

Effects of oral and intramuscular vitamin K prophylaxis on vitamin K₁, PIVKA-II, and clotting factors in breast fed infants

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Abstract

A randomised clinical trial was conducted to establish the effects of oral and intramuscular administration of vitamin K at birth on plasma concentrations of vitamin K₁, proteins induced by vitamin K absence (PIVKA-II), and clotting factors. Two groups of about 165 healthy breast fed infants who received at random 1 mg vitamin K₁ orally or intramuscularly after birth were studied at 2 weeks and 1 and 3 months of age. Although vitamin K₁ concentrations were statistically significantly higher in the intramuscular group, blood coagulability, activities of factors VII and X and PIVKA-II concentrations did not reveal any difference between the two groups. At 2 weeks of age vitamin K₁ concentrations were raised compared with reported unsupplemented concentrations and no PIVKA-II was detectable. At 3 months vitamin K₁ concentrations were back at unsupplemented values and PIVKA-II was detectable in 11.5% of infants. Therefore, a repeated oral prophylaxis will be necessary to completely prevent (biochemical) vitamin K deficiency beyond the age of 1 month.

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Vitamin K deficiency is associated with haemorrhagic disease of the newborn. Three patterns of bleeding have been differentiated: early haemorrhagic disease of the newborn within 24 hours after birth, classical on days 1 to 7, and late after the first week of life.¹ Late disease is often intracranial. These haemorrhages may be fatal or cause serious morbidity. Breast feeding has an important role in the pathogenesis of classical and late disease.¹ Many countries recommend vitamin K prophylaxis after birth to prevent this hazard of vitamin K deficiency. Nevertheless, there are still controversies concerning the best way of providing effective prophylaxis, resulting in different policies. The safety of oral and parenteral vitamin K prophylaxis in the prevention of classical haemorrhagic disease of the newborn has been established, whereas the relationship between a single vitamin K dose at birth and late haemorrhagic disease of the newborn has not been clearly determined.^{1,2} Several studies have indicated that oral prophylaxis might be as effective as intramuscular administration, but these studies lack follow up beyond the first week of life.³⁻⁵ Epidemiologically, intramuscular vitamin K prophylaxis appears to have a lower incidence of failure,⁶⁻⁸ probably because of the more reliable

absorption.⁴ Oral administration has the appealing characteristics that an injection is avoided and that administration is simple, resulting in better parental acceptance.

The aim of this study was to evaluate whether oral administration of vitamin K is as effective as intramuscular administration in the prevention of vitamin K deficiency beyond the first week of life in breast fed infants. Determination of proteins induced by vitamin K absence (PIVKA-II) was used to detect biochemical vitamin K deficiency. Vitamin K is necessary for production in the liver of coagulation factors II, VII, IX, and X. The vitamin K dependent carboxylation of glutamic acid residues to γ -carboxyglutamic acid residues promotes calcium binding to these proteins which is essential for effective haemostatic function. When carboxylation is impaired because of deficiency or antagonism of vitamin K, inert precursors of prothrombin (factor II) are detected in the blood.⁹ These are known as PIVKA-II. In formula fed infants and adults PIVKA-II is not detectable, but it is found relatively frequently in breast fed infants without vitamin K prophylaxis at birth.¹⁰ Although biochemical markers of vitamin K deficiency are only of limited value in assessing clinical relevance, they provide a most sensitive way to determine which group of infants is at risk for haemorrhagic disease of the newborn.

Subjects and methods

A total of 331 infants, delivered spontaneously vaginally, in the University Hospital of Nijmegen or at home under midwife guidance, were enrolled. Inclusion criteria were: gestational age of 37 weeks or more, birth weight over the 2.3rd centile, and Apgar score of 7 or more at 5 minutes. The mother had to be healthy and not be taking vitamin K, anticoagulants, antibiotics, or antiepileptic drugs. All mothers intended to breast feed their child. After the parents had given informed consent the neonates received vitamin K prophylaxis on the first or second day of life. The newborns were randomly allocated to one of the two treatment groups. One group (n=165) received 1 mg vitamin K₁ orally (1 mg/ml phytomenadione, Konakion, Hoffman-La Roche). The other group (n=166) received 1 mg vitamin K₁ intramuscularly (Konakion 2 mg/ml). Some characteristics of the infants are represented in table 1. No feature was significantly different between the groups at the beginning or at other times during the study (χ^2 and Student's *t* test).

