**LOW VITAMIN K1 INTAKE IN HEMODIALYSIS PATIENTS**

**Maria Fusaro[1*], Claudia D'Alessandro[2], Marianna Noale[3], Giovanni Tripepi[4], Mario Plebani[5], Nicola Veronese[6], Giorgio Iervasi[1], Sandro Giannini[7], Maurizio Rossini[8], Giovanni Tarroni[9], Sandro Lucatello[10], Alberto Vianello[11], Irene Santinello[12], Luciana Bonfante[12], Fabrizio Fabris[7], Stefania Sella[7], Antonio Piccoli[12], Agostino Naso[12], Daniele Ciurlino[13], Andrea Aghi[7], Maurizio Gallieni[14], Adamasco Cupisti[2]**

We thank the **LOW VITAMIN K1 INTAKE IN HEMODIALYSIS PATIENTS** Study Investigators, who provided patient clinical care and collected clinical data. They were the following: **Davide Miozzo, Silvia Lazzaris, Daniela Cambi, Mirella Zabeo, Francesco Barbisoni,**

Affiliations:

[1*] Institute of Clinical Physiology, National Council of Research, Pisa, Italy;

[2] Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy;

[3] National Research Council (CNR), Neuroscience Institute, Padua, Italy

[4] Clinical Epidemiology and Physiopathology of Renal Diseases and Hypertension, CNR, Institute of Biomedicine, Reggio Calabria, Calabria, Italy;

[5] Laboratory Medicine Unit, Department of Medicine, University of Padua, Italy;

[6] Department of Medicine (DI-MED), Geriatric Section, University of Padua, Italy;

[7] Department of Medicine, Clinica Medica 1, University of Padova, Padova, Italy;

[8] Rheumatology Unit, Department of Medicine, University of Verona, Italy;

[9] Nephrology Unit, ULSS 1 Belluno, Italy;

[10] UOC Nephrology and dialysis, ULSS 13, Dolo, Italy;

[11] UOC Nephrology and dialysis, ULSS 2, Feltre, Italy;

[12] Nephrology Unit, University of Padua, Padua, Italy;

[13] Renal Unit, IRCCS Multimedica, Sesto San Giovanni, Milan, Italy.

[14] Nephrology and Dialysis Unit, Ospedale San Carlo Borromeo, Department of Clinical and Biomedical Sciences "Luigi Sacco", University of Milano – Italy;

**Corresponding Author:**

Maria Fusaro

Institute of Clinical Physiology,

National Council of Research,

Via G. Moruzzi 1, 56124, Pisa, PI, Italy.

dante.lucia@libero.it

## ABSTRACT

### Background & Aims

Vitamin K acts as a coenzyme in the γ-carboxylation of vitamin K-dependent proteins, including coagulation factors, osteocalcin, matrix Gla protein (MGP), and the growth arrest-specific 6 (GAS6) protein. Osteocalcin is a key factor for bone matrix formation. MGP is a local inhibitor of soft tissue calcification. GAS6 activity prevents the apoptosis of vascular smooth muscle cells. Few data on vitamin K intake in chronic kidney disease patients and no data in patients on a Mediterranean diet are available. In the present study, we evaluate the dietary intake of vitamin K1 in a cohort of patients undergoing hemodialysis.

### Methods

In this multi-center controlled observational study, data were collected from 91 patients aged > 18 years on dialysis treatment for at least 12 months and from 85 age-matched control subjects with normal renal function. Participants completed a food journal of seven consecutive days for the estimation of dietary intakes of macro- and micro-nutrients (minerals and vitamins).

### Results

Compared to controls, dialysis patients had a significant lower total energy intake, along with a lower dietary intake of proteins, fats, carbohydrates, fibres, and of all the examined minerals (Ca, P, Fe, Na, K, Zn, Cu, and Mg). With the exception of vitamin B12, vitamins intake followed a similar pattern, with a lower intake in vitamin A, B1, B2, C, D, E, folates, K1 and PP. These finding were confirmed also when normalized for total energy intake or for body weight.

In respect to the adequate intakes recommended in the literature, the prevalence of a deficient vitamin K intake was very high (70% to 90%) and roughly double than in controls. Multivariate logistic model identified Vitamin A and iron intake as predictors of vitamin K deficiency

### Conclusions.

Hemodialysis patients had a significantly low intake in vitamin K1, which could contribute to increase the risk of bone fractures and vascular calcifications. Since the deficiency of vitamin K intake seems to be remarkable, dietary counselling to HD patients should also address the adequacy of vitamin K dietary intake and bioavailability. Whether diets with higher amounts of vitamin K1 or vitamin K supplementation can improve clinical outcomes in dialysis patients remains to be demonstrated.

**Keywords**

Hemodialysis, dialysis, phylloquinone, menaquinone, diet, nutrition.

## INTRODUCTION

Vitamin K is a fat-soluble vitamin, which includes a series of vitamers: phylloquinones (vitamin K1) and menaquinones (vitamin K2). Vitamin K1 is found mainly in green leafy vegetables while vitamin K2 is mainly synthesized by intestinal bacteria and is found in butter and fermented cheeses [1].

