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## **Vitamin E status and quality of life in the elderly: influence of inflammatory processes.**

Lucile Capuron, Aurélie Moranis, Nicole Combe, Florence Cousson-Gélie,  
Dietmar Fuchs, Véronique de Smedt-Peyrusse, Pascale Barberger-Gateau,  
Sophie Layé

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1 **Short Communication**

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4 **Vitamin E Status and Quality of Life in the Elderly:**  
5 **Influence of Inflammatory Processes**  
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10 Lucile Capuron<sup>1</sup>, Aurélie Moranis<sup>1</sup>, Nicole Combe<sup>2</sup>, Florence Cousson-Gélie<sup>3</sup>, Dietmar Fuchs<sup>4</sup>,  
11 Véronique De Smedt-Peyrusse<sup>1</sup>, Pascale Barberger-Gateau<sup>5</sup>, Sophie Layé<sup>1</sup>  
12

13  
14  
15 <sup>1</sup> Laboratory of Psychoneuroimmunology, Nutrition and Genetics (PSYNUGEN), INRA 1286 –  
16 University Victor Segalen Bordeaux 2, CNRS 5226, 146 rue Léo Saignat, Bordeaux, F-33076  
17 France;

18 <sup>2</sup> ITERG, University Bordeaux 1, Avenue des Facultés, Talence, F-33405, France;

19 <sup>3</sup> Laboratory of Psychology ‘Health and Quality of Life’ EA 4139, University Victor Segalen  
20 Bordeaux 2, 3 Ter Place de la Victoire, Bordeaux, F-33076, France;

21 <sup>4</sup> Division of Biological Chemistry, Biocenter, Innsbruck Medical University, Innsbruck, Austria;

22 <sup>5</sup> Inserm, U897, University Victor Segalen Bordeaux 2, Bordeaux, F-33076 France  
23

24  
25 **Address for correspondence:** Dr Lucile Capuron, Laboratory of Psychoneuroimmunology,  
26 Nutrition and Genetics (PSYNUGEN), INRA 1286 – University Victor Segalen Bordeaux 2, CNRS  
27 5226, 146 rue Léo Saignat, Bordeaux, F-33076 France.

28 Email: [lucile.capuron@bordeaux.inra.fr](mailto:lucile.capuron@bordeaux.inra.fr)  
29

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34

## Abstract

Chronic low grade inflammation is a characteristic of aging that may lead to alterations in health status and quality of life. In addition to intrinsic biological factors, recent data suggest that poor nutritional habits may largely contribute to this condition. The present study aimed at assessing mental and physical components of quality of life and at determining their relationship to vitamin E status, inflammation and tryptophan (TRP) metabolism in the elderly. Sixty-nine elderly subjects recruited from the Three-City (3C) cohort study participated in the study. Quality of life was assessed using the medical outcomes study (MOS) 36-item short-form health survey (SF-36). Biological assays included the measurement of plasma vitamin E (alpha-tocopherol), inflammatory markers, including interleukin (IL)-6 and C reactive protein (CRP), and TRP metabolism. Results showed that participants with poor physical health status, as assessed by the SF-36, exhibited lower circulating concentrations of alpha-tocopherol together with increased concentrations of inflammatory markers. Similarly, poor mental health scores on the SF-36 were associated with lower concentrations of alpha-tocopherol, but also with decreased concentrations of TRP. These findings indicate that nutritional status, notably as it relates to vitamin E, is associated with immune function and quality of life in the elderly.

## 55 Introduction

56

57 Impaired quality of life, associated with mood and physical symptoms, is frequent in the  
58 elderly. Approximately 7-40% of older persons report mental dysfunctions, especially in the form  
59 of mood and cognitive alterations, and these contribute considerably to their social and occupational  
60 dysfunction<sup>(1,2)</sup>. With the growing elderly population, there is a risk of a recrudescence of aged-  
61 related behavioural symptoms and reduced wellbeing. Thus, the promotion of healthy lifestyles and  
62 the prevention of impaired quality of life in the elderly represent a major public health concern.

