Plasma Retinol Concentration Is Mainly Driven by Transthyretin in Hemodialysis Patients.

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Abstract

Background

Micronutrients deficiencies in hemodialysis patients are due to low dietary intakes and intradialytic losses for hydrophilic micronutrients. Conversely, lipophilic non-dialyzable compounds might accumulate due to a lack of elimination through renal metabolism or dialysis. Other compounds have complex metabolism: their concentration is not explained by these phenomenons.

Study design

Monocentric observational longitudinal study

Subjects

123 hemodialysis patients

Main outcome measure

Plasma concentration of lipophilic micronutrients: retinol and its two cotransporters transthyretin and retinol binding protein 4, tocopherol, and carotenoids (α- and β-carotene, β-cryptoxanthin, lycopene, lutein, zeaxanthin) and all factors associated with one-year mortality

Results

Within the 123 patients of the study, median age [IQR] was 77.5[69.5–84.5] years and 58.5% were male. Median retinol plasma concentration was 4.07[2.65-5.51]µmol/L, and 91.9% of patient had high plasma retinol concentrations. In monovariate analysis, retinol levels were inversely correlated with mortality (HR=0.57[0.45-0.72]; $p<0.001$). This effect remained significant after adjustment with several parameters. Nevertheless, the correlation between retinol and mortality disappeared as soon as transthyretin was added in the statistical model, suggesting an effect of transthyretin as confusing bias.

Median tocopherol plasma concentration was 34.8[28.3-42.9]µmol/L and 72.4% of patients had high plasma tocopherol concentration. Neither tocopherol plasma levels, nor carotenoids concentrations were correlated with death in multivariate analysis.

Conclusions

In hemodialysis patients, the correlation between retinol plasma concentration and mortality represents the nutritional status but not a direct biological effect of retinol. Retinol is only a surrogate predictor of mortality. It might not represent vitamin A levels, but likely the transthyretin level. Plasma retinol levels should be interpreted cautiously in hemodialysis patients.

Keywords: chronic hemodialysis, retinol, transthyretin, micronutrients, tocopherol, vitamin A

Conflict of Interest Statement and Funding sources: None

Introduction

Protein energy wasting syndrome affects 30 to 60% of hemodialysis patients (HD) and contributes to high morbidity and mortality rates in this specific population [1]. Yet, in daily practices, very little concern is made about micronutrients (*i.e.* vitamins and trace elements) which are implicated in many metabolic functions including regulation of oxidative stress, or modulation of the immune system [2].

Micronutrients deficiencies in HD patients are due to low dietary intakes, but also from intradialytic losses for small hydrophilic micronutrients, and conversely micronutrients accumulation, to a lack of elimination through renal metabolism or dialysis techniques for some lipophilic or non-dialyzable compounds [2,3]. Nevertheless, some compounds have more complex metabolism and their plasma concentration is not only explained by these simple phenomenons.

In the lack of supplementation, some micronutrient plasma levels are low in HD patients compared to healthy subjects: thiamine (vitamin B1), niacin (vitamin B3), pyridoxine (vitamin B6), folates (vitamin B9), ascorbic acid (vitamin C), vitamin D,selenium, zinc and manganese [2, 4].Other micronutrient plasma levels, on the contrary, are high, especially retinol (vitamin A)but also cadmium, chromium, copper, lead, and vanadium [4, 5].Finally, for some micronutrients, like tocopherol (vitamin E), plasma levels have been described as low, normal or high according to different studies. [2].

In HD patients, plasma retinol levels have been described to be inversely related to mortality rate in two observational prospective studies [5, 6]. However, pathophysiological explanations for the patients' benefit of survival in case of high concentrations of plasma retinol are not clear. In another study, high tocopherol levels were associated with a better survival, but this effect disappeared with adjustment with other parameters [7]. To our best knowledge, no

study has assessed the link between carotenoids plasma concentration and survival in HD patients.

