

1 **Total Plasma Carotenoid and Mortality in the Elderly:**

2 **-Results of EVA Study-**

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20 Abstract

21 Carotenoids are pigments brought by fruit and vegetable consumption. While high intake of
22 fruits and vegetables were found to be associated with lower mortality, our objective is
23 investigate if total plasma carotenoid, via their antioxidant properties, are associated with
24 mortality risk in a free-living elderly population. The EVA study (“Epidemiology of Vascular
25 Ageing”), (n=1389, 59-71 years) is a 9-year longitudinal study with 6 waves of follow-up.
26 Association between baseline total plasma carotenoid and mortality were determined by Cox
27 proportional hazards regression analyses. Low total plasma carotenoid level was significantly
28 associated with all-cause mortality in men but not in women. After controlling for potential
29 confounding factors, mortality risk increased significantly in men (p=0.03) with plasma
30 carotenoid in the lowest quintile compared to men with plasma carotenoid in the highest (2.89
31 [1.20; 6.97]). A significant association between mortality by cancer and low plasma
32 carotenoid level variable was also found in men (unit=1 $\mu\text{mol/L}$, RR=1.89 [1.14; 3.12],
33 p=0.01). Associations between total plasma carotenoid and mortality risk remained
34 statistically significant after taking into account: 1) plasma selenium level, which previously
35 was found associated with mortality in this population and 2) TBARS level considered as an
36 indicator of oxidative stress. By showing, prospectively, in a general healthy elderly
37 population, that total plasma carotenoid levels were independently associated with mortality
38 risk in men, our study suggests that total plasma carotenoid levels could be a health indicator
39 in elderly populations.

40 .

41 Introduction

42 Carotenoids are natural pigments, synthesized by plants and micro-organisms, but not
43 by animals nor by humans. These pigments are found in food, especially in fruits and
44 vegetables. Large epidemiological studies suggest a protective effect of high intake of fruits
45 and vegetables and all-cause mortality⁽¹⁻⁵⁾. Consumption of fruits and vegetable could have a
46 protective effects on stroke and coronary heart diseases⁽⁶⁻¹⁰⁾. Concerning cancer, benefits of
47 fruits and vegetables intake are more controversial and the potential protective effect seems
48 depend of the type of cancer. Some studies did not showed evidence of strong association
49 with ovarian cancer⁽¹¹⁾, breast cancer⁽¹²⁾, overall colon rectal cancer^(13, 14), renal cell
50 carcinoma⁽¹⁵⁾. However some studies suggest potential benefits of fruits and vegetable
51 consumption for some other cancer such as cancer of upper aero digestive tract⁽¹⁶⁾, or lung
52 cancer^(17, 18).

53 More information is needed to ascertain the association between the intake of single
54 nutrients, such as carotenoids, and the risk of all cause mortality.

55 The hypothetical protective role of carotenoids could come from their antioxidant
56 properties⁽¹⁹⁾. Literature on the implication of free-radicals in the aging process is well
57 documented^(20, 21) but the relationship between total plasma carotenoid and mortality in free-
58 living elderly populations via their antioxidant roles has not been previously studied. Other
59 underlying mechanisms such as inflammation mechanisms or immunomodulatory
60 mechanisms could also be mentioned⁽¹⁹⁾.

61 Our objective is to explore the relationships between total plasma carotenoid at
62 baseline and 9-year mortality risk in a healthy elderly population

63

63 **Experimental Methods:**

64 Study population

65 The EVA study is a 9-year longitudinal study with 6 follow-up periods^(22, 23). During
66 the first two years 1991-1993 (EVA0), 1389 volunteers (575 men and 814 women, age range:
67 59-71 years) residing in the town of Nantes (western France) were recruited from electoral
68 rolls, and to a lesser extent, via information campaigns. All subjects were community
69 residents and underwent a complete examination in the EVA study centre where they spent
70 half a day. The last follow-up of the EVA study (EVA6) was conducted between June 2000
71 and December 2001. The study protocol was approved by the Ethical Committee of
72 University Centre Hospital of Kremlin-Bicêtre (Paris). Signed informed consent was obtained
73 from all participants at enrolment.