63 Nutritional factors have been recently involved in pathways likely to influence mood and  
64 wellbeing. This idea is supported by a growing number of data indicating the protective effects of  
65 nutritional factors, including antioxidants, on mood symptoms, cognitive decline and impaired  
66 quality of life in the elderly<sup>(3,4)</sup>. Recent data suggest that the mechanisms by which micronutrients  
67 influence health and quality of life involve immunological processes<sup>(5)</sup>. Alpha ( $\alpha$ )-tocopherol is the  
68 most bioavailable form of vitamin E. This natural antioxidant is lipid-soluble, and due to this  
69 property, it exerts preferentially its antioxidant activity in lipid-rich membranes, **which concerns**  
70 **immune cells**. In terms of immunomodulatory properties,  $\alpha$ -tocopherol was shown to exert anti-  
71 inflammatory actions, including the modulation of T cells function and prostaglandin-E2 production  
72 by macrophages and the reduction pro-inflammatory cytokine synthesis from activated  
73 macrophages and monocytes<sup>(5,6)</sup>. The current recommended dietary intake of vitamin E is 15mg  
74 per day of  $\alpha$ -tocopherol<sup>(7)</sup>. However, this standard appears not to be reached in the aged population,  
75 a condition that may facilitate the development of immune alterations. Inflammation is a  
76 fundamental characteristic of aging. In the aged organism, the chronic, low-grade, activation of the  
77 innate immunity is associated with an over-expression of inflammatory factors, including pro-  
78 inflammatory cytokines (e.g., tumor-necrosis-factor (TNF)- $\alpha$ , interleukin (IL)-6), to the detriment  
79 of anti-inflammatory factors<sup>(8)</sup>. Not only involved in age-related inflammatory processes and  
80 disorders, pro-inflammatory cytokines appear also to play a role in the pathophysiology of mood  
81 and cognitive disorders, including depression<sup>(9,10)</sup>. The alteration of tryptophan (TRP) metabolism  
82 through the induction of the enzyme indoleamine-2,3-dioxygenase (IDO) upon chronic immune  
83 activation represents a mechanism by which inflammation induces mood symptoms. IDO can be  
84 induced in a variety of immune cells, such as monocyte-derived macrophages and microglia, by  
85 inflammatory cytokines, including most notably IFN- $\gamma$ <sup>(11)</sup>. This enzyme catalyzes the rate-limiting  
86 step of TRP conversion into kynurenine (KYN) and then quinolinic-acid, thereby reducing the  
87 availability of TRP for conversion into serotonin. *In vivo*, the activity of IDO is reflected by the  
88 relative concentrations of KYN and TRP, **with increased KYN/TRP ratio indicating increased IDO**

89 **activity**. Interestingly, older age has been associated with increasedIDO activity and TRP  
90 degradation, consistent with the notion that immune activation is more prominent/sustained in the  
91 elderly<sup>(12,13)</sup>. Altogether, these data support the hypothesis that vitamin E status may participate in  
92 aged-related alterations in health and quality of life, through effects on immune function and  
93 inflammatory pathways. The purpose of this study was to assess quality of life (mood and physical  
94 components) and to determine its relationship to vitamin E status, inflammation and TRP  
95 metabolism in a population of elderly subjects.

96  
97

## 98 **Subjects and Methods**

99

### 100 **Participants**

101 Participants (N=69) were recruited from the Three-City (3C) study, an epidemiological cohort study  
102 of 9,294 aged, not institutionalized, persons living in Bordeaux, Dijon and Montpellier recruited  
103 since 1999 (PI: A. Alperovitch, INSERM U708). The general methodology of the 3C study was  
104 published elsewhere<sup>(14)</sup>. Participants in the present study were drawn from the Bordeaux site at 7-  
105 year follow up. **Subjects with known or acute signs of inflammatory disease, with dementia or**  
106 **taking statins or medications likely to influence immune parameters were excluded. This study was**  
107 **conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures**  
108 **involving human subjects were approved by the Consultative Committee for the protection of**  
109 **Persons in Biomedical Research of the Kremlin-Bicêtre University Hospital (Paris). Written**  
110 **informed consent was obtained from all subjects.**

111

### 112 **Assessment of Quality of Life**

113 Quality of life was assessed using the medical outcomes study (MOS) 36-item short-form  
114 health survey (SF-36)<sup>(15)</sup>, a well validated self-report questionnaire which assesses the physical and  
115 mental components of quality of life through eight health concepts: physical functioning, physical  
116 role functioning, bodily pain, general health, vitality, social functioning, emotional role functioning  
117 and mental health. According to standard procedures, two summary scores ranging from 0 to 100  
118 (worst to best health) were calculated corresponding respectively to physical health and mental  
119 health. These scores were weighted, norm-based and expressed as t-scores with mean=50 (SD=10).

120

### 121 **Biological Measurements**

122 Fasting blood samples were collected between 8.00am and 9.30 am the same day as the  
123 assessment of quality of life. Plasma were stored at -80°C until thawed for the biological assays.

