CAFFEINE AND DEMENTIA

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CAFFEINE AND DEMENTIA

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Investigação realizada no Serviço de Higiene e Epidemiologia da Faculdade de Medicina da Universidade do Porto, sob orientação do Professor Doutor Nuno Lunet e co-orientação da Professora Doutora Ana Azevedo.
Esta dissertação teve como base dois manuscritos. No primeiro artigo colaborei activamente na operacionalização das hipóteses, na análise e na interpretação dos dados. No segundo artigo colaborei activamente na definição das hipóteses, recolha, análise e interpretação dos dados. Fui responsável pela redacção das versões iniciais de ambos os manuscritos.


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Aos co-autores dos trabalhos incluídos nesta dissertação, pelo seu valioso contributo.

Aos meus Amigos, sem os quais esta caminhada não seria possível com o mesmo ânimo.

Aos meus Pais, pelos valores que partilham e pela segurança que me transmitem.
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1. Dementia and cognitive impairment

Dementia is a chronic, debilitating condition, characterized by the deterioration of cognitive functions in several domains, in the absence of impairment of consciousness and persisting for a period of at least 6 months (1). Memory impairment is a typical, early and prominent feature in dementia, especially Alzheimer's disease (AD), followed by executive function deterioration, apraxia, aphasia and/or agnosia (1). Dementia is increasingly recognized as a manifestation of a degenerative process and not a disease itself. The cognitive and behavioural symptoms constitute only the "tip of the iceberg", and the underlying pathological processes, although yet to be fully understood, may involve different degenerative pathways.

The existence of several diagnostic criteria for dementia should be kept in mind when analyzing and interpreting the findings from epidemiological studies. This applies in particular to the two most representative forms of dementia: AD and vascular dementia, where existing diagnostic criteria have been demonstrated not to be interchangeable (2-4). Mild cognitive impairment is a recently defined construct, aiming to identify the transitional state between non-pathological cognitive changes associated with aging and the earliest clinical features of dementia (5). This distinction is however not without difficulties and the criteria used to define mild cognitive impairment have evolved throughout the years (6); it is nevertheless an increasingly used construct in clinical practice and in epidemiological studies.

Despite the nosological heterogeneity and potential for misclassification arising when dealing with dementia, this is by far the most common neurodegenerative disease and constitutes a burdensome condition, especially at older ages (7). The trend for an increase in overall burden of disease associated with dementia in the next few decades is therefore largely determined by the social and demographic changes contributing to population ageing, especially in developing countries undergoing the "epidemiological transition" (8, 9). Notwithstanding the multidisciplinary commitment and the investment made in research, there are no disease modifying treatments for dementia approved up to date (7), highlighting the importance of defining earlier clinical stages of dementia, identifying modifiable risk factors and implementing preventive strategies.
1.1. Clinical diagnosis and classification of mild cognitive impairment and dementia

Several types of dementia have been described: AD, vascular dementia (VaD), Lewy body, fronto-temporal dementia, and dementia associated with Parkinson’s disease. Dementia may also be secondary to the human immunodeficiency virus (HIV-associated dementia) or other infectious agents, and this may assume particular importance in younger adults in specific world regions. Other rarer causes of dementia include Huntington’s disease, prion disease, and head trauma dementia. AD is the most frequent subtype, corresponding to about 55% of all diagnoses in individuals aged above 65-years (10). Next in frequency is VaD, a frequent condition, especially in older people (11, 12), estimated to represent 15% of all cases (10), as illustrated in figure 1:

![Figure 1: Distribution according to dementia subtype](image)

AD – Alzheimer’s Disease, VaD – Vascular Dementia
Source: Dugu et al, 2003 (10)

Although AD can be identified with a considerable degree of accuracy, at present, there is no consensus on how to define “mixed” dementia in a clinical setting. Moreover, overlapping symptomatology, pathophysiology, and comorbidity make the distinction between VaD and AD often difficult, and a differential diagnosis is further complicated by the fact that many patients have concomitant AD and cerebrovascular disease (13). In an elderly community-based autopsy study in the United Kingdom, AD was the primary
pathological diagnosis in 59% of cases and VaD in 16% (14); nevertheless, when analyzing pathological findings regardless of the primary diagnosis, AD features were present in 61% of cases and cerebrovascular pathology in 54%, highlighting the relevance of "mixed" dementia (14). The distinction between isolated AD, VaD, and mixed dementia coexisting in the same patient, remains a controversial issue and one of the most difficult diagnostic challenges in this field (15).

Aiming the early detection of individuals at risk of dementia and seeking to delay the onset or progression of the disease, there has been an attempt to define earlier clinical stages of dementia. The consensual expression used to define this transitional state is Mild Cognitive Impairment (MCI), which is a conceptual rather than an operational definition, and the construct of this entity has evolved throughout the years. Initially, the term was used to reflect memory impairment with preserved non-memory cognitive performance and functional abilities (16). More recently, MCI was acknowledged to include impairment of cognitive domains other than memory (6), but it is presently unclear whether it should be strictly considered an early stage of a specific disease (AD being the paradigm), or rather, part of a broader syndrome. The criteria used to define MCI is a crucial methodological point to account for when comparing data as it may be responsible for heterogeneous findings across studies.

A recent study conducted in Portugal by Nunes et al (17) concluded that elderly individuals with cognitive complaints but normal performances on neuropsychological testing suffered a higher decline in total hippocampal volume during a 3.5-year follow-up. This suggests that cognitive complaints alone in elderly patients may preclude neurodegenerative changes and that “pre-MCI” could also be an interesting clinical and research concept to pursue (17).

1.1.1. Mild cognitive impairment

According to the original criteria dated from 1999, the definition of MCI relied heavily on memory as the main cognitive function affected, in the absence of functional dependence regarding daily life activities (5).

Two years later, Petersen et al (6) extended the definition and proposed the creation of four categories of MCI: amnestic versus non-amnestic and single versus multiple domains. This broader concept, however, does not specify the cognitive domains
to be assessed when defining MCI, apart from memory, nor which neuropsychological instruments should be applied (6).

A diagnostic algorithm developed by Peterson et al (18) in 2004, requires an expression of concern regarding cognitive abilities from the patient or informant as the first step to define MCI. Clinicians should then assess whether the complaint effectively reflects cognitive impairment abnormal for age, along with normal daily functional abilities. Clinical history, mental status examination and neuropsychological testing are the main instruments available to perform this evaluation and demonstration of abnormal cognitive functioning for age, which may be quantified by reference to standard deviation from scores obtained by normal subjects within the same age range. The patient should be considered to have MCI if the previous assessment suggests that cognitive performance levels are neither normal nor compatible with dementia, and the individual is able to perform most daily activities. This algorithm does not impose a defined mental status and neuropsychological approach and it is the clinician that ultimately defines the degree of functional disability of the individual (19). The European Alzheimer’s Disease Consortium/Alzheimer’s Disease Cooperative Study (EADC/ADCS) criteria were recently revised to allow for a mild decline in complex daily life activities when assessing MCI, extending the former concept of intact functional performance for the diagnosis of this condition (20).

In 2003, a group of clinicians and epidemiologists proposed a set of new working criteria to define MCI (21), in an attempt to provide clearer guidelines for clinical and research purposes. These criteria require a stepwise assessment of three diagnostic features:

1) Definition of “not normal”, but also “not demented”, according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) or the International Classification of Mental and Behavioral Disorders (ICD-10) for a dementia syndrome;

2) Cognitive decline indicated by subject and/or informant report and objective cognitive tests

3) Preserved basic activities of daily living with some minimal impairment in complex instrumental functions

The need for subjective memory complaints may impair the sensitivity of criteria because patients with borderline dementia may have impaired awareness or deny
cognitive problems, leaving the informants as the most reliable source of information. The 10/66 Dementia Research Group study has recently reported that in less developed countries the informants were less likely to report cognitive decline and social impairment (22), reflecting the social and cultural determinants of acknowledgement of this condition. The diversity of neuropsychological instruments used to evaluate cognitive performance and the different thresholds considered to define MCI also contribute to the arbitrary nature of this diagnosis. This was demonstrated by Larrieu et al (23) and Ritchie et al (20) in two separate longitudinal studies, in which the variability in defining MCI was partly attributed to differences in neuropsychological tests and cutoff scores used.

The lack of an operational definition of MCI adapted to the general population, as well as the fact that sample assessment at a single point in time may reflect a cohort effect are important concerns when considering estimates of incidence and prevalence of MCI (21).

The difficulties in defining and diagnosing this entity are thus considerable, given the insidious manifestations, gradual progression of degenerative conditions and cultural issues. Nevertheless, MCI is a construct increasingly used in clinical practice and research, and is currently being considered for inclusion in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (24).

1.1.2. Alzheimer’s Disease

Brain imaging techniques have shown that AD patients have characteristic anatomical features distinguishing them from normal controls, namely atrophy of the temporal cortices, including the amygdala, hippocampus, and inferior temporal lobes, as well as of the anterior cingulate cortex (25).