In this study, we aimed 1/ to assess plasma concentrations of lipophilic micronutrients: retinol (and its physiological partners: retinol binding protein 4 (RBP4) and transthyretin), tocopherol, and carotenoids; and 2/ to analyze if these plasma concentrations are predictive of subsequent mortality in HD patients.

Methods

Subjects and study design

We conducted a retrospective longitudinal study on all patients from our HD center from July 2014 to July 2015. All patients with data available regarding micronutrients were included, except for pregnant women or patients aged <18 years. Written information was provided to all patients, and all gave consent for their personal data to be used for research purposes. According to French law, it is neither necessary nor possible to obtain approval from an ethical committee (in French CPP, Comité de Protection des Personnes) for this type of non-interventional study. Moreover, CPPs are not entitled to issue waivers of approval for this type of study. Nevertheless, this study obtained approval from the Health Research Data Processing Advisory Committee (in French CCTIRS, Comité consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé; ref 15.684bis) and the French National Commission for Data Protection and Liberties (CNIL).

Clinical, biological, and hemodialysis parameters

The following data were collected from the patients' medical files: age, gender, diabetes mellitus, nephropathy, height, post-dialysis weight, vascular access. Evaluation of daily urine output was based on oral questioning of the patients and was therefore semi-quantitative: ≥500 mL/d or <500 mL/d. Body mass index (BMI) was calculated as post-dialysis weight after the mid-week session in kilograms divided by squared height in meters. Survival was assessed for all patients at one year.

Dialysis parameters were recorded at the mid-week session, and biological analyses were all performed at the start of this hemodialysis session. Dialysis dose was estimated by a single-pool Kt/V (spKt/V), as recommended by Daugirdas et al. [8]. The ESRD adapted Charlson Comorbidity Index was performed for each patient [9].

Biological data

Albumin, transthyretin, predialysis creatinine and urea as well as C-reactive protein which are performed in routine practice in our hemodialysis center were recorded from patient's medical files. Transthyretin was dosed using an immunoturbidimetry assay (ADVIA® 1800 Clinical Chemistry System, Siemens, France). Albumin was dosed in serum using bromocresol green assay (ADVIA® 1800 Clinical Chemistry System, Siemens, France).

Lipophilic vitamins and carotenoids quantification

The assays of carotenoids (α- and β-carotene, β-cryptoxanthin, lycopene, lutein, zeaxanthin) and vitamins were carried out by high-pressure liquid chromatography (HPLC) after extraction by organic solvents [10].

RBP4 quantification was performed using dedicated ELISA kits (Quantikine Elisa, R&D systems, France) according to the manufacturer's instructions.

Statistical analyses

Kaplan Meier tests were performed to assess association between transthyretin and mortality, as well as retinol and mortality. Univariate linear models were used to assess association between retinol and transthyretin, retinol and RBP4, and predialysis serum creatinine and RBP4. Cox models were used to determine factors associated with mortality. In a first step, variables with a statistical p-value of <0.10 in the univariate analysis were considered eligible for inclusion in the multivariate analysis. In a second step, using a descending stepwise method, variables with a $p<0.05$ in the multivariate analysis were retained within the final model. Results are shown as their medians [IQR] or percentages. Statistical analyses were performed with IBM SPSS 15.0 software.

Results

Studied population

A total of 123 HD patients was included in this observational study. Median age [IQR] was 77.5 [69.5–84.5] years and 58.5% of patients were male (Table 1). Within the studied population, 49.6% of patient had diabetes mellitus. All patients had end-stage renal failure and had been treated with conventional hemodialysis with high-flux membranes for 28.6 [12.4–76.6] months. Residual diuresis of ≥500 mL was present in 53.3% of patients.

The etiology for the primary cause of renal failure was diabetic nephropathy in 30.1% of patients, vascular nephropathy in 26.8%, chronic interstitial nephritis in 11.4%, non-diabetic glomerular disease in 4.1%, autosomal dominant polycystic kidney disease in 1.6%, other in 4.9%, and unknown in 21.1%.

