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Chronic Low-Grade Inflammation in Elderly Persons Is Associated with Altered Tryptophan and Tyrosine Metabolism: Role in Neuropsychiatric Symptoms

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Background: Neuropsychiatric symptoms are common complaints of elderly persons. Recent data suggest that chronic low-grade inflammation, a fundamental characteristic of aging, plays a role. Effects might rely on the influence of inflammation on the activity of two enzymatic pathways, the indoleamine-2,3-dioxygenase (IDO) and the guanosine-triphosphate-cyclohydrolase-1 (GTP-CH1) pathways, which are involved in the biosynthesis of monoamines. The present study assessed this possibility in 284 healthy elderly subjects drawn from the Three-City cohort.

Methods: Assays included the measurement of serum interleukin-6 and C-reactive-protein, as inflammatory markers; tryptophan, kynurenine, and their ratio as index of IDO activity; and neopterin, phenylalanine, tyrosine, and nitrite, as markers of GTP-CH1 activity. In addition, structured assessments of depressive symptomatology, fatigue, and general behavioral/neurovegetative symptoms were performed.

Results: As expected, age correlated significantly with concentrations of immune markers and neuropsychiatric symptoms. Increased inflammation was related to reduced tryptophan concentrations and increased kynurenine levels, suggestive of IDO-induced increased tryptophan catabolism. In addition, inflammation was associated with increases in neopterin and nitrite levels and in phenylalanine concentrations at the expense of tyrosine. Interestingly, increased tryptophan catabolism was associated with the depressive symptoms of lassitude, reduced motivation, anorexia, and pessimism. In contrast, variations in markers of GTP-CH1 activity correlated more with neurovegetative symptoms, including sleep disturbance, digestive symptoms, fatigue, sickness, and motor symptoms.

Conclusions: These findings show that chronic low-grade inflammation in aging is associated with alterations in enzymatic pathways involved in monoamine metabolism and suggest that these alterations might participate in the pathophysiology of neuropsychiatric symptoms in elderly persons.

Key Words: Aging, enzymatic pathways, inflammation, neuropsychiatric symptoms, tryptophan, tyrosine

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Neuropsychiatric symptoms, including mood and cognitive alterations, represent common complaints of elderly persons. According to a recent meta-analysis, the prevalence of major depression ranges from .9% to 42% among healthy elderly Caucasians, and clinically relevant depressive symptom cases vary between 7.2% and 49% (1). The occurrence of mental dysfunction in elderly persons interferes with social and occupational functions and is associated with a greater risk of medical comorbidity (2–4). Although multiple etiologies might underlie the development of neuropsychiatric symptoms in elderly persons, recent data suggest the involvement of immune processes (5–8). Normal aging is a situation characterized by a chronic low-grade inflammatory state, with an overexpression of circulating inflammatory factors, including proinflammatory cytokines such as interleukin (IL)-6, IL-1, and tumor necrosis factor-α, to the detriment of anti-inflammatory factors (9,10). Accordingly, the notion of "inflammaging" has been proposed to refer to this fundamental inflammatory characteristic of aging (11,12). In the brain, this condition manifests by the chronic activation of perivascular and parenchymal macrophages/microglia expressing proinflammatory cytokines, while the number of astrocytes increases (13–15). This situation is accompanied by an increase in the brain production of reactive oxygen species, leading thus to a higher brain susceptibility to neuronal damage and death (16).

During the last decade, proinflammatory cytokines have been repeatedly linked to cognitive decline and mood disorders. At the experimental level, a rich database substantiates the capacity of proinflammatory cytokines to impact on neurotransmitter and neuroendocrine functions and to induce behavioral symptoms referred to as sickness behavior (17,18). At the clinical level, strong support for this observation comes from data obtained in our group and others, indicating that cytokine treatment in medically ill patients is responsible for the development of major depression and related neuropsychiatric symptoms in up to 45% of patients (6,19,20). More recently, we have shown that increased concentrations of inflammatory markers were associated with reduced quality of life in elderly persons (21). Mechanisms by which inflammation contributes to neuropsychiatric alterations in elderly persons might involve several nonexclusive biochemical pathways. Both in vitro and in vivo, immune cascades have been shown to significantly modulate the metabolism of neurotransmitters known to play a role in the regulation of mood and cognitive processes, including serotonin, norepinephrine, and dopamine. These monoamine neurotransmitters are synthesized within the brain from their precursors, the large neutral amino acids, tryptophan and tyrosine. Upon immune activation, alterations in the metabolism of tryptophan and tyrosine might rely on the activation of two enzy-

