ELSEVIER

Biochimica et Biophysica Acta 1570 (2002) 27–32

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.bba-direct.com

Differential lipoprotein transport pathways of K-vitamins in healthy subjects

Leon J. Schurgers, Cees Vermeer *

Department of Biochemistry and Cardiovascular Research Institute, Maastricht University, P.O. Box 616, 6200 MD Maastricht, The Netherlands

Received 23 July 2001; received in revised form 10 December 2001; accepted 13 December 2001

Abstract

Vitamin K is a group name for K_1 (phylloquinone) and K_2 (menaquinones). Both forms contribute to the tissue vitamin K status. Following intestinal absorption, the serum transport of these lipophilic compounds to their target tissues takes place via lipoproteins. In previous studies we have found that K_1 is preferentially accumulated in the liver, whereas menaquinones have a more widespread distribution pattern. Here we have tested whether these differences may be explained by the different liposolubility of the various K-vitamers, resulting in their association with different lipoprotein particles. Six healthy male volunteers received a mixture containing 2 μmol of each of three K vitamers (K_1, MK-4, and MK-9) dissolved in corn oil. Blood was obtained at baseline and at different time intervals after intake for the measurement of vitamin K in serum and in the lipoprotein fractions. During the first 4 h after intake all K-vitamins were found to be associated predominantly with the triacylglycerol-rich lipoprotein (TGRLP) fraction. Since the TGRLP fraction is mainly cleared by the liver, this suggests that initially most of the K-vitamins are transported to the liver. In contrast to K_1, however, both menaquinones investigated were also found in TGRLP and low-density lipoprotein, whereas MK-4 was even present in high-density lipoprotein. This explains why menaquinones may have a different distribution profile and suggests a relatively large impact of menaquinones on extra-hepatic vitamin K status than generally assumed. Moreover, the very long half-life time of MK-9 in the circulation indicates that it may form a more constant source of vitamin K than are either K_1 or MK-4. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Vitamin K; Phylloquinone; Menaquinone; Absorption; Lipoproteins; Plasma transport

1. Introduction

Vitamin K is a group name for a class of compounds which share a 2-methyl-naphthoquinone ring system, substituted at the 3-position with a variable aliphatic side chain [1,2]. Most common forms found in the human diet are phylloquinone (vitamin K_1) and menaquinones (vitamin K_2). Whereas phylloquinone is a single compound containing a mono-unsaturated side chain of four isoprenoid residues, menaquinones may be subdivided further on the basis of the number of isoprene residues (all of which are unsaturated) in the aliphatic side chain. According to the general nomenclature menaquinones are denominated as MK-*n*, where *n* stands for the number of isoprene residues present. Menaquinones most commonly found in food are MK-4, which is regarded as a short chain menaquinone, and the long chain menaquinones MK-7, MK-8, and MK-9. Although all K-vitamins are fat-soluble, their lipophilic character increases substantially at increasing chain length; this may have consequences for their uptake, plasma transport, tissue storage, and biological half-life time [3].

The function of all K-vitamins in mammals is that they serve as a cofactor for the enzyme γ-glutamylcarboxylase during the biosynthesis of a special class of proteins, the so-called Gla-proteins [4]. Well known examples of Gla-proteins are a number of clotting factors produced by the liver, including prothrombin and factors VII, IX, and X [5]. During the last two decades, however, gammaglutamyl-carboxylase has been detected in a wide range of non-hepatic tissues, and an increasing number of Gla-proteins of extrahepatic origin have been characterized [1,5]. Most of these proteins have regulatory functions, for instance in tissue mineralization (osteocalcin and matrix Gla-protein) [6,7] and cell growth (growth-arrest-specific gene-6 protein, Gas6) [8].

It is well known that intestinal absorption of K_1 takes place via the enterocytes, which facilitate its incorporation

* Corresponding author. Fax: +31-43-388-4160.
E-mail address: c.vermeer@bioch.unimaas.nl (C. Vermeer).