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Table 1 Comparison of the two study groups of healthy breast fed infants; results are mean (SD)

	Oral group	Intramuscular group
No of infants	165	166
Sex (% male)	55.8	47.0
Gestational age (weeks)	39.8 (1.3)	40.1 (1.2)
Birth weight (g)	3424 (439)	3417 (440)
Apgar score at 5 min	9.7 (0.5)	9.7 (0.6)
Arterial cord blood pH	7.26 (0.07)	7.26 (0.07)
Age at vitamin K administration (hours)	19.9 (12.7)	13.6 (13.5)
Follow up assessments:		
Age at 1st venepuncture (days)	14.1 (1.2)	14.0 (1.3)
Age at 2nd venepuncture (days)	30.5 (1.8)	30.5 (1.8)
Age at 3rd venepuncture (days)	88.6 (5.6)	89.5 (5.6)

If the child was still exclusively breast fed, blood was sampled at 2 weeks and 1 and 3 months of age. In other words, when the mother stopped breast feeding follow up was terminated. A sample of 5 ml of blood was drawn by venepuncture and divided: 2 ml of citrated blood (in silicone coated tubes containing 10% (v/v) of sodium citrate 3.8%) and 3 ml of coagulated blood with no additive. After measuring blood coagulability (Thrombotest, Nijegaard and Co), the citrated blood was centrifuged (5000 rpm for 10 minutes) and the plasma stored at -70°C until coagulation parameters were determined. The coagulated blood was protected from the light immediately after sampling, centrifuged (3000 rpm for 5 minutes) and the serum stored at -20°C for vitamin K₁ determination. All samples were coded to provide blind analysis.

Activities of clotting factors VII and X were measured by chromogenic substrate assay, with substrate S2765 (Coa-set FVII kit, Kabi Diagnostica) and substrate S2337 (Coatest FX kit, Kabi) respectively. These methods are not sensitive to PIVKA factors.

PIVKA-II was assayed by an enzyme linked immunosorbent assay, using a monoclonal antibody previously described (Eitest mono P-II Eisai).¹¹ This antibody reacts with decarboxylated prothrombin (PIVKA-II) quantitatively and does not cross react with native prothrombin.

PIVKA-II concentrations are expressed in arbitrary units (AU)/ml, so that 1 AU corresponds with 1 μg of purified prothrombin. In severe vitamin K deficiency PIVKA-II can amount to more than 20 AU/ml. The detection limit of 0.10 AU/ml was used as the upper normal limit, defining PIVKA-II concentrations higher or lower than that concentration as PIVKA-II positive or negative, respectively.

Vitamin K₁ was extracted from 1 ml serum samples by a two step high performance liquid chromatographic (HPLC) procedure, according to the method of Lamber *et al.*^{12,13} A few modifications were applied. The assays were performed with two Spectra Physics SP8800 systems equipped with Rheodyne 7125 manual injectors, with 100 μl sample loops. Vitamin K₁₍₂₅₎ was used as an internal standard. The first HPLC step was used for pre-separation of vitamin K₁ and K₁₍₂₅₎ on a microspher-Si column (Chrompack). Locations of vitamin K₁ and internal standard were detected with a variable wavelength detector model 770 (Spectra Physics) set at 248 nm. Final separation and quantification were performed during the second HPLC step, using a microspher-C18 column (Chrompack) and postcolumn chemical reduction with tetramethylammonium-octahydrodiborate for fluorescence detection ($\lambda_{\text{ex}}=325$ nm, $\lambda_{\text{em}}=450$ nm) with a Waters 470 fluorescence detector (Waters, Millipore). Concentrations were calculated from relative peak heights of vitamin K₁ versus known amount of internal standard. Recovery of vitamin K₁ from standard solutions added to normal serum was $85 \pm 5\%$, with a detection limit of 45 pg/ml.

In all PIVKA-II positive samples and in about 30% of other samples alanine aminotransferase was determined (normal <40 U/l).

For statistical calculations χ^2 , Student's *t*, Mann-Whitney U, and Wilcoxon's one sample tests and Spearman's rank correlation coefficient were used where appropriate.

The study was approved by the local medical ethical committee.

Results

None of the infants had clinical symptoms of bleeding diathesis or severely disturbed coagulation parameters.

