

Cell Signalling and Vessel Remodelling

Beneficial effects of vitamins D and K on the elastic properties of the vessel wall in postmenopausal women: a follow-up study

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Summary

Matrix-Gla Protein (MGP) is a strong inhibitor of vascular calcification, the expression of which is vitamin D dependent. MGP contains five γ -carboxyglutamic acid (Gla)-residues which are formed in a vitamin K-dependent carboxylation step and which are essential for its function. Hence vascular vitamin K-deficiency will result in undercarboxylated, inactive MGP which is a potential risk factor for calcification. In the present study we describe the effects of vitamin K₁ and D supplementation on vascular properties in postmenopausal women. In a randomized placebo-controlled intervention study, 181 postmenopausal women were given either a placebo or a supplement containing minerals and vitamin D (MD-group), or the same supplement with vitamin K₁ (MDK-group). 150 participants completed the study and analysis was performed on 108 participants. At baseline and after three years, vessel wall characteris-

tics, including compliance coefficient (CC), distensibility coefficient (DC), intima-media thickness (IMT) and the Young's Modulus (E) were measured to assess the effect of the supplements on the change of these parameters. The results showed that the elastic properties of the common carotid artery in the MDK-group remained unchanged over the three-year period, but decreased in the MD- and placebo-group. Comparing the MDK- and placebo-group, there were significant differences in decrease of DC (8.8%; $p < 0.05$), CC (8.6%; $p < 0.05$), and in increase of PP (6.3%; $p < 0.05$) and E (13.2%, $p < 0.01$). There were no significant differences between the MD-group and placebo. No significant differences were observed in the change of IMT between the three groups. It is concluded that a supplement containing vitamins K₁ and D has a beneficial effect on the elastic properties of the arterial vessel wall.

Keywords

Elastic properties, vessel wall, vitamin K, postmenopausal women, ultrasound

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Introduction

Vitamin K is a cofactor in the post-translational carboxylation of selective protein-bound glutamate residues which are converted into gamma-carboxy glutamate (Gla) (1). To date, only a dozen mammalian Gla-containing proteins (shortly: Gla-proteins) have been identified. Vitamin K deficiency results in the

synthesis of under-carboxylated, biologically inactive Gla-proteins. Examples of Gla-proteins are several blood coagulation factors (all synthesized in the liver), osteocalcin (synthesised in bone) and matrix Gla-protein (MGP), which is synthesized in a number of non-hepatic tissues, notably cartilage and the arterial vessel wall. Today MGP is regarded as a major inhibitor of soft tissue calcification (2).

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Vascular calcification is an important factor that contributes to considerable morbidity and mortality, notably in diabetics and in hemodialysis and atherosclerotic patients (3). Calcification may occur either in the tunica intima in association with atherosclerosis or in the tunica media where it is known as Mönckeberg's sclerosis (4). Medial vascular smooth muscle cells (VSMCs) synthesize most of the MGP in the vessel wall (5). It was found that the overall arterial expression of MGP was decreased in Mönckeberg's sclerosis suggesting that low levels of MGP may predispose to calcification (6). However, in VSMCs adjacent to the calcium salt deposits, MGP mRNA expression was substantially elevated which may represent a response to the locally increased calcium concentration in order to enhance calcium clearance (5). Using monoclonal antibodies against MGP Schurgers, et al. (7) demonstrated that MGP also accumulates around the calcified areas.

Although the precise sequence of events in the association between MGP-expression and calcification still needs to be elucidated, a number of animal studies have established unequivocally that MGP is a potent inhibitor of calcification. The vascular phenotype of the transgenic MGP null mouse showed massive calcification of the large arteries within 4 weeks after birth (8). The fact that comparable results were obtained in normal rats after treatment with the vitamin K-antagonist warfarin demonstrated that Gla-residues are essential to the calcification inhibitor function of MGP (2).

Whereas vitamin K is involved in the posttranslational processing of MGP, vitamin D has a role in the regulation of MGP gene expression. Fraser, et al. have shown that the MGP promoter contains a vitamin D response element that is responsible for a 2-3 fold enhancement of MGP expression after vitamin D binding (9). However, to our knowledge, a direct association between vitamin D deficiency and arterial media calcification has not yet been demonstrated. With respect to vitamin K, it was shown in postmenopausal women that low vitamin K₁ intake is a risk factor for aortic calcification (10). In an independent population-based study among 4500 elderly subjects, an inverse correlation was demonstrated between vitamin K₂ intake and aortic calcification, myocardial infarction and sudden cardiovascular death (11). Based on these findings, it has been suggested that in a substantial part of the population the vitamin K status of the arterial vessel wall is inadequate to support full MGP carboxylation (7). We wish to put forward the hypothesis that local vitamin K deficiency forms a risk factor for vascular hardening, increasing stiffness, and loss of elastic properties.

