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

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Plasma retinol in hemodialysis patients

1 **Plasma retinol concentration is mainly driven by transthyretin in hemodialysis patients.**

2
3 **Bataille S, Landrier JF, Astier J, Cado S, Sallette J, Serveaux M, Burtey S, Cohen J, Tournier C, Tournaire F, Darmon P**

4

5 **Abstract**

6 **Background**

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

12 **Study design**

13 Monocentric observational longitudinal study

14 **Subjects**

15 123 hemodialysis patients

16 **Main outcome measure**

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

20 **Results**

21 Within the 123 patients of the study, median age [IQR] was 77.5[69.5–84.5] years and 58.5%
22 were male. Median retinol plasma concentration was 4.07[2.65-5.51] μ mol/L, and 91.9% of
23 patient had high plasma retinol concentrations. In monovariate analysis, retinol levels were
24 inversely correlated with mortality (HR=0.57[0.45-0.72]; $p < 0.001$). This effect remained
25 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.

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

29 **Conclusions**

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

35

36

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

38

39 **Conflict of Interest Statement and Funding sources:** None

40

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41 **Introduction**

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

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

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

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

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

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

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75 **Methods**

76 Subjects and study design

77 We conducted a retrospective longitudinal study on all patients from our HD center from July
78 2014 to July 2015. All patients with data available regarding micronutrients were included,
79 except for pregnant women or patients aged <18 years. Written information was provided to
80 all patients, and all gave consent for their personal data to be used for research purposes.
81 According to French law, it is neither necessary nor possible to obtain approval from an
82 ethical committee (in French CPP, Comité de Protection des Personnes) for this type of non-
83 interventional study. Moreover, CPPs are not entitled to issue waivers of approval for this type
84 of study. Nevertheless, this study obtained approval from the Health Research Data
85 Processing Advisory Committee (in French CCTIRS, Comité consultatif sur le traitement de
86 l'information en matière de recherche dans le domaine de la santé; ref 15.684bis) and the
87 French National Commission for Data Protection and Liberties (CNIL).

88

89 Clinical, biological, and hemodialysis parameters

90 The following data were collected from the patients' medical files: age, gender, diabetes
91 mellitus, nephropathy, height, post-dialysis weight, vascular access. Evaluation of daily urine
92 output was based on oral questioning of the patients and was therefore semi-quantitative:
93 ≥ 500 mL/d or < 500 mL/d. Body mass index (BMI) was calculated as post-dialysis weight
94 after the mid-week session in kilograms divided by squared height in meters. Survival was
95 assessed for all patients at one year.

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

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101 Biological data

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

107

108 Lipophilic vitamins and carotenoids quantification

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

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

114

115 Statistical analyses

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

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126 **Results**

127 Studied population

128 A total of 123 HD patients was included in this observational study. Median age [IQR] was
129 77.5 [69.5–84.5] years and 58.5% of patients were male (Table 1). Within the studied
130 population, 49.6% of patient had diabetes mellitus. All patients had end-stage renal failure and
131 had been treated with conventional hemodialysis with high-flux membranes for 28.6 [12.4–
132 76.6] months. Residual diuresis of ≥ 500 mL was present in 53.3% of patients.

133 The etiology for the primary cause of renal failure was diabetic nephropathy in 30.1% of
134 patients, vascular nephropathy in 26.8%, chronic interstitial nephritis in 11.4%, non-diabetic
135 glomerular disease in 4.1%, autosomal dominant polycystic kidney disease in 1.6%, other in
136 4.9%, and unknown in 21.1%.

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

140 Nutritional parameters are reported in Table 1. Briefly, median BMI was 25.8 [22.5-
141 29.3]kg/m², and median albumin was 38.0 [34.0-40.0] g/L.

142

143 Transthyretin and RBP4

144 Median transthyretin was 270 [200-330] mg/L and median RBP4 was 90.8 [59.3-115.8] mg/L.

145 Only 37.3% of patients had a normal transthyretin level (*i.e.* ≥ 300 mg/L); 82.8% had plasma
146 concentrations of RBP4 above the higher normal threshold, 17.2% had normal levels and no
147 patient had low levels. Albumin was also correlated with transthyretin ($r^2=0.49$; $p<0.001$).

148 RBP4, which accumulates during chronic renal failure, was correlated with transthyretin
149 ($r^2=0.31$; $p<0.001$) and with predialysis serum creatinine ($r^2=0.19$; $p<0.001$).

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150

151 Retinol plasma concentration

152 Median retinol plasma concentration was 4.07 [2.65-5.51]µmol/L (Table 1). Among the 123
153 patients 91.9% had plasma retinol concentrations above the higher normal threshold, only
154 8.1% had normal levels and 0.8% had a low level.

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

158 In monovariate analysis, lower retinol plasma levels were correlated with mortality (HR=0.57
159 [0.45-0.72]; $p<0.001$) (Table 1, Figure 1). This effect remained significant after adjustment
160 with several parameters (Table 3), but the correlation between retinol and mortality
161 disappeared as soon as transthyretin was added to the statistical model, suggesting an effect of
162 transthyretin as confusing bias.