0304-4165/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0304-4165(02)00147-2

into chylomicrons. These chylomicrons enter the circulation via the lymphatic system, and most of the vitamin is delivered to the liver through receptor-mediated uptake of chylomicron remnants by hepatic parenchyma cells [2,9]. It seems probable that the other K-vitamins are taken up via a similar process, but limited data for menaquinone metabolism is available at this time. Moreover, it is unknown how the extra-hepatic tissues are provided with vitamin K. A possible route would be that part of the K-vitamins are re-packed into the low-density lipoproteins (LDL) which form a major transport system from the liver to peripheral tissues. From animal experiments is has become clear that the liver is the main target for uptake and accumulation of vitamin K_1, whereas most extra-hepatic tissues (e.g., vessel wall, bone, testis, pancreas, kidney, and lung) preferentially accumulated menaquinone [10,11].

Recently, it has been shown that postprandially, vitamin K_1 is predominantly carried in the triacylglycerol-rich lipoprotein fraction (TGRLP), and little is carried by the LDL or HDL fraction [12]. In the study described in this paper we have hypothesized that the vitamin K transport in the blood stream to various tissues will take place via circulating lipoproteins, and that the lipophilicity of the various K-vitamers is an important factor in their distribution over the different lipoprotein fractions. To test this hypothesis, human volunteers received a single oral dose of a mixture of K_1, MK-4, and MK-9, and we have measured the appearance and disappearance of these vitamins in serum. Subsequently, we have recorded their distribution over the various lipoprotein fractions as a result of changes in postprandial lipoprotein concentration of vitamin K.

2. Subjects and methods

2.1. Subjects and study protocol

In this study we have worked with a panel of six healthy male volunteers recruited at the University of Maastricht. Their mean age was 33.5 ± 6.3 years, and their body mass index was 24.3 ± 2.0 kg/m^2. All participants were apparently healthy, and their serum lipid profiles were within the normal range. Neither medications nor vitamin supplements (other than the experimental ones) were taken throughout the study. The experimental protocol started at 8 am after an overnight fast. At that time the participants received a breakfast containing vitamin K (2 μmol of each of K_1, MK-4 and MK-9) dissolved in 30 g of fat (corn oil), which was spread on toast with marmalade. The blank meal (baseline curve to obtain at corresponding time points serum vitamin K concentrations as a function of the low-vitamin K meal) consisted of 30 g of fat without vitamin K given at breakfast; values obtained during this control phase were subtracted from those obtained during the experimental phase. To exclude influences in vitamin K metabolism due to additional K-intake by the diet, participants were only allowed to eat food low in vitamin K (toast, marmalade, bananas, apples) at lunch, and to drink orange juice and water at libitum. After 18:00 h and during the rest of the entire study consumption of vitamin K-rich foods (spinach, broccoli, Brussels sprouts and kale) was not allowed. Unless indicated otherwise, blood was drawn by venipunctures at 0, 2, 4, 6, 8, 24, and 48 after intake. Serum was prepared and 1-ml aliquots were kept frozen until serial vitamin K determination. The study design was approved by the local Medical Ethics Committee, and informed consent was obtained from all subjects according to the institutional guidelines.

2.2. Materials

MK-4 was a kind gift from Eisai (Tokyo, Japan), K_1 and MK-9 were obtained from Hoffmann-La Roche (Basel, Switzerland). A set of purified menaquinones (MK-4 through MK-10) was supplied by Roche and served as reference compounds for the identification of menaquinones. Corn oil was from CPC Bestfoods (Heilbronn, Germany) and contained 60 pmol/g of vitamin K_1 and no detectable amounts of menaquinones.