124 **Vitamin E status** – Plasma vitamin E concentrations were measured according to the  
125 method of Menke et al <sup>(16)</sup>. Briefly, after addition of 2,6-di-tert-butyl-p-cresol, vitamin E was  
126 extracted from 100µL plasma with hexane and separated by high-performance-liquid-  
127 chromatography.

128 **Inflammatory markers** -Assays included the measurement of C-reactive protein (CRP) and  
129 the pro-inflammatory cytokine, IL-6. **These markers were selected on the basis of previous reports**  
130 **indicating their involvement in neuropsychiatric symptoms in aged populations or with metabolic**  
131 **disorders** <sup>(17, 18)</sup>. Plasma concentrations of IL-6 were assayed by quantitative enzyme-linked-  
132 immunosorbent-assay (ELISA) techniques based on appropriate and validated sets of monoclonal  
133 antibodies (R&D Systems). CRP was measured by enzyme-immunoassay (EIA, Chemicon,  
134 Millipore, France). Inter- and intra-assay variability is reliably <10%.

135 **TRP catabolism** - Free TRP and KYN plasma concentrations were determined by high-  
136 performance-liquid-chromatography, as described elsewhere <sup>(19)</sup>.

137

### 138 **Statistical Analysis**

139 Relationship between vitamin E status, inflammatory markers and TRP levels was estimated  
140 using the Bravais-Pearson (*R*) coefficient for continuous variables. Separate multivariate linear  
141 regression analyses entering biological parameters in separate models adjusting for age and gender  
142 were used to assess the association of vitamin E, inflammatory markers and TRP metabolism with  
143 the SF-36 physical and mental health summary scores. Finally, dichotomous analyses were  
144 performed stratifying participants into distinct subgroups on the basis of their mental and physical  
145 health status (poor versus good), as assessed by the SF-36. Good mental and physical health statuses  
146 corresponded respectively to a summary mental health score and a summary physical health score  
147 above the median of the study population (respectively  $\geq 73$  and  $\geq 67$ ). Analyses of variance with age  
148 as covariate (ANCOVA) were performed to compare biological parameters across subgroups. Three  
149 participants had a missing value for one question of the SF-36. Accordingly, the missing value was  
150 replaced using the algorithm described by the authors <sup>(15)</sup>. All probabilities were two-tailed, with the  
151 level of significance set at  $p < 0.05$ .

152

153

### 154 **Results**

155

156 Sixty-nine elderly subjects (46 women, 23 men) participated in the study. The mean age **and**  
157 **body mass index (BMI)** of participants were respectively 78.9 years (SD= 4.9) and **27.7 kg/m<sup>2</sup>**  
158 **(SD= 4.3)**. The mean physical health and mental health summary scores were respectively 61.7

159 (SD= 22.6) and 67.4 (SD= 18.9). Overall, there was no significant relationship between gender,  
160 BMI and quality of life scores (all  $p>0.05$ ). Age, however, correlated significantly with both the  
161 physical and mental components of quality of life (respectively,  $R= -.407$ ,  $p<0.001$  and  $R= -.363$ ,  
162  $p<0.01$ ), with greater age corresponding to lower quality of life.

163 As expected, IL-6 levels correlated significantly with CRP levels ( $R= .377$ ,  $p<0.01$ ).  
164 Interestingly, IL-6 levels also correlated with vitamin E levels; with higher IL-6 levels  
165 corresponding to lower vitamin E concentrations ( $R= -.277$ ,  $p<0.01$ ). CRP concentrations were  
166 negatively correlated with levels of TRP ( $R= -.270$ ,  $p<0.05$ ), the latter being also correlated to KYN  
167 levels ( $R= .443$ ,  $p<0.001$ ). In addition, there was a trend for a relationship between IL-6 and TRP  
168 concentrations ( $R= -.209$ ,  $p=0.09$ ). There was no significant relationship between age, BMI and any  
169 of the measured biological parameters. Nevertheless, gender was related to IL-6, TRP, KYN and  
170 vitamin E, with women displaying lower levels of IL-6, TRP and KYN and higher levels of vitamin  
171 E compared to men (all  $p<0.05$ ). Accordingly, subsequent analyses were performed controlling for  
172 the age and gender of participants.

