacologic treatments include oral nonsteroidal anti-inflammatory drugs and oral contraceptives. However, these medications have side effects and potential risks. The San Yin Jiao/Spleen 6 is an important acupuncture point located on the lower leg and has been widely used for several indications in classical and modern acupuncture, especially for gynecological diseases such as menstrual disorders, menstrual pain, vaginal discharge, urinary retention, and induction and acceleration of labor.[49] A nonrandomized uncontrolled pilot study, conducted in Italy and China, suggests that in Italian and Chinese women aged 14 to 25 years, acu-point injection with vitamin K arrests menstrual pain quickly, allows more participation in daily activities, reduces hours in bed, and reduces the amount of pain medication ingested.[50]

The hypothesized mechanism by which vitamin K injection at the Spleen 6 acupuncture point relieves dysmenorrhea is that it causes relaxation of uterine muscle spasm caused by prostaglandins.[51] It is currently not known if vitamin K is present in uterine muscle and/or if it has any biochemical role in muscle function.

Although not systematically studied, estrogen withdrawal at menopause can result in impairment in vitamin K metabolism.[32] Among early postmenopausal women not using hormonal replacement therapy, there was a higher phylloquinone level (ie, indicative of superior vitamin K status) and high percentage of undercarboxylated osteocalcin (ie, indicative of inferior vitamin K status) compared with premenopausal women. This suggests that the relationship between the amount of phylloquinone in circulation and the amount of phylloquinone available in osteoblasts to carboxylate osteocalcin can be modulated by the presence of estrogen. Along the same lines of investigation, the change in undercarboxylated osteocalcin was examined in response to 2 different regimens of hormonal replacement therapy in postmenopausal women.[52] In this study, there was a decrease in undercarboxylated osteocalcin, which the authors attributed to the effect of hormonal replacement therapy on increased triglyceride levels, which in turn elevated levels of vitamin K available to the bone for carboxylation. However, the proportion of undercarboxylated osteocalcin to total osteocalcin (ie, a higher percentage of undercarboxylated osteocalcin) actually increased, suggestive of a decline in vitamin K status in the bone in response to hormone replacement therapy. Collectively, these studies implicate the absence of estrogen as a determinant of vitamin K status in postmenopausal women, although the mechanisms are currently unknown.

Further studies need to be conducted in order to evaluate the underlying mechanism by which vitamin K acu-point injection alleviates dysmenorrhea. The role of estrogen in vitamin K metabolism also merits further investigation.

Summary

Several new roles for vitamin K beyond coagulation are emerging that could have an impact on subgroups of the population who are at increased risk for vitamin K deficiency. Thus far, the protective role of vitamin K in bone and cardiovascular health is modest, and much of the evidence is conflicting. Confusion exists in the field as mechanisms underlying the effects of vitamin K remain to be elucidated. Further research should also be conducted to determine what forms and amounts of vitamin K should be consumed to optimize health.

Author Contributions

Both authors contributed equally to the manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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