Vitamin K1 is the main source of vitamin K. Vegetables in particular contain relevant amounts of phylloquinone, although there is great variability in vitamin K1 content among different vegetables. Green leafy vegetables and cabbages have the highest content and contribute to 40-50% of total intake [2] while most of fruits, fruit juices and other vegetables, such as carrots and tomatoes, have a lower content. Since Vitamin K1 is fat-soluble, its intestinal absorption is higher when introduced with vegetable oils, as soy, cottonseed, canola or olive oil.

Compared to phylloquinone, the dietary contribution of menaquinones intake is markedly lower. Dietary sources of vitamin K2 are chicken, egg yolk, dairy products, fermented cheeses in particular, cow liver and natto, a Japanese food made from fermented soybeans, which has the highest vitamin K2 content.

Some investigators also suggested the hypothesis of a conversion of dietary phylloquinone in menaquinone-4 (MK-4): it was reported that MK-4 is present in tissues of animals fed with phylloquinone as a sole source of vitamin K [3]. Extra-hepatic tissues from rats were shown to contain more MK-4 after phylloquinone administration rather than after MK-4 administration. High concentration of MK-4 were also found in extra-hepatic human tissues [4, 5].

Vitamin K acts as a coenzyme in the γ-carboxylation of vitamin K-dependent proteins (VKDPs), including coagulation factors, osteocalcin (bone Gla protein, BGP), matrix Gla protein (MGP), and the growth arrest-specific 6 (GAS6) protein. BGP is produced by osteoblasts during bone matrix formation and its hydroxyapatite-binding capacity is dependent on the vitamin K γ-carboxylation. MGP is produced by osteoclasts, chondrocytes and vascular smooth muscle cells and is a local

inhibitor of soft tissue calcification. The γ-carboxylation determines MGP bioactivity in preventing vascular calcification [6]. In this context, GAS6 activity is also relevant since it prevents the apoptosis of vascular smooth muscle cells [7].

Up to now, no evidence exists for an average requirement of Vitamin K, and as a consequence the Recommended Daily Allowance (RDA) is not defined. Hence, an Adequate Intake (AI) has been set based on representative dietary intake data from healthy subjects. In the U.S.A., the Food and Nutrition Board at the Institute of Medicine recommends an AI of 120 µg/day for men and 90 µg/day for women [8]. Instead, the Italian Society of Human Nutrition recommends an AI of 140 µg/day below 60 years of age and 170 µg/day above 60 years [9].

When considering the AI for vitamin K, no distinction is made between the different forms. This can be justified by the fact that phylloquinone is the major form in the diet while menaquinones, collectively referred to as vitamin K2, contribute a relatively small amount to satisfying the human requirement for vitamin K.

A deficit of vitamin K, due to a low dietary intake, potentially leads to a low serum level of carboxylated Gla proteins (VKDPs) and therefore to an increased risk of vascular calcification and bone fractures [10].

In chronic kidney disease, vascular calcification and mineral and bone disorders are very common: the risk of hip fracture is four times higher and that of aortic calcification is twice higher than in the general population [11, 12]. Recent studies have demonstrated that low levels of vitamin K are predictors of hip and vertebral fractures, aortic calcification, and cardiovascular disease [11, 13].

Growing evidence indicates that hemodialysis (HD) patients are at high risk of vitamin K deficiency. To achieve a lower dietary load of potassium and phosphorus, patients with end stage renal disease limit foods representing main sources of vitamin K, especially phylloquinone [14-16]. Regardless of its cause, vitamin K deficiency could worsen the fragile clinical status of this subset of patients in terms of cardiovascular disease and mineral bone disorders. Because data on vitamin

K intake in ESRD patients on a Mediterranean diet are lacking, in the present study we evaluate the dietary intake of vitamin K1 in a cohort of clinically stable patients undergoing maintenance HD.

## METHODS

### *Participants*

This observational study, collecting information reflecting the usual clinical practice, is population based, multi-center and controlled. Demographics and clinical data were collected from the medical charts of patients aged ≥ 18 years on HD treatment for at least 12 months. Exclusion criteria were: life expectancy lower than 6 months, a history or evidence of malignancy (except for non-melanoma skin neoplasia), gastrointestinal diseases with malabsorption, liver insufficiency, acute infectious diseases, psychiatric illnesses, use of antibiotics in the week preceding the dietary questionnaire. Only 6 patients (7%) were using warfarin and they were not excluded. A control group was formed by people without chronic kidney diseases and comparable for age, gender and race; they were hospital employers, nurses, or patient's partners. All the participants gave their own informed consent to the study, which did not modify the patient's clinical management. The study is in accordance with the Helsinki Declaration.

### *Dietary assessment*

With the aim of assessing the nutritional status, participants were asked to complete a food journal of seven consecutive days for the estimation of dietary intakes of macro- and micro-nutrients (minerals and vitamins) between 2013 and 2014. An individual food journal was given to each patient. An experienced operator provided all the necessary information for a correct compilation. The food journal reported a description of the type and amount of food and beverages consumed in the different meals or during all the days of the week.

