Vitamin K Status and Vascular Calcification: Evidence from Observational and Clinical Studies[1,2]

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ABSTRACT

Vascular calcification occurs when calcium accumulates in the intima (associated with atherosclerosis) and/or media layers of the vessel wall. Coronary artery calcification (CAC) reflects the calcium burden within the intima and media of the coronary arteries. In population-based studies, CAC independently predicts cardiovascular disease (CVD) and mortality. A preventive role for vitamin K in vascular calcification has been proposed based on its role in activating matrix Gla protein (MGP), a calcification inhibitor that is expressed in vascular tissue. Although animal and in vitro data support this role of vitamin K, overall data from human studies are inconsistent. The majority of population-based studies have relied on vitamin K intake to measure status. Phylloquinone is the primary dietary form of vitamin K and available supplementation trials, albeit limited, suggest phylloquinone supplementation is relevant to CAC. Yet observational studies have found higher dietary menaquinone, but not phylloquinone, to be associated with less calcification. Vascular calcification is highly prevalent in certain patient populations, especially in those with chronic kidney disease (CKD), and it is plausible vitamin K may contribute to reducing vascular calcification in patients at higher risk. Subclinical vitamin K deficiency has been reported in CKD patients, but studies linking vitamin K status to calcification outcomes in CKD are needed to clarify whether or not improving vitamin K status is associated with improved vascular health in CKD. This review summarizes the available evidence of vitamin K and vascular calcification in population-based studies and clinic-based studies, with a specific focus on CKD patients. *Adv. Nutr. 3: 158–165, 2012.*

Introduction

Vascular calcification occurs when vessel and/or valvular tissue becomes mineralized. In the vessel wall, calcium deposition can occur in the intimal and/or medial layers. Calcification of the intimal layer is reflective of atherosclerotic heart disease. Calcium deposition in the intimal layer of the coronary arteries (known as CAC[5]) can lead to vascular occlusion. It is detectable in ~30% of adults without clinical CVD (1–4) and is incrementally predictive of future cardiovascular events and overall mortality, independent of traditional CVD risk factors (5–7). Certain patient groups, especially those with CKD, are at greater risk for CAC (8–10).

In 2004, it was determined that 11% of the general population in the United States had CKD, translating into >19 million affected people (11). CKD is defined as the presence of kidney damage with or without reduced kidney function (12). The severity of CKD is determined by a staging process that is based on an estimated glomerular filtration rate. Moderate to severe CKD (stages 3–5) is represented by an estimated glomerular filtration rate of <60, <30, and <15 mL/(min·1.73 m^2), respectively, and stage 5b encompasses those individuals who require a form of kidney replacement therapy (hemodialysis, peritoneal dialysis, or kidney transplant) (12).

At every stage of CKD, the leading cause of mortality is CVD and patients are more likely to die of a cardiac event than they are to ever require a form of kidney replacement therapy (13). CKD patients are particularly prone to medial calcification (known as Monckeberg's sclerosis), which leads to arterial stiffening, elevated systolic pressure, and increased cardiac workload (14,15). Medial calcification is predictive of cardiovascular and all-cause mortality in CKD patients, independent of intimal calcification and CVD risk

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[5] Abbreviations used: AAC, abdominal aortic calcification; BAC, breast artery calcification; CAC, coronary artery calcification; CKD, chronic kidney disease; CVD, cardiovascular disease; ESKD, end-stage kidney disease; HF, heart failure; PROSPECT, Predictors of Response to Cardiac Resynchronization Therapy study; VSMC, vascular smooth muscle cell.

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factors (16,17). Calcific uremic arteriopathy, also known as calciphylaxis, is unique to patients with ESKD and classically manifests as calcification of cutaneous and s.c. arteries with occlusive intimal proliferation and subsequent fat necrosis (18).