The decrease in number of infants studied during follow up is caused by frequent stopping of exclusive breast feeding before the age of 3 months. Some values are missing due to sampling errors. The number of samples studied is stated where different.

Results of coagulation tests are shown in fig 1. Blood coagulability and activities of clotting factors VII and X revealed no difference between the two groups at any of the ages (Mann-Whitney, $p>0.05$). As is to be expected, a rise in activity between 14 and 30 days of age was found for these coagulation parameters (Wilcoxon, $p<0.01$).

Results of PIVKA-II determination are represented in table 2. Two weeks after birth PIVKA-II could not be demonstrated in any of the 285 infants studied. At 1 month PIVKA-II was detectable in four out of 262 infants: one in

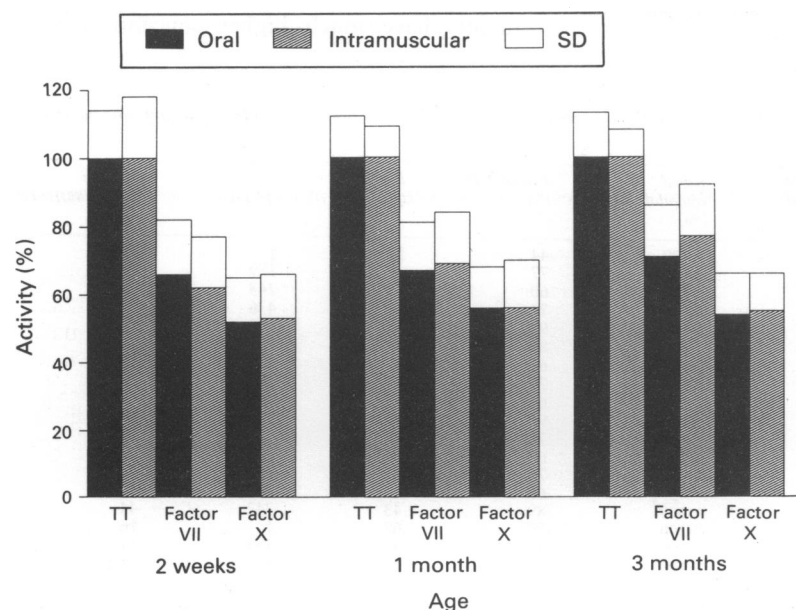


Figure 1 Median (SD) of blood coagulability (Thrombotest, TT) values and activities of clotting factors VII and X in breast fed infants at 2 weeks, 1 month, and 3 months of age after either oral or intramuscular vitamin K prophylaxis at birth. Values are expressed as a percentage of normal adult pooled plasma.

Table 2 Presence of PIVKA-II (≥ 0.10 AU/ml) in breast fed infants of different ages after either oral or intramuscular vitamin K prophylaxis at birth

	Time after birth		
	2 weeks	1 month	3 months
Oral group	0 (n=145)	3 (n=135)	7 (n=68)
Intramuscular group	0 (n=140)	1 (n=127)	8 (n=63)

the intramuscular group (0.8%) and three in the oral group (2.2%). PIVKA-II concentrations ranged from 0.10 to 0.47 AU/ml. The difference in percentages of positive samples after oral compared with intramuscular administration is not statistically significant (χ^2 , $p=0.34$). The 95% confidence intervals of the difference were -1.5 to 4.3%. At 3 months of age PIVKA-II could be detected in 15 out of 131 infants: seven in the oral group (10.3%) and eight in the intramuscular group (12.7%). The 95% confidence interval of the difference between the oral and intramuscular group was -13.3 to 8.5% ($p=0.67$). PIVKA-II concentrations ranged from 0.10 to 0.32 AU/ml.

