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► To cite this version:

Marie-Laure Ancelin, Yves Christen, Karen A. Ritchie. Is antioxidant therapy a viable alternative for mild cognitive impairment? Examination of the evidence.: Antioxidant therapy and cognitive decline. *Dementia and Geriatric Cognitive Disorders*, 2007, 24 (1), pp.1-19. 10.1159/000102567 . inserm-00169500

HAL Id: inserm-00169500

<https://inserm.hal.science/inserm-00169500>

Submitted on 10 Nov 2009

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Is anti-oxidant therapy a viable alternative for mild cognitive impairment? Examination of the evidence

Short title: Antioxidant therapy and cognitive decline

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Key Words: Oxidative stress; Antioxidants; Vitamins; *Ginkgo biloba*; Mild Cognitive Impairment; Alzheimer's disease

Abstract

Therapeutic interventions for the prodromal stages of dementia are currently being sought with a view to delaying if not preventing disease onset. Uncertainty as to whether cognitive disorder in a given individual will progress towards dementia and adverse drug side-effects has led to hesitancy on the part of drug regulators to instigate preventive pharmacotherapies. In this context, anti-oxidant therapies may provide a low-risk alternative, targeting very early biological changes. While a growing body of knowledge demonstrates both the importance of oxidative stress in the aetiology of dementia and the efficacy of antioxidant treatment in animal and cellular models, studies in humans are presently inconclusive. While some antioxidants, notably flavonoid- or vitamin- rich diets, appear to lower the relative risk for Alzheimer's disease in humans in observational studies, these results must be interpreted in the light of the biological complexity of the relationship between oxidative stress and neurodegeneration, and the methodological and theoretical shortcomings of studies conducted to date. A clearer understanding of these factors will assist in the interpretation of the results of the intervention studies which are now being undertaken; these studies being the only current means of establishing efficacy for preventive drug treatment of Alzheimer's disease.

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Introduction

Katzman and Fox [1] have estimated that there are currently around 20 million prevalent cases of dementia in the world, with a doubling of rates predicted in the next 30 - 50 years. With an average life expectancy from diagnosis of around 8 years, the disorder is associated with high levels of dependency, consumption of medical services, and care-giver morbidity, thus constituting a major public health burden. In the absence of a specific and effective treatment, intervention has focused on the prodromal stages of dementia, or mild cognitive disorder (MCI; [2]) in order to delay onset and reduce the rate of progression. Given the complexity of current aetiological models, it is highly unlikely that a single treatment strategy will be developed in the near future. However a number of intervention points are now being identified, focusing on vascular, inflammatory, endocrinological and cellular risk factors which may lower the individual risk of developing dementia. While all these approaches have been based on robust biological arguments, they remain for the most part highly contested due to adverse side effects or failure to produce an effect at all. Additionally, given the current poor predictive validity of diagnostic algorithms for identifying those subjects with cognitive impairment who will actually go on to dementia, the application of such treatments for wide-scale prevention is considered both risky and expensive. It is within this context that prevention trials for hormonal treatments and anti-inflammatory drugs have now been stopped, leaving antioxidant treatment as the main current therapeutic option, alongside other low-risk alternatives such as increasing physical activity. Antioxidant therapy could be delivered through modifications in the normal diet or through therapeutic intervention. Nonetheless, it requires supervised administration to avoid the adverse effects which could occur at very high doses [3]. It remains, however, to establish (a) their biological coherence and (b) their treatment effectiveness.

a) The biological arguments

Oxidative stress and neurodegeneration

A large body of research has consistently confirmed the implication of free radicals both in normal cerebral ageing and ageing-related pathologies. The most compelling evidence comes from genetic mutations which simultaneously prolong life-span in numerous species (notably *Caenorhabditis elegans* and the fruit fly) and modulate oxidative stress [4]. This association is particularly marked in the presence of neurodegenerative disease because (a) the neurons have

an extended membrane surface increasing the possibility of lipoperoxidation, (b) the oxygen metabolism is raised in cerebral areas and (c) their glutathione content (a natural antioxidant) is low [5].

One of the most important pathological signs of Alzheimer's disease (AD) is extracellular amyloid- β ($A\beta$) peptide deposition, which has been linked to increased ageing-related oxidative stress. Subsequent chelation of transition metal ions by $A\beta$, accumulation of toxic $A\beta$ -metal complexes, and production of reactive oxygen species (ROS), may lead to further increases in oxidative stress and neurotoxicity relevant to AD [4, 6, 7]. Current findings converge to suggest that β -amyloid produces free radicals, a process which is particularly vulnerable to oxidative stress, and that this chain probably contributes largely to cell death in AD [8]. Recent studies suggest the involvement of metals in oxidative stress in neurodegenerative disorders. Pathological brain iron deposition has been shown to be a possible source of neurotoxic ROS in AD [9, 10] linked to H63D and C282Y HFE mutations.

The process of oxidation generally involves increased levels of oxidatively altered metabolites, compensatory defense reactions, oxidative stress responses, and disturbances of the mitochondrial metabolism, which, in turn, may account for an increased leakage of ROS originating from the reaction of the respiratory chain. The different types of antioxidants can thus act by inhibition of free radical formation, direct chemical (non enzymatic) scavenging of generated free radicals by antioxidant compounds, enzymatic detoxification of accumulating ROS, and long term support and induction of cellular self-defense by initiation of gene transcription [11]. Thus while the principal action of antioxidants is generally considered to be their ability to protect cells from lesions induced by free radicals, they also appear to have a more general impact on cell regulation, notably in relation to mitochondrial energy metabolism, cell signalling and gene expression.

Biological markers

ROS are short-lived and difficult to measure in biological samples. However, there are indirect indices that can be used to examine the consequences of ROS production, including the different potential macromolecule targets for oxidation (lipids, proteins, DNA and glycols). There are also various markers of the response to oxidative stress, with two main mechanisms of defense, including antioxidant enzymes, ROS scavengers [*e.g.* superoxide

dismutase (SOD), catalase, glutathione-peroxidases (GPx)] and « chain breaking antioxidants », such as vitamin C (which blocks the creation of nitrosamines by reducing nitrites and acts as a co-oxidant for vitamin E), vitamin E and carotenes, which may modulate lipid peroxidation [12]. An association has been sought by numerous studies between these markers of oxidation from various locations [brain tissue, cerebrospinal fluid (CSF), plasma, red blood cell (RBC)] and cognitive impairment. While some changes may be specific to cognitive impairment and AD, it should be noted that others are also observed in normal brain ageing, although generally to a lesser degree [13], underlining the importance of using age-matched controls. **Table 1** summarizes the main results published in the past 10 years. While observations remain somewhat inconclusive, there does appear to be an overall association between MCI, AD and low plasma levels of vitamins E, C and carotene. However, a recent case-control study, including 65 patients with AD, 293 dementia-free subjects with cognitive decline, and 437 controls within the Rotterdam Study, reported an association between AD (but not MCI) and plasma vitamin E which disappeared after multiadjustment for age, gender, total cholesterol education, current smoking and BMI [14].