Most patients underwent at least 12 hours per week HD, distributed among three sessions. Median spKt/V [IQR] was 1.56 [1.43-1.74]. Vascular access was a native fistula in 63.4% of patients, an arteriovenous graft in 18.7% and a catheter in 17.9%.

Nutritional parameters are reported in Table 1. Briefly, median BMI was 25.8 [22.5-29.3]kg/m^2, and median albumin was 38.0 [34.0-40.0] g/L.

Transthyretin and RBP4

Median transthyretin was 270 [200-330] mg/L and median RBP4 was 90.8 [59.3-115.8] mg/L. Only 37.3% of patients had a normal transthyretin level (*i.e.* ≥300 mg/L); 82.8% had plasma concentrations of RBP4 above the higher normal threshold, 17.2% had normal levels and no patient had low levels.Albumin was also correlated with transthyretin (r^2=0.49; $p<0.001$). RBP4, which accumulates during chronic renal failure, was correlated with transthyretin (r^2=0.31; $p<0.001$) and with predialysis serum creatinine (r^2=0.19; $p<0.001$).

Retinol plasma concentration

Median retinol plasma concentration was 4.07 [2.65-5.51]µmol/L (Table 1). Among the 123 patients 91.9% had plasma retinol concentrations above the higher normal threshold, only 8.1% had normal levels and 0.8% had a low level.

Retinol plasma concentration was well correlated with its two plasma transporters transthyretin (r^2=0.72; p<0.001) and RBP4 (r^2=0.26; p<0.001) as well as with albumin (r^2=0.36; p<0.001).

In monovariate analysis, lower retinol plasma levels were correlated with mortality (HR=0.57 [0.45-0.72]; p<0.001) (Table 1, Figure 1). This effect remained significant after adjustment with several parameters (Table 3), but the correlation between retinol and mortality disappeared as soon as transthyretin was added to the statistical model, suggesting an effect of transthyretin as confusing bias.

Tocopherol

Median tocopherol plasma concentration was 34.8 [28.3-42.9]µmol/L (Table 1). 72.4% of patients had high plasma tocopherol concentration, 27.6% had normal levels and only 2.4% had low tocopherol levels. In monovariate analysis, lower tocopherol plasma levels were correlated with mortality (HR=0.96 [0.94-0.99]; p<0.003) (Table 1). This effect remained significant after adjustment with albumin and c-reactive protein, but disappeared after adjustment with other parameters (Table 3). Tocopherol was correlated with triglycerides plasma concentration (data not shown), thus, to assess the effect of free tocopherol on mortality, the relationship between the tocopherol/triglycerides ratio and mortality was studied. Median tocopherol/TG concentration was 20.7 [15.8-30.2] µmol/mmol. This ratio

was correlated with mortality in monovariate analysis, but this correlation was no longer significant after adjustment with albumin (Table 1).

Carotenoids

Plasma concentrations of carotenoids are reported in Table 1. No normal values are available for these compounds. In monovariate analysis, only lycopene (HR=0.52 [0.31-0.88]; p=0.02) and lutein (HR=0.04 [0.01-0.84]; p=0.04) were associated with mortality. However, this effect was no longer significant after adjustment with albumin.

Survival

At one year of follow-up, 26.8% of patients had died (n=33 patients). Causes of death are reported in Table 2. They were mainly cardiac (27.3%), infectious (18.2%), cancer (15.2%), withdrawal of dialysis (15.2%) and vascular causes (12.1%).

Besides lipophilic vitamins and carotenoids, the parameters associated with mortality in monovariate analysis were: age (HR=1.03]1.00-1.07]; p=0.04), adapted Charlson comorbidity index (HR=1.19 [1.07-1.31]; p=0.001), presence of a catheter for the vascular access (HR2.99 [1.45-6.17]; p=0.003), albumin (HR=0.88 [0.83-0.93]; p<0.001), transthyretin (HR=0.90 [0.87-0.94]; p<0.001), predialysis creatinine (HR=0.99 [0.99-0.99]; p=0.002) and predialysis urea (HR=0.93 [0.88-0.99]; p=0.03). C-reactive protein level was at the limit of significance.