When completed, the diary was handed back to the operator who interviewed the patients with the aim to accurately verify the data reported in the diary, making any needed corrections or

integrations. The actual amount of food consumed was recorded by weighing or through the use of photographic images of real size portions included in the Atlas Photo Food by the Scotti-Bassani Institute [17].

The processing of data collected from the diaries allowed to estimate the average daily intake of Vitamin K1, using data on the content of vitamin K1 supplied by the United States Department of Agriculture (USDA), referring to over 900 foods including foods also typical of the Mediterranean diet [18].

We also estimated the energy and nutrients intake, namely protein, carbohydrates, lipids, unsaturated fatty acids, saturated fats, cholesterol, calcium, phosphorus, magnesium, iron, zinc, copper, fiber, and vitamins A, C, B1, B2, PP, D. Data were obtained using the food composition Tables of the Italian National Institute of Nutrition and of the European Institute of Oncology Database Edition 2008 [19, 20].The daily energy and nutrients intake were reported as daily average of the 7-day food records. When appropriate, intakes were normalized by body weight or energy intake. Adjustment for total energy intake is usually appropriate in epidemiologic studies to control for confounding, reduce extraneous variation, and predict the effect of dietary interventions [21].

***Statistical analysis***

Data are expressed as mean ± standard deviation (SD), or median and inter-quartile range (Q1-Q3), for quantitative variables, and frequency percentages for discrete variables. Normal distribution of continuous variables was tested using the Shapiro-Wilk test.

The differential distribution for categorical variables among HD patients and controls was analyzed considering the $\chi^2$ test or the Fisher's exact test. Quantitative variables were compared among groups of subjects (HD patients vs controls) considering the Generalized Linear Model (GLM), after testing for homoschedasticity (Levene's test) or the non-parametric Mann-Whitney test.

Associations between variables measured in the study and low vitamin K intake were analyzed by univariate and multivariate regression. Variables associated with the low vitamin K intake were

identified using univariate logistic regression. Any significant predictors with $p \leq 0.10$ were then introduced into a multivariate model using the stepwise selection method. Statistical significance was assumed for p-value<0.05. Analyses were carried out using SAS Software 9.3 (SAS Institute, Cary, NC, USA).

## RESULTS

Ninety-one HD patients and 85 healthy controls, comparable for age and gender, were included in the analysis (**Table 1**). Some features of the studied HD patient group are reported in Table 1: weight and BMI were significantly lower in patients than in controls.

**Table 2** shows the daily dietary intakes of macronutrients, minerals and vitamins. HD patients showed a significant lower total energy intake with a lower dietary intake of proteins, fats, fibres and carbohydrates compared to controls. HD patients had significantly lower dietary intake for all the examined minerals (Ca, P, Fe, Na, K, Zn, Cu, and Mg) than healthy controls (**Table 2**). Vitamins intake followed a similar pattern, having HD patients significantly lower intake in vitamin A, B1, B2, C, D, E, folates, and PP, although no differences came to light for vitamin B12, as reported in **Table 2**. As a whole, HD patients ate less than controls, according to a lower body mass. However, differences in energy and protein intake are attenuated, but not blunted, when expressed as normalized by body weight (**Table 2**).

Similarly, HD patients showed an intake of vitamin K1 significantly lower than controls (**Table 3**). This finding was confirmed also when normalized for total energy intake or for body weight (**Table 3**). We also calculated vitamin K intake after exclusion of the 6 patients on warfarin treatment and results did not change.

Regarding the recommended intakes, an insufficient intake of vitamin K1 was more common in HD patients than in control subjects (**Table 4**). In respect to the AI suggested in 2001 by the National Research Council, the prevalence of a Vitamin K intake lower than recommended resulted very

high (89.2%) and roughly double than in controls. According to the Italian RDA value, a low Vitamin K intake was recorded in 71% of HD patients. Finally, when a cut-off value of 1 μg Vitamin K1 for kg of body weight is assumed [2], Vitamin K deficiency was observed in 49.5% of HD patients and in 24.7% of controls ($p<0.001$)**.**

**Table 2** also shows low vitamin D intake for both groups, according to Italian RDA values. Multivariate logistic model identified Vitamin A and iron as predictors of vitamin K deficiency according to Italian RDA values (**Table 5**) and according to AI Dietary Reference Intakes of the Institute of Medicine (U.S.) Panel on Micronutrients. (**Table 6**). Comparison between the two multivariate regression logistic models is shown in **Table 7**.

## DISCUSSION

In this study, we assessed the dietary intake of Vitamin K1 in a group of stable patients with CKD undergoing maintenance HD. As a whole, the proportion of people with insufficient intake of vitamin K was approximately two fold in HD patients compared to healthy controls. Further adjustments for energy and body weight did not modify this finding. Our data showed that HD patients had a remarkable low vitamin K1 intake, since they reported a 2-fold lower median intake of dietary vitamin K1 than that suggested by RDAs for Italian population. This finding is in agreement with other observations that patients with chronic kidney disease undergoing HD are at high risk of vitamin K1 deficiency [14-16].