The present study forms part of a large intervention trial in which we investigated the effects of minerals (calcium, magnesium, and zinc) and vitamins D and K on bone mineral density and vascular properties. Here we report the effects of minerals + vitamin D, and minerals + vitamins D and K supplementation on the vascular properties during a three-year follow-up period. Measures of functional vascular properties are the distensibility

coefficient (DC) and compliance coefficient (CC) as they characterize the arterial stiffness of the vessel wall, which may be modified by medial calcification. The intima-media thickness (IMT) is assumed to represent an endothelial adaptive response to physiological and pathophysiological processes (12-14) and is regarded as an indicator of atherosclerosis (15, 16). The ratio of diameter to IMT corrects the functional parameter DC for the tissue volume involved, resulting in a parameter for the structural elasticity, known as the Young's Modulus (E) (17). The present study also included the effects of vitamins K₁ and D on changes in IMT and E. We selected a group of healthy postmenopausal women to assess the effects of vitamin K₁ and D supplementation on changes in vessel wall characteristics because it has been suggested that changes in vessel wall properties occur faster in postmenopausal women between 45 and 60 years than in men of the same age (18).

Methods

Subjects

The participants were enrolled in a 3-year double-blind placebo-controlled clinical trial in which the effects of minerals, vitamins D and K were investigated on bone mineral density and vessel wall characteristics. The participants of this trial were recruited by newspaper advertisements. Inclusion criteria were: apparently healthy women, Caucasian race, between 50 and 60 years old and at least 2 years postmenopausal. Exclusion criteria were: use or recent use (< 1 year) of oral anticoagulants, corticosteroids, hormone replacement therapy and alcohol consumption of > 2 glasses/day. Furthermore, persons using multivitamins or food supplements recently (< 1 year) were excluded from participation. In this way, the effects of the intervention could be studied without wash-out effects. In total 188 women met the criteria for participation and were randomized into our study. Information on cardiovascular risk factors, current health status, medical history, drug use and smoking behaviour was collected before the start of the study. No medical history of important systemic diseases was reported. Within this trial participants were seen every 3 months to check for physical health and compliance of the treatment by short questionnaires; on a number of these occasions bone densitometry (DXA) and blood sampling were performed. The vascular examination only took place at baseline and at the end of the study after 3 years. Baseline measurements were performed between November 1997 and March 1998, and the follow-up examination took place between December 2000 and March 2001. All participants gave written informed consent and the trial was approved by the University Hospital medical ethics committee.

Study design

The subjects were randomized into three groups. Of the total group of 188 women, 7 women were referred for treatment out-

side the study because abnormalities were found during the baseline measurements. In the first group (n=60) participants received a placebo (maltodextrine, i.e. placebo group), in the second group (n=58) participants received a supplement containing 500 mg calcium (natural calcium complex derived from milk), 10 mg zinc, 150 mg magnesium and 8 µg vitamin D₃ (minerals + vitamin D = MD-group), and in the third group (n=63) participants received a supplement containing the same constituents as the MD-group but with additional 1 mg vitamin K₁ (minerals + vitamins D+K = MDK-group). In the present study, we compared the changes of vessel wall characteristics between treatment groups and placebo to elucidate the effects of vitamins K and D on the vessel wall characteristics.

The randomization of the participants to the three groups was performed according to a computer-generated randomization list and the randomization codes were kept apart from the study site during the trial. The participant randomization codes were allocated sequentially in the order in which the participants were enrolled. One investigator who supervised the whole study was responsible for the enrollment and assignment of participants to the respective groups. Because the three different types of supplements were similar in appearance and taste, participants and investigators were not aware of group assignment. Participants were allowed to choose between a supplement in the form of a tasteless powder (to be mixed with water before intake) or in the form of chocolate-coated tablets with a crunchy malt core. The percentage of subjects who used the powder or tablets was equally distributed across the three groups. Participants were instructed to take one sachet with powder or three tablets per day during evening hours, preferably after the meal. Also, they were advised to maintain their usual diets and to avoid taking supplements containing either calcium, vitamin D, or K throughout the study. Novartis Consumer Health SA (Nyon, Switzerland) prepared and provided all supplements. After randomization, the women received the first batch of supplements and were supplied with a new batch of supplements every six months.