74 Data collection

75 Vital statistics and date and cause of death were collected throughout the 9 years of
76 follow-up. At each of the EVA steps, and at the end of the last year of study, the **vital** status of
77 individuals for whom we had no feedback was collected from town hall civil registries. The
78 cause of death was determined with the help of both the subject's general practitioner and
79 family.

80 **At baseline, the** general questionnaire allowed us to obtain information on socio-
81 demographic factors such as sex, age, educational level (\leq primary school / \geq high school),
82 plus lifestyle habits like smoking habit (current, ex-smokers / non-smokers) and alcohol intake
83 (\geq 20ml / $<$ 20ml per day). In addition, height and weight were measured. Two independent
84 measures of systolic and diastolic blood pressure were taken with a digital electronic
85 tensiometer (SP9 Spengler) after a 10-minute rest. Cognitive performances were assessed
86 using the Mini Mental Status Examination (MMSE)⁽²⁴⁾. Blood samples were collected
87 between 8:30 am and 9:30 am after a 12 hour fast. Total plasma cholesterol, and plasma
88 glucose levels were measured using standard methods.

89 Health characteristics considered in this analysis were MMSE score, BMI, diabetes
90 status (plasma glucose level \geq 7.80 mmol/L, use of anti-diabetic drugs or diabetes medical
91 history), dyslipidemia (total cholesterol \geq 6.20 mmol/L, use of lipid-lowering drugs or
92 dyslipidemia medical history), hypertension (systolic or diastolic blood pressure \geq 140 or \geq 90
93 mm Hg respectively, or use of hypertensive drugs or hypertension medical history), history of
94 vascular diseases (self-reported history of myocardial infarction, angina pectoris, stroke).

95 Laboratory procedure

96 Spectrophotometric assay of plasma carotenoids

97 After precipitation of plasma proteins with ethanol, carotenoids were extracted with hexane
98 and measurements of absorbance on the hexane phase at 350, 450 and 550nm were performed
99 (spectrophotometer Uvikon 860, Kontron, Rotkreuz, Switzerland). Concentrations were
100 calculated on the basis of a molecular extinction factor at 450 nm of 134,000 L/mol/cm.
101 Absorbance values at 350 and 550 nm were used to correct the absorbance obtained at 450 nm
102 by applying an adequate equation. Coefficients of intra- and inter-assay variations were 5.4%
103 and 4.9%, respectively.

104 Thiobarbituric acid-reactive substances (TBARS) and plasma selenium determination
105 Plasma levels of TBARS were determined by a fluorometry method as described by Richard
106 *et al.* ⁽²⁵⁾ and described previously ⁽²⁶⁾. Selenium was determined in serum using
107 electrothermal atomic absorption spectrometry (Perkin Elmer 5100 ZT, Norwalk, CT, USA)
108 according to Arnaud *et al.* ⁽²⁷⁾ and described previously ⁽²²⁾.

109

110 Statistics

111 Survival was analyzed with actuarial methods, and Wilcoxon tests were used to
112 compare survival between total plasma carotenoid quintile groups. **Association between** total
113 plasma carotenoid **and** mortality were determined by Cox proportional hazards regression
114 models in which year of age during the study was used as time axis, with left truncation at the
115 age of study entry. **Multivariate analyses were adjusted for potential confounding variables**
116 **and similar analyses were repeated after additionally taking into account TBARS levels and**
117 **plasma selenium levels (analysed as continuous variables)**. The proportional hazards
118 assumption was verified by adding a time-dependent variable to the model ⁽²⁸⁾. In these
119 analyses, total plasma carotenoid level was considered by quintiles defined in each sex and
120 was also considered as a continuous variable when the strength of analyses was too small to
121 allow a categorical treatment. Results of Cox multivariate regressions were expressed by
122 **Hazard Ratio (HR)** with their confidence interval (CI) at 95 %. All interactions between total
123 plasma carotenoids and other variables were tested. Statistical analyses were performed using
124 SAS software version 9.1 (SAS Institute, Inc. Cary, North Carolina).

