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Association of increased carotid intima–media thickness and lower plasma levels of vitamin C and vitamin E in old age subjects: implications for Alzheimer's disease

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Abstract In light of the recent advances regarding the role of vascularity in Alzheimer's disease (AD) pathophysiology, the relationship between plasma levels and activities of the major antioxidant molecules and the carotid intima–media thickness (C-IMT) of older persons with no or very mild cognitive impairment was evaluated. The underlying hypothesis is that the IMT may be an indirect index of vascular damage in persons with low levels of plasma antioxidants. Plasma levels of vitamins A, C, E, of uric acid as well as activities of the plasma antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured. Plasma levels of vitamins C and E significantly decreased among participants from the first to the fourth IMT quartile, with a linear slope only for vitamin C. Compared to participants in the lowest vitamin C quartile, the probability to have IMT >1.2 mm significantly decreased among persons from the second to the fourth quartile independent of confounders. In conclusion, only vitamin C plasma levels appear to be selectively associated with the risk of increasing C-IMT. An adequate vitamin C status might be particularly important for protection against AD and other clinical manifestations of vascular and cognitive ageing.

Keywords Vascular diseases · Cognitive impairment · Healthy ageing · Vitamins · Intima–media thickness

Introduction

Phenomena-like French paradox (Renaud and Ruf 1994), obesity paradox (Dehlendoff et al. 2014) and dropping dementia rates (Prince et al. 2012) still coexist with projections of 115 million people with dementia in 2015 (Prince et al. 2013) and the related enormous socio-economic burden of Alzheimer's disease (AD) (Larson et al. 2013). AD epidemiology, the absence of a cure against AD and the still difficult early diagnosis constitute three major challenges which make AD a unique social and medical issue of these decades. The recent development of AD research towards prevention strategies and the pathophysiological role of the vascular component have opened new investigation possibilities, in which oxidative stress-related mechanisms appear to confirm their pivotal position (recently reviewed in Polidori and Pientka 2012). The evidence of a link between vascular disease and AD was reported originally in a comprehensive review published by de la Torre (2002). The link was at that time under investigation since over ten years and supported by evidence that vascular risk factors and indicators of vascular disease are strongly associated with AD among over 7,000 older persons with and without dementia participating in the Rotterdam Study (Breteler et al. 1998, 2000). Several epidemiological studies in the last decade confirmed the

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relevance of the relationship between vascular disease and AD. However, reasons and mechanisms of such a relationship remain under debate. Meanwhile, there is an established epidemiological link between hypercholesterolemia and hypertension at midlife and increased risk for AD (reviewed in Polidori et al. 2012). Epidemiological risk assessment and clinical management of the axis vascular disease risk-dementia is extremely complex and cannot go beyond the attentive evaluation of subclinical and early clinically overt atherosclerosis. Atherosclerosis is known to cause ischemia- and inflammation-related oxidative stress as well as subsequent free radical production-related reperfusion and to be linked to dementia and AD (Luzzi et al. 2010). The conclusive statement for healthcare professionals from the American Heart Association/American Stroke Association cites that "Vascular contributions to cognitive impairment and dementia are important. There is a need for prospective, quantitative, clinical–pathological-neuroimaging studies to improve knowledge of the pathological basis of neuroimaging change and the complex interplay between vascular and Alzheimer disease. Studies of intensive reduction of vascular risk factors in high-risk groups are another important avenue of research". Despite this need, however, there is still a substantial lack of assessment and management of vascular risk factors in the general population and in AD.

The involvement of oxidative stress at the neurovascular level includes several potential mechanisms. Neuronal mitochondria, known to be especially vulnerable to vascular oxidative damage in AD, undergo oxidation-mediated damage such as DNA oxidative changes with subsequent alterations of form and function, and endothelial cells might be the target for the toxic action of heavily aggregated proteins, glia-derived cytokines and stimuli-inducing oxidative and metabolic stress in AD brains (Salmina et al. 2010). Sustained hypoperfusion promoting oxidative stress of brain tissues might also stimulate secondary damage via the overexpression of inducible and neuronal-specific nitric oxide synthase and endothelin-1, and the continuous accumulation of oxidative stress products such as peroxynitrite accumulation may in turn accelerate the damage and compromise the blood–brain barrier (reviewed in Mariani et al. 2005). In the presence of vascular risk factors, a threshold for cerebral hypoperfusion might be critically attained (de la Torre 2000) in a way that resulting oxidative stress might damage the neurovascular unit and endothelium of the brain vessels, the latter producing in turn ROS able to favour Aβ formation and nourishing a vicious circle in which also plaque-induced ROS formation damages the neurovascular unit and the endothelium. In general, free radical production does play a critical role in vascular diseases and in AD and surely even more so in the combination of the two, especially according to ongoing research thoroughly reviewed by our group (Polidori et al. 2012).