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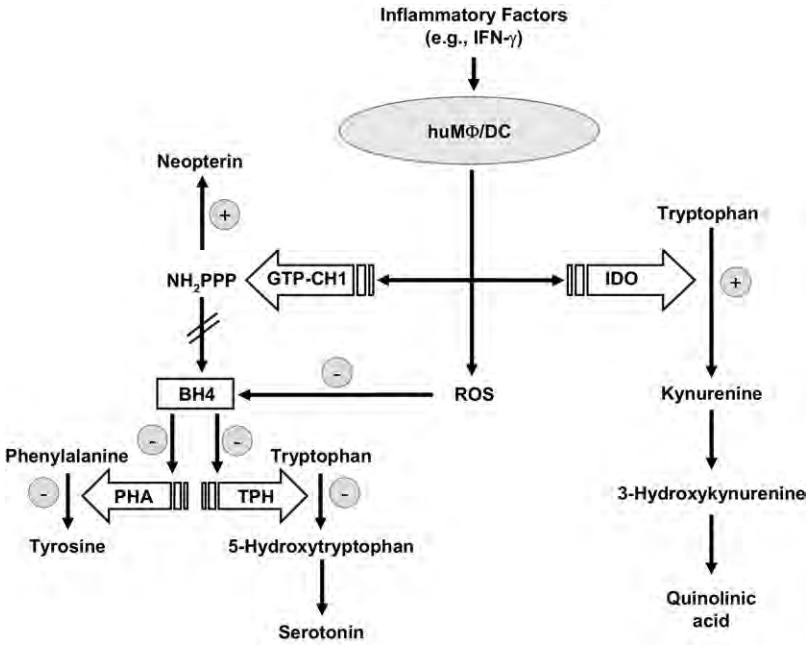

Figure 1. Effects of inflammatory factors on indoleamine-2,3-dioxygenase (IDO) and guanosine-triphosphate-cyclohydrolase-1 (GTP-CH1) pathways. Inflammatory factors (e.g., interferon [IFN]-γ) induce the expression of the enzymes GTP-CH1 and IDO in monocytes/macrophages (huMΦ) and dendritic cells (DC). The GTP-CH1 produces 7,8-dihydroneopterin triphosphate (NH_2PPP), which is further converted by pyruvoyl tetrahydropterin synthase to form tetrahydrobiopterin (BH4), the cofactor of amino acid monoxygenases including phenylalanine-hydroxylase (PHA) and tryptophan-hydroxylase (TPH). The TPH forms 5-hydroxytryptophan, which is further converted to serotonin by aromatic-L-amino-acid-decarboxylase. The IDO degrades the essential amino acid tryptophan into kynurenine. Kynurenine is then degraded into different neuroactive metabolites, including 3-hydroxykynurenine and quinolinic acid. Human monocyte-derived huMΦ and dendritic cells possess only low pyruvoyl tetrahydropterin synthase activity and thus are unable to release large amounts of BH4 but produce neopterin instead. Concomitantly, reactive oxygen species (ROS) are released within the oxidative burst reaction. The ROS can destroy BH4 released from other cells, leading thus to impairment in enzymic reactions that require BH4 (e.g., conversion of phenylalanine to tyrosine by PHA, formation of L-3,4-dihydroxyphenylalanine from tyrosine, conversion of tryptophan by TPH). Consequently, the production of the biogenic amines dopamine, norepinephrine, epinephrine, and serotonin is diminished.

matic pathways, corresponding respectively to the indoleamine-2,3-dioxygenase (IDO) pathway and the guanosine-triphosphate-cyclohydrolase-1 (GTP-CH1) pathway (Figure 1).

Indoleamine-2,3-dioxygenase can be induced by inflammatory cytokines, including most notably interferons (IFN), in a variety of immune cells such as monocyte-derived macrophages and microglia (22). When activated, IDO catalyzes the rate-limiting step of tryptophan conversion into kynurenine. Kynurenine is then degraded into different neuroactive metabolites, including 3-hydroxykynurenine, quinolinic acid, and kynurenic acid (23). In vivo, the ratio of kynurenine/tryptophan (Kyn/Trp) reflects tryptophan breakdown and is considered to represent one estimate of IDO activity (24). Although activation of the IDO pathway is particularly relevant in conditions of inflammation, it is noteworthy to mention that the major site of tryptophan conversion into kynurenine is the liver via the enzyme tryptophan 2,3-dioxygenase, which is induced by corticosteroids.