Measurements

The primary outcome measures for the purpose of this study were the vessel wall characteristics of the common carotid artery measured with ultrasound (7.5 MHz, ATL Mark V). The ultrasonic vessel wall tracking system (WTS) to determine arterial wall properties has been described in detail previously (19, 20). It provides estimates of the arterial end-diastolic adventitia-adventitia diameter (d) and the change in diameter (distension) from diastole to systole (Δd) normalized for the end-diastolic diameter (Δd/d) for each captured heart beat. In parallel with diameter change measurements, arterial blood pressure was recorded at the level of the brachial artery by means of a semi-automated oscillometric device (DINAMAP). Pulse pressure (PP), defined as systolic minus diastolic blood pressure, was

determined by averaging the three measurements nearest to the distension measurements.

The intima-media thickness (IMT) was measured simultaneously at the same location (2-3 cm proximal to the bifurcation) of the common carotid artery where the diameter and diameter changes were measured. Only the IMT of the posterior wall was assessed because here the reflections from the blood-intima and media-adventitia transition are distinctly visible, whereas at the anterior wall, the trailing edge of the adventitial reflections may obscure the medial and intimal signal. At the end of the session, recorded IMT-files were processed employing the wall thickness program. The threshold for the derivative was maintained at 0.025 (21). Each heart beat within a recording resulted in an estimate of wall thickness; the median of the estimates per recording was used for further evaluation. From d, Δd, PP and IMT, we have calculated the vascular distensibility coefficient (DC), the compliance coefficient (CC) and the elasticity coefficient (E) according to the following equations:

$$DC = (2d\Delta d + \Delta d^2)/(d^2PP)$$

$$CC = \pi(2d\Delta d + \Delta d^2)/4PP$$

$$E = d / (IMT * DC)$$

The examinations were performed in a quiet room with a controlled temperature of 21 ± 2 °C. The measurements were made at the same moment of the day to avoid diurnal variations and after a 10-15 minute supine rest to stabilize blood pressure levels. Participants were asked to refrain from smoking and the consumption of alcohol-, caffeine-, or quinine-containing beverages at least 3 hours prior to the examination. To save time we have investigated only the right common carotid artery. To our knowledge no significant differences between the wall properties of the right and the left common carotid artery have ever been reported. The same investigator performed all examinations at the start and the end of the study and for each participant several repeated measurements (5-7) were made during one session, the average of which was calculated and used for the analysis of the data. The reproducibility of the method is around 10% for the distension, DC, CC (20) and IMT (22).

Before the vascular examination, height and weight of each participant were measured with standardized equipment to estimate the body mass index (weight/height²).

Statistical analysis

The sample size was calculated on the assumption that the desired minimal detectable effect was a 15% reduced decrease in distensibility of the MDK-group compared to the placebo group with a 90% power and a 0.05 level of significance. With the assumption of a dropout rate of 10% per year we calculated that 180 subjects had to be included. Statistical analysis was performed using the Statistical Package SPSS (SPSS Corp,

Baseline-characteristics	Placebo (n=40)	MD-group (n=30)	MDK-group (n=38)
Age (yr)	54.1 ± 3.0	55.9 ± 2.8*	55.4 ± 2.8
Weight (kg)	69.5 ± 11.9	70.6 ± 11.1	66.3 ± 9.5
Height (m)	1.65 ± 0.05	1.65 ± 0.07	1.63 ± 0.06
BMI (kg/m ²)	25.6 ± 4.3	26.0 ± 4.4	25.1 ± 3.1
Postmenopausal age (yr)	4.6 ± 3.7	7.6 ± 5.1**	5.1 ± 4.3
Non-smokers (%)	75.0	73.9	85.0
Diameter (µm)	7162 ± 562	7314 ± 582	7173 ± 411
Distension (µm)	372 ± 118	353 ± 83	332 ± 83
Pulse Pressure (mmHg)	51.9 ± 11.1	52.9 ± 10.1	53.7 ± 14.3
Diastolic Blood Pressure (mmHg)	73.5 ± 7.0	73.9 ± 8.7	73.3 ± 8.6
Heart Rate (beats/min)	60.8 ± 9.2	63.1 ± 8.9	60.6 ± 6.6
CC (mm ² /kPa)	0.64 ± 0.23	0.61 ± 0.20	0.56 ± 0.17
DC (MPa ⁻¹)	15.8 ± 5.2	14.5 ± 4.0	14.0 ± 4.0
IMT (mm)	0.63 ± 0.11	0.64 ± 0.10	0.61 ± 0.08
Diameter / IMT	11.7 ± 1.9	11.6 ± 1.8	12.0 ± 1.7
E (kPa)	822 ± 300	852 ± 266	941 ± 298