Although the cellular and molecular events leading to calcium deposition in vascular tissue continue to be explored, it is understood to be a highly regulated process. Animal and molecular studies have demonstrated that vitamin K is involved in the development and progression of vascular calcification, as mediated by the carboxylation of matrix gla protein (MGP) (19). MGP, which is synthesized by VSMC, functions as a calcification inhibitor (20–22). In mice, targeted deletion of the *MGP* gene results in rapid and complete arterial calcification, resulting in death by 6 wk (23). For MGP to inhibit soft-tissue calcification, vitamin K is required as an enzymatic cofactor in the γ-carboxylation of the protein. It has been suggested that a lack of functional MGP, rather than the amount of total MGP, may increase risk for vascular calcification (24). This is supported by the observation that vitamin K antagonism with warfarin, which antagonizes the vitamin K-dependent carboxylation of MGP, also leads to rapid arterial calcification in rats (20). Furthermore, diets high in vitamin K have been shown to reverse aortic calcification and improve arterial elasticity in warfarin-treated rats (22), suggesting that the calcification in response to warfarin treatment is due to the inhibition of the vitamin K-dependent γ-carboxylation of MGP. In addition, MGP isolated from atherosclerotic plaque of aging rats was found to be incompletely carboxylated, thereby inhibiting its ability to function as a calcification inhibitor (25). Although vitamin K's role in vascular calcification has been demonstrated in animal and in vitro studies, the evidence from human studies is less clear. The aim of this review was to examine the human evidence of vitamin K's role in vascular calcification, first in population-based studies and then in patients with CKD, a group at greater risk for vascular calcification (26,27).

Current status of knowledge

Population-based studies

Population-based studies are studies of free-living, nonclinic-based participants who were not selected with respect to the disease of interest, allowing for the study of natural history of disease incidence and progression. Population-based studies are more generalizable to the general population than clinic-based studies, although participant selection procedures should be considered (28).

Observational studies. The majority of population-based studies reporting on the association between vitamin K status and vascular calcification have relied on vitamin K intake as the measure of status (summarized in **Table 1**). The primary form of vitamin K in most diets is phylloquinone (vitamin K1), which is found primarily in green leafy vegetables and vegetable oils (29). The current RDA for vitamin K in the US are 90 and 120 μg/d for adult women and men, respectively, based on median intakes according to NHANES (1988–1994) (30). In a small (n = 113), nested, case-control study of Dutch community-dwelling postmenopausal women, Jie et al. (31) reported that women with abdominal aortic calcification (AAC) reported lower intakes of phylloquinone (190 $\pm$ 15 μg/d) compared to women without (244 $\pm$ 15 μg/d). However, subsequent observational studies have not supported this finding. Among 4473 men and women (mean $\pm$ SD age = 67 $\pm$ 8 y) in the Rotterdam Study, there were no significant differences in phylloquinone intake across categories of AAC severity (32). In 564 healthy Dutch postmenopausal women participating in the Predictors of Response to Cardiac Resynchronization Therapy study(PROSPECT) study (mean $\pm$ SD age 67 $\pm$ 5 y old), the prevalence of CAC did not significantly differ across quartiles of phylloquinone intake (33). In a separate analysis of PROSPECT participants that measured breast artery calcification (BAC), phylloquinone intake did not differ significantly between women with BAC [adjusted mean (95% CI) = 209 (194–225) μg/d] and women without [adjusted mean (95% CI) = 211 (206–216) μg/d]. Among U.S. military personnel, Villines et al. (34) did not find phylloquinone intake to be associated with presence or severity of CAC. Because this was a younger cohort (39–45 y old) at low risk for CVD, the expected extent of CAC would be less than for other groups, so any association with vitamin K intake may have been more difficult to detect. In these studies, self-reported phylloquinone intakes were semiquantitatively estimated using validated FFQ (35–38). The reported phylloquinone intakes in studies outside the US (31–33,39) were on average more than double the current U.S. recommendations [mean $\pm$ SD = 217 $\pm$ 92 μg/d (33); 211 $\pm$ 73 μg/d (39); and 249 $\pm$ 126 μg/d (32)]. It is plausible that overall nutrient adequacy may have blunted the ability to detect an association. However, intakes among U.S. military personnel were in line with U.S. recommendations and were also not associated with CAC (mean $\pm$ SD = 115 $\pm$ 79 μg/d) (34). Self-reported dietary intakes are imprecise and there are inherent limitations to estimating nutrient intakes from FFQ (40). Because phylloquinone is found in healthy foods (green vegetables), self-reported intakes may be subject to overreporting (41). Phylloquinone intake is a marker of a heart-healthy diet (42), so it may be difficult to disentangle whether it or generally healthy lifestyle behaviors are associated with calcification outcomes, even when statistical adjustment is made (43).