To the best of our knowledge, this is the only study available about dietary vitamin K intake in HD patients in a Mediterranean country, and including a control group. This is a relevant point since the study by Cranenburg et al. [14] was made in the Netherlands, where dietary habits are quite different from Mediterranean countries. The type of diet is an important aspect also in patients undergoing HD, considering that vitamin K is highly dependent from dietary habits. Oriental countries, for instance, have higher vitamin K intake due to a higher use of some foods, namely

cabbage kimchi, spinach, spring onions, and soybean oils, lacking in Western countries [22]. Our findings about HD patients are in agreement with previous reports, suggesting that HD patients introduce significantly less vitamin K1 than a reference population, independently from dietary habits [14]. A possible explanation of these findings is that haemodialysis patients on good nutritional status are advised to limit both sodium and potassium intake, the latter abundant in the same foods rich in vitamin K1, as green leafy vegetables [23]. This is in agreement with the low intake of fibres, vitamin A and C, and folate as well. A second possible explanation is that loss of appetite and food restrictions in the attempt to limit inter-dialysis weight gain are quite common in this population, affecting the total energy and nutrients intake [24]. In essence, they eat less and this fact is confirmed by the finding that HD patients had a significantly lower intake of macro- and micro-nutrients than controls. Although our data may seem underestimated, we believe that data collection was accurate and these data might reflect the real intake. Accordingly, our data are very similar to those reported by Martins et al [25] in elderly HD population (namely an energy intake of 18±7 vs. 21±8 kcal/kg/day, and of protein 0.8±0.4 vs. 1.0±0.4 g/kg/day in HD and non-HD days, respectively). In addition, the Authors reported no differences in energy intake between HD and non-CKD elderly subjects. Therefore, the question arises as to whether a low nutrient intake really occurs in elderly subjects. [26]

No correlation was reported between vitamin K intake and markers of vitamin K status [14]. However, markedly reduced vitamin K intake was also reported in a cohort of kidney transplant recipients with a median glomerular filtration rate of 61 ml/min, who did not have significant food intake limitations [27]. Total vitamin K intake was below the recommended level in 50% of patients and lower vitamin K intake was associated with less consumption of green vegetables.

Assessing the vitamin K status could be very important in HD patients because it has a key role in the activation of Gla-proteins involved in bone and tissue calcification. Mineral bone disorders and vascular calcifications are central issues in the management of chronic kidney disease. Vitamin K deficiency leads to an impairment in MGP and BGP activation and could indeed contribute to

worsening the cardiovascular and bone fracture risks in these patients [10]. In a multivariable-adjusted model, we identified Vitamin A (OR 0.996) and iron (OR 0.616) as predictors of vitamin K deficiency. Interestingly, another fat soluble vitamin, the vitamin A metabolite retinoic acid, down-regulates MGP gene expression in different rat and human cell lines [28], indicating that diet can have different effects on vitamin K-dependent proteins activity. Iron intake as predictor of vitamin K deficiency is a novel finding with no clear explanation, which should be further studied. The potential benefit of vitamin K supplementation has been shown in studies involving populations without kidney disease [29-30]. However, these studies have focused on the amount of vitamin K necessary for an adequate synthesis of blood coagulation factors, but not for the metabolism of the other vitamin K-dependent proteins, which might be different. Based on the current knowledge, our study confirms that supplementation is probably needed in HD patients, to achieve an optimal intake of vitamin K [31].

The main limitation of the study is that no specific determinations of vitamin K status and of VKDPs levels or activity (e.g. osteocalcin, MPG, INR) were performed, and that only estimation of VitK1 intake was reported. Conversely, the strength of our work is the presence of a control group, and the use of a 7-day dietary recall that was not used in previous investigations. There is great variability in the content of vitamin K1 in food, so short-term food journals are not suitable to gain reliable information and appropriate estimations. This is even more relevant in dialysis patients, whose diet is greatly influenced by dialysis treatments.

It is well known that a potential underestimation of energy and nutrients intake may occur using the food journal. Even if the food record analysis represent the most valid and simple tool for nutrients and calories estimation, the occurrence of a conscious or unconscious underreporting is largely known. We tried to limit this bias collecting an interview at the time of delivery of the 7-day food journal in order to verify, as described in the method section, the sizes of the portion, the type of food and any missing data. Furthermore, vitamin K1 and protein intake were normalized to the energy intake in order to limit the effect of a potential underestimation. Additional methods helping

in the assessment of energy and proteins intake, such as body weight changes over time before the dietary questionnaire and nPCR obtained from urea kinetics were not recorded in this study. The 7-day recall has been demonstrated to be more effective in collecting dietary habits in respect to other methods (i.e. 3-day recall) [32]. Dietary habits of HD patients may change significantly from dialysis to non dialysis day and in the weekend days, so the 7-day recall is particularly reliable to detect the average weekly intake of nutrients.

In conclusion, patients with chronic kidney disease undergoing haemodialysis had a significantly low intake in vitamin K1, which could contribute to increase the risk of bone fractures and vascular calcifications. Since the deficiency of vitamin K intake seems to be remarkable, dietary counselling to HD patients should also address the adequacy of vitamin K dietary intake and bioavailability. The retrospective nature of our study does not allow to adequately address all the issues related to vitamin K intake. In particular, data about nPCR and body weight fluctuations might be of importance to better understand the real dietary intake. In addition, we looked at vitamin K intake for the possible relationship with fractures and vascular calcifications in dialysis patients, but these clinical entities are complex and several other nutrients related to bone metabolism might be involved, such as calcium, phosphorus, vitamin A, vitamin K and vitamin D.