2.3. Analytical techniques

Triacylglycerol and cholesterol were determined by standard enzymatic techniques (Boehringer Mannheim, Germany) using a Beckmann Synchron CX 7-2 autoanalyser (Beckmann, Fullerton, CA). Vitamin K concentrations were assessed by high-performance liquid chromatography, with on-line electrochemical reduction of the effluent and fluorescence detection [3]. The lipoproteins in serum were separated on a 1006–1250 g/l KBr density gradient [13]. Serum samples (3.5 ml) were adjusted to a density of 1250 g/l with solid KBr in polyallomer tubes and stacked up with three 3-ml layers of KBr/NaCl solutions in 1 mM EDTA (pH 7.4) of 1063, 1019 and 1006 g/l, respectively. The gradients were centrifuged for 22 h at 36 000 rpm in a Beckman SW 40 rotor. In this way the following lipoproteins were obtained in separate fractions: TGRLP (including the chylomicrons, the chylomicron remnants, and the very low-density lipoprotein (VLDL) fraction), the intermediate-density lipoprotein (IDL), LDL, high-density lipoprotein (HDL), as well as the lipoprotein-free (LPF) fraction.

2.4. Data handling

Serum vitamin K concentrations during 48 h after oral ingestion were recorded at indicated intervals. At each time point mean values ± S.E. for the six participants were calculated and plotted as a function of time. Blank values (no vitamin K ingested) were subtracted throughout this study.

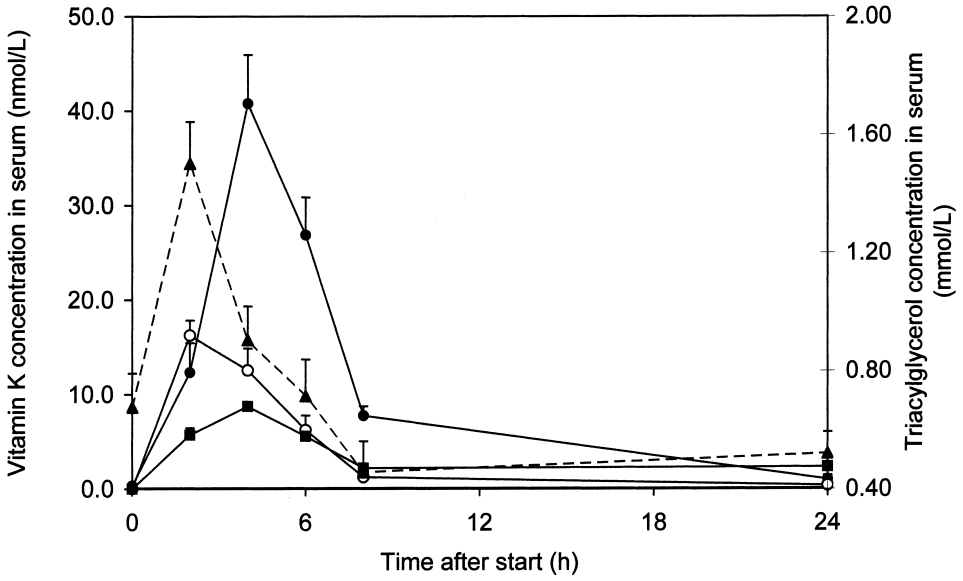

Fig. 1. Serum vitamin K following the oral intake of 2 μmol of oil-solubilized preparations. Also the triacylglycerol concentration of the TGRLP fraction is drawn to show the correlation between the K-vitamin and triacylglycerol. Points are mean values from six volunteers; error bars represent S.E.M. ●, K_1; ○, MK-4; ■, MK-9; ▲, TG-TGRLP.

3. Results

Serum profiles for lipid components were prepared after the vitamin K-rich meal only. Cholesterol and triacylglycerol concentrations in total serum at baseline level were within the normal range for all subjects. Lamon Fava et al. showed that vitamin K did not affect the plasma lipoprotein profile and that serum vitamin K and cholesterol are not correlated [12]. Therefore, cholesterol in the lipoprotein subfractions was not analysed. Peak values for serum triacylglycerol concentrations were observed around 2 h postprandially, and were within the normal range. They remained elevated until 8 h after the start and at 24 h all values had returned to baseline. The profile of serum triacylglycerol in the TGRLP fraction was comparable with that in total serum. The triacylglycerol content of the LDL and HDL fraction remained constant in the experiment.