Microscopically, the deposition of amyloid plaques is a characteristic finding, even in the earlier stages of AD, spreading to areas outside the entorhinal cortex as the disease progresses (26). The intracellular accumulation of neurofibrillary tangles composed of hyperphosphorylated tau is another neuropathological feature of AD (26). In 1991, Braak and Braak described an early selective deposition of neurofibrillary tangles involving the entorhinal region in AD (27), providing the rationale for neuropathological criteria to stage AD according to the stereotypic spread of these tangles in the central nervous system. These abnormal depositions are clearly involved in a cascade of pathological processes
involving inflammation, disturbed calcium homeostasis, free radical toxicity, and ultimately synaptic loss (7). Glutamate excitotoxicity is a relevant mechanism in this cascade, as happens in other neurodegenerative conditions. N-methyl D-aspartate (NMDA) molecules are a type of glutamate receptor and their activation leads to a significant increase in intracellular calcium concentration, which ultimately promotes apoptosis and neurotoxicity, through the generation of free radicals, oxidative stress and mitochondrial dysfunction (28). The cholinergic system is primordially affected in AD. As the disease progresses, noradrenergic and serotonergic system deficiencies develop and have also been associated with further cognitive deterioration and behavioural abnormalities (29).

Regardless of the recent advances in the knowledge of AD pathology, the clinical phenotype of this condition is largely non-specific and the definite diagnosis is ultimately neuropathological. Several criteria have been proposed, essentially based on the density of plaques and/or tangles, namely by the National Institute of Aging (30), the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) (31) and the Reagan Institute (32).

Despite the intense research on biological markers and neuroimaging techniques as potential aiding diagnostic tools, the recognition of AD in everyday practice and epidemiological surveys still relies essentially on clinical criteria (33).

Two sets of criteria have been developed for the clinical diagnosis of AD: the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), and the consensus criteria developed by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA). In addition to these, the widely used International Statistical Classification of Diseases and Related Health Problems (10th revision) also proposes a definition for Alzheimer’s disease.

The DSM-IV are less extensive (table 1) than NINCDS-ADRDA, requiring only deficits in two or more cognitive domains, with a progressive course, and after excluding other potential causes, namely other systemic or psychiatric illnesses and delirium (34).
Table 1. DSM-IV criteria for AD

<table>
<thead>
<tr>
<th>Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Criteria to define Alzheimer's Disease (AD)</td>
</tr>
<tr>
<td>Multiple Cognitive Deficits</td>
</tr>
<tr>
<td>Criterion A</td>
</tr>
<tr>
<td>A1. Memory impairment</td>
</tr>
<tr>
<td>A2. One or more of the following:</td>
</tr>
<tr>
<td>Aphasia</td>
</tr>
<tr>
<td>Apraxia</td>
</tr>
<tr>
<td>Agnosia</td>
</tr>
<tr>
<td>Disturbed executive function</td>
</tr>
<tr>
<td>Criterion B</td>
</tr>
<tr>
<td>Cognitive deficits in criteria A1 and A2 each:</td>
</tr>
<tr>
<td>Cause impairment in social or occupational functioning</td>
</tr>
<tr>
<td>Are not due to a CNS disease</td>
</tr>
<tr>
<td>Are not due to a medical disorder</td>
</tr>
<tr>
<td>Do not occur solely during the course of delirium</td>
</tr>
<tr>
<td>Criterion C</td>
</tr>
<tr>
<td>Gradual and continued cognitive decline</td>
</tr>
<tr>
<td>Criterion D</td>
</tr>
<tr>
<td>Other systemic neurologic and psychiatric illnesses should be eliminated</td>
</tr>
<tr>
<td>Criterion E</td>
</tr>
<tr>
<td>AD should not be diagnosed in the presence of delirium</td>
</tr>
</tbody>
</table>

Source: Sadock, 2008 (35)

The NINCDS-ADRDA is a more comprehensive set of criteria, proposing three categories of diagnoses: 1) definitive, when characteristic features are obtained from a neuropathological examination, 2) probable, when other likely causes of dementia are excluded, 3) possible, when other causes of dementia cannot be thoroughly excluded or atypical findings explained. According to this classification, the probable diagnosis of AD relies on the evidence of deficits in two or more areas of cognition, confirmed by neuropsychological evaluations (36). In parallel, it requires the exclusion of other causes of cognitive impairment, accounting for features that make the diagnosis of probable AD unlikely, such as sudden onset, focal neurological findings, seizures or gait disturbances early in the course of the illness (36). Table 2 illustrates the definition of a probable diagnosis of AD, according to the NINCDS-ADRDA:
Table 2. National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable Alzheimer’s Disease

Criteria to define Alzheimer’s Disease (AD)

I. Criteria for the clinical diagnosis of probable AD:
- Dementia established by clinical examination and documented by the Mini-Mental State Examination, Blessed Dementia Scale, or some similar examination and confirmed by neuropsychological tests
- Deficits in two or more areas of cognition
- Progressive worsening of memory and other cognitive functions
- No disturbance of consciousness
- Onset between ages 40 and 90, most often after age 65; and
- Absence of systemic disorders or other brain diseases that could account for dementia

II. A probable AD diagnosis is supported by:
- Progressive deterioration of specific cognitive functions such as language (aphasia), motor skills (apraxia) and perception (agnosia)
- Impaired activities of daily living and altered patterns of behaviour
- Family history of similar disorders, particularly if confirmed neuropathologically; and
- Laboratory results of lumbar puncture as evaluated by standard techniques; normal pattern or nonspecific changes in EEG, such as increased slow-wave activity; and evidence of cerebral atrophy on CT with progression documented by serial observation

III. Other clinical features consistent with the diagnosis of probable AD include:
- Plateaus in the course of progression of the illness
- Associated symptoms of depression, insomnia, incontinence, delusions, illusions, hallucinations, catastrophic verbal, emotional, or physical outbursts, sexual disorders, weight loss; other neurological abnormalities in some patients, especially with more advanced disease and including motor signs such as increased motor tone, myoclonus or gait disorders
- Seizures in advanced disease and
- CT normal for age

Source: Sadock, 2008 (35)

For the diagnosis of AD, the ICD-10 requires the fulfillment of the general criteria for dementia presented in table 3, and the exclusion of other possible causes for dementia or systemic disorders, alcohol or drug abuse (37). This classification defines 4 categories for AD: early onset – before 65 years; late onset – after 65 years; atypical or mixed type; unspecified (37).
Table 3. Definition of dementia according to the 10th revision of International Statistical Classification of Diseases and Related Health Problems (ICD-10).

<table>
<thead>
<tr>
<th>Criteria to define dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G1</strong></td>
</tr>
<tr>
<td>A decline in memory, which is more evident in the learning of new information, although, in more severe cases the recall of previously information may also be affected. The impairment applies to both verbal and nonverbal material. The decline should be objectively verified by obtaining a reliable history from an informant, supplemented if possible by neuropsychological tests or quantified cognitive assessments.</td>
</tr>
<tr>
<td><strong>G2</strong></td>
</tr>
<tr>
<td>A decline in other cognitive abilities characterized by deterioration in judgement and thinking, such as planning and organizing, and in the general processing of information. Evidence for this should ideally be obtained by an informant and supplemented, if possible, by neuropsychological tests or quantified objective assessments. Deterioration from a previously higher level of performance should be established.</td>
</tr>
<tr>
<td><strong>G3</strong></td>
</tr>
<tr>
<td>Awareness of the environment is preserved during a period sufficiently long to allow the unequivocal demonstration of the symptoms in criterion G1</td>
</tr>
<tr>
<td><strong>G4</strong></td>
</tr>
<tr>
<td>There is a decline in emotional control or motivation, or a change in social behavior manifest as at least one of the following: emotional lability, irritability, apathy, coarsening of social behavior.</td>
</tr>
<tr>
<td><strong>G5</strong></td>
</tr>
<tr>
<td>For a confident diagnosis, the symptoms in criterion G1 should have been present for at least 6 months; if the period since the manifest onset is shorter, the diagnosis can only be tentative.</td>
</tr>
</tbody>
</table>

Source: Sadock V, 2008 (35)

The NINCDS-ADRDA and the DSM-IV criteria are, however, the prevailing diagnostic standards in AD research (7), but both are likely to exclude broad populations of patients in very early stages of the disease, currently being labeled as having MCI (4). Despite the recent proposals for criteria incorporating data concerning cerebrospinal fluid analysis of amyloid beta or tau proteins as well as other biological markers (4), validation studies are still needed (4), and the DSM-IV and NINCDS-ADRDA criteria remain the mainstay of AD possible and probable diagnosis.
1.1.3. Vascular Dementia

The term “vascular cognitive impairment” refers to all clinical phenotypes where cognitive impairment is attributable to cerebrovascular disease, encompassing different forms of blood vessel pathology, spectra of risk factors, time-installation profile and regional distribution of brain vascular lesions. Vascular dementia represents a subset of this broad phenotype.