Looking at the relationship between nutrients intake (vitamin K and the other CKD-MBD related nutrients) and patient outcomes such as fractures and vascular calcification requires a larger patient population, possibly studied in a prospective study. Also, a randomized trial could allow evaluating whether diets with higher amounts of vitamin K1 or vitamin K supplementation can improve clinical outcomes in patients undergoing hemodialysis.

## References


1. Shearer, M.J.; Newman, P. Metabolism and cell biology of vitamin K. *Thromb. Haemost.* **2008**, *100*, 530-547.

2. Booth, S.L.; Suttle, J.W. Dietary intake and adequacy of vitamin K. *J. Nutr.* **1998**, *128*, 785–788.

3. Sakamoto, N.; Kimura, M.; Hiraike, H.; Itokawa, Y. Changes of phylloquinone and menaquinone-4 concentrations in rat liver after oral, intravenous and intraperitoneal administration. *Int. J. Vitam. Nutr. Res.* **1996**, *66*, 322-328.

4. Thijssen, H.H.W.; Drittij-Reijnders, M. J. Vitamin K status in human tissues: tissue-specific accumulation of phylloquinone and menaquinone-4. *Br. J. Nutr.* **1996**, *75*, 121– 127.

5. Thijssen, H.H.W., Drittij-Reijnders, M. J.; Fischer, M.A.J.G. Phylloquinone and menaquinone-4 distribution in rats: synthesis rather than uptake determines menaquinone-4 organ concentrations. *J. Nutr.* **1996**, *126*: 537– 543.

6. Vermeer, C. Vitamin K: the effect on health beyond coagulation - an overview. *Food. Nutr. Res.* **2012**, 56.

7. Krueger, T.; Westenfeld, R.; Ketteler, M.; Schurgers, L.J.; Floege, J. Vitamin K deficiency in CKD patients: a modifiable risk factor for vascular calcification? *Kidney Int.* **2009**, *76*, 18-22.

8. Institute of Medicine (US) Panel on Micronutrients. Recommended Dietary Intake for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc, 2001, National Academy Press, Washington, D.C.

9. SINU Società Italiana di Nutrizione Umana. *LARN - Livelli di Assunzione di Riferimento di Nutrienti ed energia per la popolazione italiana*. IV Revisione. SICS Ed, Milan, Italy, 2014; pp. 1-656.

10. Fusaro, M.; Crepaldi, G.; Maggi, S.; Galli, F.; D'Angelo, A; Calò, L.; Giannini, S.; Miozzo, D.; Gallieni, M. Vitamin K, bone fractures and vascular calcifications in chronic kidney disease: an important but poorly studied relationship. *J. Endocrinol. Invest.* **2011**, *34*, 317-323.

11. Rodriguez-Garcia, M.; Gomez –Alonso, C.; Naves-Diaz, M.; Diaz-Lopez, J.B.; Diaz-Corte, C.; Cannata-Andia, J.B. Asturias Study Group. Vascular calcifications, vertebral fractures and mortality in haemodialysis patients. *Nephrol. Dial. Transplant.* **2009**, *24*, 239-246.

12. Alem, A.M.; Sherrad, D.J.; Gillen, D.L.; Weiss, N.S.; Beresford, S.A.; Heckbert, S.R.; Wong, C.; Stehman-Breen, C. Increased risk of hip fracture among patients with end-stage renal disease. *Kidney Int.* **2006**, *70*, 1358-1366.

13. Fusaro, M.; Noale. M.; Viola, V.; Galli, F.; Tripepi, G.; Vajente, N.; Plebani, M.; Zaninotto, M.; Guglielmi, G.; Miotto, D.; Dalle Carbonare, L.; D'Angelo, A.; Naso, A.; Grimaldi, C.; Miozzo, D.; Giannini, S.; Gallieni, M.; for the VItamin K Italian (VIKI) Dialysis Study Investigators. Vitamin K, vertebral fractures, vascular calcifications and mortality: Vitamin K Italian (VIKI) Dialysis Study. *J. Bone Miner. Res.* **2012**, *27*, 2271-2278.

14. Craneburg, E.C.M.; Schurgers, L.J.; Uiterwijk, H.H.; Beulens, J.W.J.; Dalmeijer, G.W.; Westerhuis, R.; Magdeleyns, E.J.; Herfs, M.; Vermeer, C.; Laverman, G.D. Vitamin K intake and status are low in hemodialysis patients. *Kidney Int.* **2012**, *82*, 605-610.

15. Pilkey, R.M.; Morton, A.R.; Boffa, M.B.; Noordhof, C., Day, A.G.; Su, Y.; Miller, L.M.; Koschinsky, M.L.; Booth, S.L. Subclinical vitamin K deficiency in haemodialysis patients. *Am. J. Kidney Dis.* **2007**, *49*, 432-439.