In the same samples we have measured the concentrations of K_1, MK-4, and MK-9 (Fig. 1). Maximal concentrations in serum were reached at 2.5, 4, and 5 h postprandially for MK-4, K_1, and MK-9. All three vitamins showed complex pharmacokinetics with multiphasic disappearance curves, but their half-life times differed substantially. Whereas K_1 and MK-4 had virtually disappeared from the circulation after the overnight period, the clearance of MK-9 was remarkably slower: after 24 h its serum concentration was still about 25% of the peak value, and it remained detectable until the last measurement at 48 h after intake. The estimated half-life time for MK-9 during the latter phase was about 60 h.

In Fig. 2 we have plotted the postprandial distribution of K_1, MK-4 and MK-9 over the lipoprotein fractions as a function of time; the recoveries as a percentage of total serum vitamin K are given in Table 1. The clearance from the blood stream of K_1 in the TGRLP fraction was nearly complete after 8 h, and we have measured whether other lipoprotein fractions contributed to the vitamin K trans-

Table 1
Recoveries of K-vitamins after serum fractionation

K-vitamer	Time after ingestion (h)						
	0	2	4	6	8	24	48
Total serum K1 (nmol/l)	1.49	15.2	46.8	31.4	10.1	2.63	1.02
Sum of K1 in fractions (nmol/l)	1.36	11.9	38.7	28.3	10.5	3.05	0.86
Recovery (%)	91.7	78.6	82.7	90.1	103.9	116.1	84.8
Total serum MK-4 (nmol/l)	n.d.	18.1	14.0	6.94	1.41	0.43	n.d.
Sum of MK-4 in fractions (nmol/l)	n.d.	13.7	12.5	8.21	2.11	0.41	n.d.
Recovery (%)		76.0	89.2	118.2	149.6	95.3	
Total serum MK-9 (nmol/l)	n.d.	3.65	5.58	3.56	1.41	1.51	1.11
Sum of MK-9 in fractions (nmol/l)	n.d.	3.44	4.17	2.80	1.16	1.41	0.73
Recovery (%)		94.2	74.7	78.7	82.0	91.5	62.1

Mean values of six subjects. Vitamin K in total serum was before fractionation. The sum of all fractions include: TGRLP, IDL, LDL, HDL, and the lipoprotein free fraction. n.d., not determined.