Menaquinones (collectively referred to as vitamin K2) differ structurally from phylloquinone in their side-chain length and saturation. They are primarily found in meat and dairy-based foods and fermented soybeans (known as *natto*, commonly consumed in Japan). In most diets, menaquinones generally contribute less to total dietary vitamin K intakes than phylloquinone (29,44). Consumption of foods high in menaquinone tends to vary geographically; they appear to be more commonly consumed in non-Western diets. Circulating levels of menaquinone-7, e.g., are reported to be higher than phylloquinone in Japanese women, indicating a

Table 1 Population-based observational studies of vitamin K intake and vascular calcification[1]

Reference	Participants	Design	Outcome measure	Results
Studies of phylloquinone intake				
(31)	113 postmenopausal women (60–69 y old)	Nested case-control	AAC	Women with AAC had lower phylloquinone intake ($P < 0.05$)
(32)	4807 men and women (62% female, ≥55 y old)	Cross-sectional	AAC	No association between phylloquinone intake and AAC
(34)	807 military personnel (18% female, 39–45 y)	Cross-sectional	CAC	No association between phylloquinone intake and CAC
(39)	1689 women (49–70 y old)	Cross-sectional	BAC	No association between phylloquinone intake and BAC
(33)	560 postmenopausal women	Cross-sectional	CAC	No association between phylloquinone intake and CAC
Studies of menaquinone intake				
(32)	4807 men and women (62% female, ≥55 y old)	Cross-sectional	AAC	Lower odds of AAC in highest tertile of menaquinone intake (P-trend < 0.001)
(39)	1689 women (49–70 y old)	Cross-sectional	BAC	No association between menaquinone and BAC
(33)	560 postmenopausal women	Cross-sectional	CAC	Lower prevalence of CAC in highest quartile of menaquinone intake (P-trend = 0.03)

[1] AAC, abdominal aortic calcification; BAC, breast artery calcification; CAC, coronary artery calcification.

higher intake of this form of vitamin K in this region (45). Geleijnse et al. (32) reported a lower odds of severe AAC among Dutch men and women in the highest tertile of menaquinone intake (>32.7 μg/d, included menaquinones-4 through 10) [OR (95%CI) = 0.48 (0.32–0.71)] but no difference with respect to moderate AAC. In the PROSPECT cohort, Dutch women in the highest quartile of menaquinone intake (mean ± SD = 48.5 ± 9.0 μg/d, energy adjusted) had a significantly lower prevalence of CAC [prevalence ratio (95%CI) = 0.80 (0.65–0.98)] (33). However, in the study of BAC in PROSPECT, menaquinone intake did not significantly differ between women with BAC and women without [adjusted mean (95% CI) = 29 (27–30) and 29 (28–30) μg/d, respectively] (39). When interpreting these associations, it is important to note that the range of menaquinone intake reported in these studies was narrow compared to phylloquinone and the differences between tertiles/quartiles was small [i.e., 10–30 μg/d difference between highest and lowest groups (32,33)]. It is uncertain if intake differences of this magnitude would result in considerable differences in vascular tissue accumulation of menaquinone in humans. Because menaquinone intake data were derived from FFQ, there are inherent limitations to these estimates (40). Dietary sources of menaquinones are not reflective of overall healthy diet and lifestyle patterns, so menaquinone intake data may not be prone to overreporting and residual confounding to the same extent as are phylloquinone intake data. However, food composition data available for certain menaquinones are limited and circulating menaquinone concentrations are usually nondetectable, so validating dietary menaquinone intake data is problematic (42).