16. Holden, R.M.; Morton, A.R.; Garland, JS.; Pavlov A.; Day A.G.; Booth, S.L. Vitamins K and D status in stages 3-5 chronic kidney disease. *Clin. J. Am. Soc. Nephrol.* **2010**, *22*, 387-395.

17. Fantuzzi, A.L.; Chiuchiù M.P.; Bedogni, G. *Atlante fotografico delle porzioni degli alimenti.* Istituto Scotti Bassani: Milano, Italy, 2005. http://www.scottibassani.it/

18. USDA National Nutrient Database for Standard Reference, Release 24, 2011, http://ndb.nal.usda.gov/

19. Tabelle di Composizione degli alimenti. INRAN. Edizione 1993 http://nut.entecra.it/646/tabelle_di_composizione_degli_alimenti.html

20. Banca dati di composizione degli alimenti per studi epidemiologici in Italia. Istituto Europeo di Oncologia. Revisione del 2008. http://www.bda-ieo.it/wordpress/en/

21. Willett, W.C.; Howe, G.R.; Kushi, L.H. Adjustment for total energy intake in epidemiologic studies. *Am. J. Clin. Nutr.* **1997**, *65(4 Suppl),* 1220S-1228S; discussion 1229S-1231S.

22. Kim, E.S.; Kim, M.S.; Na, W.R.; Sohn, C.M. Estimation of vitamin K intake in Koreans and determination of the primary vitamin K-containing food sources based on the fifth Korean National Health and Nutrition Examination Survey (2010-2011). *Nutr. Res. Pract.* **2013**, *7*, 503-509.

23. Beto, J.A.; Bansal, V.K. Medical nutrition therapy in chronic kidney failure: integrating clinical practice guidelines. *J. Am. Diet. Assoc.* **2004**, *104*, 404-409.

24. Kopple, J.D.; Greene, T.; Chumlea, W.C.; Hollinger, D.; Maroni, B.J.; Merrill, D.; Scherch, L.K.; Schulman, G.; Wang, S.R.; Zimmer, G.S. Relationship between nutritional status and the glomerular filtration rate: results from the MDRD study. *Kidney Int.* **2000**, *57*, 1688-1703.

25. Martins A.M., Dias Rodrigues J.C., de Oliveira Santin FG, Barbosa Brito Fdos S, Moreira AS, Lourenço RA, Avesani CM. Food intake assessment of elderly patients on hemodialysis. J Ren Nutr. 2015 May;25(3):321-6).

26. Shapiro BB, Bross R, Morrison G, Kalantar-Zadeh K, Kopple JD. Self-Reported Interview-Assisted Diet Records Underreport Energy Intake in Maintenance Hemodialysis Patients. J Ren Nutr. 2015 Jul;25(4):357-63).

27. Boxma, P.Y.; van den Berg, E.; Geleijnse, J.M.; Laverman, G.D.; Schurgers, L.J.; Vermeer, C.; Kema, I.P.; Muskiet, F.A.; Navis, G.; Bakker, S.J.; de Borst, M.H. Vitamin K intake and plasma desphospho-uncarboxylated matrix Gla-protein levels in kidney transplant recipients. *PLoS One.* **2012**, *7*, e47991.

28. Kirfel, J.; Kelter, M.; Cancela, L.M.; Price, P.A.; Schüle, R. Identification of a novel negative retinoic acid responsive element in the promoter of the human matrix Gla protein gene. *Proc. Natl. Acad. Sci. U S A.* **1997**, *94*, 2227-32.

29. Schurgers, L.J.; Shearer, M.J.; Hamulyak, K.; Stocklin, E.; Vermeer, C. Effect of vitamin K intake on the stability of oral anticoagulant treatment: dose-response relationships in healthy subjects. *Blood* **2004**, *104*, 2682-2689.

30. Koos, R.; Mahnken, A.H.; Muhlenbruch, G.; Brandenburg, V.; Pflueger, B.; Wildberger, J.E.; Kühl, H.P. Relation of oral anticoagulation to cardiac valvular and coronary calcium assessed by multislice spiral computed tomography. *Am. J. Cardiol.* **2005**, *96*, 747-749.

31. Westenfeld, M.D.; Krueger, T.; Schlieper, G.; Cranenburg, E.C.M.; Magdeleyns, E.J.; Heidenreich, S.; Holzmann, S.; Vermeer, C.; Jahnen-Dechnet, W.; Ketteler, M.; Floege, J.; Schurgers, L.J. Effect of vitamin K2 supplementation on functional vitamin K deficiency in hemodialysis patients: a randomized trial. *Am. J. Kidney Dis.* **2012**, *59*, 186-195.

32. Dwyer, J.T. Dietary assessment. In *Modern Nutrition in health and disease;* Shils, M.E.; Olson, J.A.; Shike, M., Eds.; Lea & Febiger, Philadelphia, USA, 1994: pp. 842-860.