Abnormal free radical-related lipid peroxidation is accompanied by a poor status of some antioxidant molecules in patients with atherosclerosis and cardiovascular diseases and F2-isoprostanes, robust biomarkers of lipid peroxidation in humans, are inversely correlated with increased intima–media thickness (IMT) of the carotid arteries (Polidori et al. 2007). An increase of the IMT is the earliest stage of atherosclerosis and is an independent risk factor for cardiovascular events (Tribble 1999; Lorenz et al. 2007; Lester et al. 2009). Several studies have investigated the possibility that carotid steno-occlusive disease may predispose to reduced cognitive function and multiple measures of carotid atherosclerosis have been shown to be associated with prospective risk of dementia (Wendell et al. 2012). In addition, lipid peroxidation, micronutrient status and endothelial function appear to be tightly connected mechanisms of neuronal damage in cognitive impairment with and without dementia (Dias et al. 2014).

Evidence from population studies has shown that both dietary (Klipstein Grobusch et al. 2001), plasma, or serum level of vitamin E (e.g. α-tocopherol) (Riemersma et al. 1996), vitamin C (ascorbic acid) (Tribble 1999; Riemersma 2001) and of the antioxidant enzyme superoxide dismutase (SOD) (Isogawa et al. 2009) are inversely associated with early atherosclerosis and cardiovascular mortality rate. Although RCTs of antioxidant compounds against vascular and neurodegenerative diseases led to largely conflicting results, the role of oxidative stress-lowering strategies cannot be considered disproven yet, due to severe methodological and design study limitations (Tribble 1999; Mecocci and Polidori 2012). Therefore, studies are particularly needed to identify the relationship between atherogenesis and degree of antioxidant defence prior to the clinical manifestation of overt atherosclerosis and neurodegeneration. B-mode ultrasound is, among several non-invasive imaging techniques (CT, echocardiography, MRI, carotid artery imaging), a well-established method to evaluate atherosclerosis of peripheral arteries. By means of ultrasonographic assessment, the thickness of the intima–media complex of easily accessible arteries can be calculated and has been also advocated as surrogate marker for less accessible vessels.

For its independent relationship with cardiovascular disease beyond standard risk factors and as non-invasive, nonionizing radiation test, carotid IMT (C-IMT) is recommended for risk assessment in asymptomatic adults with a class IIa level of evidence (2010 ACCF/AHA Guideline for assessment of cardiovascular risk in asymptomatic adults). We conducted the present study with the aim of exploring the relationship between plasma levels of the

major antioxidant molecules of the organism and the carotid IMT in a consecutive sample of old age subjects with no or very mild cognitive impairment.

Subjects and methods

Subjects

One hundred ninety-two subjects (127 F, 65 M) aged 65 years and older (78.5 ± 4.3 years) admitted for routine check-up to the Outpatient Clinic of the Department of Gerontology and Geriatrics at the University Hospital of Perugia, Italy, were consecutively included in the study. The study conforms to the principles outlined in the Declaration of Helsinki as approved by the local Ethics Committee and all study participants gave their signed consent to participate. All participants underwent comprehensive geriatric assessment, including performance of the Mini-Mental State Examination (MMSE) and collection of information on comorbidities, drug therapy, smoking habit and physical activity level. Subjects with a body mass index (BMI) <18.5 or >30 kg/m^2, current infections, existing diagnosis of chronic infection, carotid atherosclerotic plaque or major organ failure, as well as those taking antioxidant or iron supplements and consuming <100 or >400 g fruits and vegetables per day were excluded from the study. The rationale for the applied selection criteria was to minimize confounding factors.

IMT assessment

All participants underwent high-resolution B-mode ultrasonography (ESAOTE MAGAS GP, Genova Italy) for the measurement of IMT of the carotid artery using a 7.5 MHz array transducer. They were placed in a supine position and longitudinal, anterior, anterior-lateral and posterior-lateral projections were used to image the left and right carotid arteries. Briefly, IMT was determined as the distance between the blood to intima and media to adventitia interfaces along a 1-cm length at one centimetre of distance from the carotid bifurcation (Sidhu and Allan 1997). The average value of three measurements of the IMT at the common carotid artery (CCA) was calculated for each subject. One physician, blinded to the biochemical measurements, carried out all ultrasound examinations.