Although nutritional biomarkers are not susceptible to the biases inherent to dietary intake questionnaires (46,47), population-based studies of vitamin K status biomarkers and vascular calcification are fairly limited. There is currently no one biomarker considered to be a robust indicator of vitamin K nutritional status. Circulating phylloquinone levels are thought to reflect overall status but are highly correlated with TG [due to it being transported on TG-rich lipoproteins (48)] and fluctuate according to recent intakes (29,49). In a cross-sectional analysis of older men and women, lower plasma phylloquinone was associated with higher CAC, adjusted for TG and potential confounders (50). The undercarboxylated fractions of vitamin K-dependent proteins, which can also be measured in circulation, are considered functional indicators of vitamin K status of certain tissues. These measures are elevated when vitamin K status is low (49,51). Undercarboxylated prothrombin (PIVKA-II) reflects hepatic vitamin K status and changes according to vitamin K intake (52). However, it is generally not used as a marker of subclinical vitamin K deficiency in population-based studies, because changes in PIVKA-II do not reflect changes in clotting function in generally healthy adults (52). Undercarboxylated osteocalcin (ucOC) (the primary vitamin K-dependent protein in bone) has been utilized as a measure of vitamin K status of bone in several population-based studies (53–55). Jie et al. (31) found women with AAC had higher levels of undercarboxylated (free) OC in serum, reflective of lower vitamin K status compared to women without AAC. However, ucOC may not necessarily reflect vitamin K status in vascular tissue. MGP is the most-studied vitamin K-dependent protein implicated in the regulation of vascular calcification. Although assays that measure the total MGP in circulation (regardless of its carboxylation status) have been available for some time (56), data are conflicting as to whether the total circulating MGP pool reflects atherosclerotic calcification (56–58). Because only the carboxylated form of MGP can function as a calcification inhibitor, distinguishing the uncarboxylated and carboxylated fractions of MGP in circulation has been suggested, to clarify the role of the functional forms of MGP in vascular calcification (19). Recently assays that measure different fractions of MGP in circulation have been developed, only one of which [the dephosphorylated

uncarboxylated MGP, (dp)ucMGP] appears to reflect vitamin K status (59). This was confirmed by a post hoc analysis of archived blood samples from a vitamin K supplementation study, which found (dp)ucMGP was inversely correlated with plasma phylloquinone, positively correlated with ucOC, and was reduced following 3 y of phylloquinone supplementation (500 μg/d) in community-dwelling older adults. However, (dp)ucMGP was not associated with CAC in this cohort, cross-sectionally or longitudinally (51). The associations of other circulating forms of MGP [dephosphorylated carboxylated MGP ((dp)cMGP) and total ucMGP (regardless of phosphorylation status) (59)] with vascular calcification have not yet been reported in population-based studies. The biochemistry and physiology of MGP continues to be explored and the relationship between vitamin K status and vascular calcification will be clarified by ongoing longitudinal analyses of vitamin K biomarkers in larger population-based cohorts.

Intervention studies. There are currently 2 reported intervention trials of vitamin K on cardiovascular outcomes, only one of which directly measured vascular calcification. In a 3-y, double-blinded, randomized controlled trial, 500 μg/d of phylloquinone supplementation was associated with reduced CAC progression in 388 older men and women free of clinical CVD. Although the effect of supplementation was not significant in intent-to-treat analysis, men and women with preexisting CAC who received phylloquinone supplementation had 6% less CAC progression compared to the controls over 3 y, which was significant (60). In a 3-y follow-up analysis of patients treated with statins, those who had a myocardial infarction had 25% more CAC progression compared to myocardial infarction-free participants (61), so the clinical relevance of the observed effect of vitamin K remains to be clarified. Because calcification progresses more rapidly in persons with preexisting CAC (62,63) and in vitro studies show increased MGP expression in calcified VSMC (64,65), it is plausible that vitamin K may affect CAC progression more than it does disease onset. In an earlier, double-blind, randomized supplementation trial in 108 postmenopausal women, those who were randomized to receive a supplement containing 1000 μg/d phylloquinone in addition to minerals and 320 IU cholecalciferol had better carotid artery distensibility, compliance, and elasticity after 3 y compared to women who received the mineral supplement alone or the mineral supplement with cholecalciferol (66). Although no direct measures of calcification were included in this study, the authors speculated their findings may be due in part to vitamin K's potential role in reducing calcium deposition in the vessel wall.

CKD

Vascular calcification is highly prevalent in certain patient populations, especially in those with CKD. Although studies in this area are still limited, there is growing interest in a potential role for vitamin K in modifying the progression of calcification in patients with CKD, because vascular calcification is common in this patient group.