Table 1: Baseline characteristics (mean ± standard deviation) in the placebo group (n=40), MD-group (n=30) and MDK-group (n=38). *significant different from placebo (p<0.05), ** significant different from placebo and MDK-group (p<0.05). Abbreviations used: body mass index (BMI), compliance coefficient (CC), distensibility coefficient (DC), intima-media thickness (IMT), Elasticity (E).

Chicago, IL). Results are presented as means ± standard deviation (SD), unless indicated otherwise. Only participants who had completed the study were included in the primary outcome analysis. Furthermore, participants who had started to use medication directly affecting the vessel wall during the study and participants with atherosclerotic plaques in the common carotid artery, were excluded from analysis. Also, participants with a high variability in their results were excluded (arterial translation of > 2 mm and beat-to-beat variation in distension of > 20%). Namely, a beat-to-beat variation in distension of > 20% would only be explainable by difficulties in the recording technique rather than by changes in blood pressure. Furthermore, for an arterial translation of > 2 mm it is highly unlikely that this motion is restricted to the plane of observation. Out of plane motion induces false diameter and distension measurements.

The primary outcome analyses were repeated to exclude the possibility of bias by drop-outs and excluded subjects. In these repeat analyses, the missing values of drop-outs and excluded subjects were replaced by mean values of outcome variables of the total population of 108 participants. A paired t-test was used to evaluate the change in the vessel wall characteristics over the three years within each group. We considered a level of p<0.05 to be statistically significant. For every participant, the percentage change from baseline in all parameters was calculated and the mean change from baseline was calculated per group. Primary outcome analysis consisted of comparison of the change in DC, CC, PP, IMT, d/IMT and E between the MD-group and placebo and between the MDK-group and placebo using linear regression analysis. In this analysis, the change in vascular parameters relative to baseline was used as dependent variable and the treatment groups and several covariates were used as explanatory variables. Baseline values of age, weight,

smoking (yes or no), heart rate and mean arterial pressure were chosen as covariates because their influence on the change in vascular properties or response to the supplementation could not be excluded.

Results

Baseline characteristics

Baseline characteristics of the selected participants are presented in Table 1. The MD group differed slightly but significantly from the placebo and the MDK group with respect to age and the number of years since menopause. No significant differences were observed in the baseline values of the vessel wall characteristics. Figure 1 shows the flow of participants through each stage for the separate groups. Of the 181 subjects who entered the study, 31 discontinued their participation during the course of the study and were not available for the follow-up measurement. Of the remaining 150 participants who completed the study, we excluded 42 subjects from the analysis: 4 women started to use medication known to have direct effects on the vessel wall (ACE-inhibitors, β-blockers or Calcium-antagonists), in 11 subjects atherosclerotic plaques in the common carotid artery were clearly visible and they were referred for treatment outside the study, and 27 subjects had a high variability in their repeated measurements (arterial translation of > 2 mm and beat-to-beat variation in distension of > 20%). Analysis was performed on 108 participants who completed the study and who did not have any of the above mentioned interfering factors. There were no significant differences in baseline values of the vessel wall characteristics between the 108 selected participants and those who discontinued treatment or