125

126 **Results**

127 **Of the 1389 study participants included in the analyses, 1283 had measurements of total**
128 **plasma carotenoid and complete information on covariables. Characteristics according to sex**

129 were described in Table 1. During the 9-year follow-up, 93 death occurred with a higher rate
130 in men than in women (n=61 in men n=32 in women, $p<10^{-4}$). A higher mortality rate was
131 observed in current and former smoker, in regular alcohol consumer, in participants with low
132 concentration of plasma selenium and with high BMI and participants with diabetes,
133 hypertension and cardiovascular diseases (results not showed).

134 Total plasma carotenoid level was significantly higher in women ($m=3.08$ (SD=1.33)
135 $\mu\text{mol/L}$) than in men ($m=2.19$ (SD=0.99) $\mu\text{mol/L}$) (table 1) and a discrepancy in the
136 distribution was observed between men and women (figure 1).

137
138 Means of total plasma carotenoid were significantly higher in surviving individuals
139 ($m=2.75$ (SD=1.27) $\mu\text{mol/L}$) than in those who died (2.12 (SD=1.12) $\mu\text{mol/L}$, $p<10^{-4}$). This
140 association was found to be gender dependant. The relationship was found to be significant
141 for men ($m=2.24$ (SD=0.97) $\mu\text{mol/L}$ vs. $m=1.76$ (SD= 0.94) $\mu\text{mol/L}$, $p=0.0002$) but not for
142 women (3.09 (SD=1.34) $\mu\text{mol/L}$ vs. 2.83 (SD=1.11) $\mu\text{mol/L}$, $p=0.27$). Comparison of
143 survival distributions among total plasma carotenoid quintiles shows that mortality increased
144 in subgroups with the lowest percentile groups of total plasma carotenoid in men but not in
145 women (figures 2).

146 Bivariate cox proportional hazard regression (table 2) model showed that men in the lowest
147 quintile of total plasma carotenoid had a significantly higher risk of mortality than men in the
148 highest (HR_{Q1 vs. Q5} = 4.08 [1.77; 9.45]). No significant association was found in men who had
149 a plasma carotenoid level within Q2, Q3 or Q4 compared to subjects in Q5 (Q2 vs. Q5:
150 HR=1.69 [0.65; 4.36], Q3 vs. Q5: HR=1.07 [0.38; 3.05], Q4 vs. Q5: HR=1.24 [0.46; 3.34]).
151 The global p-value in men was $p=0.0003$. No significant association was found in women,
152 $p=0.20$ (Q1 vs. Q5: HR=0.61[0.18; 2.11], Q2 vs. Q5: HR=1.73 [0.68; 4.39], Q3 vs. Q5:
153 HR=0.59 [0.17; 2.03], Q4 vs. Q5: HR=0.74 [0.23; 2.33]).

154 At baseline, a significant association was observed between concentration of plasma
155 carotenoid and education level in women (lower concentrations observed in women with low
156 education level) and marital status in men (higher concentration in married men). In both
157 sexes, a lower total plasma carotenoid concentration was also observed in participants who
158 were regular alcohol consumers, in participants with diabetes, hypertension, cardiovascular
159 disease history and a higher concentration was observed in dyslipidemic participants. Plasma
160 carotenoids concentrations were also negatively correlated with BMI and positively correlated
161 with plasma selenium in both sexes and with TBARS levels in women.