The definition of the cognitive syndrome and the establishment of its vascular cause are critical elements in the concept and diagnosis of vascular dementia (VaD), for which there are four main sets of criteria available: DSM-IV, ICD-10, National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l’Enseignement en Neurosciences (NINDS-AIREN) and Alzheimer’s Disease Diagnostic and Treatment Centers (ADDTC) (38).

To define VaD, the DSM-IV criteria require the same general symptoms as for AD, but clinical or laboratory evidence is needed for a vascular cause for dementia (34). The latter requirements, however, are difficult to materialize because this set of criteria does not specify which brain imaging findings or focal neurological signs and symptoms should be valued.

The ICD-10 criteria define vascular dementia as the presence of all the general criteria for dementia and evidence of focal brain damage in the neurological examination (37). Information from history or complementary tests of relevant cerebrovascular disease provides additional evidence of causal relationship.

The NINDS-AIREN criteria have been the most widely adopted in pharmacologic drug trials and epidemiological studies (39). This is considered the most conservative classification since it requires imaging findings of vascular brain injury as well as focal signs on neurologic examination to define VaD (2). The ADDTC criteria also rely on radiological findings but do not require focal neurological signs and are therefore considered quite liberal (2).

Table 4 describes the main clinical criteria used to define VaD.
Table 4. Diagnostic criteria for vascular dementia.

<table>
<thead>
<tr>
<th>Diagnostic Criteria</th>
<th>Dementia</th>
<th>Evidence of vascular brain injury</th>
<th>Evidence of causal relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSM-IV</td>
<td>Memory loss sufficient to interfere with daily functioning</td>
<td>Stepwise deteriorating course Focal neurological signs and symptoms</td>
<td>Evidence from history, neurological examination, or laboratory tests of cerebrovascular disease, judged to have a causal relation with dementia</td>
</tr>
<tr>
<td>ICD-10</td>
<td>Unequal distribution of deficits in higher cognitive functions</td>
<td>Clinical evidence of focal brain damage, with at least one of the following: Unilateral spastic weakness of the limbs, unilateral increased tendon reflexes, extensor plantar response, pseudobulbar palsy</td>
<td>Evidence from post medical history, neurological examination, or laboratory tests of cerebrovascular disease, judged to have a causal relation with dementia</td>
</tr>
<tr>
<td>NINDS-AIREN (Probable)</td>
<td>Memory loss + Impairment in two other cognitive domains</td>
<td>Focal neurological signs Imaging findings of cerebrovascular disease</td>
<td>Onset of dementia within 3 months after a stroke or abrupt deterioration or stepwise progression of cognitive impairment</td>
</tr>
<tr>
<td>ADDTC (Probable)</td>
<td>Multidomain cognitive impairment sufficient to interfere with daily activities</td>
<td>Two or more strokes outside cerebellum</td>
<td>One infarct with temporal relationship to onset of cognitive impairment (in alternative to the former criteria of evidence of vascular brain injury)</td>
</tr>
</tbody>
</table>

DSM-IV - Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; ICD-10 - 10th revision of International Statistical Classification of Diseases and Related Health Problems; NINDS-AIREN - National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l’Enseignement en Neurosciences; ADDTC - Alzheimer’s Disease Diagnostic and Treatment Centers

Adapted from Chui et al, 2007 (38)

Pohjasvaara et al (2) evaluated the use of different clinical definitions of VaD in case finding, in a series of 107 poststroke patients fulfilling the Diagnostic and Statistical Manual of Mental Disorders, Thirth Edition (DSM-III) criteria for dementia. These authors reported that defining VaD according to the different existing criteria, when compared to a DSM-III diagnosis, originated distinct frequency estimates. DSM-III and ICD-10 criteria had a concordance of 100%; the DSM-IV criteria were the most liberal and NINDS-AIREN the most restrictive classifications (2). Gold et al described a sensitivity of 50% for the DSM-IV criteria contrasting with 20% for probable VaD according to the NINDS-AIREN, in a clinicopathological validation study; the specificity for each set was 84% and 93%, respectively. The ICD-10 criteria showed diagnostic accuracy performance similar to the
observed for the NINDS-AIREN (sensitivity, 20%; specificity, 94%) (3). The existence of several non-interchangeable criteria for the diagnosis of vascular dementia raises important concerns when estimating the burden of disease in different settings or interpreting etiological research, stressing the need for standardization.

1.2. Neuropsychological Evaluation

Neuropsychological assessment enables the characterization and quantification of the effects of brain damage on intellectual, motor or emotional functions. It is therefore an essential step in the diagnosis of dementia, contributing to the identification of specific cognitive domains affected, and establishing a differential diagnosis with other important neuropsychiatric syndromes (40).

These instruments are also important to monitor the progression of disease and treatment efficacy (41).

There are several individual tests available to evaluate particular cognitive domains or behaviors (40), but the existing diagnostic criteria for dementia and MCI do not impose the use of specific tools for cognitive assessment.

The choice of tests or batteries from the large number of existing neuropsychological instruments is ultimately an individual clinical decision, largely governed by the available time and hypothesis-testing strategies.

Tables 5 and 6 summarize the most widely used neuropsychological tests and batteries (40, 42-44).
<table>
<thead>
<tr>
<th>Neuropsychological Domain</th>
<th>Neuropsychological Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attention</td>
<td>Digit Span</td>
</tr>
<tr>
<td></td>
<td>Letter Cancellation</td>
</tr>
<tr>
<td></td>
<td>Trails A Test</td>
</tr>
<tr>
<td>Language</td>
<td>Boston Naming Test</td>
</tr>
<tr>
<td></td>
<td>Boston Diagnostic Aphasia Examination</td>
</tr>
<tr>
<td></td>
<td>Western Aphasia Battery</td>
</tr>
<tr>
<td></td>
<td>Verbal Fluency Test</td>
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<tr>
<td></td>
<td>Verbal IQ scale of the WAIS-R</td>
</tr>
<tr>
<td>Memory</td>
<td>Wechsler Memory Scale</td>
</tr>
<tr>
<td></td>
<td>Rey Auditory Verbal Learning Test</td>
</tr>
<tr>
<td></td>
<td>California Verbal Learning Test</td>
</tr>
<tr>
<td></td>
<td>Benton Visual Retention Test</td>
</tr>
<tr>
<td></td>
<td>Camden Memory Test</td>
</tr>
<tr>
<td>Visuospatial skills</td>
<td>Rey-Osterrieth Complex Figure</td>
</tr>
<tr>
<td></td>
<td>Raven’s Progressive Matrices</td>
</tr>
<tr>
<td></td>
<td>Benton Visual Retention Test</td>
</tr>
<tr>
<td></td>
<td>Block Design subtest of WAIS-R</td>
</tr>
<tr>
<td>Executive function</td>
<td>Wisconsin Card Sort Test</td>
</tr>
<tr>
<td></td>
<td>Stroop Test</td>
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<tr>
<td></td>
<td>Trails B Test</td>
</tr>
<tr>
<td></td>
<td>Porteus Maze Test</td>
</tr>
<tr>
<td>Verbal Fluency</td>
<td>Isaacs Set Test</td>
</tr>
<tr>
<td>Intelligence</td>
<td>WAIS-R</td>
</tr>
<tr>
<td></td>
<td>Wechsler Intelligence Scale for Children</td>
</tr>
<tr>
<td></td>
<td>New Adult Reading Test</td>
</tr>
<tr>
<td>Motor speed</td>
<td>Finger Tapping</td>
</tr>
<tr>
<td>Educational achievement</td>
<td>Grooved Pegboard</td>
</tr>
<tr>
<td></td>
<td>Wide Range Achievement Test</td>
</tr>
</tbody>
</table>

WAIS-R – Wechsler Adult Intelligence Scale – Revised
<table>
<thead>
<tr>
<th>Neuropsychological battery</th>
<th>Main utilization(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAIS, WAIS-R, WAIS-III</td>
<td>Originally devised as a test of adult “intelligence”, and not of neuropsychological functioning</td>
</tr>
<tr>
<td>Halstead-Reitan</td>
<td>Originally devised to determine cognitive effects of brain injury Evaluates memory, abstract thought, language, sensory-motor integration, imperception, and motor dexterity</td>
</tr>
<tr>
<td>Luria-Nebraska</td>
<td>Susceptible to confounding effects by abilities not directly tested by the scales Limited utility in language-impaired patients</td>
</tr>
<tr>
<td>CAMDEX</td>
<td>Designed as a diagnostic and measurement instrument for dementia in the elderly</td>
</tr>
<tr>
<td>CERAD</td>
<td>Designed for the diagnosis of Alzheimer’s disease</td>
</tr>
</tbody>
</table>

WAIS = Wechsler Adult Intelligence Scale (R = Revised); CAMDEX = Cambridge Mental Disorders of the Elderly Examination; CERAD = Consortium to Establish a Registry for Alzheimer’s Disease;

Source: Martin, 2006 (41); Santana, 2005 (45)