Biochemical analyses

All subjects under fasting conditions underwent blood withdrawal in a 10-ml heparinised tube. Blood was immediately centrifuged, and plasma was stored frozen (with 5 % metaphosphoric acid to stabilize vitamin C levels in the relative analysis aliquots) at −80 °C until analysis. Plasma samples were coded and analysis was carried out in a blind fashion. Plasma levels of vitamin C and uric acid were measured by high-performance liquid chromatography (HPLC) with electrochemical detection (Kutnik et al. 1987). Plasma levels of vitamins A and E were measured after extraction with methanol and exane by HPLC with UV detection (Nierenberg and Nann 1992). Activities of the plasma antioxidant enzymes superoxide dismutase (SOD) (L'Abbé and Fisher 1990) and glutathione peroxidase (GPx) (Flohé and Gunzler 1984) were measured spectrophotometrically.

Statistical analysis

Participants' characteristics were described grouping them according to IMT quartiles. Variables with symmetric distribution were reported as means and standard deviations (SD). Variables with asymmetric distribution were summarized as medians and interquartile ranges. Statistical comparisons across groups were performed using the ANOVA analysis of variance with Bonferroni post hoc test or the Chi-square test, as appropriate. The Mantel–Haenszel Chi-square test was used for trends among IMT quartiles. The significance level used for 2-sided tests was $p < 0.05$. The associations between IMT quartiles and plasma levels or activity of antioxidants were evaluated using multivariate regression model based on generalized linear model procedure. The probability of a IMT >1.2 mm among participants stratified by quartiles of vitamin C and E plasma levels was tested using multivariate logistic analysis. All analyses were performed using the SAS statistical package, version 8.2 (SAS Institute, Cary, NC).

Results

Demographics and clinical characteristics of the participants for the entire sample and according to IMT quartile distribution are presented in Table 1. Overall, participants had IMT 1.2 ± 0.2 mm (mean ± SD), with the lowest and the highest estimates ranging from 0.70 to 1.50 mm, respectively. Participants were over 75 years old, mainly women, living almost fully independent in ADL and IADL and showing a borderline performance at MMSE. Of note, the IADL score tended to decline across IMT strata ($p < 0.003$), while the prevalence of smokers was very low in the entire sample and almost superimposed across IMT quartiles. There was no difference with respect to the number of comorbidities and current drug therapy across IMT quartiles. Hypertension and CVD were the most prevalent diseases, with three on four participants (76.6 %) taking low dose antiplatelets, while less than 10 and 5 % of

Table 1 Participants' characteristics of the entire sample and according to carotid IMT quartile distribution

	Entire sample	IMT Quartiles				
	($n = 192$) 0.70–1.50 mm	1° ($n = 51$) 0.70–1.10 mm	2° ($n = 50$) 1.20–1.20 mm	3° ($n = 50$) 1.25–1.30 mm	4° ($n = 41$) 1.40–1.50 mm	p
Carotid IMT (mm), M ± SD	1.2 ± 0.2	0.9 ± 0.1	1.2 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	<0.0001*
General characteristics						
Age (years), M ± SD	78.5 ± 4.3	78.5 ± 3.4	79.2 ± 5.1	78.4 ± 4.7	77.5 ± 3.6	0.3439
Woman, [*n* (%)]	127 (65.0)	33 (64.7)	31 (62.0)	36 (72.0)	27 (66.0)	0.7535
Smoking habit, [*n* (%)]	7 (3.6)	2 (3.9)	2 (4.0)	1 (2.0)	2 (4.9)	0.4968
Independence in IADL, M ± SD	7.1 ± 1.6	7.5 ± 1.2	7.1 ± 1.7	6.9 ± 1.8	6.6 ± 1.6	0.0667*
Independence in ADL, M ± SD	5.7 ± 0.9	5.85 ± 0.6	5.64 ± 1.2	5.86 ± 0.6	5.53 ± 1.1	0.2267
MMSE score, M ± SD	26.9 ± 3	26.2 ± 2.7	26.3 ± 2.6	25.6 ± 3.3	25.5 ± 2.9	0.3574
Drugs and diseases						
Hypertension, [*n* (%)]	76 (39.6)	12 (23.5)	20 (40.0)	24 (48.0)	20 (48.8)	0.0379*
CVD, [*n* (%)]	78 (40.6)	20 (39.2)	22 (44.0)	18 (36.0)	18 (43.9)	0.8244
Diabetes, [*n* (%)]	18 (9.4)	7 (13.7)	1 (2)	5 (10)	5 (12.2)	0.1915
Stroke, [*n* (%)]	6 (3.1)	1 (1.9)	2 (4.0)	2 (4.0)	1 (2.4)	0.9089
Statins, [*n* (%)]	11 (5.7)	2 (3.9)	2 (4.0)	4 (8.0)	3 (7.3)	0.7401
Antiplatelets, [*n* (%)]	147 (76.6)	40 (78.4)	32 (64.0)	40 (80.0)	35 (85.4)	0.0859
Oral hypoglycaemics, [*n* (%)]	9 (4.7)	2 (3.9)	0 (0)	2 (4.0)	5 (12.2)	0.0514
Plasma levels						
Vitamin C (μM), M ± SD	37.0 ± 13.5	54.8 ± 12.40	33.4 ± 5.1	30.5 ± 5.9	27.7 ± 6.1	<0.0001*
Uric acid (μM), M ± SD	245.4 ± 80.5	275.6 ± 81.8	242.1 ± 83.9	247.5 ± 76.0	209.3 ± 65.9	0.0011*
Vitamin E (μM), M ± SD	45.1 ± 10.0	47.9 ± 8.8	45.4 ± 9.6	44.71 ± 9.8	41.9 ± 11.7	0.0422*
Vitamin A (μM), M ± SD	2.5 ± 0.5	2.6 ± 0.4	2.5 ± 0.51	2.48 ± 0.5	2.5 ± 0.6	0.6314
SOD activity (U/ml), M ± SD	25.8 ± 6.1	27.3 ± 6.5	24.8 ± 5.8	25.6 ± 5.6	25.3 ± 5.2	0.1789
GPX activity (U/ml), M ± SD	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.02	0.10 ± 0.08	0.10 ± 0.02	0.5100
Cholesterol (mg/dl), M ± SD	198.3 ± 13.1	197.2 ± 13.8	197.6 ± 15.3	198.1 ± 10.6	200.9 ± 11.2	0.5516