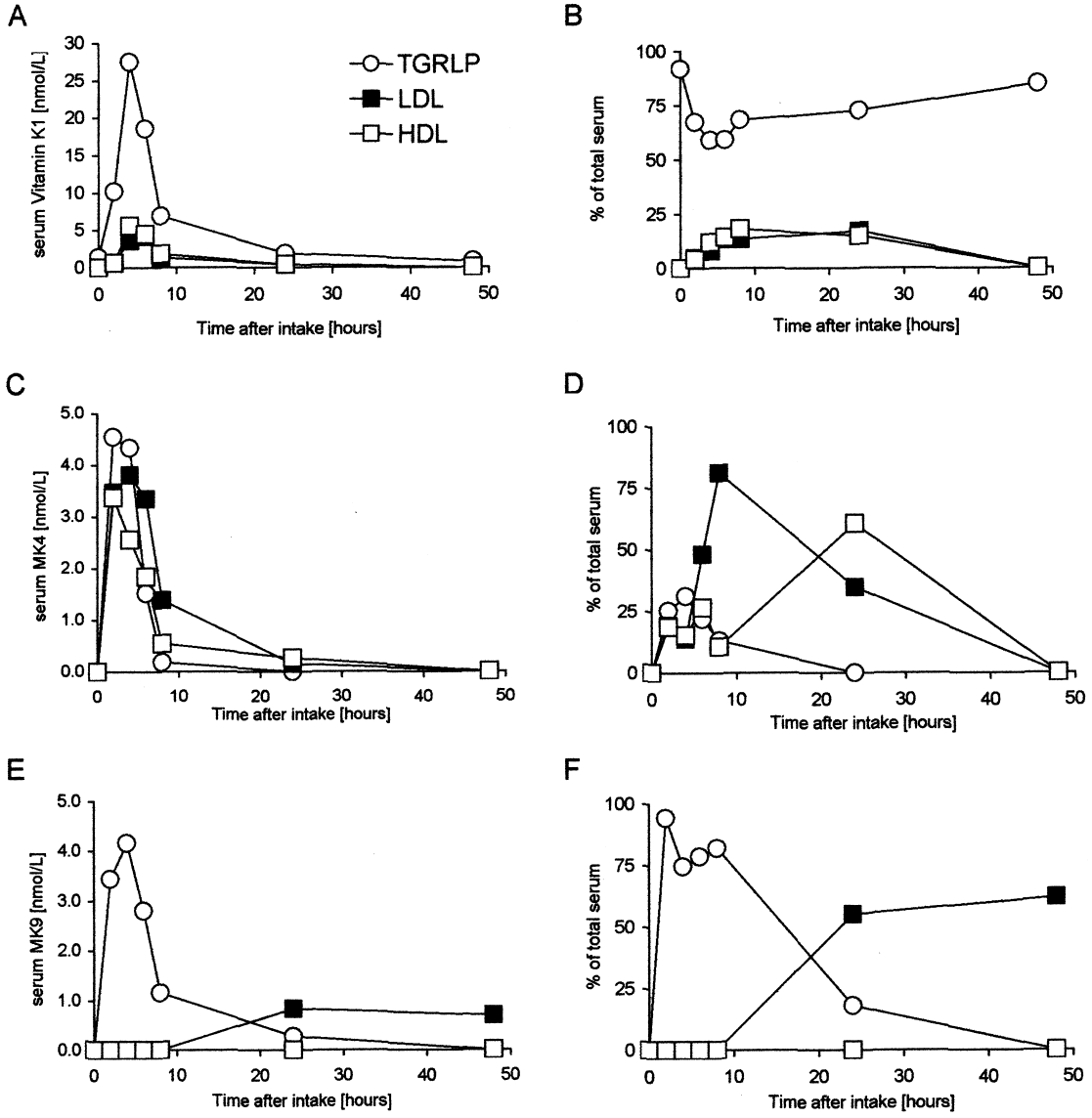

Fig. 2. Serum vitamin K in lipoprotein fractions 0–48 h after intake. A, C, and E represent serum vitamin K in lipoprotein fractions of, respectively, vitamin K_1, MK-4, and MK-9 in nmol/l. B, D, and F represent the serum lipoprotein profile of K_1, MK-4, and MK-9 as a percentage of the sum of all lipoprotein fractions. ○, TGRLP; ■, LDL; □, HDL.

port. As is shown in Fig. 2A, the K_1 concentration in the LDL and HDL fractions was elevated during the first 24 h after K_1 intake, but had returned to baseline at 48 h. If expressed as a percentage of total serum vitamin K_1, LDL never contained more than 17% and HDL less than 18% of total, whereas the remaining K_1 was recovered in the TRLG fraction (Fig. 2B). MK-4, on the other hand, was equally distributed over the TGRLP, LDL, and HDL fractions (Fig. 2C). The clearance of MK-4 present in the TGRLP fraction followed that of triacylglycerol. Expressed as a percentage, 19% of total serum MK-4 was present in the LDL fraction at 2 h, and up to 81% at 8 h after ingestion (Fig. 2D). The pharmacokinetic behaviour of circulating MK-9 was different from that of K_1 and MK-4: its appearance in the circulation and incorporation into lipoproteins was slower than for both other vitamins (Fig. 2E). MK-9 appeared in the LDL fraction only at 8 h after intake, and remained detectable therein until at least 48 h. At that time the MK-9 in LDL was 55% of the total serum concentration (Fig. 2F). At no time point MK-9 was found in the HDL fraction.