Tests for screening of cognitive function are useful instruments for a time-limited mental status evaluation. The *Mini Mental Status Examination* (MMSE) is the best known and most extensively used of these tools, in both epidemiological studies and clinical practice. It includes questions on orientation, registration, attention and calculation, recall, language and visual construction (46). It is a valid test to define cognitive impairment, has high test-retest reliability, and the variation in the attained score in evaluations conducted in different moments is a good indicator of clinically significant cognitive decline (7). Although the average administration time is only 10 minutes (45), this test does not assess executive dysfunction, which is a major limitation in the evaluation of cognition. In addition, it suffers from a strong ceiling effect and it is not suitable to identify slight declines when the levels of cognition are high (47).
The Clock-Drawing test is mainly used to assess visuo-constractive skills, although it also provides valuable information on attention, episodic and semantic memory as well as executive and spatial capacities (48). There are several scoring scales for the Clock-Drawing test, assessing different cognitive components in distinct ways. There is presently a growing interest in the potential of this test as a screening bedside tool for cognitive impairment (49, 50); it is an easy and quick test to apply, with the objective of detecting moderate to severe cognitive impairment, although diagnostic validity is dependent on age and level of education (48). Other examples of screening tests are the Blessed Dementia Scale and the Alzheimer’s Disease Assessment Scale (ADAS) (45). The ADAS was initially proposed as a practical instrument to monitor the efficacy of AD treatments, covering the cognitive areas most likely to be impaired in this form of dementia, with the notable exception of frontal dysfunction (44). It is commonly used to assess cognitive dysfunction (ADAS-Cog), as well as non-cognitive domains, in individuals with AD and other dementias. Screening tests to be applied by telephone have been developed to be used in epidemiological surveys, of which the Telephone Interview for Cognitive Status (TICS) is the most notable example (51); some authors suggest that TICS provides a valid alternative to the MMSE and that scores obtained in both screening tests can be linked directly (52).

These screening tests cannot be used as an isolated instrument for the diagnosis of dementia, but they are often useful as an initial approach, preceding a more comprehensive and formal neuropsychological assessment.

Factors such as language, reading ability, socio-economic status, level of education and culture can strongly influence test performance. These instruments frequently have to be translated and also modified for a better adjustment to culturespecific references in different countries. The vocabulary, information and comprehension test components are the most often changed (40).

Neuropsychological evaluation instruments in the Portuguese population

The most widely used test to screen for cognitive dysfunction, the MMSE, has been validated for the Portuguese population in 1994 by Guerreiro et al (53), establishing cutoff values according to formal education levels. A recent study conducted in the urban area of Lisbon by Morgado et al (54) proposed different cutoff values, reflecting cultural and social
progresses over the past 20 years in Portugal. For the time being, the former criteria are still adopted when screening for cognitive impairment using the MMSE.

Table 7: MMSE cutoff values used to define cognitive impairment for Portuguese population, according to the education level

<table>
<thead>
<tr>
<th>MMSE score cutoff values validated for the Portuguese population (Guerreiro et al, 1994) (53)</th>
<th>MMSE scores cutoff values recently proposed, based on an urban population survey (Morgado et al, 2009) (54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Education</td>
<td>Cognitive Impairment</td>
</tr>
<tr>
<td>Illiterate</td>
<td>≤ 15 points</td>
</tr>
<tr>
<td>1-11 years</td>
<td>≤ 22 points</td>
</tr>
<tr>
<td>≥ 12 years</td>
<td>≤ 27 points</td>
</tr>
</tbody>
</table>

The ADAS-Cog screening test has a mean duration of administration of 20-30 minutes and is translated and validated for the Portuguese population, for different age groups and education levels (55).

The *Bateria de Lisboa para Avaliação de Demência* (BLAD) is the only comprehensive neuropsychological evaluation instrument designed and validated for the Portuguese population (56). It is more time-consuming than the ADAS, with a mean administration time of 90 minutes and requires specific training of the interviewers (45). The BLAD is composed of a number of tests that enable the characterization of levels of functioning localized in specific cortical areas and is useful in detecting executive dysfunction, which is frequently impaired in AD and other dementias (45). It is also suitable for the evaluation of populations with heterogeneous educational levels and constitutes the most widely recommended comprehensive instrument for the corroborative diagnosis of dementia in Portugal (45).

It must be stressed that cognitive assessment should always be complemented by an evaluation of depressive symptoms and other neuropsychiatric manifestations. It is also essential to assess the ability to perform daily life activities, as this is one of the criteria necessary to define dementia. The former objective can be accomplished by using the *Neuropsychiatric Inventory*, the *Cornell Scale for Depression in Dementia* and the *Geriatric*
Depression Scale. The Instrumental Activities of Daily Living Scale and the Disability Assessment for Dementia Scale (57) are two of the most commonly used scales to characterize everyday functional performance. All of the referred scales and instruments are translated into Portuguese and available for clinical and research purposes (55).

2. Burden of disease associated with dementia and mild cognitive impairment

Dementia is an increasingly frequent condition, associated with high morbidity and mortality.

Several multicentric studies have been conducted to estimate frequency measures in different regions of the globe, despite the use of distinct methodological approaches. Diagnostic criteria discrepancies between studies are among the most relevant differences to account for when comparing the results.

The EURODEM – Prevalence Research Group study estimated the frequency of dementia subtypes in Europe, using studies conducted in the 1990s in Finland, Denmark, Sweden, the Netherlands, United Kingdom, Italy, Spain and France. Dementia was diagnosed based on DSM-III-R, but the criteria to define AD and VaD varied across centers. The age-standardized prevalences in individuals 65 years and older were 6.4% for dementia (all types), 4.4% for AD and 1.6% for VaD.

The prevalence of dementia increased continuously with age, reaching 28.5% for individuals aged 90 years and older (56), with AD being the main contributor to the steep increase observed (58), showing that population ageing will contribute to an increasing burden of AD and presumably other dementia forms (59). The prevalence of dementia was higher in women than in men and this difference was particularly relevant in the older subjects (80-84 years: 11% in men vs 12.6% in women; 85-90 years: 12.8% in men vs 20.2% in women%) (58). The same collaborative study of population-based cohorts estimated age-specific incidence rates, as illustrated in table 8 (60).
Table 8. Incidence of dementia according to the collaborative study of population-based cohorts EURODEM (pooled rates).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Incidence rate, per 1000 person-years (95% CI)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men and women</td>
</tr>
<tr>
<td>65-69</td>
<td>2.4 (1.4-5.5)</td>
<td>2.5 (1.4-4.9)</td>
<td>2.4 (1.8-4.3)</td>
</tr>
<tr>
<td>70-74</td>
<td>6.4 (4.5-9.3)</td>
<td>4.7 (3.2-6.8)</td>
<td>5.5 (4.3-7.1)</td>
</tr>
<tr>
<td>75-79</td>
<td>13.7 (12.1-19.7)</td>
<td>17.5 (13.4-19.8)</td>
<td>16 (13.8-18.6)</td>
</tr>
<tr>
<td>80-84</td>
<td>27.6 (22.5-35.1)</td>
<td>34.1 (30.3-40.8)</td>
<td>30.5 (27.5-35.3)</td>
</tr>
<tr>
<td>85-90</td>
<td>38.8 (30.5-51.9)</td>
<td>53.8 (44.8-62.4)</td>
<td>48.6 (41.1-54.5)</td>
</tr>
<tr>
<td>+90</td>
<td>40.1 (23.7-58.5)</td>
<td>81.7 (61.9-91.0)</td>
<td>70.2 (54.4-77.4)</td>
</tr>
</tbody>
</table>

The incidence of dementia increased with age up to 85 years, after which rates increased only in women and reached a plateau in men (60).

Regarding subtypes of dementia, the incidence of AD was higher than the observed for VaD, regardless of age, despite the large variability in the diagnostic criteria used in the different study centers (60).

A Delphi consensus study aimed to derive a quantitative estimate of prevalence of dementia around the world, through qualitative assessment of the best available evidence (9). This study was published in 2005 and prevalence was estimated for all regions in the world, for 5-year age groups from 60 to 84 years and for those aged 85 years and older (9). Studies of good methodological quality were frequent in North America, Europe, Japan and Australia while evidence from well-planned representative epidemiological investigations were scarce in South America, Africa and some regions of Asia. Table 9 illustrates the distribution of prevalence according to 14 World Health Organization (WHO) regions.