Discussion

In this study, we confirmed that hemodialysis patients display high plasma retinol and RBP4concentrations, and that the highest concentrations of retinol were correlated with an improved survival in monovariate analysis. A new insight of our study was that this correlation disappears when retinol concentration was adjusted with transthyretin concentration, which suggests that high retinol plasma concentration is only a surrogate marker of higher concentration of transthyretin, which reflects a good nutritional status of hemodialysis patients.

To understand this new insight, one must be familiar with retinol physiology. Dietary sources of vitamin A include its provitamin, beta-carotene which is found in vegetable sources, and its active forms -retinol, retinal and retinoic acid- which are found in animal sources. Retinol storage is mainly in the liver stellar cells as retinyl esters. In the plasma, retinol which is a lipophilic compound is linked to a transport complex consisting of RBP4 and transthyretin -also known as prealbumin - forming a complex characterized by an equimolecular ratio of 1:1:1 [11].

RBP4 is a single polypeptide chain protein, synthesized in the liver, which binds and transports retinol to the target organs. It is a strong signal for stellar cells to remove retinol into the plasma and therefore, retinol plasma concentration is highly regulated by RBP4[12]. During renal failure, RBP4, which has a renal degradation, accumulates. The high concentration of retinol is mainly due to RBP4 accumulation which is a signal for liver cells to remove retinol in the plasma. In our study, median retinol plasma concentration was 4 times higher than normal values, which was in accordance with previous literature reporting retinol concentrations to be 3 to 4 times higher in HD patients than in healthy subjects [13-15].

RBP4 is a 21 kDa molecule and is thus considered as a middle size (>500Da) protein bound uremic toxin [16, 17]. In hemodialysis patients, convective therapy using online

hemodiafiltration improved RBP4 removal compared to hemodialysis with high-flux membranes, as attended for a middle size uremic toxin, whereas membrane permeability only provided a slight removal increase [18]. Nevertheless, a 4-hour high volume post-dilution hemodiafiltration session only partly removes accumulated RBP4 with a reduction ratio of less than 30% [18]. Thus, plasmatic RBP4 concentrations in HD patients are usually 2 to 2.5 times higher than in healthy subjects [16]. Our observation was very similar: median RBP4 concentration was of 90.8 [59.3-115.8] mg/L where upper normal threshold of the dosing technique was 48.6mg/L.

In HD patients, transthyretin concentration is representative of the nutritional status, exhibiting significant relationships with energy and protein intake as well as with fat stores and lean body mass. In addition to its relationship with nutritional status, transthyretin is involved in the inflammatory response and its serum concentration is negatively correlated with inflammatory markers. Serum transthyretin concentrations lower than 300 mg/l were associated with an increased risk of morbidity and mortality independently from serum albumin [11].

In malnourished HD patients, the serum transthyretin/RBP4 ratio is thus highly modified because of a high RBP4 concentration and low transthyretin concentration [13]. We show that in this specific population, retinol is better correlated with transthyretin (r^2=0.72; $p<0.001$) than RBP4 (r^2=0.26; $p<0.001$). Thus, in patients with a low transthyretin and a high RBP4 level, transthyretin, which is necessary to remove retinol from its storage in liver cells, could be the limiting factor for this removal [19]. Another explanation could be that transthyretin which reflects the daily dietary intakes is correlated with vitamin A intakes [3], but this latter is not likely because in plasma, retinol concentration is high.

Confusion is often made between plasma concentrations of micronutrients and micronutrient status. Regarding vitamin A, plasma retinol concentration is not representative of the overall

vitamin A stores which are mainly located in the liver. Interestingly, vitamin A accumulation hepatotoxicity might occur with a normal retinol plasma level [20] and in patients with hepatitis C virus, liver fibrosis has been associated with retinol plasma levels increase but lower liver storage of vitamin A [21]. In this latter study, no correlation was found between hepatic tissue retinyl palmitate-a retinyl ester- and plasma retinol concentration. Thus, to assess retinol whole body stores, a liver biopsy analysis is required [21].