According to estimates from the Chronic Renal Insufficiency Cohort Study, >60% of CKD patients have detectable CAC, the severity of which increases as CKD progresses (67). In one cross-sectional study, >30% of patients with stage 3–5 CKD already had severe CAC (>400 Agatson units) despite the absence of a cardiac history (68). Accelerated calcification in patients with CKD relates in part to the mineral bone disorder that is observed in these patients and is characterized by abnormalities in phosphorus homeostasis. The regulation of phosphate is primarily mediated by renal excretion in healthy participants; in CKD, a positive phosphate balance occurs and multiple studies have linked abnormal phosphorus levels with severity and progression of calcification (69,70–72). Excess phosphate uptake into VSMC via a sodium-phosphate cotransporter is thought to be a key initiating event in the process of vascular calcification (73,74). Elevated intracellular and extracellular phosphate stimulates expression of proteins involved in bone formation [e.g., Cbfa-1, BMP-2, and -4, and OC] and downregulates the expression of VSMC contractile proteins (75,76).

Observational studies of CKD patients

A number of studies have determined the vitamin K status of individuals with CKD; however, studies linking vitamin K status to clinical outcomes or surrogate measures of calcification are lacking. Dietary vitamin K intake may be compromised in patients with CKD for at least 2 reasons. First, the renal diet is restricted with respect to potassium-rich foods that otherwise may be a good source of vitamin K, and secondly, this population has a high frequency of anorexia and gastrointestinal symptoms that result in a compromised overall energy intake (77). Usual dietary vitamin K intake was assessed in a cohort of patients with stage 3–5 CKD by a FFQ validated for vitamin K intake in the general population (78,79). The mean phylloquinone intake was 130 $\pm$ 103 μg/d, with higher intakes largely reflected by overall energy intake and better clinical nutritional status (78).

It has become evident that a high prevalence of subclinical vitamin K deficiency exists in hemodialysis and peritoneal dialysis patients and in individuals with earlier stages of CKD (78,80–82). The percentages of participants that meet the criteria for subclinical vitamin K deficiency based on very low circulating concentrations of phylloquinone are: CKD (6%), hemodialysis (29%), and peritoneal dialysis (24%) (78,80,81). Individuals with CKD consistently demonstrate marked elevations in serum TG and low levels of HDL-cholesterol (83). In all cohort studies of CKD patients, higher levels of phylloquinone strongly and independently associate with higher levels of TG, which may confound any association between circulating phylloquinone and CKD-related health outcomes in this particular group. Between 60 and 90% of patients with CKD fulfill criteria for subclinical vitamin K deficiency on the basis of circulating ucOC (78,81). However, the spectrum of mineral and bone disorders and potential for accumulation of OC fragments

in individuals with low kidney function may render it a less useful measure of vitamin K status in this population. The strongest predictors of elevated percent ucOC are parameters related to the CKD-mineral bone disorder (higher phosphorus and parathyroid hormone levels) (80). In contrast to healthy populations, PIVKA-II may therefore be a superior marker of vitamin K deficiency in the CKD population given that it is not affected by kidney function. Ninety-four percent of CKD patients had PIVKA-II levels > 2 g/L, consistent with dietary deficiency (78). No study has yet linked abnormalities in these particular biomarkers of vitamin K status with any measure of calcification in CKD.

Warfarin use could be considered a surrogate for impaired vitamin K status, because warfarin antagonizes the vitamin K epoxide reductase enzyme, thereby limiting the carboxylation of vitamin K-dependent proteins. The frequency of warfarin use reported by single-center and population-based studies in ESKD patients approaches 20% in North America (84,85). Despite the frequency of warfarin prescription, there are few studies that have evaluated the adverse consequences of its use in patients with CKD. One study has demonstrated that long-term warfarin exposure was independently associated with greater severity of aortic valve calcification in dialysis patients (86) and anecdotal evidence has linked warfarin use to the pathogenesis of calcific uremic arteriopathy in patients with ESKD (18). Calciphylaxis is associated with exceedingly high morbidity and mortality in dialysis patients (87).