p values are from Chi-square test or ANOVA analysis of variance, as appropriate

ADL activity of daily living, *IADL* instrumental activity of daily living, *MMSE* mini-mental state examination, *IMT* intima–media thickness, *CVD* cardio vascular disease, *SOD* superoxide dismutase, *GPX* glutathione peroxidase, *OHD* oral hypoglycaemic drug

* p value <0.05 from Mantel-Haenszel test for trend

them had diabetes or stroke, respectively. Statins (5.7 %) and oral hypoglycaemic drugs (4.7 %) tended to be more prescribed among participants belonging to the third and fourth IMT quartiles ($p = 0.7$ and $p = 0.05$, respectively). Plasma levels of vitamin C, uric acid and vitamin E significantly decreased among participants from the first to the fourth IMT quartile, with a linear slope only for vitamin C. Plasma levels of vitamin A, cholesterol and SOD activity were similar across IMT strata (Table 1). The associations between IMT quartile distribution and both vitamin C and E plasma levels are presented in Table 2. Compared to participants with the lowest IMT, those belonging to the higher quartiles showed declining levels of Vitamin C independent of age, gender, smoking habit, hypertension, CVD, stroke, diabetes, antiplatelets, statins, uric acid levels. Only participants with the highest IMT showed

Table 2 Association between carotid IMT quartile distribution and plasma levels and activity of antioxidants

IMT quartile	Vitamin C		Vitamin E		Vitamin a		SOD	
	β + SE	*p*	β + SE	*p*	β + SE	*p*	β + SE	*p*
1°	Ref	–	Ref	–	Ref	–	Ref	–
2°	−20.32 ± 1.63	<0.0001	−2.48 ± 1.08	0.2360	−0.079 ± 0.10	0.4598	−2.45 ± 1.22	0.0473
3°	−23.10 ± 1.62	<0.0001	−2.58 ± 2.07	0.2149	−0.092 ± 0.10	0.3840	−1.58 ± 1.22	0.1944
4°	−25.07 ± 1.75	<0.0001	−4.97 ± 2.26	0.0294	−0.117 ± 0.11	0.3090	−1.82 ± 1.32	0.1703

Models are adjusted for gender, smoking habit, age, hypertension, cardiovascular disease, diabetes, stroke, statins, antiplatelets, uric acid or cholesterol levels, as appropriate

significant lower levels of vitamin E compared to those in the lowest IMT quartiles independent of confounders, including age, gender, smoking habit, hypertension, CVD, stroke, diabetes, antiplatelets, statins, uric acid and cholesterol levels. Finally, we tested the probability of having IMT >1.2 mm among participants stratified by quartile distribution of vitamins C and E plasma levels (Fig. 1, panel a and b). Compared to participants with the lowest vitamin C quartile, the probability to have IMT >1.2 mm significantly decreased among persons from the second to the fourth quartile independent of confounders (Fig. 1, panel a). The probability of having IMT >1.2 mm was not influenced by vitamin E plasma levels when confounders were taking into account (Fig. 1, panel b).

Finally, although no statistically significant difference was observed in MMSE scores among vitamin C strata and vitamin C levels among MMSE score strata, there was a tendency of CC-IMT values to decline across MMSE quintiles ($p = 0.09$ adjusted for age and sex).