The relationship with enzyme activity is more controversial, although some studies suggest an interactive effect with the ApoE4 allele as also reported for brain malondialdehyde (MDA) levels [15, 16]. One longitudinal study reported that after 5 years, AD patients had poorer cognitive performance and significantly increased erythrocytic MDA and SOD with decreased GPx, compared to baseline [17]. The increase in MDA level was reported as a marker that differentiates AD from normal aging, since it was elevated in AD patients and not correlated with age, in contrast with SOD activity, whose increase appears to be a valid marker for ageing. In AD an increase in lipid oxidation markers is commonly observed in parallel with a lowering of polyunsaturated fatty acids. Alterations are also observed in markers of protein and DNA oxidation and glyco-oxidation. Similar but less marked changes are also occasionally observed in plasma. This has thus led to the finding of a markedly poor antioxidant status and function in AD patients compared to controls. Regarding the association of oxidation markers with cognitive performance in non-demented patients, elevated isoprostane levels were found in plasma, urine, and CSF of MCI subjects as compared with controls in a cross sectional analysis [18] as well as in a small 2-year longitudinal study [19]. Lower plasma and erythrocyte SOD, and lower plasma GPx as compared with controls were also reported [20]. A significant association was reported in longitudinal but not cross-sectional analyses of cognitive decline after 4 years with low

plasma thiobarbituric acid-reactive substances and low erythrocytic GPx but not SOD [21-23].

A Critique of the biological arguments

The fact that there is a multitude of oxidants as well as antioxidants, with overlapping reactivities, renders a biochemically rigorous assessment of the implication of oxidative stress difficult. Furthermore there is considerable methodological variability (a) in the methods used to measure antioxidants and (b) the target populations with regard to age, diet, lifestyle, comorbidities and social conditions (see [24]). Measures of peripheral status, such as dietary intake and blood antioxidant levels, do not necessarily reflect the availability of antioxidants centrally in the brain and the redox balance in the CNS. Montine et al. [25] notably reported that peripheral (plasma and urine) isoprostanes and neuroprostanes do not accurately reflect their CNS levels and are not reproducibly elevated in body fluids outside of central nervous system in AD patients. Moreover different brain regions are likely to have differential susceptibility to oxidative stress. The interpretation of the results is also complex: antioxidants may be decreased due to oxidation associated with ageing and dementia onset, and the modifications in antioxidant concentrations can be primary or secondary to the diet. Antioxidants are both parallel (different antioxidants such as GPx and catalase can play nearly similar roles in different cell compartments) and serial (enzymes such as SOD and GPx operate in tandem to break down radicals into harmless products) [26]. In addition, antioxidants can compete with each other at multiple levels (*e.g.* cellular or tissue level), as well as in the gastrointestinal track, thus modifying absorption and bioavailability. Consequently, measuring individual antioxidant activities may not be useful or meaningful. Another complication is that defenses are induced by stress. Therefore, a higher level of antioxidants may indicate better protection, or alternatively, a greater need for antioxidant defenses due to increased oxidant production.

b) Treatment effectiveness of antioxidants

Theoretically progression from MCI towards neurodegenerative pathologies may be considered to result from the addition of 2 processes: a specific process of protein aggregation and a non-specific process of senescent-related oxydative stress. Thus in theory both specific and non-specific therapeutic interventions may be considered. Antioxidants are generally

considered to fall into the latter category [27], although recent findings suggest that there may be oxidative mechanisms specific to AD relative to fronto-temporal dementia [28].

Numerous antioxidants have been successfully tested in experimental animal models of AD and cell cultures; notably vitamin E [29, 30], vitamin C [30], *Ginkgo biloba* extract EGb 761 containing both flavonoids and terpenes [31-35], blueberry [36], curcumin [37], spinach [38], other nutritionally-derived antioxidants [39], melatonin [40], huperzine [41], N-acetylcysteine and glutathione [42], synthetic EUK compounds [43], selegiline [44], idebenone [45] and overall diet rich in fruit and vegetables [46].

Apart from their effects on experimental models of AD, some of these substances also appear to have a direct impact on normal cognitive functioning, notably memory, in a number of animal models. This has been demonstrated for vitamin E [47], idebenone [48], EUK compounds [49], *Ginkgo biloba* extract EGb 761 [50], red fruits and spinach [38, 46], apple juice [51] and preparations rich in dietary antioxidants [52]. Similar effects have also been noted in response to caloric restriction which reduces oxidative metabolism and is also associated with increased longevity [53]. As a treatment, however, caloric restriction not only poses problems of compliance, but would also carry more health risks than simply increasing the dietary intake of antioxidants through fruit and vegetables. Its beneficial effects also appear to be limited to an early point in the lifespan [54].

While many studies have demonstrated the protective effect of a diet rich in antioxidants with regard to cardiovascular disease [4], few studies have examined its relationship to MCI and dementia. In this review, we excluded case-control studies which are biased due to selective recall and mortality, and retrospective transversal studies which confound cause and effect to consider only prospective observational studies and clinical trials. With regard to cognitive functioning, 5 epidemiological studies have looked at vitamin supplements (**table 2a**) but not all have adjusted for major confounding factors such as depression and ApoE status. Of the 3 studies examining vitamin E and C supplements, 1 [55], found a positive association with specific cognitive tests (short term memory and verbal fluency), and the 2 others showed a link with global cognitive functioning [56, 57]. A modest association has also been demonstrated with vitamin E supplements (2 out of 4 studies) on both specific cognitive tests and global functioning [55, 56], and vitamin C on global functioning alone in two out of five studies [56, 58]. Ten studies have looked at diet and cognition, however, none took ApoE

status into account and only 1 has controlled for depression (**table 2b**). The majority of studies (8 out of 10) show a positive association either with vitamin C, vitamin E or carotenoids, mostly on global functioning tests [58-64] although there is some evidence for specific effects (abstraction, memory, visuospatial performance [65]. No study has examined the combined effects of vitamins E and C in diet.