390 **Table 1. Demographic data of the studied haemodialysis (HD) patients and controls.**

391 **Data are reported as mean ± SD or median (Q1-Q3),**

392

| | **HD patients (n=91)** | **Controls (n=85)** | **p-value[§]** |
|---|---|---|---|
| Age, years | 71 (52, 76) | 69 (55, 75) | 0,205[§] |
| Gender – Males, n(%) | 55 (60.4) | 55 (64.7) | 0,559[§] |
| Weight, kg | 70.7±15.6 | 82.8±14.7 | **< 0,0001*** |
| Height | 1,66±0,10 | 1.70±0.9 | **0,002*** |
| Body Mass Index, kg/m$^2$ | 25.3±5.2 | 28.7±4.5 | **0,001*** |
| | | | |
| Waist circumference, cm | 97.3 ± 13.6 | 100.3±13.1 | 0,308* |
| Dialysis vintage, months | 93 (67 - 121) | | |
| Dialysis technique type, n (%) | | | |
| 1. Bicarbonate dialysis | 29 (31.9) | | |
| 2. Hemofiltration | 15 (16.5) | | |
| 3. Hemodiafitration | 24 (26.4) | | |
| 4. Acetate free biofiltration | 12 (13.2) | | |
| 5. Others | 11 (12.1) | | |
| Previous transplantation, n (%) | 11 (12.1) | - | - |

393

**Table 2. Dietary intake of macronutrients, minerals and vitamins in haemodialysis (HD) patients and in controls. Data are reported as mean ± SD, or median (Q1-Q3).**

| | HD patients (n=91) | Controls (n=85) | p-value |
|---|---|---|---|
| Total energy, Kcal/d | 1442.8 (1292.0, 1442.8 ) | 1812 (1515.7, 2136.3 ) | **<0.0001** |
| Energy Intake Kcal/kg/d | 22.32 ± 6.48 | 24.80 ± 6.35 | **0,011** |
| Total protein, g/d | 52.0 (43.9, 67.0 ) | 69.4 (58.4, 80.6) | **<0.0001** |
| Protein/Weight, g/kg/d | 0.82 (0.64, 0.97) | 0.88 (0.77, 1.10) | **0.013** |
| Total fat, g/d | 50.9 (40.1, 61.6 ) | 59.0 (49.7, 73.0) | **<0.0001** |
| Carbohydrates, g/d | 196.6 (168.7, 236.9 ) | 242.4 (203.1, 294.0) | **<0.0001** |
| Fiber, g/d | 12.0 (9.6, 13.7 ) | 19.6 (16.4, 23.3) | **<0.0001** |
| Ca, mg/d | 387.2 (264.9, 583.0 ) | 545.2 (418.2, 636.9) | **<0.0001** |
| P, mg/d | 755.9 (622.5, 922.5 ) | 1005.2 (826.1, 1179.2) | **<0.0001** |
| Fe, mg/d | 6.7 (5.6, 8.6) | 8.8 (7.7, 10.7) | **<0.0001** |
| Na mg/d | 1206.3 (887.5, 1630.3) | 1492.2 (938.7, 1833.4) | **0.003** |
| K, mg/d | 1556.4 (1247.4, 2037.1 ) | 2514.5 (2158.8, 3191.1) | **<0.0001** |
| Zn, mg/d | 6.8 (5.3, 8.2) | 8.0 (6.4, 10.5) | **<0.0001** |
| Cu, mg/d | 0.9 (0.6, 1.3) | 1.2 (1.1, 2.3) | **<0.0001** |
| Mg, mg/d | 152.6 (120.7, 190.4) | 200.1 (172.0, 267.6) | **<0.0001** |
| Vit A, mcg/d | 306.0 (130.6, 306.0) | 460.8 (286.0, 566.7) | **<0.0001** |
| Vit B1, mg/d | 0.7 (0.58, 0.88) | 0.84 (0.7, 1.1) | **<0.0001** |

| | | | |
|---|---|---|---|
| Vit B2, mg/d | 0.9 (0.7, 1.2) | 1.3 (1.0, 1.4) | **<0.0001** |
| Vit C, mg/d | 47.3 (29.4, 67.8) | 67.5 (52.4, 92.7) | **<0.0001** |
| Vit D, μg/d | 1.1 (0.8, 1.1) | 1.6 (0.5, 2.4) | **0.029** |
| Vit E, μg/d | 7.4 (5.5, 9.8) | 11.5 (10.3, 13.7) | **<0.0001** |
| Folates, μg/d | 77.4 (57.9, 118.9) | 141.2 (80.7, 181.0) | **<0.0001** |
| PP, mg/d | 10.2 (7.3, 12.9) | 12.4 (8.9, 16.7) | **<0.0001** |

396

**Table 3. Dietary intake of Vitamin K1 in haemodialysis (HD) patients and in controls, as average total daily intake, or normalized per body weight and per 1000 Kcal energy intake**

**Data are reported as mean ± SD, or median (Q1-Q3).**

| | **HD patients (n=91)** | **Controls (n=85)** | **p-value** |
|---|---|---|---|
| **Vitamin K1,** μg **/d** | 71.6 (35.5, 117.1) | 129.2 (61.5, 380.5) | **<0.0001** |
| **Vitamin K1** μg **/kg b.w.** | 1.00 (0.50, 2.22) | 1.84 (1.00, 4.83) | **<0.0001** |
| **Vitamin K1** μg **/ 1000 Kcal** | 46.8 (25.1, 85.9) | 88.9 (31.0, 210.2) | **<0.0001** |