The activation of the enzyme GTP-CH1 is responsible for the production of neopterin and tetrahydrobiopterin (BH4). Neopterin is released from human activated monocytes/macrophages and therefore is considered as a marker of cell-mediated immunity (25). Tetrahydrobiopterin is a cofactor of aromatic amino acid hydroxylases (i.e., phenylalanine-hydroxylase [PHA], tyrosine-hydroxylase, tryptophan-hydroxylase) and nitric oxide synthases (NOS) (26). These enzymes contribute to the biosynthesis of monoamines, with PHA being involved in the conversion of phenylalanine to tyrosine, tyrosine-hydroxylase in the conversion of tyrosine to L-3,4-dihydroxyphenylalanine, tryptophan-hydroxylase in the conversion of tryptophan to serotonin and NOS in the conversion of arginine to nitric oxide (NO) (26). Thus, BH4 plays a fundamental role in neurotransmitter biosynthesis. In conditions of immune activation, the release of neopterin by activated human monocytic cells is initiated at the expense of BH4 activity (27). Because of the inducibility of GTP-CH1 by proinflammatory cytokines, activation of BH4 is believed to associate more with acute inflammatory processes, whereas the inhibitory role of neopterin seems to be more relevant in situations of chronic inflammation (26). Consistent with this notion, increases in phenylalanine concentrations and in the ratio of phenylalanine/tyrosine (Phe/Tyr)—indicative of reduced PHA activity— have been reported in patients afflicted with various chronic inflammatory conditions and were found to correlate with neopterin levels (26,28,29). Tetrahydrobiopterin is particularly sensitive to oxidative stress and damage, which might explain the potent alteration of this factor during chronic inflammatory states. It is also highly probable that aging and age-related antioxidant deficiencies might exacerbate this phenomenon.

Relevant to the development of neuropsychiatric symptoms, BH4 deficiencies were found in patients with psychiatric disorders, including major depression (30,31) and schizophrenia (32,33). Interestingly, increased blood neopterin concentrations were found to correlate with a greater incidence of depressive episodes (34). Similarly, and consistent with the hypothesis that IDO activation might also contribute to the development of inflammation-related neuropsychiatric symptoms, data obtained in medically ill patients undergoing cytokine therapy indicate significant relationships between cytokine-induced tryptophan and kynurenine alterations

and the occurrence of depressive symptoms (35–37). Altogether, these data suggest that chronic low-grade inflammation in aging might underlie tangible alterations in enzymatic pathways involved in monoamine metabolism, which consequently might lead to the development of subtle neuropsychiatric symptoms.

The aim of this study was to assess the relationship between age-related low-grade inflammation and the activity of the enzymatic pathways, IDO and GTP-CH1, and to determine the impact of this relationship on neuropsychiatric symptomatology in a sample of elderly subjects.

Methods and Materials

Participants

Participants were recruited from a subsample of the Three-City (3C) study, an epidemiological cohort study that included 9294 persons, 65 years of age and over, not institutionalized, and living in Bordeaux, Dijon, and Montpellier in 1999–2000. The general methodology of the 3C study was published elsewhere (38). Participants in the present study ($n = 284$) were drawn from the Bordeaux site at 7-year follow up, the only site where concomitant inflammatory assays and detailed neuropsychiatric assessments were performed. Subjects with known or acute signs of inflammatory disease, neurological or psychiatric disorder, diabetes, and/or taking statins or medications likely to influence immune parameters were excluded from the study on the basis of self-reports.

The protocol of the 3C study was approved by the Consultative Committee for the Protection of Persons participating in Biomedical Research of the Kremlin-Bicêtre University Hospital (Paris). All participants signed an informed consent.

Biological Assays

Fasting blood samples were collected between 8:00 AM and 9:30 AM for the measurement of serum inflammatory markers and amino acids. Samples were stored at −80°C until thawed for biological assays. Serum concentrations of IL-6 were assayed by Multiplex (Bioplex, Bio-Rad, Hercules, California). C-reactive protein (CRP) was measured by immunoturbidimetric method Tina-Quant (gen.3) on analyzer Roche/Modular (Roche Diagnostics, Basel, Switzerland). Inter- and intra-assay variability was reliably $< 10\%$. Neopterin concentrations were measured by enzyme-linked immunosorbent assay (BRAHMS Diagnostics, Berlin, Germany). For an estimate of NO production, the stable NO metabolite nitrite (NO_{2-}) was determined in the cell-free culture supernatants by the Griess reaction assay (Promega, Madison, Wisconsin) (39). Free tryptophan and kynurenine serum concentrations as well as concentrations of phenylalanine and tyrosine were determined by high-performance liquid chromatography, as described elsewhere (24,40). The ratios of Kyn/Trp and Phe/Tyr were calculated as indexes of tryptophan breakdown and PHA activity respectively.