To date none but 1 [66] of 7 epidemiological studies which have evaluated individual vitamin treatments (C or E; **table 2c**) have shown consistent impact with regard to reduction of AD incidence. The 3 studies examining multivitamin supplements found no effect on AD incidence, but 2 of them did not control for ApoE genotype [66]. The association of vitamins E and C have demonstrated a lowering of risk for all dementias (incidence and prevalence) in 2 studies out of 4 [66, 67] and in 1 study for prevalent vascular dementia [56]. Of the 5 studies examining dietary vitamin content (**table 2d**), three suggest a reduction of incident AD risk in relation to vitamin E intake [64, 68], which could be specific to ApoE4 non-carriers [69], vitamin C [68] and possibly also carotene and flavonoid, at least in the case of smokers who are recognized as having a high oxidative burden [68].

G Biloba extract EGb 761 has been examined within the context of French longitudinal study of hip fracture (EPIDOS Study). Multivariate analysis showed that women developing dementia were less exposed to vasodilators (OR 0.31). Separating EGb 761 users from the other vasodilators gave similar odds-ratios, but did not reach statistical significance [70]. More recently, the analyses of the PAQUID study concluded that EGb 761 (but not vasodilators) increases the survival time without dementia [71].

The protective effects of alcohol, notably wine which is rich in flavonoids, with regard to dementia risk have been examined within a number of population cohorts, Paquid [72-74], the Eugeira Study [75], the Copenhagen City Heart Study [76], the Rotterdam Study [77], and the CardioVascular Health Study [78, 79]. A comparable association was also reported in healthy women with some cognitive functions, especially those related to verbal knowledge and phonemic fluency (see [80]). These studies all found at least a small protective effect against dementia with mild to moderate alcohol consumption, although the Eugeira study found this effect disappeared when place of residence was entered into the regression model (persons in institutions are usually forbidden alcohol but commonly have dementia). Few studies have

made the distinction between wine and other sources of alcohol, for while the flavonoids are free radical scavengers and neuroprotective [81], ethanol itself produces neurotoxic oxidative stress. Of the few studies which have made this distinction, 2 have shown a beneficial effect specific to wine [76, 79], the third a beneficial effect no matter what type of alcohol [77], whereas Commenges et al. [74] only found a beneficial effect in terms of dementia prevention when the total of all flavonoid consumption was considered and not specifically for wine. ApoE status was taken into account by only 4 studies, showing a protective effect in ApoE4 non-carriers with regard to cognitive impairment [82] and dementia [78, 79], although not systematically [83]. All studies have, however, been open to bias, particularly with regard to refusal rates, the unknown characteristics of unavailable or deceased subjects, and failure to distinguish former drinkers from long-term abstainers. The methods used to establish dementia onset have also often been questionable.

Clinical trials

Vitamin E (2000 IU/day), and to a lesser extent selegiline (10 mg/day) have been claimed to significantly slow the progression of moderate AD to severe dementia within a 2- to 3-year trial (median time = 670 and 655 days respectively, compared to 440 days with the placebo group) [84]. It should be noted, however, that the combination of these diminishes the effect (585 days) [84]. This randomized controlled trial (RCT) of vitamin E, used a very high dose (*i.e.* more than 30-fold the recommended daily dose), so it is not possible to determine whether antioxidant intake in this range is associated with a lower risk of AD in individuals without dementia. Furthermore the effect of ApoE status was not determined and the assumptions of the statistical tests were violated such that other factors, notably socio-economic status, may have explained the results [85, 86]. A meta-analysis carried out by Tabet et al. [87] thus concluded that there presently exists « insufficient evidence of efficacy of vitamin E in the treatment of people with AD ».

More recently, a larger 3-year RCT was performed by the same research group, including 769 patients with MCI randomly assigned to receive 2,000 IU vitamin E, 10 mg donepezil or placebo daily. Vitamin E showed no benefit on MCI and did not significantly slow the progression of MCI to AD, irrespective of the ApoE4 status [88]. The detailed analysis of psychometric testing also showed no significant difference between vitamin E and placebo, for memory, executive and visuospatial performances and a slight improvement for language but with no clinical relevance.

G biloba extract EGb 761 has been demonstrated to produce a significant effect on the Alzheimer's Disease Assessment Scale - cognitive subscale and other cognitive batteries in several RCT, notably that of Le Bars et al. [89] or Kanowski and Hoerr [90] on mild to severe AD patients and multi-infarct dementia patients. Another 6-month RCT only showed better cognitive performance for a subgroup of demented patients with neuropsychiatric symptoms [91], but the lack of deterioration of the placebo patients may have compromised the sensitivity of the trial to detect a treatment effect. Another study of AD, vascular dementia and MCI found no significant effect on Syndrome Kurz Test (psychometric functioning) [92, 93]. A meta-analysis found an overall beneficial effect with regard to cognitive functioning, while pointing out, however, some inconsistent results [94].

Clinical trials with other antioxidants on AD patients have shown limited and unconfirmed positive effects, for example N-acetylcysteine [95], α -lipoic acid [96], idebenone [97-101], metal chelators [102], and clioquinol [103, 104].

In healthy subjects, trace elements and multivitamin supplements did not appear to have beneficial effect on cognitive functioning in 220 elderly women after 6 months of treatment [105]. EGb 761 appear to have a beneficial effect on cognitive functioning in normal subjects in several studies (including Mix and Crews [106]), but not in another [107]. Finally there is some evidence to suggest that the micronutrients in fruit and vegetables, notably vitamins E and C may have variable effects on cerebral functioning [108, 109]. Nevertheless, the recent RCT of the Age-Related Eye Disease Study did not support a beneficial effect of antioxidants (vitamin C 500 mg; vitamin E 400 IU; β -carotene 15 mg) on cognition in 2166 older adults after 6.9 years of treatment [110]. At this point in time it is difficult to determine the true value of these clinical observations, also bearing in mind that negative results are often not published.

Shortcomings in previous studies

In relation to the above observations, it appears globally that vitamins could be more protective against cognitive decline than against AD incidence with a greater effect of dietary intake than supplement use (which was revealed to be of very low efficiency for AD prevention or treatment). Vitamin E was as efficient as, or slightly more efficient, than vitamin C, especially when considering diet intake and AD. Supplementation with both vitamin C and E appeared superior to supplementation with either vitamin alone in AD or

cognition studies, and multivitamin supplement use did not reveal efficient. However, it must be remembered that studies of supplement use are not directly comparable with studies of normal food intake for a number of reasons.