163

164 Tocopherol

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

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

176

177 Carotenoids

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

182

183 Survival

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

187 Besides lipophilic vitamins and carotenoids, the parameters associated with mortality in
188 monovariate analysis were: age (HR=1.03 [1.00-1.07]; p=0.04), adapted Charlson
189 comorbidity index (HR=1.19 [1.07-1.31]; p=0.001), presence of a catheter for the vascular
190 access (HR=2.99 [1.45-6.17]; p=0.003), albumin (HR=0.88 [0.83-0.93]; p<0.001), transthyretin
191 (HR=0.90 [0.87-0.94]; p<0.001), predialysis creatinine (HR=0.99 [0.99-0.99]; p=0.002) and
192 predialysis urea (HR=0.93 [0.88-0.99]; p=0.03). C-reactive protein level was at the limit of
193 significance.

194

195

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196 Discussion

197 In this study, we confirmed that hemodialysis patients display high plasma retinol and
198 RBP4 concentrations, and that the highest concentrations of retinol were correlated with an
199 improved survival in monivariate analysis. A new insight of our study was that this
200 correlation disappears when retinol concentration was adjusted with transthyretin
201 concentration, which suggests that high retinol plasma concentration is only a surrogate
202 marker of higher concentration of transthyretin, which reflects a good nutritional status of
203 hemodialysis patients.

204 To understand this new insight, one must be familiar with retinol physiology. Dietary sources
205 of vitamin A include its provitamin, beta-carotene which is found in vegetable sources, and its
206 active forms -retinol, retinal and retinoic acid- which are found in animal sources. Retinol
207 storage is mainly in the liver stellar cells as retinyl esters. In the plasma, retinol which is a
208 lipophilic compound is linked to a transport complex consisting of RBP4 and transthyretin
209 -also known as prealbumin - forming a complex characterized by an equimolecular ratio of
210 1:1:1 [11].

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

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

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

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

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

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

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

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

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

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

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

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

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279 Practical application

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

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- 339

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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 for albumin		
		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

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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

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Diuresis \geq 500 mL/d	-	53.3%	48.5%	0.76 [0.38-1.51]	0.43		
Vascular access [§]							
Native fistula	-	63.4%	45.5%	2.99 [1.45-6.17]	0.003	2.98 [1.43-6.20]	0.004
AV graft	-	18.7%	21.2%				
Catheter	-	17.9%	33.3%				
Weight (kg)	-	70.5 [59.8-79.4]	65.8 [53.4-74.4]	0.98 [0.96-1.01]	0.16		
BMI (kg/m ²)	-	25.8 [22.5-29.3]	24.5 [21.5-29.3]	0.95 [0.88-1.02]	0.14		
Biological values							
Albumin (g/L)						-	
Transthyretin (mg/L)						0.91 [0.86-0.96]	
Predialysis creatinine (μ mol/L)	>40	38.0 [34.0-40.0]	34.0 [29.0-38.0]	0.88 [0.83-0.93]	<0.001	0.99	-
Predialysis urea (mmol/L)	>300	270 [200-330]	190 [130-260]	0.90 [0.87-0.94]	<0.001	0.99	0.001
C-reactive protein (mg/L)	-	594 [465-775]	516 [409-616]	0.99 [0.99-0.99]	0.002	1.00	0.09
C-reactive protein (mg/L)	<0.5	18.8 [14.6-22.3]	17.0 [13.5-20.4]	0.93 [0.88-0.99]	0.03	0.96	0.19
C-reactive protein (mg/L)		8.3 [2.7-25.0]	14.0 [7.4-38.1]	1.00 [1.00-1.01]	0.05	0.96 [0.89-1.02]	

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

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

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

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

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Plasma retinol in hemodialysis patients

347 **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%)

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350 **Table 3: Association between plasmatic concentrations of liposolubles vitamins retinol**
 351 **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	-	-

352 ~~Model 1: Monovariate analysis.~~

353 ~~Model 2: Adjusted with albumin.~~

354 ~~Model 3: Adjusted with albumin, age, gender, and diabetes mellitus.~~

355 ~~Model 4: Adjusted with albumin, age, gender, diabetes mellitus, Charlson comorbidity index~~
 356 ~~score, and vascular access.~~

357 ~~Model 5: Adjusted with albumin, age, gender, diabetes mellitus, Charlson comorbidity index~~
 358 ~~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	-	-

359 ~~CI: confidence interval.~~

360 ~~Model 1: Monovariate analysis.~~

361 ~~Model 2: Adjusted with albumin.~~

362 ~~Model 3: Adjusted with albumin and c-reactive protein.~~

363 ~~Model 4: Adjusted with albumin, c-reactive protein, age, gender, and diabetes mellitus.~~

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364 Model 5: Adjusted with albumin, c-reactive protein, age, gender, diabetes mellitus, Charlson
365 comorbidity index score, and vascular access.
366 Model 6: Adjusted with albumin, c-reactive protein, age, gender, diabetes mellitus, Charlson
367 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).**

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375 **Figures**

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