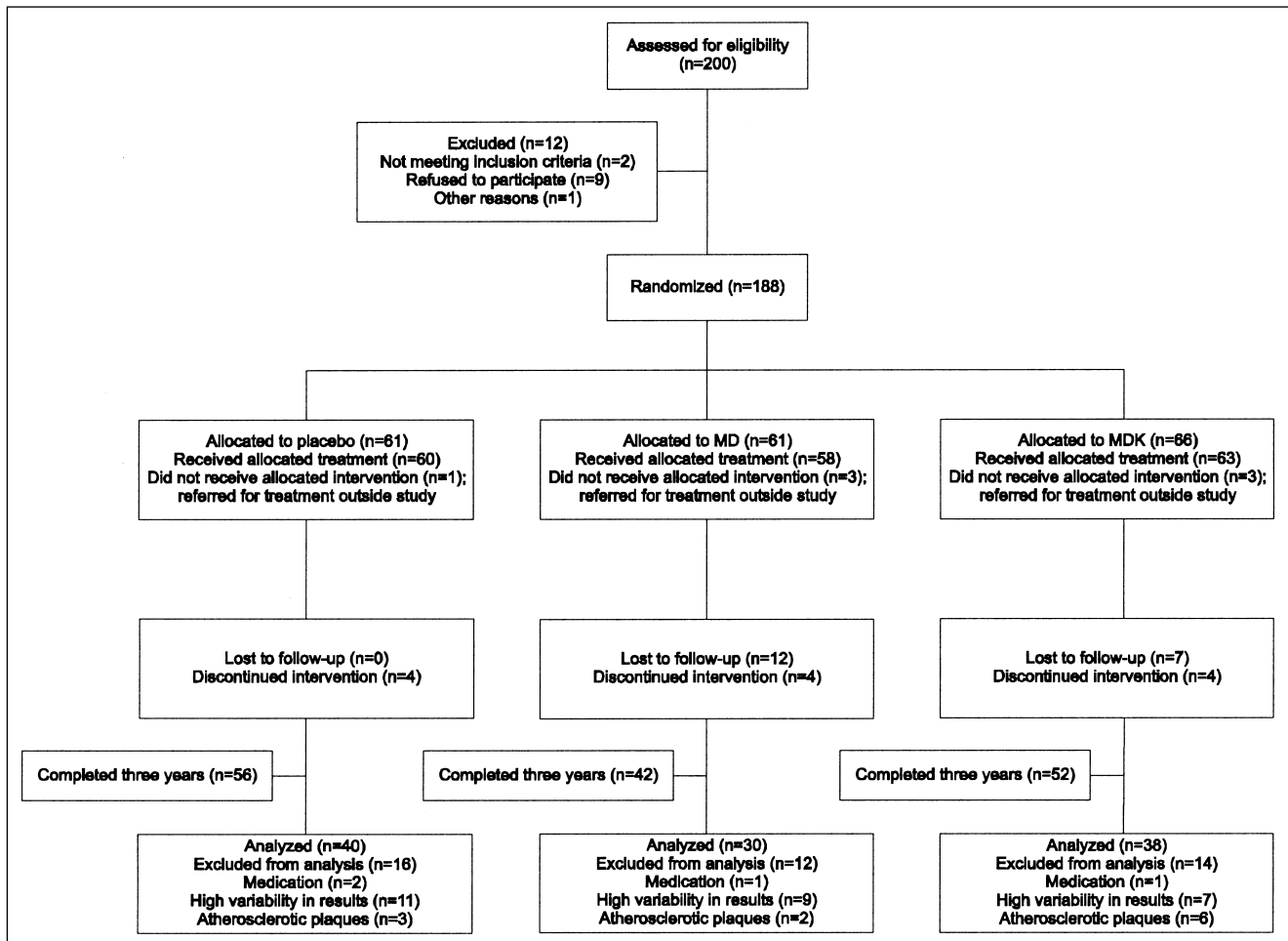


Figure 1: Flow diagram of participants through the different stages of the study.

were excluded (n=73). The only side-effect of the allocated intervention reported to the investigator were complaints of mild constipation in a few participants of the MD-group (n=4) and the MDK-group (n=3). No further adverse events occurred during the study.

Vascular parameters of elasticity

Table 2 summarizes per group the differences between the mean values at baseline and the end of the study for all vascular parameters with their paired-levels of significance. Changes in percentages between t=0 and t=3 yr are given as well. The DC and CC in the placebo group decreased significantly with 10% and 6%, respectively. The PP, on the other hand increased by 7%, but the increase did not reach the level of significance. In the MD-group, DC decreased significantly with 7% and CC decreased with 4%, while the PP increased with 6%, however these latter two changes did not reach the level of significance. In the MDK-group, the DC, CC and PP remained constant over the three years period, with even a tendency for the CC to