4. Discussion

Unlike the fat-soluble vitamins A and D, vitamin K has no specific plasma carrier protein, but is transported in plasma mainly by lipoproteins [9,12]. Following dietary uptake, vitamin K is absorbed in the intestine and enters the circulation via the lymphatic system as part of the chylomicrons [9]. In the circulation chylomicrons are catabolized by the action of lipoprotein lipase (LPL) resulting

in chylomicron remnants [14], which are then cleared mainly by the liver via an apoE receptor-specific uptake [15,16].

In our study it appeared that during the first 8 h following intake of a mixture of equal amounts of the three K-vitamins, the serum contained much higher concentrations of K_1 than MK-4 or MK-9. Several explanations may be given for this observation: poor intestinal absorption of menaquinones or rapid uptake of menaquinones from chylomicrons by the tissues. In previous studies in which we compared the absorption of orally ingested K_1 and menaquinones, it was found that both in experimental animals and in human volunteers the absorption of two menaquinones (MK-4 and MK-7, respectively) was far more complete than that of K_1 [3,17]. In these studies MK-7 was taken as a representative of the long-chain menaquinones. Although no data are available for the efficacy of intestinal MK-9 uptake in humans, the previous studies do not support poor intestinal absorption as an explanation for their relatively low circulating concentrations. The fact that in all experiments the serum concentration of MK-4 reached its maximum well before that of K_1 and MK-9 may result, therefore, from a quicker tissue uptake and clearance of MK-4 from the blood stream, whereas the very long half-life time for MK-9 points to a relatively slow tissue uptake of this vitamer. To provide an underlying mechanism for these observations we hypothesized that the different pharmacokinetic behaviour of the three vitamers is related to their different hydrophobicity: whereas the most water-soluble MK-4 may be packed relatively loosely, the highly lipo-soluble MK-9 may be present in the core of the lipoprotein particles, with an intermediate position for K_1. This hypothesis was tested by measuring the vitamin K content of the various lipoprotein fractions.

Already in 1974 Shearer et al. showed that the major transport of vitamin K in plasma takes place via the TGRLP fraction [9]; recently this was confirmed by Lamon-Fava et al., who hypothesized that during the hydrolysis of triacylglycerol by lipoprotein lipase, K_1 remains bound to the CR [12]. It is well known that after hydrolysis, the resulting fatty acids are readily taken up by endothelial cells, and the fact that the serum profile of MK-4 closely follows that of triacylglycerol in the TGRLP fraction suggests that MK-4 is taken up by the endothelium in parallel with the fatty acids. A similar principle has been reported for tocopherol [18]. During the formation of CR by LPL, excess surface components are transferred to HDL. The fact that – in contrast to the more hydrophobic K_1 and MK-9 – at 2 h after ingestion MK-4 is already present in the HDL fraction, further supports the hypothesis that MK-4 is mainly associated with the outer side of the lipoprotein particle, where it is available for substitution. HDL on its turn can transfer such hydrophobic components to all other lipoproteins [19,20]. A second pathway for lipoprotein uptake of MK-4 would be the direct exchange from TGRLP to LDL, such as has been demonstrated for retinyl esters [21]. An alternative explanation for our observation that MK-4 peaks earlier in TGRLP than K_1 does may be earlier and more efficient intestinal absorption, rather than quicker tissue uptake. In previous animal experiments MK-4 was absorbed substantially better than phylloquinone, when given in the same form [17]. Furthermore, the MK-4 present in the TGRLP fraction is rapidly cleared by the liver which than governs the MK-4 tissue distribution via its incorporation into lipoprotein particles. Such a mechanism was shown to be the case for vitamin E which has a specific binding protein in the liver cytoplasm. This protein preferentially binds α-tocopherol and incorporates this homologue into nascent VLDL, leading to a completely different lipoprotein profile for α-tocopherol and γ-tocopherol which differ only in a single methyl group. The minor structural difference results in a mainly extrahepatic distribution of α-tocopherol, whereas γ-tocopherol disappears rapidly from plasma by hepatic absorption, and is thought to be excreted via the bile [22]. Until now no vitamin K-receptor protein has been found on the cellular membrane, however. Only for MK-4 a nuclear vitamin K2 binding protein was reported to be present in human osteoblasts [23]. Further studies have to be conducted to test this hypothesis. With respect to clearance rate, Konishi et al. showed that 12 h after ingestion of ^{14}C-labelled K_1 or MK-4, only 9% of K_1 and as much as 74% of MK-4 was excreted in the bile [24]. The authors concluded that MK-4 is much more extensively metabolized than K_1. However, biliary excretion is often species specific and therefore cannot be extrapolated to humans.