Assessments

Neuropsychiatric assessments were performed between 1 and 4 days before the collection of blood samples. Depressive symptoms were assessed with the observer-rated Montgomery-Asberg Depression Rating Scale (MADRS), a 10-item scale assessing severity of depressive symptoms including sadness, inner tension, concentration difficulties, inability to feel, pessimistic thoughts, suicidal thoughts, reduced sleep, reduced appetite, and lassitude (41). The MADRS scale was administered over the phone in 36 participants, due to geographic reasons (subject living too far), and thus the score for MADRS Item 1 (apparent sadness) was not available for those subjects. Therefore, total MADRS scores used for data analysis were obtained summing individuals scores from Items 2–10. The DSM-IV criteria for current major depression were determined with the Mini International Neuropsychiatric Interview during a semi-structured interview with a trained psychologist (42). Fatigue was assessed with the Multidimensional Fatigue Inventory (MFI), a 20-item self-rating scale measuring five dimensions of fatigue, including general fatigue, physical fatigue, mental fatigue, reduced activity, and reduced motivation (43). The total score of each subscale ranges from 4 (best) to 20 (worst). Finally, general behavioral and neurovegetative symptoms were assessed with the Neurotoxicity Rating Scale (NRS), a 39-item self-report questionnaire with questions rated 0 (not present) to 4 (extremely severe), measuring general symptoms experienced by patients with inflammatory conditions (44). For data analysis, NRS symptoms were grouped into five symptom dimensions, corresponding to the dimensions of altered sleep (difficulty getting to sleep, difficulty staying asleep, sleeping too much), sickness (fever, sick feeling, tiredness/fatigue, body aches, joint/muscle pain, headaches), cognitive symptoms (difficulty making decisions, distractibility, episodes of confusion, word-finding problems, memory problems), digestive symptoms (nausea, vomiting, reduced appetite, bowel problems), and motor symptoms (motor slowing, walking problems, tremor/shakiness) as described elsewhere (19). Age, gender, and body mass index (BMI) computed as weight/height^2 (kg/m^2) were also collected in participants.

Data Analyses and Statistics

When a value was missing for one item of a neuropsychiatric scale, the missing value was replaced by the mean value calculated for the scale or for the corresponding dimension. The BMI information was missing in one participant. Extreme values for CRP ($>$ 3 times the normal value) were obtained in three participants. Similarly, extreme values for IL-6 ($>$ 3 SD above the mean) were obtained in five participants. Accordingly, data from these subjects were considered as outliers and were not considered for data analyses. Relationship between age and immune markers and amino acids were assessed with linear regressions analyses, adjusting for gender and BMI. In addition, the age of participants was stratified on the basis of the median age of the study population (i.e., $<$ vs. $\geq$ 80 years), and concentrations of biological markers were compared across subgroups with analysis of covariance, with gender and BMI as covariates. Associations between immune markers and amino acid concentrations were estimated by partial correlation coefficients, adjusting for age, gender, and BMI. Finally, multiple regressions analyses entering biological parameters as predictors in separate models were used to assess the relationship of immune markers and amino acids with neuropsychiatric scores. Analyses were performed after adjusting for age, gender, BMI, and lifetime history of major depression. Lifetime history of major depression was entered as covariate in the analyses, because of its significant relationship with neuropsychiatric scores. All statistical analyses were performed with the software SPSS 16 for Windows (SPSS, Chicago, Illinois). Two-tailed $p < .05$ was considered statistically significant.

Results

Two hundred eighty-four subjects were included. The mean age of participants was 79.9 years (SD = 4.5 years), and the median age was 80 years. One hundred ninety-seven participants (69.4%) were women, and forty (14%) had a lifetime history of major depression. The mean BMI in the study population was 25.4 kg/m^2 (SD = 4.0). Data for biological markers and relationships with age, as assessed by multiple regression analyses controlling for gender and BMI, are shown in Table 1. Overall, there was a significant relationship be-

Table 1. Biological Markers in Study Participants and Association with Age

Biological Markers	Mean	Association with Age[a]		
		β	*t*	*p*
IL-6 (pg/mL)	7.1 (8.1)	.139	2.30	.02
CRP (mg/L)	2.8 (2.7)	.09	1.53	.12
Tryptophan (μmol/L)	62.8 (9.3)	−.138	−2.29	.02
Kynurenine (μmol/L)	2.5 (.7)	.198	3.34	.001
Kyn/Trp (μmol/mmol)	40.7 (13.2)	.258	4.40	.0001
Neopterin (nmol/L)	8.5 (4.4)	.180	3.02	.003
Nitrite (μmol/L)	12.7 (10.8)	.049	.81	.42
Phenylalanine (μmol/L)	92.8 (26.9)	−.088	−1.45	.15
Tyrosine (μmol/L)	100.4 (17.6)	−.101	−1.70	.089
Phe/Tyr	.9 (.2)	−.018	−.30	.76

Data are shown as mean (SD). Mean values are reported excluding eight outliers for biological markers.

IL-6, interleukin-6; CRP, C-reactive protein; Kyn/Trp, kynurenine/tryptophan; Phe/Tyr, phenylalanine/tyrosine.

[a]Multivariate linear regression analysis controlling for gender and body mass index. Age was entered as continuous variable in regression models.

tween the age of participants and serum concentrations of IL-6, tryptophan, kynurenine, Kyn/Trp, and neopterin. The older participants were, the lower were tryptophan levels, and the higher were IL-6, kynurenine, and neopterin concentrations and the ratio of Kyn/Trp. In addition, there was a trend for a relationship between age and tyrosine concentrations, with increased age corresponding to decreased tyrosine levels. Analyses stratified on the basis of the median age of study participants were consistent with these findings (Figure 2).