Analyses of (dp)ucMGP in case-control studies have reported it to be higher among patients with diseases characterized by vascular calcification, including CKD (59), and (dp)ucMGP was found to be positively associated with aortic calcium score in 107 patients with CKD (88). Conversely, Schleiper et al. (89) reported that the circulating (dp)cMGP and (dp)ucMGP were both lower in patients with ESKD compared to controls, but neither measure correlated with vascular calcification in the ESKD patients. An alternate monoclonal-antibody ELISA that measures total-ucMGP (whether or not it is phosphorylated) is also available (59), and this measure of total ucMGP was reported to be inversely associated with CAC in a small sample of hemodialysis patients (n = 40) (90). Plasma (dp)cMGP and the total ucMGP (measured using this monoclonal assay) do not respond to changes in vitamin K status (59,89); therefore, it is difficult to extrapolate the reported associations between these measures and calcification to vitamin K status. At this point, the clinical utility of circulating MGP measures in CKD patients remains uncertain and may be clarified using larger cohorts of CKD patients who are well characterized for vascular calcification and related outcomes.

Intervention studies

There are currently no intervention trials evaluating vitamin K in the prevention of vascular calcification in CKD patients. Therefore, the role of vitamin K in CKD patients remains be clarified by future randomized controlled trials targeting this high-risk patient population.

Other patient populations

Warfarin is commonly used to manage chronic cardiovascular conditions, such as atrial fibrillation, venous thromboembolism, and valvular stenosis (91). It stands to reason that warfarin users represent a group who may be at greater risk for vascular calcification. Several case-comparison analyses [one of which contained over 1100 participants (92)] reported greater valvular calcification in warfarin users compared to nonusers (92–94). However, a small cross-sectional study of 70 warfarin patients (mean age = 68 y) attending an anticoagulation clinic found no association between warfarin treatment duration and CAC (95). It is has been suggested that although the valvular calcification and CAC processes overlap, the 2 may not be exactly the same (96), so the 2 outcomes may differ with respect to warfarin treatment. This would be clarified by examining the association between warfarin treatment duration with vascular and valvular calcification together in larger longitudinal studies.

Patients with diabetes (type 1 and 2), hypercholesterolemia, and cardiac valve disease are also at higher risk for vascular calcification (96,97). In the limited available studies of these patients, vitamin K status primarily has been estimated using the newly developed (dp)ucMGP assay. (dp)ucMGP was found to be elevated (indicative of lower vitamin K status) in patients with aortic stenosis compared to healthy controls but was not significantly correlated with stenosis severity (98). In a separate analysis, the same authors found (dp)ucMGP to be elevated in patients with chronic HF compared to healthy controls, positively associated with HF severity, and markedly higher in patients who died of HF progression over 2.9 y compared to survivors. Because this study's sample size was modest (n = 179, and 12 deaths due to HF progression) and vascular calcification was not directly quantified, it is uncertain if the HF outcomes were related to calcification or not (99). Parker et al. (100) reported higher total ucMGP was associated with higher odds of mitral annular calcification in diabetics and lower odds of mitral annular calcification in persons without diabetes. Because the total-ucMGP measure used in this study does not reflect vitamin K status (59), the relevance of vitamin K to these findings is uncertain. Overall, vitamin K's role in vascular calcification in these patients has not been well studied. Assessing vitamin K status using multiple biomarkers in observational longitudinal studies and well-designed randomized trials in these patients would provide important insight into whether vitamin K also has a role in vascular calcification in clinic-based populations in addition to CKD.

Conclusion

Overall, the available observational population-based evidence, based on dietary intake measures, suggests menaquinone intake may be more likely to protect against vascular calcification than phylloquinone intake. Yet currently, the only intervention studies have examined the effect of phylloquinone and provide evidence that phylloquinone supplementation is relevant to vascular calcification (60,66). However, confirmatory studies are needed. Furthermore,

because there are no published intervention studies of menaquinone with a measure of vascular calcification as an outcome, any differential effect of phylloquinone compared to menaquinone will be clarified by future trials designed to compare the effects of the different vitamin K forms on vascular calcification. Within the patient populations, individuals with CKD represent a rapidly growing segment at increased risk for vitamin K deficiency. There are currently no clinical practice guidelines that recommend routine vitamin K supplementation for individuals with CKD outside of patients exposed to long-term oral antibiotics (101). Future trials should specifically address this at-risk group. In addition to CKD, vascular calcification is a characteristic of other chronic health conditions (96), in which the role of vitamin K merits exploration.

Acknowledgments

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