The number of people estimated to have dementia in 2001 was 24.3 million, worldwide (9).
Table 9: World Health Organization (WHO) mean estimates for prevalence of dementia, according to world region and age-group

<table>
<thead>
<tr>
<th>WHO region</th>
<th>60-64 yrs</th>
<th>65-69 yrs</th>
<th>70-74 yrs</th>
<th>75-80 yrs</th>
<th>80-84 yrs</th>
<th>+85 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>EURO A</td>
<td>0.9 (0.1)</td>
<td>1.5 (0.2)</td>
<td>3.6 (0.2)</td>
<td>6.0 (0.2)</td>
<td>12.2 (0.8)</td>
<td>24.8 (1.0)</td>
</tr>
<tr>
<td>EURO B</td>
<td>0.9 (0.1)</td>
<td>1.3 (0.1)</td>
<td>3.2 (0.3)</td>
<td>5.8 (0.3)</td>
<td>12.2 (0.3)</td>
<td>24.7 (2.3)</td>
</tr>
<tr>
<td>EURO C</td>
<td>0.9 (0.1)</td>
<td>1.3 (0.1)</td>
<td>3.2 (0.2)</td>
<td>5.8 (0.2)</td>
<td>11.8 (0.5)</td>
<td>24.5 (1.8)</td>
</tr>
<tr>
<td>AMRO A</td>
<td>0.8 (0.1)</td>
<td>1.7 (0.1)</td>
<td>3.3 (0.3)</td>
<td>6.5 (0.5)</td>
<td>12.6 (0.5)</td>
<td>30.1 (1.1)</td>
</tr>
<tr>
<td>AMRO B</td>
<td>0.8 (0.1)</td>
<td>1.7 (0.1)</td>
<td>3.4 (0.2)</td>
<td>7.6 (0.4)</td>
<td>14.8 (0.6)</td>
<td>33.2 (3.5)</td>
</tr>
<tr>
<td>AMRO D</td>
<td>0.7 (0.1)</td>
<td>1.5 (0.3)</td>
<td>3.8 (0.4)</td>
<td>6.2 (1.1)</td>
<td>11.1 (2.0)</td>
<td>28.1 (5.2)</td>
</tr>
<tr>
<td>EMRO A</td>
<td>0.9 (0.3)</td>
<td>1.8 (0.1)</td>
<td>3.6 (0.3)</td>
<td>6.6 (0.2)</td>
<td>13.9 (1.3)</td>
<td>23.5 (2.3)</td>
</tr>
<tr>
<td>EMRO B</td>
<td>1.2 (0.3)</td>
<td>1.9 (0.2)</td>
<td>3.9 (0.3)</td>
<td>6.6 (0.4)</td>
<td>13.9 (1.3)</td>
<td>23.5 (2.3)</td>
</tr>
<tr>
<td>EMRO D</td>
<td>0.6 (0.1)</td>
<td>1.4 (0.1)</td>
<td>2.6 (0.3)</td>
<td>4.7 (0.6)</td>
<td>10.4 (1.2)</td>
<td>22.1 (3.5)</td>
</tr>
<tr>
<td>WPRO A</td>
<td>0.6 (0.1)</td>
<td>1.8 (0.2)</td>
<td>3.7 (0.4)</td>
<td>7.0 (0.9)</td>
<td>14.4 (1.9)</td>
<td>28.2 (3.9)</td>
</tr>
<tr>
<td>WPRO B</td>
<td>1.0 (0.1)</td>
<td>1.7 (0.2)</td>
<td>3.4 (0.2)</td>
<td>5.7 (0.5)</td>
<td>10.6 (1.2)</td>
<td>17.6 (2.7)</td>
</tr>
<tr>
<td>SEARO B</td>
<td>0.4 (0.1)</td>
<td>0.9 (0.1)</td>
<td>1.8 (0.2)</td>
<td>3.7 (0.4)</td>
<td>7.2 (1.2)</td>
<td>14.4 (2.7)</td>
</tr>
<tr>
<td>SEARO D</td>
<td>0.3 (0.1)</td>
<td>0.6 (0.1)</td>
<td>1.3 (0.2)</td>
<td>2.3 (0.5)</td>
<td>4.3 (1.0)</td>
<td>9.7 (1.9)</td>
</tr>
<tr>
<td>AFRO D</td>
<td>0.5 (0.3)</td>
<td>1.0 (0.4)</td>
<td>1.9 (0.9)</td>
<td>3.8 (1.7)</td>
<td>7.0 (3.6)</td>
<td>14.9 (7.2)</td>
</tr>
</tbody>
</table>


Region-specific estimates for the incidence of dementia in individuals aged 60 years and over was also obtained through the Delphi consensus study, ranging from 10.5/1000 person-years for North America and 8.8/1000 person-years for Western Europe, to 4.3/1000 person-years for India and South Asia and 3.5/1000 person-years for Africa.

According to these estimates, the number of individuals diagnosed with dementia will double every 20 years, reaching 81.1 million by 2040; by this time, 71% of the estimated cases will occur in the developing world (8). The WHO projections suggest that by 2025 about three-quarters of the estimated 1.2 billion people aged 60 years and older will live in developing countries (8), reflecting demographic and epidemiological transitions promoting population ageing. At present, countries in Latin America such as Venezuela and Argentina already bear an important burden of over 5% prevalence of dementia. This contrasts with the lowest estimates for sub-Saharan Africa and India (8), although shorter
survival after dementia in these countries, lack of awareness and social support systems as well as inadequate diagnostic assessment, may have led to an underestimation of the frequency of dementia in these settings (8).

Dementia is characterized by major cognitive and functional impairment and is a chronic and progressive disorder, leading to high levels of dependence in the advanced stages of the disease. The age-standardized Disability-Adjusted Life Years (DALYs) for AD and other dementias in 2004 was 260/100,000 in the United States of America, 259/100,000 in the UK, 268/100,000 in Australia and 247/100,000 in Japan (61). These estimates were strikingly lower in most African and South American countries; for example 135/100,000 in Angola, 120/100,000 in Mozambique, 184/100,000 in Colombia or 187/100,000 in Mexico. According to the WHO baseline scenario projections for 2030, Alzheimer’s disease and other dementias will become the third leading cause of DALYs in high-income countries (59).

Although dementia is a leading cause of death, it is often under recognized as a terminal illness (62). The European collaborative study of dementia evaluated the prognosis of this condition, assessing the time to institutionalization and the mortality (63). Jagger et al (63) described that prevalent cases had over twice the risk of death compared to noncases in four years, despite the imprecision of the estimates [Relative Risk (RR): 2.36; 95% confidence interval (95%CI), 0.18-32.45] and the use of a sample with a mixture of cases having been diagnosed close to the baseline evaluation or longer ago. Subjects with dementia were also more likely to reside in institutional care at baseline than controls, independently of age and gender (63). Mitchell et al (62) described a mortality of 54.8% over an 18-month period in a group of nursing home residents with advanced dementia; in addition, 40.7% of patients underwent at least one burdensome intervention (hospitalization, emergency room visit, parenteral therapy or tube feeding) in the last three months of life, illustrating the morbidity associated to this degenerative condition.

The social and economical repercussions of the above mentioned trends and projections are thus immense and constitute an alert for the need to stimulate organized social interventions whilst, at the same time, investing in research focusing on prevention strategies and disease-modifying treatments may contribute to reduce the morbidity and mortality burden.
Evidence on the epidemiology of MCI is beginning to emerge, but there is a considerable variability in the frequency estimates presented in the literature, also reflecting the lack of operational diagnostic criteria.

Incidence estimates are scarce; the most robust were obtained in a three-year follow up of a Finnish population aged 60-76 years at baseline, which yielded an incidence rate of 25.9/1,000 person-years (64). Prevalence estimates in studies that include currently accepted MCI criteria range from 12% to 18%, among non-demented individuals aged 65 years and over (65-67).

Regarding the four MCI clinical subtypes, Busse et al. (68) describe a higher prevalence of single domain MCI in comparison with multiple domain MCI (11.6% vs 7.6%) and the nonamnestic MCI type was as frequent as amnestic MCI (9.2% vs 10.0%), when applying the original criteria (using a cutoff of 1.0 standard deviation from the neuropsychological tests scores). In another population-based study, Lopez et al. (65) reported a similar overall MCI prevalence (19%), but inverse findings regarding MCI subtype frequencies, with multiple domain MCI being more prevalent than single domain MCI (16% vs 6%). This may be partly due to differences in the neuropsychological instruments used and cognitive domains assessed, choice of different cutoffs in neuropsychological instruments and socio-cultural characteristics of the subjects evaluated, as acknowledged by Petersen et al. (18).

2.1. Burden of disease associated with dementia, in Portugal

In addition to population ageing (69, 70), Portugal presents a high prevalence of illiteracy and low formal education levels (69), which is a well established risk factor for dementia (71). In 2001, the overall illiteracy prevalence in Portugal was 9%, especially among the elderly (reaching 6.3% in men and 11.5% in women) and in the Alentejo and interior of the Centro regions (69).

A 2-year longitudinal study conducted by Nunes et al in 2005 showed an overall incidence of dementia of 6.8/1,000 person-years, higher in the rural area of Arouca (9.1/1,000 person-years) than in the urban setting of São João da Madeira (3.9/1,000 person-years). Participants were aged 55-79 years old at baseline and dementia was defined according to the DSM-IV criteria (72).
In 1994, Garcia et al published an estimate of the number of individuals with dementia in Portugal, according to the EURODEM and the 1991 Censos data (73). The total number of subjects estimated to have dementia was 92,470, corresponding to a prevalence of 1.7%, and approximately half of these patients (n=48,706) were estimated to have AD (73).