Overall, immune markers and amino acid levels were correlated (Table 2). More specifically, IL-6 and CRP levels correlated positively with nitrite, neopterin, and kynurenine concentrations and with Kyn/Trp. Neopterin concentrations were positively correlated with kynurenine and Kyn/Trp and negatively with tryptophan and tyrosine levels. Tryptophan concentrations correlated with kynurenine and tyrosine levels and with the ratios of Kyn/Trp and Phe/Tyr, with increased tryptophan levels corresponding to increased kynurenine and tyrosine concentrations and decreased Kyn/Trp and Phe/Tyr ratios. The ratio of Kyn/Trp was also negatively associated with tyrosine levels and positively associated with the ratio of Phe/Tyr. Concentrations of phenylalanine correlated positively with concentrations of tyrosine.

Neuropsychiatric scores on the MADRS, MFI, and NRS scales and associations with age are presented in Table 3. Overall, symptoms of fatigue were significantly associated with age, with older age associating with higher total fatigue scores and subscores of general fatigue, physical fatigue, reduced activity, reduced motivation, and mental fatigue with borderline significance. In addition, there was a significant relationship between age and MADRS scores on items assessing reported sadness and reduced appetite, and there was a trend for an association with the item of lassitude, with older age corresponding to higher score on the item. Finally, increased age was associated with increased cognitive and motor symptoms as assessed by the NRS scale.

Separate linear regressions analyses adjusting for age, gender, BMI, and lifetime history of major depression revealed significant relationships between biological markers and neuropsychiatric scores (Table 4). With respect to MADRS scores, significant predictions were obtained for the items of reduced appetite and lassitude. Higher scores of reduced appetite were associated with higher neopterin and IL-6 concentrations together with lower tryptophan

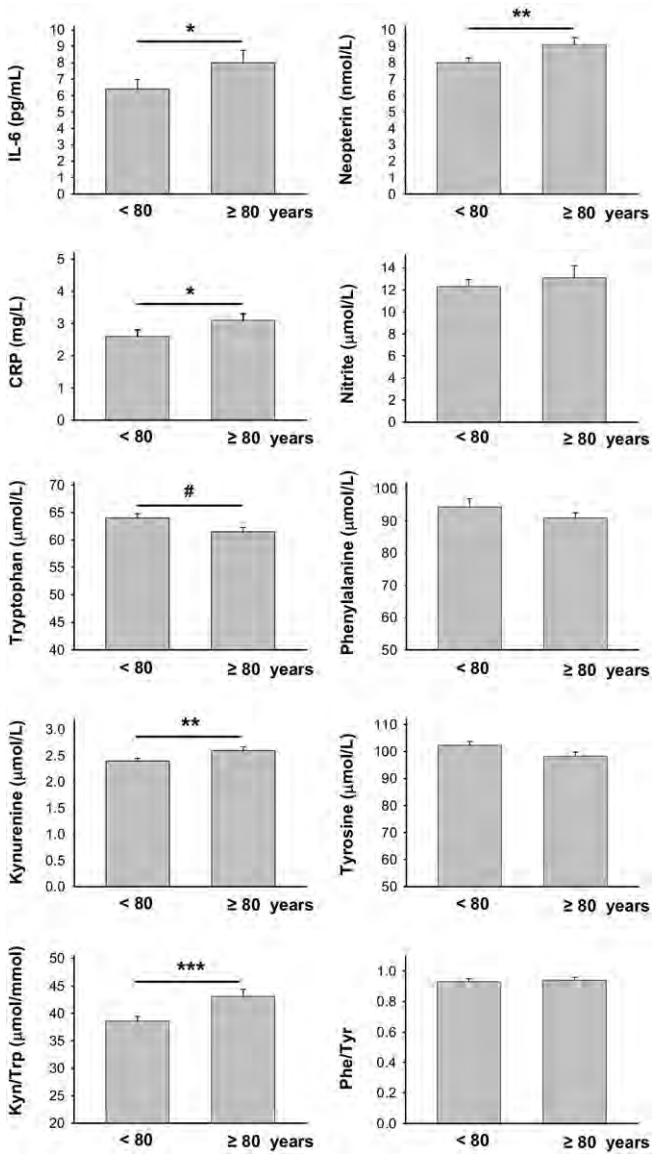

Figure 2. Concentrations of biological markers in study participants. Data were stratified on the basis of the median age of the study population (< vs. ≥ 80 years) and compared with analysis of covariance adjusting for gender and body mass index (BMI). Data are shown as mean ± SEM. ***$p < .001$, **$p < .01$, *$p < .05$, #$p = .06$. IL-6, interleukin-6; CRP, C-reactive protein; Kyn/Trp, kynurenine/tryptophan; Phe/Tyr, phenylalanine/tyrosine.