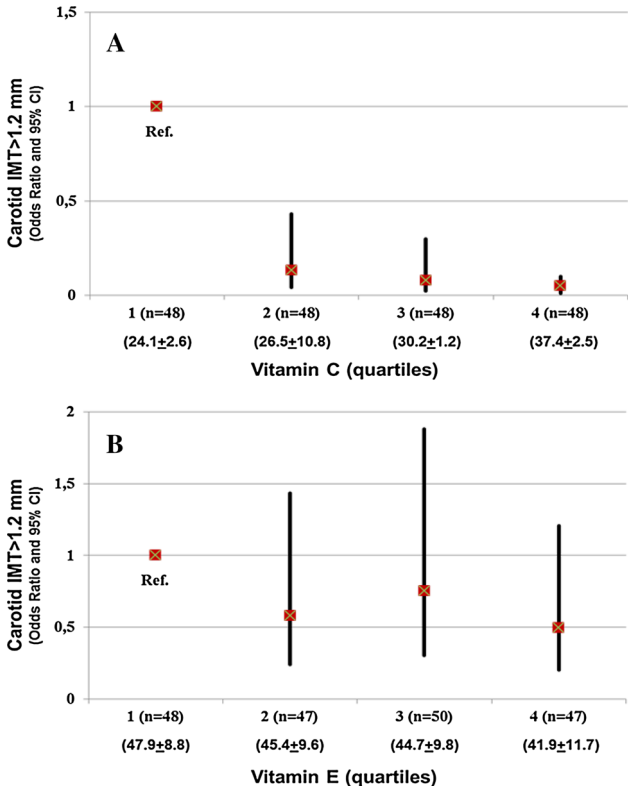

Fig. 1 The odds ratio of carotid IMT >1.2 mm in older subjects in the middle and the highest quartile of vitamin C (panel **a**) and vitamin E (panel **b**) compared to those in the lowest quartile. Adjusted for age, gender, smoking habit, age, hypertension, cardiovascular disease, diabetes, stroke, statins, antiplatelets, oral antidiabetics, total cholesterol plus plasma levels of uric acid and vitamin E in panel **a**, while plasma levels of vitamin C in panel **b**, respectively

Discussion

The main results of this study are that (1) belonging to the higher IMT quartiles is associated with lower levels of vitamins C and E, and that (2) the probability of having IMT >1.2 mm significantly decreases among persons from the second to the fourth vitamin C but not E quartile independently of confounders. The risk of having a carotid IMT >1.2 mm—i.e. largely above the cut-off for pathologic values (European Society of Hypertension and the European Society of Cardiology 2007) has been independently associated with an increased risk for vascular disease (ACCF/AHA Guidelines 2011). Our results fully support the observations of large longitudinal cohort studies on vitamin C and cardiovascular risk. An investigation on participants of the NHANES II study ($n = 8{,}453$) concluded that individuals with vitamin C levels ≥45.4 μM had a significantly 21–25 % lower risk of CVD-related deaths and a significant 25–29 % lower risk of all-cause mortality compared to the participants with low levels of vitamin C <23 μM (Simon et al. 2001). In the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk study (Boekholdt et al. 2006) a 33 % lower risk of developing coronary heart disease was shown for subjects with the highest plasma vitamin C levels compared to the lowest (mean 27.6 μM) over six years of follow-up. Long in advance to these results, the EPIC-Norfolk study had found that plasma vitamin C concentrations in 8,860 men and 10,636 women were

inversely correlated to mortality from all causes and CVD (Khaw et al. 2001). In this study, each 20 μmol/L increase in plasma vitamin C was associated with a 20–30 % risk reduction for all-cause and CVD mortality.

Vitamin C is a remarkable water-soluble antioxidant concentrated predominately in citrus fruits, strawberries and vegetables (e.g. spinach and broccoli) and found in many supplement formulations (LPI Micronutrient Information Center). The adequate functioning of the human organism in general, and of the brain in particular, is highly dependent on vitamin C, but humans are completely dependent on dietary sources due to the evolutionary process leaving us a gene incapable of producing the enzyme gulonolactone oxidase needed to yield ascorbic acid from glucose (Nishikimi et al. 1994). Thus, humans require a constant stream of ascorbic acid from the diet and rely on robust "carrier" transport and "barrier" integrity mechanisms to meet the brain's demand. Vitamin C is the most powerful water-soluble antioxidant of the organism, and key to prevent oxidative lipid damage in biological systems (Frei et al. 1989). It forms the first line of antioxidant defence under many types of oxidizing conditions. It can rapidly intercept free radicals in the aqueous phase before they attack lipids (Berger et al. 1997). As an antioxidant, vitamin C also provides protection against oxidative stress-induced cellular damage by neutralization of lipid hydroperoxyl (LHP) radicals and by protecting proteins from alkylation by electrophilic lipid peroxidation activity (for review, see Harrison et al. 2010).