162 Association between total plasma carotenoid levels and 9-year risk mortality was
163 analysed after adjustment for all factors associated with mortality or /and with total plasma
164 carotenoid in each sex separately, results are presented in Table 2. The multivariate Cox
165 hazard proportions regression models showed that low levels of plasma carotenoid was
166 associated with higher mortality risk in men but not in women after controlling for age
167 education level, marital status, smoking habits, alcohol intake and health factors (diabetes,
168 hypertension, cardiovascular antecedent, dyslipidemia, BMI). The hazard ratio of 9-year
169 mortality in men with plasma carotenoid levels in the lowest quintile compared to men with
170 plasma carotenoid levels in the highest quintile was 2.94 [1.21; 7.17]. No significant
171 association was found for men who had a plasma carotenoid level within Q2, Q3 or Q4
172 compared to subjects in Q5 (Q2 vs. Q5: HR=1.33 [0.50; 3.50], Q3 vs. Q5: HR=0.98 [0.34;
173 2.82], Q4 vs. Q5: HR=1.22[0.45; 3.28]) suggesting a threshold effect.

174 Similar analyses were performed after adjusting for 1) TBARS levels which could be
175 considered as biological markers of oxidative stress.2) plasma selenium level which was
176 found to be associated with all-cause mortality in both sexes. After these supplemental
177 adjustments, total plasma carotenoid levels still remained associated with 9-year mortality risk
178 in men: global p-value=0.04 after adjustment for TBARS (Q1 vs. Q5: HR=2.67 [1.08; 6.61]
179 and global p-value =0.04 after adjustment for plasma selenium (Q1 vs. Q5: HR=2.52 [1.03;
180 6.21] . Total plasma carotenoid remained unrelated to mortality in women.

181
182 Cause of death was determined by subject's general practitioner for 88.1% of subjects.
183 Cancer was the first leading cause of death (n=45, 44.5%). Men who died by cancer had a
184 significantly lower total plasma carotenoid mean compared to those in surviving individuals
185 (m=2.34 (SD=0.97) $\mu\text{mol/L}$ vs. m=3.09(SD= 1.34) $\mu\text{mol/L}$, p=0.0002). Results of Cox
186 models showed a significant association between total plasma carotenoid level analysed as
187 continuous variables and cancer mortality risk in men (unit=1 $\mu\text{mol/L}$, HR=1.85 [1.14; 3.03],
188 p=0.01) but not in women (HR=1.07 [0.75; 1.56], p=0.67). After taking into account the
189 socio-demographic, life habits and health variables this association in men remained
190 significant with a HR= 1.72 [1.02; 2.86], p=0.04.

191

191 **Discussion**

192 The present study shows that low total plasma carotenoid level was significantly
193 associated with all-cause mortality and mortality by cancer, in men but not in women after
194 controlling for the main potential confounding factors. We also highlighted that this
195 association was independent of plasma selenium level, that was found to be significantly
196 associated **with** all cause mortality in this population ⁽²²⁾.

197 The EVA study included a large number of volunteers, whose educational status and
198 cognitive function levels are known to be linked with mortality risks and this proportion is
199 higher in the EVA cohort than in the average French elderly population. Despite this
200 selection, total **plasma carotenoid** concentrations were within the same ranges as those of
201 different European populations.

202 **The lower total plasma carotenoid concentrations in men compared to women in our**
203 **cohort has been described in several epidemiological studies ^(29, 30) especially for β -carotene**
204 **^(31, 32). Regarding to the large discrepancy in the distribution of total plasma carotenoid**
205 **concentration between the two sex, as the threshold of the lowest quintile in women (<2.0**
206 **$\mu\text{mol/L}$) was about the median in men, the lack of evidence of association between total**
207 **plasma carotenoid concentration and mortality in women could be explained by the fact that it**
208 **is only participants with very low levels who have higher risk of mortality. Additionally, the**
209 **higher number of death observed in men than in women (61 versus 32) could also participate**
210 **to the non evidence of an association in women.** This difference **of concentrations of plasma**
211 **carotenoid** between men and women could result from a higher fruit and vegetable intake in
212 women than in men explainable by **exogenous factors such as** socio-cultural factors **leading to**
213 **better dietary habits in women** - a gender difference - but we can not exclude the effect of
214 **endogenous factors in the** hormonal differences or lipid and nutrient transport differences ⁽³³⁾-
215 a sex difference -. Anyway, the differences **between men and women for** carotenoid
216 distributions and mortality **rate** led us to conclude that only stratified analyses on sex should
217 be undertaken to investigate and better understand relationships between plasma carotenoid
218 level and mortality risk.