Vitamin K₁ plasma concentrations decreased significantly during follow up in both groups (Wilcoxon, $p<0.001$); fig 2). At 2 weeks of age the mean (SD) concentration of 1608 (873) pg/ml in the intramuscular group (n=64) was

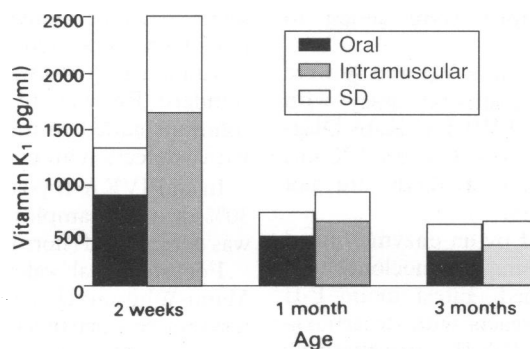


Figure 2 Mean (SD) vitamin K₁ plasma concentrations in breast fed infants at 2 weeks, 1 month, and 3 months of age after either oral or intramuscular vitamin K prophylaxis at birth.

significantly higher than that of 815 (414) pg/ml in the oral group (n=74) (Mann-Whitney, $p<0.0001$). At 1 month of age the concentration was still significantly higher in the former group: 615 (272) pg/ml (n=84) v 391 (207) pg/ml (n=94); $p<0.0001$. Remarkably, at 3 months of age concentrations were still slightly different: 329 (186) pg/ml (n=57) v 268 (174) pg/ml (n=62); $p=0.03$. Plasma concentrations of vitamin K₁ did not correlate with sex, gestational age, birth weight, Apgar score, or arterial cord blood pH, nor with blood coagulability, activities of factor VII or X, or PIVKA-II concentration.

In table 3 individual results of PIVKA-II, blood coagulability, factors VII and X, vitamin K₁, and alanine aminotransferase determinations are shown for the infants positive for PIVKA-II. Values for blood coagulability and factors VII and X were not different from infants negative for PIVKA-II. Similarly, vitamin K₁ concentrations were not extremely low. At 3 months of age mean (SD) vitamin K₁ concentration in the infants positive for PIVKA-II was 221 (74) pg/ml compared with 312 (191) pg/ml in the infants negative for PIVKA-II (Mann-Whitney, $p=0.16$). Results of the alanine aminotransferase determinations indicate that liver dysfunction is not a major cause for the appearance of PIVKA-II. No relevant correlations between the concentration of PIVKA-II and other parameters could be detected.

Discussion

Concentrations of vitamin K₁ in blood vary widely, according to the diet of the subject and the method used. The reference range reported for fasting adults, measured by a technique comparable with ours, ranges from 62–980 pg/ml.¹⁴ The reference range for neonates and infants is still unknown. Mean plasma concentrations in healthy breast fed infants of 1 month of age without vitamin K prophylaxis are reported to be 500–700 pg/ml.^{10–15} Formula fed infants have much higher concentrations of

Table 3 Results of PIVKA-II, blood coagulability clotting factors VII and X, vitamin K₁ and alanine aminotransferase in PIVKA-II positive infants

Group	Age (months)	PIVKA-II (AU/ml)	Blood coagulability* (%)	Factor VII* (%)	Factor X* (%)	Vitamin K ₁ (pg/ml)	Alanine aminotransferase† (U/l)
Oral	1	0.47	90	44	57	‡	21
Oral	1	0.16	70§	77	47	189	15
Oral	1	0.12	>100	60	43	243	6
IM	1	0.10	>100	74	51	436	23
IM	3	0.32	>100	99	52	190	20
IM	3	0.31	100	85	61	220	58
IM	3	0.29	>100	61	52	238	31
Oral	3	0.28	>100	70	47	140	16
Oral	3	0.22	>100	66	51	125	13
IM	3	0.19	>100	95	74	328	17
IM	3	0.14	65§	78	48	357	26
IM	3	0.14	>100	62	54	235	8
Oral	3	0.13	70§	74	48	126	20
Oral	3	0.12	57§	66	49	263	12
IM	3	0.11	>100	89	43	242	44
IM	3	0.11	>100	72	67	‡	16
Oral	3	0.11	>100	89	46	184	11
Oral	3	0.10	>100	67	63	357	18
Oral	3	0.10	—	64	37	‡	19

*Blood coagulability and factors VII and X are expressed as a percentage of normal adult pooled plasma.

†Alanine aminotransferase: normal value <40 U/l.

‡Missing value due to insufficient volume of serum.

§Activity less than mean -2 SD of PIVKA-II negative infants of that age.

IM=intramuscular.