- (a) Individual supplements and multivitamin preparations are in themselves very different. Multivitamin preparations contain very low doses of vitamin, *i.e.* 30 IU vitamin E and around 60 mg vitamin C, corresponding approximately to the daily recommended dose, while individual supplements typically contain doses of up to 400-2,000 IU of vitamin E and 500 - 1,000 mg or more of vitamin C. The absence of an association between multivitamin use and AD in contrast to the positive associations reported with combined vitamin E and C supplementation suggests that lower doses are insufficient to produce a protective effect [66, 67], as also reported for coronary heart disease [111, 112].
- (b) There are some arguments for a higher efficacy of vitamins taken in combination rather than alone. Vitamin C is water soluble and rapidly excreted after ingestion, which could explain why it is not active when taken alone. Its effect may be limited to the reduction of the lipid-soluble vitamin E after the latter has been oxidized. The major antioxidative action of α -tocopherol is operative when free radicals are formed at relatively high rates, *i.e.* under strong oxidative conditions, whereas this could evolve into pro-oxidant under mild oxidative conditions [113]. Ascorbic acid alone could exacerbate iron-dependent radical reactions in the brain and vitamin C (500 mg/day) may act as a pro-oxidant in the body [114]. This ability of antioxidants to act as pro-oxidants could explain the superiority of the combined supplementation with vitamin E and C over the supplementation with high doses of vitamin E alone [115].
- (c) There are several differences between supplement preparations and diet. Dietary antioxidant intake is much lower than that of supplements. Cooking and storage may also destroy vitamins and dietary vitamin intake is lower in institutions than at home. Nonetheless, vitamin dietary intake appears more efficient in preventing cognitive decline than vitamin supplementation [62, 66-68]. Antioxidants from food are always simultaneously consumed with other nutrients that might modify absorption or facilitate their bioavailability. For vitamin E, the activity could be different from the function of tocopherol composition [116]. The primary variant in vitamin E supplements is α -tocopherol, whereas dietary vitamin E is primarily γ -tocopherol which may have stronger antioxidant and anti-inflammatory properties than α -tocopherol or act synergistically. Morris et al. [64] recently suggested that the

combined intake of various tocopherol forms (especially α - and γ - tocopherol, or α -tocopherol equivalents) may be more important than α -tocopherol alone in the protective relations with AD and cognitive decline. Taken together with the fact that intake of antioxidants from food reflects long-term intake, whereas supplement intake is generally of shorter duration, this could explain why high lifetime intake from food would more likely be related to AD decrease [68] than short term high intake of supplements, especially if there is a crucial period for optimal effect.

- (d) In most studies only 1 evaluation of diet is undertaken (usually qualitative not quantitative), which raises the possibility that the observed findings could be due to unreliable reporting or dietary changes in the course of the survey. In the CHAP (Chicago Health and Aging Project) study, 9% of the participants reported taking a vitamin E supplement in 1994 compared with 19% in 1997 [69]. This calls into question whether a negative finding may be due to an insufficient period of use for protective benefit or due to persons taking vitamin supplement in response to cognitive problems (bias of indication), or with a reversal of such behaviour as the illness progresses.
- (e) Food-frequency questionnaire (FFQ) assessment requires sustained motivation, attention and memory. Hence, patients with unimpaired cognitive functioning may be expected to provide more complete and accurate information.
- (f) There is little precision in dietary assessment. The validity of questionnaires for assessing vitamin intake from subjectively recalled food intake is marginal and poorly related to blood levels (r around 0.40 in validation studies of dietary vitamin E and 0.15, for vitamin C, or α - or β - carotene, by questionnaires versus blood concentrations, and even lower, near to 0, for β -tocopherol [64, 117]), but this could also be related to the fact that vitamin intake does not correlate highly with seric concentration, *e.g.* due to metabolism.
- (g) Imprecision in dietary assessment is greater for dietary than for supplemental vitamin intake: Morris et al [69] reported that the correlation between the FFQ and the average of repeated 24-hr recalls was 0.41 for total vitamin E (*i.e.* lower than for supplements, 0.67) and 0.46 for total vitamin C (0.60 with supplements).

Another source of inconsistency in the findings of clinical studies may be due to differences in study design:

- (a) Large studies of long duration are required to detect small effects of lower doses and to allow a higher number of statistical comparisons to avoid detection of associations due to chance.
- (b) Dietary intake of antioxidants has low inter-individual variability. In the CHAP, analyses were based on the quintiles of dietary intake of vitamin E that were approximately 1 IU/day (0.7 mg, a part or the equivalent of less than a tablespoon of peanut butter). Consequently, the small minority of patients with extremely low and high values disproportionately influenced the statistical model.
- (c) The criteria for cognitive assessment and diagnosis of dementia are highly variable, most studies having used tests of global cognition which cover a limited number of functions, have ceiling effects and are insensitive to the subtle changes of MCI.
- (d) There are several confounding factors influencing both the availability of anti-oxidants and oxidative status and cognitive function which have not been systematically controlled for. If small to moderate protective effects are expected (as for coronary heart disease, [111, 112]), it will be difficult for observational studies to provide a definitive answer because persons who choose to use vitamin supplements are likely to differ from non-users, *e.g.* with respect to personal and lifestyle characteristics that are difficult to assess but may affect risk of disease.
- (e) Age differences in successive cohorts need to be considered, as increased age is associated with increased oxidative stress (see [5])
- (f) Studies vary in the proportion of institutionalized persons included and in institutions nutritional status is poorer and cognitive impairment is common [118]. Poor cognitive status itself may be a risk factor for malnutrition even in subjects without MCI.
- (g) Illness and chronic conditions (*e.g.* renal insufficiency, physical disability, inability to chew adequately) as well as drug interaction for polymedicated elderly may affect intake of foods and the absorption and metabolism of nutrients and contribute to the risk of food insufficiency among the elderly [119]. This is especially true for depression (*e.g.* anorexia) which has been rarely evaluated and controlled for.
- (h) Differences in socio-demographic variables may influence the outcome of studies for example education differences linked to both cognitive impairment and diet, sex differences in diet [62].
- (i) Smoking may be associated with poor diet and may increase the load of free radicals and metabolic demand for antioxidants as well as sensitivity to the effects of antioxidants. In the Rotterdam study [68] no association was found between vitamin

E, β -caroten or flavonoid and AD among never smokers and a strong association among current smokers.

- (j) The effect of antioxidants on prevention of vascular dementia, stroke and atherosclerosis are other mechanisms by which dietary antioxidants may also reduce the risk of dementia associated with vascular dysfunction.
- (k) The statistical relationship between vitamins E and C and cognitive functioning could be incidental if nutrients also contain other protective substances. There is notably a possible co-variation of vitamin C or E and vitamin B (B6, B12, folates, which could be neuroprotective) and an inverse association with homocystein, a risk factor for both AD and vascular dementia [120, 121].
- (l) Apo E status may bias results as oxidative-stress-induced injury and protection by antioxidants in AD are related to ApoE genotype [15, 122]. In the CHAP population, vitamin E intake from foods was associated with reduced risk of developing AD, only among Apo E4-negative subjects [69]. The same observation has been made in relation to dementia or cognitive functioning and alcohol [78, 79, 82]. Although ApoE4 non-carriers could be more susceptible to oxidative stress and thus to antioxidant treatment, this has only been controlled for in the most recent studies.