Vitamin C plays a role in the function of endothelial nitric oxide synthase (eNOS) by recycling the eNOS cofactor, tetrahydrobiopterin, relevant to arterial elasticity and blood pressure regulation (Traber and Stevens 2011). In addition to the more well-known functions of vitamin C, such as synthesis and deposition of collagen in the basement membrane and antioxidant defence against oxygen and other radicals, roles include stimulating endothelial proliferation, inhibiting apoptosis, and sparing endothelial cell-derived nitric oxide to help modulate blood flow (May et al. 2013). While the position of vitamin C in vascular pathology is well established, this might have much larger implications in age-related diseases, as more recently vascular comorbidities including atherosclerosis and increased intima–media thickness are being acknowledged as important factors in age related dementias (de la Torre et al. 2000; Polidori et al. 2001, 2004; Knopman et al. 2001; Wendell et al. 2009; 2012). Some of the vitamin C-mediated protective "vascular" mechanisms negatively affecting dementia onset and progression may include: (1) reducing carotid intima–media-thickness; (2) reducing lipid peroxidation and (3) reducing endothelial dysfunction (for review, see Harrison et al. 2010).

When 563 elderly men were randomly allocated to one of four treatment groups in a clinical trial that included dietary intervention, omega-3 supplementation, both or neither, carotid IMT progression over a three-year term was reduced in those undergoing dietary intervention that included daily vitamin C intake (Ellingsen et al. 2009). Vitamin E (Hodis et al. 2002) or combined antioxidants (Zureik et al. 2004) in the dietary intervention groups were not successful. These data suggest that vitamin C has a role in modifying vascular risk factors and vascular disease, which could represent mechanisms by which atherosclerosis might reduce dementia risk in people carrying this vascular risk profile (Luzzi et al. 2010). As far as cognitive performance in our study is concerned, the absence of statistically significant differences in MMSE scores among groups do not allow, unfortunately, the exploration and detection of an influence of the vitamin C/C-IMT relationship on cognitive status. However, the trends to significance of decreasing CC-IMT values across MMSE quintiles might warrant further research in patients with different degrees of cognitive performance and C-IMT.

In conclusion, higher vitamin C plasma levels appear to be protective against elevated carotid IMT values in the older persons. In light of the increased risk for cardiovascular disease, cerebrovascular disease and cognitive impairment shown by patients with increased IMT, the achievement of a better antioxidant status in general and of vitamin C in particular should be always encouraged through natural nutrition in the elderly.

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Conflict of interest There are no conflicts of interest to disclose.

References

Aronow WS, Fleg JL, Pepine CJ, Artinian NT, Bakris G, Brown AS, Ferdinand KC, Ann Forciea M, Frishman WH, Jaigobin C, Kostis JB, Mancia G, Oparil S, Ortiz E, Reisin E, Rich MW, Schocken DD, Weber MA, Wesley DJ, Harrington RA, Bates ER, Bhatt DL, Bridges CR, Eisenberg MJ, Ferrari VA, Fisher JD, Gardner TJ, Gentile F, Gilson MF, Hlatky MA, Jacobs AK, Kaul S, Moliterno DJ, Mukherjee D, Rosenson RS, Stein JH, Weitz HH, Wesley DJ, ACCF/AHA (2011) Expert consensus document on hypertension in the elderly: a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents developed in collaboration with the American Academy of Neurology. American Geriatrics Society, American Society for Preventive Cardiology,

American Society of Hypertension, American Society of Nephrology, Association of Black Cardiologists, and European Society of Hypertension J Am Soc Hypertens 5:259–352

Berger TM, Polidori MC, Dabbagh A, Evans PJ, Halliwell B, Morrow JD, Roberts LJ II, Frei B (1997) Antioxidant activity of vitamin C in iron-overloaded human plasma. J Biol Chem 272:15656–15660

Boekholdt SM, Meuwese MC, Day NE, Luben R, Welch A, Wareham NJ, Khaw KT (2006) Plasma concentrations of ascorbic acid and C-reactive protein, and risk of future coronary artery disease, in apparently healthy men and women: the EPIC-Norfolk prospective population study. Br J Nutr 96:516–522

Breteler MM (2000) Vascular involvement in cognitive decline and dementia. Epidemiologic evidence from the Rotterdam Study and the Rotterdam Scan Study. Ann N Y Acad Sci 903:457–465