increase (+3%). Figure 2 illustrates the change in DC and CC of the three groups. After adjustment for baseline heart rate, mean arterial pressure, age, weight and smoking, there were significant differences between the MDK- group and the placebo with respect to DC (8.8%, 95% CI: 1.9 to 21.4), CC (8.6%, 95% CI: 1.8 to 20.3), and PP (-6.3%, 95% CI: -17.1 to -0.7). In the same analysis no differences were found between the MD- and placebo-group with respect to DC (2.5%, 95% CI: -6.3 to 14.8), CC (2.2%, 95% CI: -6.3 to 13.8), and PP (-0.11%, 95% CI: -12.1 to 5.6). The repeat analyses in which mean values were allocated to drop-outs and excluded subjects, showed the same trends as those described above; as to be expected differences between the MDK-group and placebo in change of DC, CC and PP were smaller, but they remained statistically significant.

Intima-media thickness

Changes of the intima-media thickness relative to baseline are also portrayed in Table 2. The IMT increased in all three groups: 9% in the placebo (p<0.01), 10% in the MDK-group (p<0.01),

Table 2: Change in vessel wall characteristics (mean \pm standard deviation and change in percentages) in the placebo group (n=40), MD-group (n=30) and MDK-group (n=38) after three years of treatment. *significant different from placebo ($p < 0.05$); P-value in parentheses with respect to baseline. Abbreviations used: compliance coefficient (CC), distensibility coefficient (DC), intima-media thickness (IMT), Elasticity (E).

Change between T=0 and T=3 years (paired t-test)	Placebo (n=40)	Placebo (n=40)	MD-group (n=30)	MD-group (n=30)	MDK-group (n=38)	MDK-group (n=38)
	mean \pm standard deviation	mean percent change	mean \pm standard deviation	mean percent change	mean \pm standard deviation	mean percent change
Diameter (μm)	+196 \pm 295 ($p < 0.01$)	+2.8%	+154 \pm 179 ($p < 0.01$)	+2.2%	+131 \pm 226 ($p < 0.01$)	+1.8%
Distension (μm)	-21 \pm 61 ($p < 0.05$)	-4.3%	-12.6 \pm 47 ($p = 0.15$)	-2.4%	-3.9 \pm 49 ($p = 0.63$)	+0.3%
Pulse Pressure (mmHg)	+2.7 \pm 9.9 ($p = 0.09$)	+6.5%	+2.8 \pm 10.1 ($p = 0.14$)	+6.4%	-0.2 \pm 7.6 ($p = 0.89$)*	+0.2%
DC (MPa^{-1})	-1.8 \pm 3.4 ($p < 0.01$)	-9.6%	-1.4 \pm 3.0 ($p < 0.05$)	-7.1%	-0.4 \pm 3.0 ($p = 0.43$)*	-0.8%
CC (mm^2/kPa)	-0.05 \pm 0.1 ($p < 0.01$)	-5.9%	-0.04 \pm 0.1 ($p = 0.10$)	-3.7%	+0.01 \pm 0.11 ($p = 0.75$)*	+2.7%
IMT (mm)	+0.05 \pm 0.08 ($p < 0.01$)	+8.6%	+0.02 \pm 0.09 ($p = 0.32$)	+4.0%	+0.06 \pm 0.06 ($p < 0.01$)	+9.8%
Diameter / IMT	-0.56 \pm 1.5 ($p < 0.05$)	-3.8%	-0.14 \pm 1.5 ($p = 0.63$)	-0.02%	-0.85 \pm 1.1 ($p < 0.01$)	-6.5%
E (kPa)	74 \pm 279 ($p = 0.12$)	+13.2%	68 \pm 254 ($p = 0.17$)	+13.7%	-27 \pm 236 ($p = 0.50$)*	+0.03%

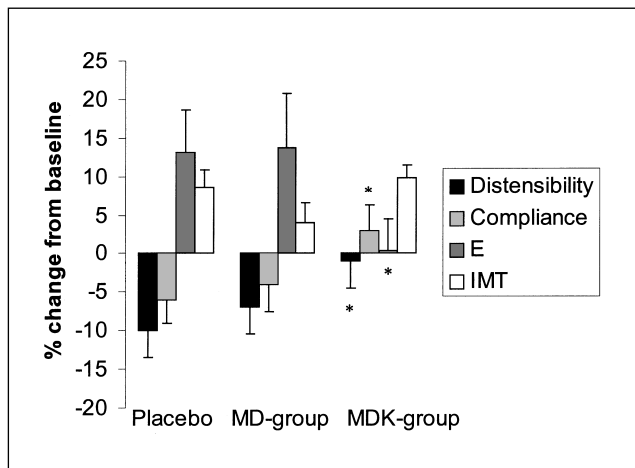


Figure 2: Mean percent change (\pm standard error of the mean) from baseline (0%) in distensibility coefficient, compliance coefficient, intima-media thickness and the Young's Modulus (E) of the common carotid artery in the placebo group (n=40), MD-group (n=30) and MDK-group (n=38) after three years of treatment. *significant different from placebo ($p < 0.05$).