Design of epidemiological studies and RCTs and related outcomes

Both *in vitro* and *in vivo* experimental evidence converges to support a positive role of antioxidants on neuronal functioning. Epidemiologic observational studies also suggest that antioxidants can protect against cognitive decline [55-69]. Nevertheless, none of the clinical trials performed to date have demonstrated a significant major clinical benefit in patients with cognitive decline. These findings are similar to observations made in relation to cardiovascular disease. In prospective, observational studies of primary prevention, the use of vitamin E supplements for ≥ 2 yrs (400 IU/day) has been associated with a 20 - 40% reduction in the risk of coronary disease [111, 112]. The published RCT focused, however, on persons with existing coronary disease (secondary prevention) and gave inconsistent results. Thus the weight of evidence is against short-term benefit from vitamin E supplements among patients with cardiovascular disease being treated with multiple pharmacologic agents. The long-term benefit of vitamin E supplementation for primary prevention remains unclear [123]. Current findings discourage the use of high doses of vitamin E for prevention in the elderly, especially given recent negative findings concerning vitamin E [3, 124, 125] and at high dosage (> 400 IU/day), an increase in all-cause mortality (for meta-analysis [3]). In a large

RCT enrolling 7,030 patients with vascular disease or diabetes mellitus, an increase in the risk of heart failure was observed, but not major cardiovascular events and deaths or cancer incidence and deaths [124]. On the other hand, in a larger RCT including 39,876 healthy women receiving 600 UI of vitamin E for 10.1 years, no significant effect was observed on cancer incidence or death or cardiovascular events, in contrast to a significant 24% reduction in cardiovascular death [125]. However, previous studies do not address the question of whether lower doses of vitamin E taken earlier, before the onset of neuropathological changes, might be helpful in the primary prevention of AD. Regarding vitamin C, there is little evidence to support the existence of a benefit of vitamin C supplementation beyond the range of the typical American diet or the current recommended dietary allowance of 90 mg for men and 75 mg for women (35 mg higher for smokers) and minimal effects might be expected from supplementation as tissues become saturated at about these levels of intake.

Of all the factors discussed above, short treatment duration (from 3 weeks to 3 years for RCT, *i.e.* too short for an effect to be detected), the age of patients (mean age >70 yrs) and the therapeutic administration window (probably far from the optimal period before the onset of neurodegeneration) most likely explain the discrepancies between studies. Epidemiological studies showing a beneficial effect of dietary antioxidants were those larger in size and involving younger subjects with a longer follow up [64, 68]. A recent study found no association between midlife intake and risk of late-onset AD 30 years later, however, dietary intake was only evaluated at baseline [126]. Animal models also suggest an age-dependent effect of antioxidant diet. Feeding rats with extracts of strawberry, spinach, or blueberry for 8 months from 6 months of age was effective in reversing age-related deficits in several neuronal and behavioural variables. However, feeding middle-aged mice (18 months of age) with vitamin E, strawberry extract or melatonin for 6 months had no effect on their psychomotor performance, although supplemental glutathione was effective [127]. This suggests that the timing of treatment is probably critical with no possibility of improvement once the neurodegenerative process has started, several years before cognitive alteration is apparent. This is also suggested by recent results from a transgenic mouse model of AD showing that only early treatment with vitamin E lead to a significant reduction in A β levels and amyloid deposition [128]. A critical period has also been suggested for estrogen which may act as a preventive agent for neurodegeneration, but could probably not act as a restorative agent in already degenerated neural tissue [129].

Current intervention studies

Antioxydants are more likely to be preventive rather than curative in relation to cognitive impairment and for this reason it is their impact on MCI rather than dementia which is of interest. A study of vitamin E supplement and MCI (the PREADVISE trial) has recently been incorporated into the SELECT Study [130] and EGb 761 use and cognitive performance is being examined in 3 studies; the Ginkgo Evaluation of Memory trial and the NIH Oregon Study in the USA, and GuidAge in France. However, despite the large size of these studies, they may not provide conclusive results, principally because it has not been possible to examine different doses. They also make the assumption that a time frame targeting older age will capture the protective effect, which may not be the case, as optimal neuroprotective effect may occur at a much earlier point in the life cycle, well before the first signs of cognitive impairment. Furthermore, in so far as the normal diet includes efficient antioxidants, notably flavonoids, it will not be easy to distinguish the specific effects of the medication. This means above all that it will be difficult to interpret negative results. Positive results, on the other hand, will provide evidence of efficacy but will be able to indicate neither the most efficient dose range nor the age at which treatment with vitamin E or EGb 761 should commence. There is thus still a need for new intervention trials and observational investigations based on large cohorts studied over longer periods of time, with several methods for assessing antioxidant exposure and controlling for many confounders to prove or disprove the hypothesis that exposure to antioxidants can prevent cognitive impairment likely to lead to neurodegenerative disease. Combinations of vitamins, minerals and herbal antioxidants could offer greater potential benefit than any single antioxidant, especially if the agents work in different cellular compartments or have complementary mechanisms of action. Nevertheless, it is not clear how to optimize the dose of each component or assure that when they are mixed they will not have an interacting toxicity or loss of efficacy. Saver and Kalafut [131] noted that testing 7 combinations of potential treatments for AD would yield 127 hierarchical, serial clinical trials enrolling 63,500 patients requiring 286 years. This practical problem needs to be kept in mind especially in light of the fact that, for the most part, patent protection is unattainable and therefore, few resources are available for the scientific study of alternative treatments. Large-scale testing of many compounds in humans is also complex, since reduction of oxidative damage is not a valid clinical outcome. An additional problem is that it is difficult to justify the treatment of normal subjects without any signs of dementia, over long periods. In practice lifestyle changes are difficult to initiate and to sustain for benefits

expected to occur only in late life. Another problem is linked to the cost of such exhaustive studies (Ginkgo Evaluation of memory and GuidAge require the follow-up of 3,000 people over 5 years on a single dose of EGb 761 [132]; testing 3 doses and several age cohorts is 10 times more costly). The ideal intervention trial is thus extremely difficult to perform. Despite the limitations of the current trials, their outcome may have important implications for clinical practice as in the face of the steady increase in the number of persons in the population with MCI and dementia, and the suspension of Cox 2 inhibitors and estrogen trials, vitamin and *G Biloba* treatment is currently the most promising avenue of risk-free intervention.