The frequency distribution of dementia subtypes in Portugal is unknown. Nevertheless, given the high impact of cerebrovascular disease in the country it is likely that VaD has a strong expression (74). Regarding the burden of dementia in Portugal, the World Health Organization reported an age-standardized Disability-Adjusted Life Years (DALYs) of 254/100,000, concerning the year 2004. This estimate is comparable to those for the United Kingdom and the United States of America in the same year (61), showing that dementia is a major public health issue also in Portugal.

3. Risk factors for MCI and dementia

3.1. MCI and progression to dementia – risk factors

Few studies addressed the determinants of MCI. Tervo et al (64) identified older age, low educational level, Apolipoprotein (APOE) ε4 allele carrier state and treated hypertension as the most prominent variables associated with a higher risk of MCI (64). In the longitudinal Cardiovascular Health Study Cognition Study low educational level, cortical atrophy, magnetic resonance imaging-identified infarcts and depression were found to be the main risk factors for MCI (75).

In contrast, the progression to AD has been a matter of intense research and debate, although no consensus exists as to whether MCI should be viewed as an entity with multiple etiological explanations or constrained to identify only patients with prodromal AD. Overall, the conversion rate from MCI to dementia in clinical samples is reported to range between 10% and 20%, regardless of age, considering follow-up periods of one to three years (5). Different subtypes of MCI may be associated with the development of specific forms of dementia; amnestic subtypes of MCI were shown to be associated with a higher risk of AD (7); in the study by Busse et al (68), progression to non-AD dementia was more frequent in non-amnestic MCI and for all other types of MCI the most common form of dementia diagnosed at follow-up was AD. A correct characterization of the
cognitive domains affected may therefore help to determine outcome, and in fact deficits in verbal memory and psychomotor speed/executive function abilities were shown to strongly predict conversion to AD in a prospective study conducted by Tabert et al in an MCI group of individuals (76).

Predictors of progression to AD in individuals with MCI have been identified: APOE ε4 allele carrier state (77) and reduced hippocampal volumes derived from magnetic resonance images (78) are the two main risk factors identified.

Cerebral spinal fluid markers, Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) patterns of metabolism and molecular imaging techniques are among other variables currently being assessed in what is considered to be an expanding field of research (79, 80).

3.2. Dementia – risk factors

Family history is a major non-modifiable risk factor for AD, reflecting the contribution of genetic determinants. In the rare familial forms of AD with onset before the age of 60, point mutations in the amyloid precursor protein (APP, chromosome 21), the presenilin 1 (PS1, chromosome 14) and the presenilin 2 (PS2, chromosome 1) genes cause autosomal dominant transmission of the disease. In non-familial AD, which constitutes the vast majority of cases, the major genetic risk factor identified are related with the APOE ε4 gene polymorphism (81).

Aging and female gender are two additional well-established non-modifiable risk factors for AD (7, 82), independently from education (82). This gender difference was initially suggested to be associated with postmenopausal estrogen deficiency, but the large prospective Women's Health Initiative Memory Study (WHIMS) confirmed that hormone replacement therapy (HRT) provided no benefit for global cognition among women aged 65 years or older (83); in fact, women assigned to HRT had a slightly but significantly lower average cognitive function compared with those assigned to placebo (84).

Current therapies for AD are mainly symptomatic, focusing on treating cognitive or behavioral symptoms and disease-modifying treatments are the main target of molecular research, especially focused on amyloid deposition (85), hyperphosphorilated tau protein (86) and oxidative stress (87). In parallel, epidemiological research has tried to identify modifiable risk factors for the development of dementia, since there are at present no
approved disease-modifying treatments and primary prevention could play a crucial role in controlling the outbreak of dementia. Education level was the first modifiable exposure shown to be inversely associated with the risk of dementia (7). Lifestyles and habits have also received considerable attention, and, among these, some of the traditional determinants of cardiovascular disease have been described as important risk factors for dementia and AD, in addition to VaD. Timing of exposure is an extremely important aspect to consider, especially because neurodegenerative conditions are chronic and progressive, with pathological changes developing over years before symptoms and deficits are experienced; the same generally happens for conditions emerging as a consequence of vascular risk factors, and it is thus reasonable to assume that exposure patterns in midlife may contribute to determine the risk of dementia at an older age (88), even though the latency period is essentially unknown. Table 10 summarizes some of the available epidemiological evidence associating midlife exposure to cardiovascular risk factors and a higher risk of dementia at an older age.
<table>
<thead>
<tr>
<th>Vascular factor</th>
<th>1st author</th>
<th>Year</th>
<th>Country</th>
<th>Type of study</th>
<th>Sample characteristics</th>
<th>Outcome(s)</th>
<th>Results</th>
<th>Control of confounding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>Whitmer (89)</td>
<td>2005</td>
<td>USA</td>
<td>Retrospective cohort</td>
<td>Members of the Kaiser Permanent Medical Care Program of Northern California Baseline population: 6,945 Mean age at baseline: 41 years Follow-up (mean): 34 years</td>
<td>Dementia (ICD-9)</td>
<td>Hypertension vs no hypertension HR=1.24 (1.22-1.68)</td>
<td>Age at start of case ascertainment, race, education, sex</td>
</tr>
<tr>
<td></td>
<td>Kivipelto (60)</td>
<td>2005</td>
<td>Finland</td>
<td>Prospective cohort</td>
<td>Participants from the CAIDE study Baseline population: 1,666 Follow-up (mean): 21 years Completeness of follow-up: 73%</td>
<td>Dementia (DSM-IV) and Alzheimer’s disease (NINCDS-ADRDA)</td>
<td>SBP &gt; 140 vs &lt; 140 mmHg OR=1.97 (1.03-3.77)</td>
<td>BMI, total cholesterol level, age, sex, education, follow-up time</td>
</tr>
<tr>
<td></td>
<td>Launer (51)</td>
<td>2000</td>
<td>USA</td>
<td>Retrospective cohort</td>
<td>Participants from the Honolulu Heart Program (Japanese-American men) Baseline population: 3,703 Follow-up (mean): 20 years</td>
<td>Alzheimer’s disease and vascular dementia (DSM-III)</td>
<td>DBP ≥ 90-94 vs 80-89 mmHg; OR=3.8 (1.6-8.7) DBP ≥ 95 vs 80-89 mmHg; OR=4.3 (1.7-10.6) SBP ≥ 160 vs 110-139 mmHg; OR=8.8 (2.6-11.6)</td>
<td>Age, education, apolipoprotein epsilon allele, smoking and alcohol intake</td>
</tr>
<tr>
<td></td>
<td>Klander (92)</td>
<td>1998</td>
<td>Sweden</td>
<td>Prospective cohort</td>
<td>Men residing in Uppsala (born in 1920-1924) Baseline population: 959 Follow-up (mean): 20 years</td>
<td>MMSE and Trail-Making Test scores</td>
<td>DBP (mmHg), Cognitive Score, Mean (SD)¹: 70-79, ± 0.17 (0.71) 75-80, ± 0.00 (0.02) 80-89, ± 0.04 (0.7) 90-100, ± 0.02 (0.8) ≥ 105, -0.33 (0.82)</td>
<td>Age, educational and occupational levels</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Kivipelto (60)</td>
<td>2005</td>
<td>Finland</td>
<td>Prospective cohort</td>
<td>Participants from the CAIDE study Baseline population: 1,666 Follow-up (mean): 21 years Completeness of follow-up: 73%</td>
<td>Dementia (DSM-IV) and Alzheimer’s disease (NINCDS-ADRDA)</td>
<td>Total cholesterol ≥ 251 vs &lt; 251 mg/dl; OR=2.09 (1.16-3.77)</td>
<td>SBP, BMI, age, sex, education, follow-up time</td>
</tr>
<tr>
<td></td>
<td>Whitmer (89)</td>
<td>2005</td>
<td>USA</td>
<td>Retrospective cohort</td>
<td>Members of the Kaiser Permanent Medical Care Program of Northern California Baseline population: 6,945 Mean age at baseline: 41 years Follow-up (mean): 34 years</td>
<td>Dementia (ICD-9)</td>
<td>Total cholesterol ≥ 240 mg/dl vs &lt; 240 mg/dl HR=1.42 (1.22-1.68)</td>
<td>Age at start of case ascertainment, race, education, sex</td>
</tr>
<tr>
<td></td>
<td>Schneider (93)</td>
<td>2004</td>
<td>Israel</td>
<td>Prospective cohort</td>
<td>Members of the Israeli Ischemic Heart Disease project (men) Follow-up (mean): 35 years Completeness of follow-up: 72.8%</td>
<td>Diabetes vs no diabetes</td>
<td>OR=2.83 (1.40-5.71)</td>
<td>Age, area of birth, socioeconomic status, mean cholesterol, mean HDL cholesterol, mean DBP and SBP, BMI, smoking</td>
</tr>
</tbody>
</table>

CAIDE = Cardiovascular Risk Factors, Aging and Dementia; ICD = International Classification of Diseases; DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; DSM-III = Diagnostic and Statistical Manual of Mental Disorders, Third Edition; NINCDS-ADRDA = National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association; DBP = diastolic blood pressure; SBP = systolic blood pressure; BMI = Body Mass Index; MMSE = Mini Mental State Examination; OR = odds ratio; HR = hazard ratio; ¹ results are presented as OR or HR (95% confidence interval).
Table 10 (cont.). Cohort studies assessing the relation between midlife exposure to vascular risk factors and the risk of dementia.