levels. In addition, the ratio of Kyn/Trp was significantly associated with MADRS scores of reduced appetite. Similarly, higher MADRS scores of lassitude were associated with lower tryptophan and phenylalanine concentrations. Moreover, there was a trend for a relationship between reduced tryptophan concentrations and increased MADRS scores of pessimistic thoughts. On the MFI scale, the dimensions of general fatigue and reduced motivation were associated with nitrite, kynurenine, and tyrosine concentrations. More precisely, increased MFI scores of general fatigue were significantly associated with lower nitrite levels, and reduced motivation correlated with higher concentrations of kynurenine or tyrosine. With regard to scores on the NRS scale, significant predictions were found with the dimensions of sleep, sickness, and digestive symptoms. Increased scores in the neurovegetative dimensions of altered sleep and digestive symptoms were associated with in-

Table 2. Correlations Among Biological Markers

	IL-6	CRP	Neopterin	Tryptophan	Kynurenine	Kyn/Trp	Nitrite	Phenylalanine	Tyrosine	Phe/Tyr
IL-6	—									
CRP	.335[a]	—								
Neopterin	.123[b]	.138[b]	—							
Tryptophan	−.115[c]	−.041	−.276[a]	—						
Kynurenine	.141[b]	.187[d]	.601[a]	.261[a]	—					
Kyn/Trp	.197[d]	.199[d]	.778[a]	−.254[a]	.853[a]	—				
Nitrite	.345[a]	.131[b]	.089	.015	.111[c]	.100[c]	—			
Phenylalanine	.029	.024	−.028	.112[c]	.098	.041	−.015	—		
Tyrosine	.062	.026	−.183[d]	.357[a]	.020	−.177[d]	.023	.325[a]	—	
Phe/Tyr	.001	.029	.109[c]	−.120[b]	.098	.172[d]	−.034	.789[a]	−.300[a]	—

Partial correlation coefficients, adjusted for age, gender, and body mass index. Abbreviations as in Table 1.
[a] $p < .001$.
[b] $p < .05$.
[c] $p < .10$.
[d] $p < .01$.

creased phenylalanine concentrations and Phe/Tyr ratio. In addition, lower tryptophan levels predicted increased scores of digestive symptoms. Moreover, higher scores of sickness symptoms were significantly associated with increased Phe/Tyr ratio and phenylalanine concentrations with borderline significance. Finally, there was a trend for a relationship between motor symptoms and the ratio of Phe/Tyr.

Table 3. Neuropsychiatric Scores[c]

		Association with Age[a]		
Scales	Score	β	*t*	*p*
Depressive Symptoms (MADRS scale)[b]				
MADRS scale (total score)	5.6 (5.1)	.105	1.72	.09
Reported sadness	.7 (1.2)	.125	2.03	.04
Inner tension	.7 (1.0)	.045	.73	.47
Reduced sleep	1.4 (1.6)	−.036	−.57	.57
Reduced appetite	.5 (1.1)	.171	2.75	.006
Concentrations difficulties	.7 (1.2)	.011	.18	.86
Lassitude	.5 (.9)	.103	1.66	.09
Inability to feel	.3 (.7)	.060	.96	.34
Pessimistic thoughts	.5 (.8)	.010	.16	.87
Suicidal thoughts	.3 (.6)	.087	1.39	.16
Fatigue Symptoms (MFI scale)[c]				
MFI total score	50.2 (13.6)	.251	4.21	.0001
General fatigue	11.2 (3.6)	.209	3.50	.001
Physical fatigue	10.6 (3.8)	.237	3.94	.0001
Mental fatigue	8.7 (3.3)	.115	1.90	.06
Reduced activity	10.5 (3.5)	.239	3.96	.0001
Reduced motivation	9.2 (3.3)	.164	2.72	.007
General Behavioral and Neurovegetative Symptoms (NRS scale)[c]				
Altered sleep	2.2 (1.9)	−.037	−.61	.54
Sickness	5.4 (3.6)	−.007	−.12	.90
Cognitive symptoms	3.8 (2.9)	.156	2.61	.01
Digestive symptoms	1.2 (1.6)	−.073	−1.19	.24
Motor symptoms	2.1 (2.1)	.302	5.15	.0001

Data are shown as mean (SD).
[a] Multivariate linear regression analysis controlling for gender, body mass index, and lifetime history of major depression. Age was entered as continuous variable in regression models.
[b] The Montgomery-Asberg Depression Rating Scale (MADRS) scale was incomplete in 13 participants. The MADRS scale was administered over the phone in 36 participants, due to geographic reasons, and thus score for Item 1 (apparent sadness) was not obtained in those subjects. Total MADRS scores were therefore computed with Items 2–10.
[c] Data for the Multidimensional Fatigue Inventory (MFI) and neurotoxicity rating scale (NRS) were missing in one participant.