Critical comments relating to antioxidant effects

The positive effect of antioxidants is rarely questioned. However, a number of theoretical and practical points should be raised. The most obvious relates to the importance of oxydative mechanisms in cellular organisation, especially at the mitochondrial level, and whether free radical production is indeed a useful process. This is certainly likely to be the case with regard to the destruction of aggressive agents by phagocytes. Free radicals are not absolute enemies but rather partners in cell metabolism. In this respect it is reasonable to consider that in the case of ageing-related pathology there is likely to be a disequilibrium in favour of oxidative stress insufficiently compensated for in natural selection as it occurs over the age of reproduction. One would therefore logically expect medical science to reinforce such defences by therapeutic means in elderly subjects. It should also be emphasized that the antioxidants cited here (vitamin E, vitamin C, EGb 761, blackberry extract, diet rich in fruit and vegetables) have effects other than free radical scavenging which may be the true cause of observed beneficial effects [133].

Moreover, it is important to understand that the 2 principal antioxidants used in clinical medicine, vitamin E and *G biloba* are complex substances. Vitamin E contains 8 isoforms of tocopherol (with numerous biologically active metabolites), notably γ -tocopherol which plays an important role in peroxynitrite protection [116]. Preparations sold as dietary supplements contain principally α -tocopherol which is not the same; high doses of α -tocopherol displace γ -tocopherol. Up to now, trials of vitamin E in AD patients have used only the α form. This could explain the inefficiency of certain vitamin E supplements and not others. It is also to be noted in this context that the flavonoids have the same capacity as γ -tocopherol to scavenge peroxynitrite [116]. In the case of *G biloba*, only EGb 761 has been standardized and the

object of controlled clinical and experimental investigations, the composition and activity of the numerous other extracts currently on the market being largely unknown.

Even if antioxidants are in theory beneficial, they have to be transported to their site of action. In the case of early neurodegenerative disorders this assumes that they can pass the blood brain barrier, which is not highly permeable to vitamin E or desferrioxamine [11], whereas absorption of flavonoids is higher than previously believed [134] especially for *G biloba* extract EGb 761 [135]. The impact of therapies on natural anti-oxidative defenses should also be considered, as therapeutic interventions are likely to reduce them by feedback effects. It has been noted in this respect, however, that EGb 761 not only acts as a free radical scavenger but also enhances natural defence systems such SOD, heme oxygenase, and vitamin E. Protection by EGb 761 also involves the expression of several other genes, and their biochemical pathways: NF κ B, SIRT1 and MAPKs [136]. It also activates several genes coding for anti-oxidant activity: γ -glutamyl cysteinyl synthetase, heme-oxygenase-1, NADPH oxidoreductase, MnSOD, etc. [137, 138]. We are thus dealing with a very complex system of regulation, especially at the level of gene expression.

This latter mechanism is undoubtedly essential to our understanding of certain antioxidant effects, notably those relating to immediate effects. Generally these substances are assumed to have a therapeutic effect due to their capacity to protect against neuronal death, which, while targeting a preventive mechanism, is not immediate in its action. EGb 761 does, however, have rapid effects even on cognitive functioning [139], so that its action cannot simply be explained in terms of neuroprotection. Perhaps the influence is in fact quite unrelated to its anti-oxidative properties. One interesting hypothesis is based on observations of its effects on gene expression in vitro and in vitro using DNA arrays. This almost immediate reaction is in addition to its protective effect with regard to cell death which is only observed after several months [140]. It is possible that antioxidants exercise a regulatory role at the level of the cell and the organism which is not limited to protection against cellular insult but which also contributes to metabolic regulation and gene expression. In the case of EGb 761, the result of these complex actions is adaptation or regulation rather than a fixed effect: this substance is thus antiapoptotic in relation to models of neuronal death associated with neurodegeneration, but proapoptotic in the case of cancer. It is possible that this adaptive capacity results from its vegetal origins, that is a substance not resulting from chemical synthesis but from natural selection, itself a source of adaptation [140]. These observations lead us towards a highly

complex model of antioxidant effects, far from the simple notion of protection from cellular insult by free-radical scavenging.

While generally speaking little has been demonstrated with absolute certainty, the knowledge presently at our disposal tends to suggest that the consumption of antioxidants may have some preventive effect, in which case the following may be recommended:

- unrestricted consumption of fruit and vegetables, and moderate consumption of wine;
- in supplementing intake of Vitamin E, high doses should be avoided as these may carry cardio-vascular risks; current evidence is in favor of its use in the primary prevention of moderate AD but not MCI;
- if *Ginkgo* extract is selected as a treatment, EGb 761 is preferable as it is the only form for which precise information is available regarding efficacy and side effects; current evidence suggests that doses of 120 or 240 mg/day may be used either as primary prevention, or in cases of MCI and moderate AD;
- preparations claiming to have antioxidant properties whose composition is unknown or experimental should be avoided, particularly those which are freely available to the general public.

Competing interest: YC is an employee of IPSEN. M-L.A. and K.R. have no competing interests to disclose.

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Table 1: Studies showing significant association (positive ↑ or negative ↓) or no association (↕) between various markers of oxidant stress and AD or cognitive impairment

	Vitamin E		Vitamin C		β-Carotene		Other carotenoids	
	AD	cognitive impairment	AD	cognitive impairment	AD	cognitive impairment	AD	cognitive impairment
Plasma	↓ [20, 24, 141-148] ↕ [14, 149-151]	↓ [63, 148, 152, 153] ↕ [14, 22, 147, 154]	↓ [20, 24, 142, 144, 145, 149, 150] ↕ [141]	↓ [60, 154] ↕ [152, 153, 155]	↓ [145, 156] ↕ [20, 24, 141, 142, 150]	↓ [154]	↓ [20, 24, 144, 145, 150] ↕ [156]	↓ [21] ↕ [22, 152, 153]
RBC	↕ [143]	↕ [21]	ND	ND	ND	ND	ND	ND
CSF	↓ [157] ↕ [150]	ND	↓ [150]	ND	↕ [150]	ND	ND	ND

	Oxidation markers in AD									Antioxidant Enzymatic Activities in AD			
	Lipids						Proteins	DNA	Glyco-oxidation	SOD	Catalase	GPx	Grdase
	MDA	TBARS	IsoProstanes or NeuroProstanes	LDL oxidation	HNE or Acrolein	PUFA	(carbonyl or nitro- or di-Tyr)	single strand break or base modification	AGE				
Plasma	↑ [143, 144] ↕ [24, 141, 146, 158]	↕ [158]	↑ [18, 159] ↕ [25]	↑ [150] ↕ [151]	ND	ND	↑ [24]	ND	↑ [149]	↓ [20]	ND	↓ [20] ↑ [158]	ND
(R)BC	↑ [17]	↑ [160]	ND	ND	ND	ND	ND	↑ [145] (Lymphocytes) ↑ [161] (Leukocytes)	ND	↓ [20, 162] ↕ [143, 158] ↑ [17, 160]	ND	↓ [17] ↕ [143, 158]	ND
CSF	ND	ND	↑ [18, 163-166]	↑ [150]	↑ [167]	↓ [150]	↑ [168, 169]	↑ [170, 171]	↑ [172]	ND	ND	ND	ND
Brain	↕ [173-175]	↑ [15, 16, 173, 176, 177]	↑ [28, 164, 178, 179]	ND	↑ [180]	↓ [181]	↑ [168, 169, 173, 175] ↕ [174]	↑ [175, 182]	ND	↓ [177] ↕ [15, 174, 176, 183]	↑ [15, 176] ↓ [177, 183]	↑ [15, 176] ↕ [174, 177]	↑ [176]