The link between retinol concentration and mortality reflects only the relationship between transthyretin and mortality. In two previous studies, retinol plasma concentration was correlated with death, even after adjustment with many parameters including nutritional status and RBP4, but these studies did not analyze transthyretin levels which is a key element in retinol physiology [5, 6]. The link between retinol plasma concentration and death is not likely because retinol plasma concentration couldincorrectly reflect the vitamin A status. Moreover, these two studies hypothesize a role of low retinol in a high infection rate in HD patients, but one could argue an opposite relationship in which infection induces inflammation and reduces transthyretin concentration and thus retinol concentration [6].

Still, the lack of effect of high retinol concentrations on mortality should not be interpreted as if this substance has no effect. Roehrs et al. reported a study in which higher retinol concentrations were associated with higher superoxide dismutase and catalase activities, but these activities did not prevent lipid peroxidation, hypothesizing a pro-oxidant role of high retinol concentrations [15].

Regarding vitamin E, our findings are very similar to what has been reported in literature [7]: plasma concentration of tocopherol and tocopherol/TG are correlated with poor outcomes, but this effect does not resist to adjustment with other parameters and thus, plasma tocopherol concentration is only a surrogate marker of the measured outcome, i.e. death. Identically, plasma carotenoids levels were not independently associated with mortality.

We agree this study suffers some limitations: retrospective analysis, small size and old age of the studied population, monocentric design, lack of follow-up. Our results require confirmation in a~~n~~ large independent cohort of patients. Nevertheless, the strength of the correlation between plasma retinol and transthyretin concentrations is striking.

In conclusion, our study reports that in HD patients with low transthyretin, retinol plasma concentration ~~could~~ might not represent vitamin A levels, but likely the nutritional status of the patients. Its correlation with mortality ~~is~~ could be more linked to the nutritional status rather than a direct biological effect of retinol.

Practical application

Plasma retinol concentration is only a surrogate marker of mortality and it should be interpreted in the highlight of transthyretin concentration. Plasma retinol levels shouldbe interpreted cautiously in HD patients, as well as for tocopherol: its plasmatic concentration might not represent the whole-body vitamin A.

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341 **Tables**

342 **Table1: General characteristics and factors associated with death: univariate analysis and adjustment with albumin (n=123 patients).**

	Normal range (*or recommended value in HD patients)	**Total population (n=123)**	**Survival (n=90)**	**Death (n=33)**	**Monovariate analysis**	**Adjusted foralbumin**		
		Median[25–75] or %		Median[25–75] or %	HR	p-value	HR	p-value
Age (years)	-	77.5 [69.5-84.5]		81.4 [73.2-84.3]	1.03[1.01-1.07]	0.04	1.04[1.01-1.08]	0.03

Plasma retinol in hemodialysis patients

Male gender	-	58.5%	63.6%	0.80 [0.39-1.62]	0.52		
Diabetes mellitus	-	49.6%	42.4%	0.75 [0.38-1.50]	0.42		
Dialysis vintage (months)	-	28.6 [12.4-76.6]	34.9 [16.5 [78.1]	1.00 [0.99-1.01]	0.84		
Charlson comorbidity index score	-	6 [5-8]	7 [6-9]	1.19 [1.07-1.31]	0.001	1.22 [1.09-1.36]	<0.001