but only 4% in the MD-group ($p = 0.32$). The d/IMT ratio decreased significantly in the placebo and MDK-group by 3.8% and 6.5% respectively, while in the MD-group the ratio remained constant. The Young's Modulus (E) increased in the placebo and MD-group by 13.2% and 13.7% respectively, however, these changes did not reach the level of significance. In the MDK-group, E remained constant. Figure 2 illustrates the change in IMT and E of the three groups. In the multivariate analysis with adjustments for baseline heart rate, mean arterial pressure, age, weight, and smoking, the difference in increase of IMT between the MDK-group and placebo was 1.3% (95% CI: -3.2 to 9.2) and between the MD-group and placebo the differ-

ence was -4.5% (95% CI: -8.9 to 4.2). Hence a significant beneficial effect on the age-related increase of the IMT could not be demonstrated for either the MD- nor the MDK-supplement. However, there was a significant difference in the change of E between the MDK-group and placebo (-13.2%; 95% CI: -35.8 to -5.3), while no significant difference was observed between the MD-group and placebo (0.46%; 95% CI: -23.2 to 9.4) and no significant differences were observed between the groups in change of the d/IMT ratio.

Discussion

In the present investigation, we have demonstrated a long-term beneficial effect of a supplement containing vitamins K₁ and D on the elastic properties of the carotid artery. In contrast, no effect was found on the IMT. Vitamin D alone did not influence these variables. To our knowledge, this is the first study which shows a longitudinal beneficial effect of vitamins K₁ + D supplementation on vascular properties.

The occurrence of vitamin K-dependent proteins in the arterial vessel wall was discovered recently, and only limited data are available on a possible relation between vitamin K intake and vessel wall properties. In animals it was found that pharmacological doses of vitamin K₂ prevent the progression of atherosclerosis by suppression of plaque formation, intima-thickening and pulmonary atherosclerosis (23). One of the few studies in humans showed an inverse correlation between dietary vitamin K₁ intake and aortic calcification in postmenopausal women (10). In a recent population-based study an inverse correlation was found between vitamin K₂ intake and aortic calcification, myocardial infarction and sudden cardiovascular death (11). The latter study suggests that vitamin K₂ may be a more powerful inhibitor of arterial calcification than vitamin K₁.

Furthermore, vitamin K₂ was shown to decrease the total circulating cholesterol concentration (24). Most of the studies published so far have focused on the effects of vitamin K₂ and not of vitamin K₁.

When hypothesizing about the mechanism underlying our observations, we would like to focus on the vitamin K-dependent protein MGP. It is synthesized by the vascular smooth muscle cells (5), and accumulates in or around the elastic fibres in the tunica media (7). In this respect it is worth mentioning that transgenic MGP-deficient mice developed arterial calcifications starting in the media, but even at later stages there was no neointima formation or atherosclerosis. Rather, the type of calcification was similar to that found in Mönckeberg's sclerosis of the media such as is often seen in diabetics and hemodialysis patients. In these patients calcification starts from the elastic lamellae of the media and occurs without inflammation. DC and CC are related to the functions of elastin and collagen in the vascular media as they represent the elastic properties of the vessel wall. IMT, on the other hand, is regarded as endothelial response to pathophysiological processes as in atherosclerosis. While DC and CC represent functional characteristics of the vessel wall, the Young's Modulus (E) expresses the structural characteristics of the tissue. In this study it was shown that supplementation with vitamins D+K exerted beneficial effects on both structural and functional characteristics of the vessel wall.