402 **Table 4. Vitamin K intake in hemodialysis (HD) patients and in controls, according to**

403 **Recommended Dietary Allowance, Italy and AI Dietary Reference Intakes of the Institute of**

404 **Medicine (U.S.). Panel on Micronutrients**

405

| | **HD patients (n=91)** | **Controls (n=85)** | **p-value** |
|---|---|---|---|
| **Recommended Dietary Allowance, Italy. 2012. [9]** | | | |
| < 60 yrs old: < 140 μg/die, n (%) | 28 (30.8) | 16 (18.8) | **< 0.0001** |
| > 60 yrs old: < 170 μg/die, n (%) | 44 (48.4) | 22 (25.9) | |
| Total, n (%) | 72 (89.2) | 38 (44.7) | |
| **AI Dietary Reference Intakes of the Institute of Medicine (U.S.). Panel on Micronutrients, 2001. [8]** | | | |
| Men: < 120 μg/day, n (%) | 41 (45.1) | 27 (31.8) | **< 0.0001** |
| Women: < 90 μg/day, n (%) | 24 (26.4) | 6 (7.1) | |
| Total, n (%) | 65 (71.5) | 33 (38.8) | |

406 **Table 5. Logistic regression model for identifying predictors of vitamin K deficiency**
407 **according to Italian Recommended Dietary Allowance RDA values. Stepwise selection method**
408 **among significant variables (P < 0.10) in the univariate model.**

| Variable | Unadjusted models | | Multivariable-adjusted models | |
|---|---|---|---|---|
| | **OR (95% CI)** | **P-Value** | **OR (95% CI)** | **P-Value** |
| Vit A, μg /d | 0.994 (0.991, 0.997) | < **0.001** | 0.996 (0.992, 0.999) | **0.022** |
| Vit C, mg/d | 0.977 (0.964, 0.991) | < **0.001** | 0.999 (0.971, 1.009) | 0.305 |
| Folates, μg /d | 0.984 (0.974, 0.995) | **0.005** | 0.991 (0.979, 1.004) | 0.193 |
| Ca, mg/d | 0.996 (0.993, 0.999) | **0.004** | 0.998 (0.993, 1.002) | 0.288 |
| Fe, mg/d | 0.647 (0.503, 0.831) | **0.005** | 0.616 (0.421, 0.901) | **0.013** |

409

**Table 6. Logistic regression model for identifying predictors of vitamin K deficiency according to AI Dietary Reference Intakes of the Institute of Medicine (U.S.) Panel on Micronutrients. Stepwise selection method among significant variables (P < 0.10) in the univariate model.**

| Variable | Unadjusted models | | Multivariable-adjusted models | |
|---|---|---|---|---|
| | **OR (95% CI)** | **P-Value** | **OR (95% CI)** | **P-Value** |
| Age, years | 0.97 (0.937, 1.005) | 0.089 | 0.993 (0.939, 1.051) | 0,814 |
| Cholesterol, mg/d | 0.994 (0.988, 0.999) | **0.032** | 1.003 (0.993, 1.006) | 0,577 |
| Ca, mg/d | 0.997 (0.994, 1) | **0.027** | 0.999 (0.995, 1.003) | 0,507 |
| Fe, mg/d | 0.737 (0.599, 0.906) | **0.004** | 0.661 (0.472, 0.927) | **0,016** |
| Vitamin A, μg /d | 0.994 (0.991, 0.997) | **< 0.001** | 0.995 (0.991, 1) | **0,032** |
| Vitamin C, mg/d | 0.978 (0.965, 0.991) | **0.001** | 0.993 (0.974, 1.013) | 0,486 |
| Vitamin E μg/d | 0.856 (0.762, 0.961) | **0.009** | 1.05 (0.867, 1.271) | 0,618 |
| Folates, μg /d | 0.985 (0.975, 0.995) | **0.004** | 0.987 (0.97, 1.004) | 0,136 |

**Table 7. Comparison between the two multivariate regression logistic models.**

| Variable | LARN | | AI Dietary | |
|---|---|---|---|---|
| | **OR (95% CI)** | **P-Value** | **OR (95% CI)** | **P-Value** |
| Age, years | / | / | 0.993 (0.939, 1.051) | 0,814 |
| Vit A, μg /d | 0.996 (0.992, 0.999) | **0.022** | 0.995 (0.991, 1) | **0,032** |
| Vit C, mg/d | 0.999 (0.971, 1.009) | 0.305 | 0.993 (0.974, 1.013) | 0,486 |
| Vit E, μg /d | / | / | 1.05 (0.867, 1.271) | 0,618 |
| Folates, μg /d | 0.991 (0.979, 1.004) | 0.193 | 1.026 (0.963, 1.094) | 0,426 |
| Ca, mg/d | 0.998 (0.993, 1.002) | 0.288 | 0.999 (0.995, 1.003) | 0,507 |
| Fe, mg/d | 0.616 (0.421, 0.901) | **0.013** | 0.661 (0.472, 0.927) | **0,016** |
| Cholesterol, mg/d | / | / | 1.003 (0.993, 1.006) | 0,577 |