Plasma retinol in hemodialysis patients

Diuresis ≥500 mL/d	-	53.3%	48.5%	0.76 [0.38-1.51]	0.43		
Vascular access[§] Native fistula AV graft Catheter	- - -	 63.4% 18.7% 17.9%	 45.5% 21.2% 33.3%	2.99 [1.45-6.17]	0.003	2.98 [1.43-6.20]	0.004
Weight (kg) BMI (kg/m²)	- -	70.5 [59.8-79.4] 25.8 [22.5-29.3]	65.8 [53.4-74.4] 24.5 [21.5-29.3]	0.98 [0.96-1.01] 0.95 [0.88-1.02]	0.16 0.14		
Biological values Albumin (g/L) Transthyretin (mg/L) Predialysis creatinine (µmol/L) Predialysis urea (mmol/L) C-reactive protein (mg/L)	 >40 >300 - - <0.5	 38.0 [34.0-40.0] 270 [200-330] 594 [465-775] 18.8 [14.6-22.3] 8.3 [2.7-25.0]	 34.0 [29.0-38.0] 190 [130-260] 516 [409-616] 17.0 [13.5-20.4] 14.0 [7.4-38.1]	 0.88 [0.83-0.93] 0.90 [0.87-0.94] 0.99 [0.99-0.99] 0.93 [0.88-0.99] 1.00 [1.00-1.01]	 <0.001 <0.001 0.002 0.03 0.05	- 0.91 [0.86-0.96] 0.99 [0.99-1.00] 0.96 [0.89-1.02]	 - 0.001 0.09 0.19

Plasma retinol in hemodialysis patients

Liposolubles vitamins and carotenoids							
Retinol – vitamin A (µmol/L)						0.63 [0.47-0.85]	
Retinol Binding Protein 4 (mg/L)						0.99 [0.98-0.99]	
25-hydroxyvitamin D (ng/mL)	0.35-1.75 12.7-48.6	4.07 [2.65-5.51] 90.8 [59.3-115.8]	2.60 [2.11 [3.80] 67.2 [38.8-93.7]	0.57 [0.45-0.72] 0.98 [0.97-0.99]	<0.001 0.001	0.97 [0.94-0.99]	0.002 0.02
α+γ Tocopherol - Vitamin E (µmol/L)	≥30 18.0-29.0	30.8 [22.6-38.8] 34.8 [28.3-42.9]	28.0 [21.1-36.6] 29.2 [21.7-38.3]	0.98 [0.95-1.01] 0.96 [0.94-0.99]	0.20 0.003	1.02 [0.99-1.04]	0.03
Vitamin E/Triglycerides (µmol/g)	-	20.7 [15.8-30.2]	22.3 [18.6-33.3]	1.03 [1.01-1.05]	0.03	0.68 [0.41-1.14]	0.14
Lycopen (µmol/L)	-	0.93 [0.43-1.59]	0.56 [0.27-1.13]	0.52 [0.31-0.88]	0.02		0.15
α-Carotene (µmol/L)	-	0.11 [0.05-0.21]	0.11 [0.03-0.17]	1.02 [0.18-5.79]	0.98		
β-Carotene (µmol/L)	-	0.24 [0.12-0.33]	0.17 [0.07-0.30]	0.57 [0.10-3.12]	0.52		
β-cryptoxanthine (µmol/L)	-	0.08 [0.04-0.13]	0.06 [0.02-0.12]	0.01 [0.00-4.13]	0.14		
Zeaxanthine (µmol/L)	-	0.02 [0.01-0.03]	0.01 [0.00-0.02]	0.00 [0.00-3.81]	0.06	0.20 [0.02-2.56]	
Lutein(µmol/L)	-	0.23 [0.17-0.33]	0.20 [0.10-0.29]	0.04 [0.01-0.84]	0.04		0.22

BMI: Body mass index. AV graft: arteriovenous graft.

* Recommended values for hemodialysis patients are provided according to European Best Practice Guidelines Guideline on Nutrition [1]

§ Comparison is for (Native fistula + AV graft) *versus* Catheter.

Table 2 : Causes of death.

Death causes	**N=33**
	Number (%)
Heart disease	9 (27.3%)
Within which sudden death	7 (21.2%)
Infectious disease	6 (18.2%)
Cancer	5 (15.2%)
Cachexia leading to dialysis stop	5 (15.2%)
Peripheral vascular disease	4 (12.1%)
Within which inferior limb arteritis	2 (6.1%)
Stoke	1 (3.0%)
Mesenteric ischemia	1 (3.0%)
Hemorrhage	1 (3.0%)
Traffic accident	1 (3.0%)
Unknown	2 (6.1%)

Table 3: Association between plasmatic concentrations of liposolubles vitamins retinol and tocopherol and death at one year: multivariate analysis (n=123).