In many papers it has been suggested that the vitamin K requirement of extra-hepatic tissues is substantially higher than that of the liver. Although these conclusions are mainly based on the bone Gla-protein osteocalcin, which was found to be undercarboxylated in the majority of the population (notably elderly) (25), substantial undercarboxylation has also been reported for MGP (26). Assuming that at baseline part of the MGP was synthesized in an undercarboxylated (i.e.: inactive) form, this must be expected to form a risk factor for media sclerosis and vascular stiffening, but not for atherosclerosis. With regard to this, it is conceivable that increased vitamin K intake will increase the vascular vitamin K status and hence the production of active MGP, thus contributing to the inhibition of calcification and protection against arterial stiffening. Although this is precisely the effect observed in our study, it cannot be excluded that other vitamin K-dependent proteins are involved in maintaining vascular elasticity.

Since the MGP promotor contains a vitamin D-responsive element, it is theoretically possible that a low vitamin D status also affects the level of MGP expression. To make certain that the participants were sufficient in vitamin D, the treatment included supplementation with vitamin D (1 × RDA) together with the vitamin K₁ (8 × RDA). In a third arm of the study it was demonstrated that vitamin D alone had no effect on any of the vessel wall characteristics measured. No significant differences were observed in longitudinal changes between the placebo and the vitamin D group.

To which extent vessel wall characteristics of the carotid artery represent a measure for risk on cardiovascular disease remains to be investigated. In various studies, increased IMT of the carotid artery was shown to be a risk factor for atherosclerosis, especially if the IMT is > 1 mm. Since in our study population the IMT in all three groups remained between 0.6 – 0.7 mm, it cannot be excluded that under different conditions (e.g. in older age groups, or after prolonged treatment) an effect of vitamin K on IMT will also be seen. This may especially occur at later stages of atherosclerosis in which calcification of the lesions forms an end stage process. Less information is available on the correlation of distensibility and compliance with cardiovascular disease, although in previous studies arterial stiffness was also shown to be associated with myocardial infarction and coronary artery disease (27, 28). In a recent study of 110 end-stage renal disease patients, a strong correlation was found between calcification score and arterial stiffness, especially with E, but also with DC, CC and IMT (29). It has been suggested in this study that measurement of arterial parameters, exploring both structural and functional properties, could be helpful in the assessment of risk on cardiovascular disease and in the evaluation of risk reduction by treatment. However, extrapolation of these results to other groups may not be justified because of the particular characteristics of these patients. The fact that our data suggest a more pronounced effect of vitamin K1 supplements on (medial) calcification than on IMT thickening warrants further studies in other populations, for instance diabetics or patients with severe atherosclerosis.

One limitation of our study is the use of the brachial pulse pressure instead of the carotid pulse pressure. The brachial pulse pressure tends to deviate from the pulse pressure in the common carotid artery. One should realize, however, that intra-subject errors due to differences in the locations of blood pressure measurement and artery wall property assessment will be methodological in nature and of the same order of magnitude at the various moments of determination. Changes in heart rate and the associated changes in aorta pulse pressure will modify the brachial and carotid pulse pressure to the same extent. The basic question is whether for the 3 subgroups the brachial and carotid pulse pressure will vary in parallel and to the same extent. For young adults, the carotid pulse pressure is about 70% of that of the brachial artery, while the difference gradually vanishes with advancing age. For the considered age group the difference will be considerably less. Using the brachial pulse pressure the carotid wall parameters will be underestimated. If in the control group the pulse pressure percentage increases more than in the MDK-group, then the DC of the control group would deviate even more, thus supporting our main conclusion. Since both the control and MDK-group developed in parallel, there is no reason why the carotid arteries of the MDK-group should age faster.

A further limitation of our study is the fact that we observed a higher drop-out rate in the treatment groups than in the placebo group. Given the specific reasons for drop-out as mentioned in the results, it is unlikely that the treatment assignment was responsible for the higher drop-out rate. However, it cannot be excluded that the higher drop-out rate was due to minor differences in taste of the different supplements. Another limitation of this study was the exclusion of part of our randomized population. Extensive analysis of the collected ultrasound data lead to necessary exclusions because of technical difficulties in measuring diameter changes within regular heart cycles, as exhibited by example arterial translations of > 2 mm and beat-to-beat variations in distension of > 20%. Additional analyses were per-

formed to exclude the possibility that the high drop-out rate and exclusion of subjects had seriously biased our results. This turned out not to be the case. The study presented in this paper is a first step to corroborate the role of vitamin K in vascular tissue. Clinical trials measuring other endpoints and progression of calcification are needed to provide further evidence that vitamin K contributes to the prevention of cardiovascular disease.

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