219 Our finding was supported by results from the MacArthur studies on Successful Aging
220 ⁽³⁴⁾, in which low levels of serum β -carotene ($\leq 0.17 \mu\text{mol/L}$ =median value) were significantly
221 associated **with** the 7-year all-cause mortality in men (OR=2.30 (1.23; 4.31)) but not in
222 women (OR=0.85 (0.42; 1.75)). In the Women's Health and Aging studies (n=632, 70-79
223 years) ⁽³⁵⁾ a significant link was found in women between higher total serum carotenoid and a
224 lower risk of mortality (for 1 SD increase of log tot. carotenoid, RR=0.77 (0.64;0.84)),

225 however we noted in this study, that women's geometric means of total serum carotenoid
226 were very low ($m=1.63 \mu\text{mol/L}$). In non stratified analyses, two other studies showed an
227 associations between high levels of carotenoids compounds and lower mortality risk. First, in
228 the European study SENECA ($n=1168$, 70-75 years) ⁽³⁶⁾ in which plasma carotene
229 concentrations were significantly associated with mortality risk (for an increment of 0.39
230 $\mu\text{mol/L}$, $RR=0.79(0.70 ;0.89)$). Second, in a study on 638 independently living elderly
231 subjects aged 65-85 year ⁽²⁹⁾, analyses of tertiles of carotenoids showed a significant link
232 between all-cause mortality and xanthophylls carotenoids, but not with total serum carotenoid
233 even if tests for trends were significant ($p=0.02$). Discordance of the results according to the
234 carotenoids compounds studied could come from the fact that all carotenoids compounds have
235 not the same biological properties. Finally in another study led by Fletcher et al. on 1214
236 subjects (75-84 years) ⁽³⁷⁾, the relationship between plasma β -carotene and all-cause mortality
237 during the 4.4-year follow-up did not remain statistically significant after adjustment for
238 potential confounding factors. The absence of a significant link could be explained by a sex
239 effect, which was not reported, or by the advanced age of the population or more probably by
240 relatively higher baseline levels of plasma β -carotene in this population.

241
242 Concerning the randomized trials, two randomised controlled trials led in general
243 population have investigated supplementation effects on incidence of cancer and all-cause
244 mortality ^(33,38). In the Linxian trial conducted on 29584 adult subjects ⁽³⁸⁾ a significantly
245 lower 5-year total mortality risk occurred among those receiving supplementation with beta
246 carotene, vitamin E, and selenium. In the primary prevention trial SUVIMAX including
247 13017 French adults ⁽³³⁾ a significant protective effect of 7.5-years combined antioxidant
248 including β -carotene supplementation on all-cause mortality was observed in men but not in
249 women. In this trial, the effect of supplementation was also studied after stratification on
250 initial antioxidant plasma levels. A net benefit was observed only in men with a low status of
251 β -carotene or ascorbate but not in women ⁽³⁹⁾. However, in these combined multi antioxidant
252 supplementation studies, it is impossible to isolate the proper effect of carotenoids on
253 mortality. However, the recent meta analyses led by Bjelakovic et al. ⁽⁴⁰⁾, carried on 68
254 randomised trials with 232606 participants, showed that supplementation of beta-carotene
255 singly or combined significantly increased mortality. One explanation would be that instead
256 having role in the pathogenesis of many chronic diseases, oxidative stress may be a
257 consequence of pathological conditions. By eliminating free radicals from our organism, we
258 interfere with some essential defensives mechanisms ⁽⁴⁰⁾. In this meta-analyse authors did not

259 take into account sex as covariable which could influence the intervention effect across the
260 trials and constitutes a limitation for interpreting their results.