	~~Model Without Transthyretin~~		~~Model With Transthyretin~~	
	~~HR [95%CI]~~	~~*p*-value~~	~~HR [95%CI]~~	~~*p*-value~~
~~**Retinol**~~				
~~Model 1~~	~~0.57 [0.45-0.72]~~	~~<0.001~~	~~0.77 [0.51-1.17]~~	~~0.22~~
~~Model 2~~	~~0.63 [0.47-0.85]~~	~~0.002~~	~~0.77 [0.51-1.17]~~	~~0.23~~
~~Model 3~~	~~0.66 [0.48-0.89]~~	~~0.008~~	~~0.73 [0.47-1.13]~~	~~0.16~~
~~Model 4~~	~~0.70 [0.51-0.86]~~	~~0.03~~	~~0.63 [0.40-1.00]~~	~~0.05~~
~~Model 5~~	~~0.71 [0.50-1.00]~~	~~0.05~~	~~0.64 [0.40-1.03]~~	~~0.07~~
~~**Tocopherol**~~				
~~Model 1~~	~~0.96 [0.94-0.99]~~	~~0.003~~	~~-~~	~~-~~
~~Model 2~~	~~0.97 [0.94-0.99]~~	~~0.03~~	~~-~~	~~-~~
~~Model 3~~	~~0.97 [0.94-1.00]~~	~~0.05~~	~~-~~	~~-~~
~~Model 4~~	~~0.98 [0.95-1.01]~~	~~0.22~~	~~-~~	~~-~~

~~Model 1: Monovariate analysis.~~
~~Model 2: Adjusted with albumin.~~
~~Model 3: Adjusted with albumin, age, gender, and diabetes mellitus.~~
~~Model 4: Adjusted with albumin, age, gender, diabetes mellitus, Charlson comorbidity index score, and vascular access.~~
~~Model 5: Adjusted with albumin, age, gender, diabetes mellitus, Charlson comorbidity index score,vascular access and retinol binding protein.~~

	Model Without Transthyretin		Model With Transthyretin	
	HR [95%CI]	*p*-value	HR [95%CI]	*p*-value
Retinol				
Model 1	0.57 [0.45-0.72]	<0.001	0.77 [0.51-1.17]	0.22
Model 2	0.63 [0.47-0.85]	0.002	0.77 [0.51-1.17]	0.23
Model 3	0.60 [0.44-0.82]	0.001	0.77 [0.51-1.17]	0.22
Model 4	0.63 [0.45-0.86]	0.004	0.72 [0.46-1.13]	0.15
Model 5	0.65 [0.47-0.91]	0.01	0.63 [0.39-0.99]	0.05
Model 6	0.66 [0.46-0.96]	0.03	0.63 [0.39-1.02]	0.06
Tocopherol				
Model 1	0.96 [0.94-0.99]	0.003	-	-
Model 2	0.97 [0.94-0.99]	0.03	-	-
Model 3	0.97 [0.94-0.99]	0.03	-	-
Model 4	0.97 [0.94-1.00]	0.06	-	-
Model 5	0.98 [0.95-1.01]	0.25		

CI: confidence interval.
Model 1: Monovariate analysis.
Model 2: Adjusted with albumin.
Model 3: Adjusted with albumin and c-reactive protein.
Model 4: Adjusted with albumin, c-reactive protein, age, gender, and diabetes mellitus.

Model 5: Adjusted with albumin, c-reactive protein, age, gender, diabetes mellitus, Charlson comorbidity index score, and vascular access.
Model 6: Adjusted with albumin, c-reactive protein, age, gender, diabetes mellitus, Charlson comorbidity index score, vascular access and retinol binding protein.

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371 **Legends to figures**

372 **Figure 1**

373 **Kaplan-Meier curve for the time to all-cause mortality according to retinol plasma**

374 **concentration quartiles (n=123).**

375 **Figures**

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