Breteler MM, Bots ML, Ott A, Hofman A (1998) Risk factors for vascular disease and dementia. Haemostasis 28:167–173

de la Torre JC, Stefano GB (2000) Evidence that Alzheimer's disease is a microvascular disorder: the role of constitutive nitric oxide. Brain Res Brain Res Rev 34:119–136

Dehlendorff C, Andersen KK, Olsen TS (2014) Body Mass Index and death by stroke: No Obesity Paradox. JAMA Neurol 71:971–984

Dias IH, Mistry J, Fell S, Reis A, Spickett CM, Polidori MC, Lip GY, Griffiths HR (2014) Oxidized LDL lipids increase β-amyloid production by SH-SY5Y cells through glutathione depletion and lipid raft formation. Free Radic Biol Med 75:48–59

Ellingsen I, Seljeflot I, Arnesen H, Tonstad S (2009) Vitamin C consumption is associated with less progression in carotid intima media thickness in elderly men: A 3-year intervention study. Nutr Metab Cardiovasc Dis (NMCD) 19:8–14

Flohé L, Günzler WA (1984) Assays of glutathione peroxidase. Methods Enzymol 105:114–121

Frei B, England L, Ames BN (1989) Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci USA 86:6377–6381

Harrison FE, Green RJ, Dawes SM, May JM (2010) Vitamin C distribution and retention in the mouse brain. Brain Res 12(1348):181–186

Hodis HN, Mack WJ, LaBree L, Mahrer PR, Sevanian A, Liu CR, Liu CH, Hwang J, Selzer RH, Azen SP (2002) Alpha-tocopherol supplementation in healthy individuals reduces low-density lipoprotein oxidation but not atherosclerosis: the Vitamin E Atherosclerosis Prevention Study (VEAPS). Circulation 106:1453–1459

Isogawa A, Yamakado M, Yano M, Shiba T (2009) Serum superoxide dismutase activity correlates with the components of metabolic syndrome or carotid artery intima-media thickness. Diabetes Res Clin Pract 86:213–218

Khaw KT, Bingham S, Welch A, Luben R, Wareham N, Oakes S, Day N (2001) Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. European Prospective Investigation into Cancer and Nutrition. Lancet 357:657–663

Klipstein-Grobusch K, den Breeijen JH, Grobbee DE, Boeing H, Hofman A, Witteman JC (2001) Dietary antioxidants and peripheral arterial disease: the Rotterdam Study. Am J Epidemiol 154:145–149

Knopman D, Boland LL, Mosley T, Howard G, Liao D, Szklo M, McGovern P, Folsom AR (2001) Cardiovascular risk factors and cognitive decline in middle-aged adults. Neurology 56:42–48

Kutnink MA, Hawkes WC, Schaus EE, Omaye ST (1987) An internal standard method for the unattended high-performance liquid analysis of ascorbic acid in blood components. Anal Biochem 166:424–430

L'Abbé MR, Fischer PW (1990) Automated assay of superoxide dismutase in blood. Methods Enzymol 186:232–237

Larson EB, Yaffe K, Langa KM (2013) New insights into the dementia epidemic. N Engl J Med 369:2275–2277

Lester SJ, Eleid MF, Khandheria BK, Hurst RT (2009) Carotid intima-media thickness and coronary artery calcium score as indications of subclinical atherosclerosis. Mayo Clin Proc 84:229–233

Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M (2007) Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation 30(115):459–467

Luzzi S, Vella L, Bartolini M, Provinciali L, Silvestrini M (2010) Atherosclerosis in the evolution of Alzheimer's disease: Can treatment reduce cognitive decline? J Alzheimer's Dis 20:893–901

Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, Grassi G, Heagerty AM, Kjeldsen SE, Laurent S, Narkiewicz K, Ruilope L, Rynkiewicz A, Schmieder RE, Struijker Boudier HA, Zanchetti A, Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Kjeldsen SE, Erdine S, Narkiewicz K, Kiowski W, Agabiti-Rosei E, Ambrosioni E, Cifkova R, Dominiczak A, Fagard R, Heagerty AM, Laurent S, Lindholm LH, Mancia G, Manolis A, Nilsson PM, Redon J, Schmieder RE, Struijker-Boudier HA, Viigimaa M, Filippatos G, Adamopoulos S, Agabiti-Rosei E, Ambrosioni E, Bertomeu V, Clement D, Erdine S, Farsang C, Gaita D, Kiowski W, Lip G, Mallion JM, Manolis AJ, Nilsson PM, O'Brien E, Ponikowski P, Redon J, Ruschitzka F, Tamargo J, van Zwieten P, Viigimaa M, Waeber B, Williams B, Zamorano JL, The task force for the management of arterial hypertension of the European Society of Hypertension, The task force for the management of arterial hypertension of the European Society of Cardiology (2007) Guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J 28:1462–1536