Discussion

Results from this study clearly support the notion of inflammaging (11,12), because they indicate significant relationships between aging and circulating concentrations of immune markers. As expected, older age was associated with increased concentrations of both IL-6 and neopterin, indicative of immune activation in elderly persons. In addition, older age together with inflammation correlated negatively with tryptophan concentrations and positively with kynurenine concentrations and with the ratio of Kyn/Trp. These data are consistent with the hypothesis that immune activation in aging influences the metabolism of amino-acids. In the population under study, decreases in tryptophan concentrations and increases in kynurenine levels and in the ratio of Kyn/Trp were suggestive of increased tryptophan breakdown. These alterations were associated with immune activation, supporting the involvement of IDO in tryptophan degradation. This finding corroborates data from the literature indicating that IDO activity is increased with aging (45–47).

Besides effects on IDO enzymatic pathway, chronic inflammation in elderly persons was found to also modulate pathways related to activation of GTP-CH1, as revealed by the relationships measured among IL-6, neopterin, nitrite, and tyrosine concentrations. As mentioned in the preceding text, GTP-CH1 is responsible for the production of neopterin and BH4, which is a cofactor of NOS and aromatic amino acid hydroxylases, including PHA. In the present study, increased inflammation correlated with increased nitrite and reduced tyrosine concentrations. The inverse relationship found between tyrosine and phenylalanine (and Phe/Tyr) indicated reduced PHA activity (26). Reduced phenylalanine turnover has been documented in various medical conditions characterized by chronic inflammation (26,28,29). Nevertheless, to our knowledge, this is the first demonstration of decreased PHA activity in non-medically ill elderly subjects. Tyrosine deficiency in older subjects has been usually attributed to nutritional defects (48). Here, we show that alterations in PHA activity might significantly contribute to tyrosine deficiency in aging, a condition that is characterized by subtle, low-grade, chronic inflammatory processes. Altogether, these findings indicate that age-related chronic inflammation is associated with potent alterations in enzymatic pathways that are

Table 4. Relationship of Immune Markers and Amino Acids with Neuropsychiatric Scores

Dependant Variables	Predictors[a]	Coefficients β	*t*	*p*
Depressive Symptoms (MADRS items)[b]				
Reduced appetite	Neopterin	.226	3.73	<.001
	Tryptophan	−.221	−3.64	<.001
	Kyn/Trp	.200	3.20	<.01
	IL-6	.124	2.01	<.05
Lassitude	Tryptophan	−.160	−2.60	<.01
	Phenylalanine	−.131	−2.14	<.05
Pessimistic thoughts	Tryptophan	−.107	−1.71	.08
Fatigue Symptoms (MFI scores)[b]				
General fatigue	Nitrite	−.115	−1.94	.05
Reduced motivation	Kynurenine	.134	2.18	<.05
	Tyrosine	.131	2.15	<.05
General Behavioral and Neurovegetative Symptoms (NRS scores)[b]				
Sleep alterations	Phenylalanine	.151	2.51	<.05
	Phe/Tyr	.165	2.77	<.01
Sickness	Phe/Tyr	.126	2.12	<.05
	Phenylalanine	.105	1.75	.08
Digestive symptoms	Phenylalanine	.181	2.99	<.01
	Tryptophan	−.181	−2.96	<.01
	Phe/Tyr	.201	3.36	<.001
Motor symptoms	Phe/Tyr	.098	1.70	.09

Separate linear regression analyses. Abbreviations as in Tables 1 and 3.

[a]Predictors were entered in separate models. Analyses were performed, controlling for age, gender, body mass index, and lifetime history of major depression (these parameters were entered as forced variables in each model).

[b]Only items for which statistically significant prediction models were obtained are presented in the table.

strongly involved in the biosynthesis of neurotransmitters, including serotonin, dopamine, epinephrine, and norepinephrine. Behavioral consequences of these biochemical alterations are strongly expected, given the notorious role of these neurotransmitters in the regulation of mood, cognitive processes and neurovegetative function.