261
262 When considering the underlying causes of death, we found a significant association
263 between low total plasma carotenoid and higher cancer mortality in men. Our results should
264 be viewed with some caution, given that only 45 cancer deaths occurred. While low intake or
265 having a low serum concentration of beta-carotene was suggested to be associated with
266 elevated risk of cancer by epidemiological studies⁽⁴¹⁾ and by one large randomised trial
267 conducted in China, in the early 1980s⁽³⁸⁾, the results of recent trials call back to cautious
268 concerning the potential benefit of supplementation of carotenes, by showing a higher rate of
269 lung cancer in smoker participants who received a supplements containing beta-carotene
270 compared to those receiving placebo in the ATBC Study⁽⁴²⁻⁴⁴⁾. Then, association between
271 carotenoid and cancer seems to be specific of cancer site. Our data did not allow investigating
272 associations between total plasma carotenoid on specific cancer site. .

273
274 In a previous study, we showed a significant association between low plasma selenium
275 levels and all-cause mortality risk and cancer mortality in both sexes⁽²²⁾. In the present
276 analyses, after adjustment on plasma selenium levels, associations between total plasma
277 carotenoid and mortality risk remained statistically significant, suggesting that plasma
278 selenium and plasma carotenoid have each of them a proper protective effect on mortality
279 risk. This result was supported by the Women's Health and Aging Studies results⁽³⁵⁾.

280
281 Currently, the mechanism of this potential relationship is still under debate and, as it
282 has been described by Paiva et al., several hypotheses can explain this observation⁽¹⁹⁾. One of
283 them involves the antioxidant properties. In our study, analyses were repeated after
284 controlling on TBARS levels, a lipidoperoxidation marker; our results remained unchanged
285 suggesting that the association between total plasma carotenoid on mortality observed in our
286 cohort did not arise from an antioxidant protection. However the oxidative stress marker role
287 of TBARS seems controversial and we have to remain cautious with such a conclusion even
288 if, in a placebo controlled single blind study, Hininger et. al, showed that carotenoid
289 supplementation (lutein, lycopene, β -carotene) did not lead to a significantly measurable
290 improvement in antioxidant defences in apparently healthy subjects (n=175, 25-45 years)⁽⁴⁵⁾.
291 The other underlying mechanism by which low carotenoid could contribute to an increased
292 risk of mortality may be related to inflammation. In the MacArthur Studies of Successful

293 Aging, Hu et al. showed that serum β -carotene concentrations were inversely associated with
294 C reactive protein and interleukin 6 levels, and they showed an independent and synergic
295 effect between low β -carotene concentrations and high inflammation burden on mortality risk
296 ⁽³⁴⁾. Unfortunately, inflammation markers were not available in our study. Finally, two other
297 mechanisms were also mentioned, on one hand, a possible pro-inflammatory and
298 immunomodulatory mechanism is hypothesized by the carotenoid's activation of
299 lipoxygenases activities ⁽¹⁹⁾. On the other hand, it has been suggested that carotenoids may
300 also be involved in the activation of gene expression, which encodes the message for an
301 element of gap junction (connexin 43) required for cell to cell communication ⁽¹⁹⁾. To our
302 knowledge, neither the activity of lipoxygenases, nor measurements of connexin 43 have ever
303 been taken into account in epidemiological studies interested in the **relationship between**
304 carotenoids **and** chronic diseases or mortality in general populations. So, at this point, it seems
305 difficult to be more precise on **the mechanism by which carotenoids could act**. Finally, we
306 cannot exclude that carotenoids in our study might have been serving as markers for other
307 protective factors present in fruits and vegetables, but that are not acting as effective agents
308 themselves. Further biological research is necessary to confirm **the association between**
309 **carotenoids and mortality** particularly in elderly subjects, to better understand the action
310 mechanism, and so to be able to determine if the protective **association** of carotenoids on
311 mortality found in men but not in women is only a random effect, a sex or a gender
312 difference.