3000–4500 pg/ml, because of the relatively high concentration of vitamin K₁ in formula (60 µg/l) compared with human milk (2 µg/l).^{10 15} McNinch *et al* reported vitamin K₁ plasma concentrations in breast fed infants after 1 mg vitamin K₁ orally or intramuscularly at birth.⁴ In the oral group the peak median concentration occurred earlier, but was lower. In both groups vitamin K₁ concentrations declined rapidly, after 24 hours the mean was 23 ng/ml in the oral group and 444 ng/ml in the intramuscular group. The present study demonstrates that at 2 weeks of age vitamin K₁ concentrations are still raised; concentrations of about 1600 pg/ml after intramuscular injection and of 800 pg/ml after oral administration were found. At 1 month of age concentrations declined to about 600 and 400 pg/ml, respectively. Other reports confirm that four to six weeks after an intramuscular injection of 1 mg vitamin K₁, plasma concentrations are back at unsupplemented values.^{10 16} Nevertheless, at all ages vitamin K₁ concentrations were lower after oral administration. To our knowledge, vitamin K₁ concentrations beyond the first week of life after a single oral dose were not reported before. To determine whether the lower vitamin K₁ plasma concentration after oral administration also entails a worse protection against vitamin K deficiency, we have to compare coagulation parameters.

Blood coagulability and activities of clotting factors VII and X showed no difference between the two groups. However, these coagulation tests are not sensitive enough to detect biochemical vitamin K deficiency.¹⁷ Accordingly, blood coagulability and factors VII and X were not different in PIVKA-II positive infants compared with those who were PIVKA-II negative. In contrast to vitamin K₁ plasma concentrations and activities of clotting factors, PIVKA-II detection is a more direct reflection of vitamin K dependent carboxylation of clotting factors in the liver. As mentioned previously, detection of PIVKA-II has no clinical consequences, but it does indicate whether enough vitamin K has been available to carboxylate all vitamin K dependent proteins. Prolongation of low vitamin K intake may lead to serious complications.

In cord blood, using the same method as we did, Motohara *et al* detected PIVKA-II in 21.5% of 102 samples.¹⁸ At 3 to 5 days of age 50 to 60% of infants were PIVKA-II positive if they were breast fed and had not received vitamin K prophylaxis at birth.¹⁸ At the age of 1 month 12.3% were positive.¹⁹ Biochemical vitamin K deficiency occurs frequently in unsupplemented breast fed infants. Widdershoven *et al* compared PIVKA-II concentrations in breast fed and formula fed infants.¹⁰ At 4 days of age about 10% of both groups had PIVKA-II in their blood. At 1 month of age PIVKA-II was not detected in any formula fed infant, compared with in 5.5% of breast fed infants. At 3 months of age no formula fed infant was positive, compared with 7.5% of breast fed infants. Thus, formula feeding seems an effective way to prevent the appearance of PIVKA-II in the blood of young infants, probably due to the high intake of vitamin K₁.

PIVKA-II was not detectable in any of our infants at 2 weeks of age. This corresponds with the raised vitamin K₁ concentrations in both groups. Surprisingly, in four 1 month old infants PIVKA-II was detected. However, the concentration in the only positive infant of the intramuscular group was at the limit of detection. So, in the intramuscular group hardly any 1 month old infant had PIVKA-II detectable, while it was detectable in a few in the oral group. Exact comparison of our PIVKA-II results with those of Motohara *et al*¹⁰ and Widdershoven *et al*¹⁰ is hampered by the fact that our method is more sensitive. Their detection limit amounted to 0.13 compared with 0.10 AU/ml in our study. If we applied their detection limit as a selection criterium just two 1 month old infants would remain positive, thus strengthening the clinically relevant difference in PIVKA-II detectability between our supplemented and their unsupplemented 1 month old breast fed infants (χ^2 , $p < 0.01$). Even without correction, PIVKA-II was less frequently detected in our intramuscular group than in the unsupplemented infants of Widdershoven *et al* (1/127 *v* 4/73, $p < 0.05$).¹⁰ Our oral group did not differ significantly from the unsupplemented infants of Widdershoven *et al* (3/135 *v* 4/73, $p = 0.21$). This demonstrates that intramuscular vitamin K seems still effective at the age of 1 month, while oral vitamin K is not.