Elderly subjects are particularly vulnerable to neuropsychiatric alterations. A large database documents evidence of frequent mood and cognitive disorders, anorexia, sleep disturbance, and fatigue symptoms in older individuals (1–4, 49, 50). Consistent with these data, the present study indicates strong relationships between older age and neuropsychiatric symptoms. In support of the hypothesis that neuropsychiatric symptoms in elderly persons might rely on inflammation-related alterations in IDO and BH4 pathways, significant associations were found between biomarkers of these pathways and symptom dimensions. Accordingly, increased tryptophan catabolism, as reflected by reduced tryptophan concentrations and/or increases in kynurenine and Kyn/Trp, was associated with the dimensions of reduced appetite and lassitude on the MADRS scale and with the dimension of reduced motivation on the MFI scale. Moreover, there was a trend for a relationship between lower tryptophan concentrations and higher MADRS scores of pessimistic thoughts. In addition, a significant association was found between tryptophan concentrations and digestive symptoms on the NRS scale, probably due to the contribution of the dimension of "decreased appetite" to this construct. These findings concord with previous data obtained by our group in medically ill patients treated with IFN-α (35). In those patients, decreases in tryptophan during IFN-α therapy were found to correlate with increased MADRS scores of reduced appetite, pessimistic thoughts, suicidal thoughts, and loss of concentration. In the present study, the lack of relationship between tryptophan and the dimensions of sadness and suicidal thoughts might be due to the very low prevalence of current depression (.7%) in participants. This limitation might contribute to a lack of statistical power, due to the underexpression of specific dimensions of depressive symptomatology as assessed by the MADRS scale. Moreover, these results are consistent with the well-documented role of tryptophan and its product serotonin in symptoms of decreased mood, anorexia, and apathy (51,52). Although systemic decreases in tryptophan do not necessarily translate into reduced serotonin synthesis within the central nervous system, recent data indicate that inflammation-related increased tryptophan catabolism at the periphery is accompanied by marked increases in brain kynurenine and quinolinic acid (37). Interestingly, these alterations correlated with depressive symptoms in the same study (37).

With respect to alterations in the BH4 pathway, reduced PHA activity (as reflected by increases in the ratio of Phe/Tyr or in phenylalanine at the expense of tyrosine) was associated with neurovegetative symptoms, including sleep disturbances, sickness symptoms, and digestive and motor symptoms. The association of PHA activity with sleep and motor symptoms is consistent with the role of this pathway in the metabolism of dopamine, which is strongly involved in the regulation of such functions (53,54). Moreover, GTP-CH1 genes have been involved in pain, which can be consistent with the association found in our study between phenylalanine concentrations or Phe/Tyr and sickness symptoms (55). Finally, nitrite concentrations correlated inversely with scores of general fatigue in the present study. This result might be surprising, given that nitrite concentrations correlated positively with inflammatory markers. Nevertheless, in a context of chronic inflammation such as occurs with aging, lower nitrite levels might reflect either an imbalance in oxidant/antioxidant mechanisms with reduced antioxidant defenses or impairment in NOS activity. Nitrite is a major

storage pool of NO and therefore can be cytoprotective under physiological and pathophysiological conditions (56,57). Nitric oxide plays an essential role in vascular homeostasis, metabolic regulation, and immune processes. Within the central nervous system, NO is involved in various functions (e.g., synaptic plasticity, sleep-wake cycle regulation, food intake, hormone secretion) and is believed to exert neuroprotective effects at physiological amounts versus neurotoxic effects at higher concentrations (58). Moreover, it has been hypothesized that NO activity plays a role in the pathophysiology of chronic fatigue syndrome (59). In view of these data, the inverse correlation found in this study between nitrite levels and fatigue seems meaningful.

In addition to the already mentioned low prevalence of current major depression in study participants, limitations of the present study include the sample selection of healthy volunteers and the reliability of self-reported chronic conditions. Although we selected a priori healthy community dwellers without any diagnosed inflammatory disease, we cannot exclude the possibility that some individuals might have failed to report this condition or the use of anti-inflammatory drugs. Nevertheless, we believe that such exceptions, if clinically relevant, would have been detected in biological assessments. Despite these limitations, this study benefits from several major strengths, such as the large sample size and the population-based design.

The question arises regarding the possibility to prevent or reduce neuropsychiatric symptoms in elderly persons. We still need to determine whether nutritional interventions would be efficient in preventing or modulating neuropsychiatric symptoms in a context of pre-existing chronic low-grade inflammation such as aging or whether pharmacological treatments, targeting specifically neurotransmitter function or inflammatory processes, would be more adapted. With regard to nutritional interventions, strategies could include supplements with amino-acids, including tryptophan and tyrosine. Nevertheless, tryptophan supplements might end up being useless in a context of chronic inflammation, where the induction of IDO and then kynurenine monooxygenase might promote its degradation into the quinolinic acid pathway (23). Moreover, to be potentially effective, this treatment would require adequate amounts of vitamin B6, a cofactor of several enzymes in tryptophan catabolism. This condition might be difficult to meet in a significant number of elderly subjects. Alternatively, supplements with antioxidants might be able to modulate IDO and GTP-CH1 activities. Several compounds, including resveratrol, vitamins C and E and aspirin, were shown to reduce in vitro tryptophan degradation and neopterin production, in support of this notion (60–62).

In conclusion, we found that chronic low-grade inflammation in aging is associated with significant alterations in two enzymatic pathways involved in the metabolism of monoamines. These alterations, which manifested primarily by increased tryptophan catabolism and altered phenylalanine turnover, might represent major pathophysiological mechanisms in the development of neuropsychiatric symptoms in elderly persons.

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