<table>
<thead>
<tr>
<th>Vascular risk factor</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; author / Year / Country</th>
<th>Type of study</th>
<th>Outcome(s)</th>
<th>Results a</th>
<th>Control of confounding</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>Kiwipello (90) Finland 2005</td>
<td>Prospective cohort</td>
<td>Dementia (DSM-IV) and Alzheimer’s disease (NINCDS-ADRDA)</td>
<td>BMI&lt;30 vs BMI&lt;20 kg/m²</td>
<td>SBP, total cholesterol level, age, sex, education, follow-up time</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Participants from the CAIDE study Baseline population: 1965 Follow-up (mean): 21 years Completeness of follow-up: 73%</td>
<td></td>
<td>OR=2.09 (1.16-3.77)</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>Whitmer (69) USA 2005</td>
<td>Retrospective cohort</td>
<td>Dementia (ICD-9)</td>
<td>Diabetes vs no diabetes</td>
<td>Age at start of case ascertainment, race, education, sex</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Members of the Kaiser Permanent Medical Care Program of Northern California Baseline population: 8845 Mean age at baseline: 41 years</td>
<td></td>
<td>HR=1.48 (1.19-1.79)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Schneier (93) Israel 2004</td>
<td>Prospective cohort</td>
<td>Dementia (DSM-IV)</td>
<td>Diabetes vs no diabetes</td>
<td>Age, area of birth, socioeconomic status, mean cholesterol, mean HDL cholesterol, mean DBP and SBP, BMI, smoking</td>
</tr>
<tr>
<td></td>
<td>Been (93) Israel 2004</td>
<td>Members of the Israeli Ischemic Heart Disease project (men) Mean age at baseline: 47 years</td>
<td></td>
<td>OR=2.83 (1.40-5.71)</td>
<td></td>
</tr>
</tbody>
</table>

CAIDE – Cardiovascular Risk Factors, Aging and Dementia; ICD – International Classification of Diseases; DSM-IV – Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; DSM-III – Diagnostic and Statistical Manual of Mental Disorders, Third Edition; NINCDS-ADRDA – National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association; DBP –diastolic blood pressure; SBP – systolic blood pressure; BMI – Body Mass Index; MMSE – Mini Mental State Examination; OR – odds ratio; HR – hazard ratio; * results are presented as OR or HR (95% confidence interval).
The prospective studies with shorter follow-up periods and assessing the independent role of individual risk factors and the occurrence of AD later in life have shown an association between hypertension (94), diabetes (95, 96), high cholesterol (97) and a higher risk of AD, although the level of evidence is stronger for the first two (98). Clustering of cardiovascular risk factors have been reported to increase the risk of dementia in an additive manner so that individuals with hypertension, obesity and high total cholesterol in midlife have a risk of dementia and AD six times higher than those with no vascular risk factors (90). Supporting these results, the metabolic syndrome has also been shown to be independently associated with AD (99).

Gene-environmental interactions probably exert an important role in determining the risk of dementia and in fact, diabetic patients who are also APOE ε4 carriers have been described to have a higher risk of AD, supporting this hypotheses (100).

Smoking is presently recognized as an environmental risk factor for dementia (101). Although it was initially suggested that it could reduce the risk of AD (102), recent prospective studies show that smokers have a significantly increased risk of dementia, including AD (103, 104), especially among the APOE ε4 carriers (105). A meta-analysis (106) based on 19 prospective studies estimated a RR of 1.79 (95% CI: 1.43 to 2.23) for incident AD in current smokers in comparison with never smokers, 1.78 (95% CI: 1.28 to 2.47) for incident vascular dementia, and 1.27 (95% CI: 1.02 to 1.60) for any dementia. Compared with those who never smoked, current smokers at baseline also showed greater yearly declines in Mini-Mental State Examination scores over the follow-up period (beta=-0.13, 95% CI: -0.18 to 0.08).

Regarding alcohol consumption, evidence suggests that light and moderate intakes may have beneficial effects on cognitive health, in comparison to abstinence or heavy drinking. These conclusions are mostly based on prospective studies with follow-up periods ranging from 2 to 8 years, which describe a reduced risk of dementia and AD in alcohol drinkers in late life (107). Adding to these data, the prospective study by Anttila et al (108) also assessed MCI as an outcome, in parallel with dementia. This 23-year follow-up study confirmed a U-shaped relation between alcohol consumption in midlife and mild cognitive impairment at older age (108). No U-shaped relation was observed (108), but the APOE genotype seemed to modify the risk of dementia, as higher RR estimates were obtained for APOE ε4 carriers with concomitant higher alcohol intakes (108).

The role of physical activity in cognitive health has only recently been addressed. Two studies assessing leisure-time physical activity in midlife in relation to later risk of
dementia suggest that higher levels of activity are associated with a lower risk of AD and dementia (109, 110). There are at present no formal recommendations on the type, intensity, frequency and duration of exercise that most effectively reduced the risk of dementia later in life (111). The potential effect of diet on the prevention of dementia and cognitive decline further extends this research field (112) and has received considerable attention. Much research to date has focused on specific nutrients. High intake of antioxidants has been suggested as a potentially protective measure against neurodegeneration, since free radicals and oxidative damage have been implicated in age-related brain disease. Dietary and supplemental intake of vitamin C and E has been associated with a lower risk of AD (113, 114), and high midlife exposure to fruits and vegetables has been described to decrease the risk of dementia (115). Nevertheless, other authors reported no significant association between these two variables and the occurrence of dementia (116). Randomized controlled trials of folate, vitamin B6 and B12 supplementation have shown no protective cognitive effects (117, 118). Regarding macronutrients, dietary fat has been the most thoroughly investigated. In a longitudinal study with a follow up of 21 years, Laitinen et al (119) described an association between moderate intake of total and unsaturated fats and a reduced risk of AD and dementia when compared with to a low consumption. Conversely, moderate saturated fat intake was described to be associated with an increased risk of dementia; further studies with shorter follow-up periods reported similar findings (120, 121). Despite the fact that some reports support an association between at least some dietary habits and cognitive health on a long term, the IANA (International Academy of Nutrition & Aging) task force on nutrition and cognitive decline with aging stresses the need for further meta-analysis and prospective studies with a robust follow-up, accounting for confounding factors and standard social determinants of food habits, before specific recommendations can be made (112).

In keeping with the trend of investigating the role of modifiable risk factors and dietary habits in chronic diseases, caffeine has been investigated as a molecule with the potential to promote neuroprotection, especially in Parkinson’s disease (122, 123) but also in dementia and cognitive decline (124, 125).
4. Caffeine and Cognition: Molecular Mechanisms and Biological Plausibility

Caffeine is one of the natural chemicals in the external environment able to invade the synaptic cleft (126). It is also a widely available substance, being present in coffee, tea, soft drinks, chocolate and dietary supplements, and is worldwide the most commonly used neurostimulant (127).

The actions of caffeine at a synaptic level share the potential to induce plastic changes at the cortical network level, through adenosine dependent and independent mechanisms. Its ability to antagonize adenosine receptors has been extensively investigated as the primordial biological property conditioning a hypothetical neuroprotective role. Nevertheless, other neuropharmacological properties of caffeine which may also account for synaptic modulation have also been described.

4.1. Adenosine as a neuromodulator

The important neuromodulator action of adenosine is recognized since the work conducted by Phillis et al in the 1970’s (128), although it cannot be considered a classic neurotransmitter, because it is not stored in synaptic vesicles, its release is not Ca²⁺-dependent and it is generated from ATP by the action of extracellular enzymes (129). In fact, adenosine behaves as an extracellular signaling molecule that influences synaptic transmission without itself being a neurotransmitter (130).

Adenosine receptors (ARs) are coupled with G-proteins and can be divided into subtypes A₁, A₂A, A₂B and A₃ (126). The first two types are ubiquitous receptors with high-affinity for adenosine found in the brain as well as other tissues (129); A₂B receptors are low affinity-receptors which may be relevant in pathological conditions, and the A₃ subtype has a low density in most tissues (130).