173 Separate multivariate linear regression analyses adjusting for age and gender revealed that  
174 IL-6, CRP, TRP and vitamin E were significantly associated with the physical health summary  
175 score (respectively  $\beta= -.312$ ,  $p=0.007$ ;  $\beta= -.222$ ,  $p=0.047$ ;  $\beta= .299$ ,  $p=0.011$  and  $\beta= .317$ ,  $p=0.006$ ),  
176 indicating that the better was physical health status the lower were the concentrations of IL-6 and  
177 CRP and the higher were the levels of TRP and vitamin E. Similar analyses associating each  
178 biological parameter to the mental health summary score indicated that mental health status was  
179 positively associated with TRP levels ( $\beta= .282$ ,  $p=0.019$ ) and with vitamin E ( $\beta= .275$ ,  $p=0.022$ ).

180 Thirty-five participants were found to exhibit poor/low physical health status and 34  
181 participants exhibited good physical health status. As shown in **Table 1**, there was no significant  
182 difference in terms of gender or BMI between the two subgroups but a significant difference in age,  
183 with participants with poor/low physical health being significantly older. When controlling for age,  
184 participants with poor/low physical health status were found to exhibit significantly lower levels of  
185 vitamin E, higher concentrations of IL-6 and tended to display greater CRP levels compared to  
186 participants with high physical health status. Regarding mental health, 35 participants exhibited  
187 mental health subscores below the median of the study population, denoting poor/low mental health  
188 status. Consistent with differences found for physical health status, participants with poor/low  
189 mental health status were found to be older compared to participants with high mental health status.  
190 When controlling for age, participants with poor mental health status were found to exhibit  
191 significantly lower concentrations of vitamin E and TRP compared to participants with good mental  
192 health status.

193

194 **Discussion**

195

196 Results from this study clearly indicate an association between vitamin E status, immune  
197 processes and quality of life in the elderly. Participants with greater plasma concentrations of  
198 vitamin E ( $\alpha$ -tocopherol) exhibited lower plasma levels of inflammation together with better health  
199 status, as determined by higher scores of mental and physical quality of life on the SF36  
200 questionnaire. This finding is consistent with recent data showing an association between low serum  
201 concentrations of  $\alpha$ -tocopherol and subsequent decline in physical function in a population-based  
202 sample of community-living elders<sup>(4)</sup>. In our study, plasma concentrations of  $\alpha$ -tocopherol  
203 correlated with both the mental and physical components of quality of life, suggesting the  
204 involvement of vitamin E in multiple dimensions of health and wellbeing.

205 Our findings indicate that regulation of inflammatory processes may represent a primary  
206 pathway by which vitamin E influences health and quality of life in the elderly. Due to its  
207 antioxidant property,  $\alpha$ -tocopherol is able to modulate immune function and regulate inflammatory  
208 responses<sup>(5,6)</sup>. This effect is certainly not negligible in aging where inflammation is prominent and  
209 it might thus contribute to improve health and wellbeing in the aged population<sup>(6)</sup>. Recent data have  
210 shown that  $\alpha$ -tocopherol can suppress immune-induced TRP degradation in mitogen-stimulated  
211 peripheral blood mononuclear cells *in vitro*<sup>(20)</sup>. This mechanism could explain the positive  
212 association of plasma vitamin E with health and quality of life, given the well-known role of TRP  
213 and serotonin pathways in the regulation of mood and neurovegetative functions. In our study,  
214 however,  $\alpha$ -tocopherol did not significantly correlate with TRP concentrations. Nevertheless,  
215 similarly to  $\alpha$ -tocopherol, TRP levels were associated with both physical health and mental health,  
216 albeit this association was more pronounced in regards to mental health. This data is consistent with  
217 the role of TRP metabolism in mood and mental processes and is in line with previous results  
218 indicating a significant relationship between immune activation, reduced serum TRP and worse  
219 quality of life scores in medically-ill patients<sup>(21)</sup>. **The dichotomous analysis made to compare  
220 subgroups in regards to physical and mental health status did not allow us to measure any  
221 significant difference in KYN levels and in the ratio of KYN/TRP between subgroups.  
222 Nevertheless, the finding that decreased TRP levels were associated with increased levels of  
223 inflammatory markers is in favour of the hypothesis of increased TRP degradation upon chronic,  
224 low-grade, inflammation<sup>(11)</sup>.**

225 Lower levels of plasma  $\alpha$ -tocopherol in the elderly may either reflect insufficient dietary  
226 vitamin E intake<sup>(22,23)</sup> or increased formation of reactive oxygen species (ROS) by inflammatory  
227 processes, and thus degradation of antioxidants, including vitamin E. These possibilities merit



228 further investigation as they might involve different preventive strategies. In one case, a regular  
229 consumption of vitamin E rich compounds, and probably other antioxidants such as carotenoids and  
230 polyphenols which contribute to vitamin E regeneration, may prevent age-related alterations in  
231 immune function and quality of life. In the other case, supplementation with  $\alpha$ -tocopherol may be  
232 relevant as this treatment was shown to decrease inflammatory processes and enhance immune  
233 function in aged animals <sup>(6)</sup>.