AGE = advanced glycation end-products; GPx = glutathione-peroxidase; Grdase = glutathione reductase; HNE = 4-hydroxynonenal; LDL = low density lipoprotein; MDA = malondialdehyde; ND = not determined; PUFA = polyunsaturated fatty acids; (R)BC = red blood cells; SOD = superoxide dismutase; TBARS = thiobarbituric acid-reactive substances.

Table 2a: Vitamin supplementation and positive effect on cognition									
Reference	Study	Follow Up	Subjects and age	Information on vitamin use and duration	Cognitive evaluation	Main adjustment in analyses	Vitamin C	Vitamin E	Vitamin C + E
Mendelsohn et al. [184], 1998	MoVIESproject	Cross sectional 0 year	1,059 rural community-based elders; > 65 years	self report; 1 evaluation; no information on duration, dose and frequency of use	Boston Naming Test, learning, immediate and delayed recall, recognition, construction, verbal fluency, TMT, temporal orientation	age, education, sex	-	-	ND
Paleologos et al. [58], 1998	SITE Study	Cohort 4 years	117 elderly living in retirement communities; > 69 years	SQFFQ; 1 evaluation; no information on dose	MMSE, verbal fluency	age, education, sex, smoking, stroke, Parkinson's disease, total energy intake, current psychotropic drug use	+ (MMSE) OR=0.39 95% CI 0.18-0.84	ND	ND
Masaki et al. [56], 2000	Honolulu-Asia Aging Study	0	3,385 men; 71-93 years	mail survey at baseline and 6-8 years; long term users (both waves) > recent users (second wave > past users (first wave) > never users; no information on dosage	CASI (global score)	age, education, stroke, ApoE	+ improvement with long term use OR=0.80 95% CI 0.67-0.96	+ improvement with long term use OR=0.80 95% CI 0.67-0.96	+/- (borderline association) OR=0.85 95% CI 0.72-1.005
Grodstein et al. [55], 2003	Nurses' Health Study	0	14,968 women; 70-79 years	telephone interview every 2 years; information on duration and dose	immediate and delayed recall; verbal fluency, digit backwards (telephone cognitive evaluation)	age, education, diabetes, hypertension, heart disease, aspirin use, BMI, smoking, HRT, multivitamin use, mental health index, energy fatigue index, current antidepressant use	-	+ (immediate > delayed recall > verbal fluency; not digit backwards)	++ (immediate > delayed recall > verbal fluency; not digit backwards)
Maxwell et al. [57], 2005	Canadian Study of Health and Aging	5 years	894 ≥ 65 years	evaluation at baseline only	MMSE	age, sex, education, blood pressure, baseline MMS score and institution, smoking, drinking, self rated health, creatinine, albumine (+BMI, diabetes, hypertension, stroke cardiac symptoms and use of aspirin, NSAID, vitamin B12)	-	-	+ (in cases of ± multivitamin) OR=0.51 95% CI 0.29-0.90

ApoE = Aapolipoprotein E; BMI =body mass Index; CASI = cognitive abilities screening instrument; HRT = hormone replacement therapy; MMSE = Mini Mental State Examination; NSAID = nonsteroidal anti-inflammatory drugs; (SQ)FFQ = (semi-quantitative) food frequency questionnaire; TMT = Trail-Making Test; VaD: vascular dementia.

+ corresponds to significant association between vitamin intake and protection against cognitive impairment or dementia; when available adjusted OR or RR (with 95% CI) are indicated (results were generally expressed as protective effect against cognitive impairment or dementia at high vitamin intake, OR or RR < 1, except in some cases [59, 60], see table 2b) where they are expressed as increased cognitive impairment at low doses (OR or RR > 1); - corresponds to studies where no significant association was observed; ND = not determined.

Table 2b: Vitamin intake (diet) and positive effect on cognition

Reference	Study	Follow Up	Subjects and age	Information on use and duration	Cognitive evaluation	Main adjustment in analyses	Vitamin C	β-Carotene	Vitamin E
Gale et al. [60], 1996		0	921 > 65 years	food diary of every item of food consumed over a week; 1 evaluation	impairment in global score (Hodkinson mental test)	age, sex, social class, cardiovascular risk factors (blood pressure, cholesterol) plasma vitamin C	+	ND	ND
Jama et al. [59], 1996	Rotterdam study	0	5,182 55-95 years	SQFFQ; 1 evaluation	impairment in MMSE	age, education, smoking, total caloric intake, other antioxidants	-	+	-
Kalmijn et al. [185], 1997	Zutphen Elderly Study	3 years	476 men 69-89 years	food intake at 5-year intervals by cross-check dietary history; 1 evaluation	decline in MMSE	age, education, smoking, alcohol, energy intake	-	-	-
La Rue et al. [65], 1997	New Mexico Aging Process study	6 years	137 66-90 years	2 evaluations at 6-year interval	decline in 3 tests (abstraction, visuospatial performance, memory)	age, education, smoking, alcohol, energy intake	+	ND	+
Ortega et al. [61], 1997		0	260 65-90 years	weighed food record for 7 consecutive days; 1 evaluation	impairment in MMSE	age, sex,	+	+	-
Paleologos et al. [58], 1998	SITE Study	4 years	117 > 69 years	SQFFQ; 1 evaluation	decline in MMSE; verbal or category fluency	age, education, sex, smoking, stroke, Parkinson's disease, total energy intake, current psychotrope use	+	ND	ND
Peacock et al. [186], 2000	ARIC Study	0	12,187 48-67 years	FFQ and use of supplements; no indication of duration; 1 evaluation	impairment in 3 tests (delayed word recall, word fluency, digit symbol)	age, sex, education, ethnicity, centre, employment, marital status, caloric intake, smoking, waist hip ratio, sport, fibrinogen, carotid intima media, expiratory volume, hypertension, HRT, depression	-	-	-
Ortega et al. [63], 2002		0	120 65-91 years	weighed 5-day food record; 1 evaluation	Pfeiffer's Mental Status Questionnaire	sex	ND	ND	+