Surprisingly, at the age of 3 months a high percentage of children in both our oral and intramuscular groups had PIVKA-II detectable, indicating that neither route was completely effective by that age. Vitamin K₁ concentrations declined to values of 300 pg/ml. Although vitamin K₁ concentrations in the intramuscular group were slightly higher than in the oral group, both concentrations seem insufficient to prevent biochemical evidence of vitamin K deficiency in all infants. Other reports confirm the reappearance of PIVKA-II after a single vitamin K administration at birth. Motohara *et al* reported a decrease in PIVKA-II detectability on the third and fifth day of life after a single oral dose of 5 mg vitamin K₂ at birth.¹⁸ At 1 month of age, however, no significant reduction in PIVKA-II detectability was demonstrated unless a second oral dose was administered at 14 days of age.¹⁹ Widdershoven *et al* detected PIVKA-II in none of 48 infants of 1 month old, in one of 29 infants of 2 months old, and in one of 23 infants of 3 months old after the administration of 1 mg vitamin K₁ intramuscularly at birth.¹⁰

We failed to detect an association between PIVKA-II and vitamin K₁ plasma concentrations. Vitamin K₁ concentrations were not different in PIVKA-II positive children compared with PIVKA-II negative infants. This may be caused by the fact that PIVKA-II has a half life of about 70 hours and hence can still be present in plasma even when the vitamin K deficiency has been corrected.⁹ Moreover, due to frequent feeds, plasma concentrations of vitamin K₁ in infants vary widely. The plasma half life of tritiated vitamin K₁ has been reported to be 120–150 minutes.²⁰ Information about hepatic vitamin K stores in infants is

limited. Shearer *et al* reported that in unsupplemented term neonates hepatic vitamin K₁ concentrations were no more than about 1 ng/g liver (fresh weight).²¹ Total liver stores amounted to 0.1 µg. In adults a much higher mean concentration of 5.5 ng/g was measured, resulting in total stores of 8 µg. When the newborn had been given an intramuscular injection of vitamin K₁ (0.5–1.0 mg) endogenous hepatic values remained raised for at least one week. Besides vitamin K₁ different forms of vitamin K₂ (menaquinones 6 to 12) could be detected in the liver.²¹ In adults menaquinones even accounted for some 75–97% of total hepatic stores of vitamin K on a molar basis. In the neonate, however, menaquinones were not detectable until about 14 days postpartum. Thereafter a gradual build up was indicated and adult concentrations were attained about one month after birth.²¹ However, the extent of vitamin K₂ utilisation remains controversial. Nevertheless, besides vitamin K₁ vitamin K₂ has to be considered when assessing vitamin K status. Altogether, the plasma vitamin K₁ concentration may not adequately represent the total amount of vitamin K that is available as a cofactor for the carboxylase enzyme in the liver.

Wallin has reported evidence to maturation of the vitamin K dependent carboxylation system in fetal-neonatal rats.²² At 7 days of neonatal age adult values of carboxylase activity were reached. But activities of the two pathways that provide carboxylase with reduced vitamin K_{H2} cofactor (vitamin K epoxide reductase and vitamin K reductase) were never as high as in adult liver. In other words, it might be possible that an increased requirement of vitamin K exists in early infancy, due to immature reutilisation of vitamin K epoxide. The vitamin K₁ reference range for fasting adults can not be applied automatically to non-fasting young infants. Moreover, individual difference in enzyme maturation and therefore individual difference in vitamin K requirement could exist.

To summarise, single oral or intramuscular administration of 1 mg vitamin K₁ postnatally may not offer complete protection against late biochemical vitamin K deficiency. Correspondingly, except for one infant with classical haemorrhagic disease of the newborn,²³ most case reports of failures of vitamin K prophylaxis concern late disease.^{1, 24} Epidemiologically, intramuscular vitamin K prophylaxis appears to have a lower incidence of failure.^{6–8} In a recent survey in the British Isles, 27 cases of haemorrhagic disease of the newborn were recorded.⁸ Seven of them occurred in spite of oral vitamin K prophylaxis at birth. No failures were recorded after intramuscular administration, although there was uncertainty about intramuscular vitamin K in one case. The relative risk for babies who had received oral vitamin K compared with babies who had received intramuscular vitamin K was 13:1. The relative risk without prophylaxis was 81:1.⁸ A schedule of repeated oral doses was considered.²⁵ Taking our results as well as epidemiological evidence into account, we suggest that for complete

protection of breast fed infants against late haemorrhagic disease of the newborn, vitamin K administration should be repeated. Whether a monthly, weekly, or daily administered oral dose should be recommended deserves further investigation.

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