313 By showing, prospectively, in a general healthy elderly population, that total plasma
314 carotenoid levels were an independent associated marker of mortality in men after taking into
315 account potential confounding factors, our study suggests that total plasma carotenoid levels
316 could be a **"healthy diet" indicator in elderly populations. Further studies are necessary to**
317 **explore the mechanism which could explain the relationship.**

318

318 **Statements**

319 We report no conflict of interest.

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324 CB designed the study, contributed to the paper

325 AF designed the biological measurements

326 TNA carried out the analysis and wrote the paper

327

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Table 1 : Characteristic of 1283 participants included in the analyses according to sex

	Men (n=534)		Women n=749)		
	% or m	SD	% or m	SD	p
Socio-demographic and lifestyle habits					
Age by year	65.0	3.0	64.9	3.0	0.41
Education	Primary school		54.7		0.003
Marital status	Not married		30.2		<0.0001
Smoking status	Current or Former smoker		12.0		<0.0001
Alcohol intake	≥2 glasses/day		16.6		<0.0001
Health factors					
Diabetes			2.5		<0.0001
Hypertension			44.7		<0.0001
CVD history			8.0		0.02
Dyslipidemia			77.6		<0.0001
BMI by kg/m ²	26.6	3.4	24.7	3.9	<0.0001
MMSE* score	28.2	2.1	27.9	2.3	0.004
Biological measurements					
Plasma selenium (μmol/L)	1.08 (0.21)		1.10 (0.19)		0.26
TBARS * by (μmol/L)**	0.46	0.06	0.47	0.06	0.002
Total Plasma carotenoids (μmol/L)	2.19	0.99	3.08	1.33	<0.0001
9-year all cause mortality %	11.4		4.3		<0.0001

* MMSE : Mini mental State Examination, TBARS : thiobarbituric acid-reactive substances

**measurements available for 484 men and 687 women

Figure 1: Distributions of total plasma carotenoid level in both sexes

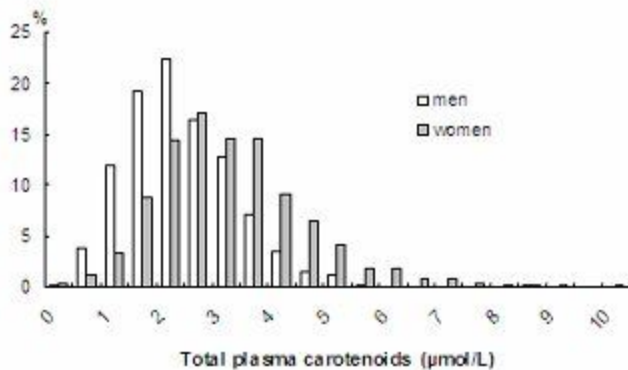


Figure 2: Survival distributions for each total plasma carotenoids quintile groups

A: exclusively in men

Quintile 1: $< 1.36 \mu\text{mol/L}$, Quintile 2: $1.36\text{-}1.86 \mu\text{mol/L}$, Quintile 3: $1.86\text{-}2.3 \mu\text{mol/L}$,

Quintile 4: $2.3\text{-}2.9 \mu\text{mol/L}$, Quintile 5: $\geq 2.9 \mu\text{mol/L}$