234 Altogether these results suggest that vitamin E status may influence quality of life in the  
235 elderly and that chronically activated inflammatory pathways may play a role in this relationship.  
236 Nevertheless, due to the correlational and cross-sectional aspects of this study, these findings cannot  
237 be interpreted in terms of causality. Other limitations to the present study include the limited sample  
238 size and the lack of operational control for potential confounders which may be linked to nutritional  
239 and immune status as well as to mental health. Despite exclusion of participants with acute  
240 inflammatory disease, we cannot rule out an effect of undiagnosed co-morbidity on nutritional and  
241 immune status. Finally, because of the absence of specific dietary data documenting on TRP intake  
242 at the time of the evaluation, we cannot exclude the possibility that, in addition to inflammatory  
243 processes, insufficient dietary intake of TRP-rich compounds may have contributed to decreased  
244 TRP concentrations in participants from this study.

245 In conclusion, the present findings document a clear association between vitamin E levels  
246 and inflammatory pathways in the elderly and suggest that their interaction may influence quality of  
247 life. Insufficient antioxidant intake and/or defences, as assessed by plasma vitamin E, appear to  
248 correlate with signs of inflammation and participate in aged-related alterations in health and quality  
249 of life.

250

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254 This study was conducted according to the guidelines laid down in the Declaration of Helsinki and  
255 all procedures involving human subjects were approved by the Consultative Committee for the  
256 protection of Persons in Biomedical Research of the Kremlin-Bicêtre University Hospital (Paris).

257 Written informed consent was obtained from all subjects.

258 Author's contribution: LC was involved in study design, statistical analysis, data interpretation and  
259 manuscript writing; AM and VDP performed laboratory measurements of inflammatory markers;

260 NC was responsible for the implementation and measurement of vitamin E; FCG was involved in  
261 study design; DF was involved in the measurement of tryptophan and kynurenine; PBG was the

262 local coordinator of the epidemiological study and was involved in study design, data interpretation

263 and manuscript editing; SL was the coordinator of inflammation measurements and was involved in  
264 study design, data interpretation and manuscript editing.  
265

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**Table 1.** Vitamin E status, inflammatory markers and TRP catabolism in participants with poor/low mental or physical health versus participants with good mental or physical health

	SF36 – Physical Health		SF36 – Mental Health	
	Poor/low	Good	Poor/low	Good
	(N=35)	(N=34)	(N=35)	(N=34)
Age	80.7 (4.9)	77.1 (4.2) **	80.4 (4.6)	77.4 (4.7) *
Sex (female/male)	24/11	22/12	25/10	21/13
Body mass index (kg/m <sup>2</sup> ) <sup>-b</sup>	26.5 (4.8)	25.1 (3.9)	26.5 (4.9)	25.1 (3.8)
<b><i>Vitamin E status</i></b>				
Alpha- tocophérol (µmol/L)	31.3 (5.7)	36.0 (7.9) **	31.7 (6.3)	35.6 (7.7) *
<b><i>Inflammatory markers</i></b>				
IL-6 (pg/mL)	5.0 (5.5)	2.8 (1.8) *	4.65 (5.4)	3.16 (2.4)
CRP (mg/L)	5.0 (5.4)	2.9 (3.8) #	4.72 (4.9)	3.15 (4.5)
<b><i>TRP catabolism</i></b>				
TRP (µmol/L)	54.9 (10.7)	59.9 (10.9)	53.3 (10.3)	61.5 (10.2) **
KYN (µmol/L)	2.2 (0.7)	2.1 (0.6)	2.1 (0.7)	2.1 (0.5)
KYN/TRP x 1000 (mmol/mol)	40.5 (13.3)	35.1 (8.1)	40.5 (13.6)	35.2 (7.6)

Data are shown as means (SD). IL-6: interleukin-6; CRP: C reactive protein; TRP:

tryptophan; KYN: kynurenine. \*\* p < 0.01; \* p < 0.05; # p = 0.07 (corrected for age)

<sup>-b</sup> this information was missing for 4 subjects.