In fasting serum low amounts of K_1 were found in the LDL and HDL fractions, with only a slight increase during the postprandial period and peak values between 4–8 h after ingestion. It is at least plausible that some of the ingested K_1 reaches these fractions by hepatic redistribution via the VLDL pathway [25]. These data suggest that the liver is the most important target tissue for vitamin K_1, whereas relatively higher fractions of MK-4 accumulate in other tissues. But also in the latter case a dominant role for the liver cannot be excluded: it could be, for instance, that the liver is equally the initial target tissue for MK-4 but that its metabolism then differs from K_1, including the possibility of a more rapid elimination of MK-4. This is consistent with previous data from our group demonstrating that K_1 was 2–5-fold more efficient than MK-4 in counteracting vitamin K-deficiency induced hypoprothrombinaemia in rats [26], and that at equal intakes of K_1 and MK-4 the former mainly accumulates in the liver, whereas MK-4 is preferentially absorbed by extra-hepatic tissues such as testis, pancreas, and kidney [10]. Also, the preferential utilization of K_1 in the liver and MK-4 in the vessel wall supports the hypothesis of tissue specific uptake of K-vitamins [27]. However, one cannot exclude the capacity of some tissues to synthesize MK-4

from K_1 [28,29]. Especially those tissues which accumulated preferentially MK-4 above K_1, turned out to be capable of synthesizing their own MK-4 by conversion of K_1 [11].

Like for K_1, serum MK-9 concentrations reached a maximum at 4 h after intake. During the first 8 h MK-9 was exclusively found in the TGRLP fraction, suggesting that the liver is the main target tissue. A major difference with both short chain K-vitamers, however, was that MK-9 remained present in the circulation until the end of the experiment, whereas K_1 and MK-4 had returned to baseline levels after 24 h. In a similar experiment (data not shown) MK-9 was even detectable at 72 h after intake. During this phase MK-9 was mainly found in LDL, which can survive in the circulation for several days. These data are compatible with an initial uptake of MK-9 by the liver, from where it is released slowly via LDL and remains available for all kind of tissues possessing LDL-receptors [30].

Taken together, our results show that the different lipophilicity of the various K-vitamers may result in substantial differences in their plasma transport and delivery to target tissues. The data provide an explanation for the previously observed preferential accumulation and utilization of K_1 in liver and MK-4 in extra-hepatic tissues. Also, they provide an explanation for the data from a recent population-based study in which it was shown that long-term intake of menaquinones (notably the long-chain ones) is inversely correlated with arterial calcification, whereas for K_1 intake the effect was much weaker [31].

Acknowledgements

The authors wish to thank Professor J. Rosing for critically reading and commenting on the manuscript.