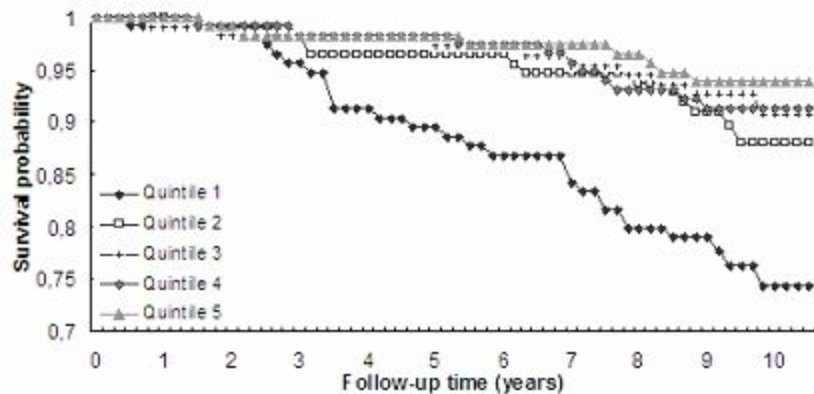


Figure 2: Survival distributions for each total plasma carotenoids quintile groups

B: exclusively in women

Quintile 1: $< 2.0 \mu\text{mol/L}$, Quintile 2: $2.0\text{-}2.60 \mu\text{mol/L}$, Quintile 3: $2.60\text{-}3.25 \mu\text{mol/L}$,

Quintile 4: $3.25\text{-}4.04 \mu\text{mol/L}$, Quintile 5: $\geq 4.04 \mu\text{mol/L}$

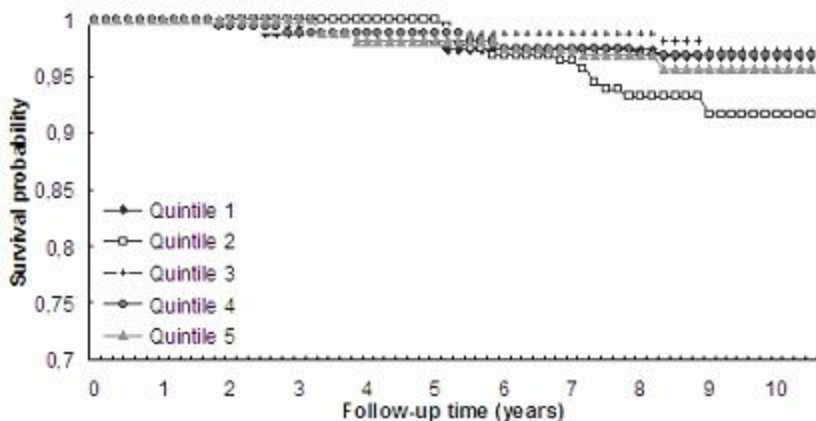


Table 2: Association between total plasma carotenoid level on all cause mortality. Results of Cox Proportional Hazards Regression Analysis

		Men						Women					
		Bivariate model			Multivariate model*			Bivariate model			Multivariate model*		
		HR	(95%CI)	p	HR	(95%CI)	p	HR	(95%CI)	p	HR	(95%CI)	p
Total plasma	Q1 vs. Q5	4.08	1.77 ;9.45		2.94	1.21 ; 7.17		0.61	0.18 ; 2.11		0.79	0.21 ; 2.90	
carotenoids	Q2 vs. Q5	1.69	0.65 ; 4.36	0.0003	1.33	0.50 ; 3.50	0.03	1.73	0.68 ; 4.39	0.20	1.87	0.70 ; 4.99	0.29
level	Q3 vs. Q5	1.07	0.38 ; 3.05		0.98	0.34 ;2.82		0.59	0.17 ; 2.03		0.69	0.20 ; 2.41	
(by quintile)	Q4 vs. Q5	1.24	0.46 ; 3.34		1.22	0.45;3.28		0.74	0.23 ; 2.33		0.82	0.26 ; 2.60	

* Model adjusted for socio-demographic factors (age, education level, marital status), lifestyle habits (smoking habits and alcohol intake) and health factors (diabetes, hypertension, cardiovascular antecedent, dyslipidemia and BMI)