Mariani E, Polidori MC, Cherubini A, Mecocci P (2005) Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview. J Chromatogr B Analyt Technol Biomed Life Sci 827:65–75

May JM, Harrison FE (2013) Role of vitamin C in the function of the vascular endothelium. Antioxid Redox Signal 19:2068–2083

Mecocci P, Polidori MC (2012) Antioxidant clinical trials in mild cognitive impairment and Alzheimer's disease. Biochim Biophys Acta 822:631–638

Nierenberg DW, Nann SL (1992) A method for determining concentrations of retinol, tocopherol, and five carotenoids in human plasma and tissue samples. Am J Clin Nutr 56:417–426

Nishikimi M, Fukuyama R, Minoshima S, Yagi K (1994) Cloning and chromosomal mapping of the human nonfunctional gene for l-gulono-gamma-lactone oxidase, the enzyme for l-ascorbic acid biosynthesis missing in man. J Biol Chem 269:13685–13688

Polidori MC, Pientka L (2012) Bridging the pathophysiology of Alzheimer's disease with vascular pathology: the feed-back, the feed-forward, and oxidative stress. J Alzheimers Dis 28:1–9

Polidori MC, Mecocci P, Frei B (2001) Plasma vitamin C levels are decreased and correlated with brain damage in patients with intracranial hemorrhage or head trauma. Stroke 32:898–902

Polidori MC, Pratico D, Savino K, Rokach J, Stahl W, Mecocci P (2004) Increased F2 isoprostane plasma levels in patients with congestive heart failure are correlated with antioxidant status and disease severity. J Card Fail 10:334–338

Polidori MC, Praticó D, Parente B, Mariani E, Cecchetti R, Yao Y, Sies H, Cao P, Mecocci P, Stahl W (2007) Elevated lipid peroxidation biomarkers and low antioxidant status in

atherosclerotic patients with increased carotid or iliofemoral intima media thickness. J Investig Med 55:163–167

Polidori MC, Pientka L, Mecocci P (2012) A review of the major vascular risk factors related to Alzheimer's disease. J Alzheimers Dis 32:521–530

Prince M, Acosta D, Ferri CP, Guerra M, Huang Y, Llibre Rodriguez JJ, Salas A, Sosa AL, Williams JD, Dewey ME, Acosta I, Jotheeswaran AT, Liu Z (2012) Dementia incidence and mortality in middle-income countries, and associations with indicators of cognitive reserve: a 10/66 Dementia Research Group population-based cohort study. Lancet 380:50–58

Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP (2013) The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement 9:63–75

Renaud S, Ruf JC (1994) The French Paradox. Circulation 90:3118–3119

Riemersma RA (1996) Coronary heart disease and vitamin E. Lancet 347:776–777

Riemersma RA, Carruthers KF, Elton RA, Fox KA (2001) Plasma ascorbic acid and risk of heart disease and cancer. Lancet 357:2136–2137

Salmina AB, Inzhutova AI, Malinovskaya NA, Petrova MM (2010) Endothelial dysfunction and repair in Alzheimer-type neurodegeneration: neuronal and glial control. J Alzheimers Dis 22:17–36

Sidhu PS, Allan PL (1997) Ultrasound assessment of internal carotid artery stenosis. Clin Radiol 52:654–658

Simon JA, Hudes ES, Tice JA (2001) Relation of serum ascorbic acid to mortality among US adults. J Am Coll Nutr 20:255–263

Traber MG, Stevens JF (2011) Vitamins C and E: beneficial effects from a mechanistic perspective. Free Radic Biol Med 51:1000–1013

Tribble DL (1999) AHA Science Advisory: Antioxidant consumption and risk of coronary heart disease: emphasis on vitamin C, vitamin E and beta-carotene: a statement fort he healthcare professionals from the American Heart Association. Circulation 99:591–595

Wendell CR, Zonderman AB, Metter EJ, Najjar SS, Waldstein SR (2009) Carotid intimal medial thickness predicts cognitive decline among adults without clinical vascular disease. Stroke 40:3180–3185

Wendell CR, Waldstein SR, Ferrucci L, O'Brien RJ, Strait JB, Zonderman AB (2012) Carotid atherosclerosis and prospective risk of dementia. Stroke 43:3319–3324

Zureik M, Galan P, Bertrais S, Mennen L, Czernichow S, Blacher J, Ducimetiere P, Hercberg S (2004) Effects of long-term daily low-dose supplementation with antioxidant vitamins and minerals on structure and function of large arteries. Arterioscler Thromb Vasc Biol 24:1485–1491