Morris et al. [62], 2002	Chicago Health and Aging Project	3.2 years	2,889 65-102 years	FFQ (139 food items) 18 months after baseline; 1 evaluation	decline in global measure from 3 tests (immediate and delayed recall, digit symbol) + 1 global (MMSE)	age, sex, education, ethnicity, smoking, alcohol, total calorie energy intake, total intake of vitamin C, A, carotene, vascular factors	-	-	+
Morris et al. [64], 2005	Chicago Health and Aging Project	6 years	3,718 ≥ 65 years	self-administered Harvard FFQ	decline in global measure from 3 tests (immediate and delayed recall, digit symbol) + 1 global (MMSE)	age, sex, education, ethnicity, total intake of vitamin C, time since baseline	ND	ND	+ (+ for α , γ not β -tocopherol)

Table 2c: Vitamin supplementation and protective effect against AD

Reference	Study	Follow Up	Subjects and age	Outcome	Information on vitamin use and duration	Main adjustment in analyses	Vitamin C	Vitamin E	Vitamin C + E	Multivitamin
Morris et al. [66], 1998	East Boston Study	cohort 4.3 years	633 ≥ 65 years	incident dementia	inspection of medication; 1 evaluation; no information on duration, dose, and frequency of use	age, sex, education	+	+	+ (half also taking vit E)	-
Masaki et al. [56], 2000	Honolulu-Asia Aging Study	0 year (cross sectional analysis from a cohort study)	3,385 men 71-93 years	prevalent dementia	mail survey; 2 evaluations at baseline and 6-8 years later; long term users (both waves > recent users (second wave) > past users (first wave) > never users; no information on dosage.	age, education, history of stroke, ApoE	- (- for VaD)	- (- for VaD)	- (+ for VaD)	ND
Laurin et al. [187], 2002	Honolulu-Asia Aging Study	cohort 5.2 years	2,369 men 71-92 years	incident dementia	mail questionnaires; 2 evaluations at baseline and 3-5 years later; long-term users (using both vitamins at both waves) > short-term users (both vitamins at 1 wave) > other users (using vitamin E or C either wave)	age, education, stroke, coronary heart disease, diabetes, atherosclerosis, lifestyle, ApoE	-	-	-	ND
Morris et al. [69], 2002	Chicago Health and Aging Project	3.9 years	815 > 65 years (65-102)	incident dementia	self administered FFQ (139 food items, intake throughout the previous year); one single evaluation	age, sex, education, ethnicity, ApoE, calory and energy intake, (total intakes of vitamin, fat, smoking, diabetes, hypertension, heart disease, stroke)	-	-	ND	ND
Luchsinger et al. [12], 2003	Washington Height Columbia Aging Project	cohort 4 years	980 ≥ 65 years	incident dementia	SQFFQ by telephone; only 1 evaluation of habitual intake during 1 yr	age, sex, education, ethnicity, smoking, ApoE	-	-	ND	ND
Zandi et al. [67], 2004	Cache County Study	cohort 3 years	4,740 > 65 years	prevalent and incident dementia	evaluation at baseline (during preceeding 2 weeks)	age, sex, education, general health status, ApoE (3MSE for incidence)	-	-	+ OR=0.22 (prevalent dementia) 95%IC 0.05-0.60 HR=0.36 (incident dementia) 95%IC 0.09-0.99]	-

Maxwell et al. [57], 2005	Canadian Study of Health and Aging	cohort 5 years	894 ≥ 65 years	Incident Dementia	evaluation at baseline	Age, sex, education, general health status, HTA, stroke, baseline 3MSE	-	-	-	-
Fillenbaum et al [188], 2005	Duke Established populations for Epidemiologic studies of the Elderly	cohort 10 years	616 65-105 years	Incident Dementia	evaluation at baseline or later (wave prior to diagnosis, 3, 6, 10 years)	Age, education, marital status, income, mobility, health service use and prescription drugs	ND	ND	-	ND

Table 2d: Vitamin intake (diet) and protective effect against incident AD

Reference	Study	Follow Up	Subjects and age	Information on vitamin use and duration	Main adjustment in analyses	Vitamin C	βCarotene	Vitamin E	Flavonoids
Engelhart et al. [68], 2002	Rotterdam Study	6 years	5,395 > 55 years	extensive validated SQFFQ (170 food items); 1 single evaluation	age, sex, edu, baseline MMSE, alcohol, smoking, BMI, total energy intake, presence of carotid plaques, use of antioxidant supplements, ApoE	+	-	+	-
						RR per 1SD increase=0.82 95%IC 0.68-0.99	(+ in smokers: RR per 1SD increase=0.49 95%IC 0.27-0.92)	RR per 1SD increase=0.82 95%IC 0.66-1.00	(+ in smokers: RR per 1SD increase=0.54 95%IC 0.31-0.96)
Morris et al. [69], 2002	Chicago Health and Aging Project	3.9 years	815 > 65 years (65-102)	self administered FFQ (139 food items, intake throughout the previous year); 1 single evaluation	age, sex, education, ethnicity, ApoE, calory and energy intake, (total intakes of vitamin, fat, smoking, diabetes, hypertension, heart disease, stroke)	-	-	+	ND
								(only in ApoE4 non-carrier) RR=0.3 95%IC 0.10-0.92	
Luchsinger et al. [12], 2003	Washington Heights Inwood Columbia Aging Project	4 years	980 > 65 years	validated SQFFQ (by telephone); 1 single evaluation of habitual intake during one year	age, sex, education, ethnicity, smoking, ApoE	-	-	-	ND
Laurin et al. [126], 2004	Honolulu-Asia Aging Study	30.2 years	2,459 men 52.4 years (SD 4.2) at baseline 1965-68; 77.4 years (4.1) at first dementia assessment (1991-93). Subjects re-examined twice for dementia 1994-6 and 1997-9.	dietary intake estimated at midlife (first examination) from a single 24-hour recall assessed by dieticians; reproducibility of dietary frequency data evaluated over 6-years (between examination 1 and 3).	age, education, smoking, alcohol, BMI, physical activity, blood pressure, year of birth, total energy intake, cholesterol concentration, history of cardiovascular disease, supplemental vitamin intake, ApoE	-	-	-	-
Morris et al. [64], 2005	Chicago Health and Aging Project	4 years	1,041 ≥ 65 years	self-administered Harvard FFQ (see Morris et al. [69], 2002)	age, sex, education, ethnicity, fat intake, ApoE, frequency of participation in cognitive abilities, time from determination of disease-free status to clinical evaluation of incident disease	ND	ND	+	ND
								RR per 5 mg/d increase=0.74 95%IC 0.68-0.88 (+ for α, γ, tocopherol not β)	