References

[1] M.J. Shearer, Lancet 345 (1995) 229–234.
[2] M.J. Shearer, Blood Rev. 6 (1992) 92–104.
[3] L.J. Schurgers, C. Vermeer, Haemostasis 30 (2000) 298–307.
[4] C. Vermeer, Biochem. J. 266 (1990) 625–636.
[5] B. Furie, B.C. Furie, New Engl. J. Med. 326 (1992) 800–806.
[6] P. Ducy, C. Desbois, B. Boyce, G. Pinero, B. Story, C. Dunstan, E. Smith, J. Bonadio, S. Goldstein, C. Gundberg, A. Bradley, G. Karsenty, Nature 382 (1996) 448–452.
[7] G. Luo, P. Ducy, M.D. McKee, G.J. Pinero, E. Loyer, R.R. Behringer, G. Karsenty, Nature 385 (1997) 78–81.
[8] K. Nagata, K. Ohashi, T. Nakano, H. Arita, C. Zong, H. Hanafusa, K. Mizuno, J. Biol. Chem. 271 (1996) 30022–30027.
[9] M.J. Shearer, A. McBurney, P. Barkhan, Vitam. Horm. 32 (1974) 513–542.
[10] J.E. Ronden, M.J. Drittij-Reijnders, C. Vermeer, H.H. Thijssen, Biochim. Biophys. Acta 1379 (1998) 69–75.
[11] H.H. Thijssen, M.J. Drittij Reijnders, M.A. Fischer, J. Nutr. 126 (1996) 537–543.
[12] S. Lamon-Fava, J.A. Sadowski, K.W. Davidson, M.E. O'Brien, J.R. McNamara, E.J. Schaefer, Am. J. Clin. Nutr. 67 (1998) 1226–1231.
[13] T.G. Redgrave, D.C. Roberts, C.E. West, Anal. Biochem. 65 (1975) 42–49.
[14] R.J. Havel, Curr. Opin. Lipidol. 6 (1995) 312–316.
[15] B.C. Sherrill, T.L. Innerarity, R.W. Mahley, J. Biol. Chem. 255 (1980) 1804–1807.
[16] M.S. Brown, J. Herz, R.C. Kowal, J.L. Goldstein, Curr. Opin. Lipidol. 2 (1991) 65–72.
[17] T.J. Koivu-Tikkanen, L.J. Schurgers, H.H. Thijssen, C. Vermeer, Br. J. Nutr. 83 (2000) 185–190.
[18] M.G. Traber, T. Olivecrona, H.J. Kayden, J. Clin. Invest. 75 (1985) 1729–1734.
[19] L.K. Bjornson, C. Gniewkowski, H.J. Kayden, J. Lipid Res. 16 (1975) 39–53.
[20] M.G. Traber, J.C. Lane, N.R. Lagmay, H.J. Kayden, Lipids 27 (1992) 657–663.
[21] D.B. Zilversmit, R.E. Morton, L.B. Hughes, K.H. Thompson, Biochim. Biophys. Acta 712 (1982) 88–93.
[22] A. Stocker, S. Zimmer, S.E. Spycher, A. Azzi, IUBMB Life 48 (1999) 49–55.
[23] K. Hoshi, K. Nomura, Y. Sano, Y. Koshihara, Biochem. Pharmacol. 58 (1999) 1631–1638.
[24] T. Konishi, S. Baba, H. Sone, Chem. Pharm. Bull. Tokyo 21 (1973) 220–224.
[25] R.J. Havel, J.P. Kane, in: C.R. Scriver (Ed.), The Metabolic and Molecular Basis of Inherited Disease, 7th Edition, McGraw-Hill, New York, 1995, pp. 1841–1851.
[26] M.M.C.L. Groenen-van Dooren, B.A.M. Soute, K.S.T. Jie, H.H.W. Thijssen, C. Vermeer, Biochem. Pharmacol. 46 (1993) 433–437.
[27] L.J. Schurgers, P.E.P. Dissel, H.M.H. Spronk, B.A.M. Soute, C.R. Dhore, J.P. Cleutjens, C. Vermeer, Z. Kardiol. 90 (2001) 57–63.
[28] J.E. Ronden, M.J. Drittij-Reijnders, C. Vermeer, H.H.W. Thijssen, Biochim. Biophys. Acta 1379 (1998) 69–75.
[29] H.H. Thijssen, M.J. Drittij Reijnders, Br. J. Nutr. 72 (1994) 415–425.
[30] M.S. Brown, J.L. Goldstein, Science 232 (1986) 34–47.
[31] J.M. Geleijnse, C. Vermeer, L.J. Schurgers, D.E. Grobbee, H.A.P. Pols, J.C.M. Witteman, Thromb, Heamost. Suppl. ISTH XVIII (2001) P473.