Regarding their distribution in the central nervous system, A₁ receptors are especially concentrated in the hippocampus and neocortex, where glutamate is used as an excitatory transmitter. Conversely, A₂A receptors are distributed locally at the highest level in the striatum and nucleus accumbens, although there is consistent evidence of their expression in the hippocampus (131). A₂A receptors are thus mainly concerned with the dopaminergic system whereas A₁ receptors in the hippocampus and neocortex are dominantly linked with the glutamatergic system. The density and functioning of each of
these subtypes (A₁ and A₂ₐ) seems to be age-dependent (130). In aged rats, the density and function of A₂ₐ receptors is increased in the hippocampus and cortex whereas the reverse happens for subtype A₁ (132); the same observation has recently been replicated in humans (133).

In neurons, A₁ and A₂ₐ receptors are expressed at both a presynaptic and postsynaptic level. The presynaptic activation of A₁ receptors has been reported to influence negatively the release of a variety of neurotransmitters, including glutamate, acetylcholine and dopamine. This suppressive effect has been evoked as the main explanation for the neuroprotective role of adenosine described in association with A₁ receptor mechanisms (134), especially in the context of a hypoxic/ischemic insult (135). Despite these observations, endogenous adenosine has been reported to constrain synaptic plasticity phenomena such as Long Term Potentiation (LTP) and Long Term Depression (LTD) through A₁ receptors (136), which inhibit NMDA receptor-mediated currents (137). For this reason, antagonism of these receptors has been suggested as a potential target in the treatment of memory disorders (138). This occurs in parallel with descriptions that A₁ receptor-agonists may acutely protect against neuronal damage caused by toxins or ischemia-reperfusion (134).

The presynaptic A₂ₐ receptors enhance neuronal activity, as opposed to A₁ receptors. These receptors are preferentially activated at high-frequency neuronal firing, such as that inducing LTP (130).

The control by adenosine of neurotransmitter release should ultimately be understood as a balance between inhibitory A₁ and facilitation A₂ₐ receptor-mediated actions (139). Adding to this, A₂ₐ receptors have also been shown to inhibit A₁ receptors functioning in nerve terminals (130). Aging therefore highlights the role of A₂ₐ receptors in the brain region most implied in the pathology of Alzheimer’s disease, not only by increasing their expression (140) but also through A₁ receptor inhibition.

In parallel with its actions on nerve terminals and neurotransmitters release control, adenosine also exerts a paramount role in neuronal modulation at a postsynaptic level. This has been described to happen through interactions with G-protein-coupled receptors, ionotropic receptors and neurotrophic factors (130).

The most widely described interaction with a metabotropic receptor is the one concerning dopamine D₂ receptors. A₂ₐARs decrease its affinity for dopamine and this mechanism has been intensely investigated, viewing new therapeutic targets for Parkinson’s disease (141, 142).
The interactions so far described between adenosine receptors and glutamate metabotropic receptors deserve considerable attention in the discussion of the cognition and neurodegeneration processes implicated in dementia. It has been shown that $A_{2A}$ARs and metabotropic glutamate 5 receptors (mGlu5Rs) co-exist in hippocampal synapses (143). It is well accepted that this subtype of glutamate receptor "sets the tone" of NMDA receptor-mediated transmission (144). An experimental study conducted in rats by Tebano et al (143) showed that $A_{2A}$ receptors exert a permissive role on mGlu5Rs-mediated effects, namely the potentiation of NMDA responses. Given the key role of NMDA receptors in both synaptic plasticity and excitotoxicity, modulating mGlu5R may help to regulate both the physiological and the pathological effects elicited by NMDA receptor stimulation in the hippocampus. The functional interaction between $A_{2A}$ receptors and mGlu5Rs in this brain region suggests that the former molecules may constitute an interesting target for therapeutic strategies concerning diseases were NMDA receptor signaling is especially involved in neurodegeneration. The blockade of $A_{2A}$ receptors may therefore limit NMDA induced neurotoxicity, recognized to be involved in AD pathophysiology. The prominent role of $A_{2A}$ receptors in preventing memory deterioration is thus probably related to the synaptic localization of this receptor in limbic areas and its ability to control glutamatergic transmission, especially NMDA receptor-dependent plasticity (145).

The interaction between adenosine and neurotrophic receptors is also an expanding field, although mechanisms are yet to be completely revealed. Brain-derived neurotrophic factor (BDNF) has been shown to promote LTP and synaptic plasticity and its action seems to be critically dependent on $A_{2A}$ receptors dynamics (146). Impairment of neuromodulation by neurotrophic factors has been implicated in the pathophysiology of many degenerative nervous system conditions, namely Alzheimer’s disease (147).

It was recently shown that $A_{2A}$ receptors play a crucial role in the development of β-amyloid induced synaptotoxicity, leading to memory dysfunction through a p38 mitogen-activated protein kinase (MAPK) dependent pathway; the blockade of these receptors could therefore constitute a potential target in AD treatment, limiting β-amyloid neurotoxicity, in addition to controlling glutamatergic synaptic transmission in limbic regions (148).
4.1.1. Caffeine and Adenosine Receptors

Adenosine dependent mechanisms have been the most extensively investigated so far. The balance between the neuroprotective role of adenosine on the one hand and the inhibition of synaptic toxicity induced by NMDA actions and β-amyloid accumulation through blockade of $A_{2A}$ receptors on the other is however still a matter of debate. It is likely that age as well as duration of exposure to caffeine constitute variables of paramount importance in determining this balance. If these aspects are better understood, adenosine modulating mechanisms should remain a very interesting target for therapeutic neuroprotective strategies.

An important aspect regarding caffeine and adenosine neuromodulation is that the effects of xanthines may depend largely on the dosage and duration of intake. Conlay et al conducted an experimental work in 1997 aiming to evaluate the relationship between the administration of caffeine and plasmatic adenosine levels (149). This study showed a dose-response relation between caffeine exposure and plasma adenosine concentration; conversely, plasma adenosine concentration decreases as exposure to caffeine is abolished, supporting a receptor-mediated effect (149). It is worth noting at this point that adenosine has been previously implicated in a non-immediate pathway of neuroprotection involving glial cells, in the setting of hypoxic/toxic insults, and therefore exposure to sufficient amounts of caffeine may enhance this neuronal defensive mechanism (150).

Duration of exposure to caffeine may also be crucial in tuning the adenosine modulating system; in fact, upregulation of $A_1$ receptors in neurons has been described with chronic intake of caffeine, which may contribute to limit the release of excitatory neurotransmitters and prevent synaptotoxicity (151). An upregulating effect occurring as a response to chronic caffeine exposure has also been described for $A_{2A}$ receptors present on the surface of platelet cells (152); it is presently unknown whether the same happens to $A_{2A}$ receptors present in neurons.

The above mentioned dose and duration-dependent consequences of exposure to caffeine differ considerably from the generally agreed upon effects of selective adenosine receptor antagonism, described in acute administration. Over the past few years, it has become apparent that the effects of acute and chronic treatment with caffeine, as well as of other adenosine receptor antagonists, are qualitatively different (151). Thus, long-term treatment with adenosine receptor antagonists can have effects that resemble those of acute administration of adenosine receptor agonists, and vice versa (151). A careful
assessment of the pattern of exposure becomes therefore crucial in epidemiological studies.

4.2. Non-purinergic mechanisms

Apart from its adenosine modulating effect, caffeine displays additional pharmacological mechanisms that may potentially interfere with cognition at a synaptic and molecular level.

Caffeine is responsible for the activation of internal ryanodine receptors, resulting in reduction of the calcium-induced calcium release (CICR) threshold. Concentration of endoplasmic calcium are thus amplified and intracellular calcium signaling pathways are activated (126). Caffeine also promotes blockade of phosphodiesterases (PDEs), resulting in intracellular cAMP accumulation and enhancement of the pathways which involve cAMP signaling (126). Finally, it also interferes in GABAergic dynamics, although the molecular pathway is not yet clear (126). GABA receptor activation generates inhibitory postsynaptic potentials and caffeine has been shown to depress GABA receptor activities in the hippocampus, through calcium mediated and calcium independent mechanisms (153, 154).

Chronic administration of xanthines also interferes with other neurotransmitters, namely 5-HT (151). It has been suggested that presynaptic serotonergic function is altered before the development of psychiatric problems such as depression in AD (155), and cortical 5-HT(1A) receptor have recently been described to mediate cognition, although in ischemic vascular dementia (156). Lai et al had also previously described that the loss of neocortical 5-HT 2A receptors could predict a faster cognitive decline in patients with AD. The serotonergic system may therefore also be modulated by xanthines and contribute to alter cognition processing pathways.
5. Objectives

Alzheimer's disease and other aging-related neurodegenerative disorders have emerged in the last decades as a major health problem in our society. The efficacy of available treatments for these disorders is at present quite limited, which enhances the importance of identifying potential risk factors whose modulation might decrease the risk or attenuate the progression of these neurodegenerative disorders.

Caffeine is widely consumed worldwide and a potential protective role regarding the development of dementia is biologically plausible. Epidemiological research on this topic, however, is relatively scarce and methodologically heterogeneous. An updated systematic review of the literature and prospective studies could both be helpful to obtain new insights on this topic.

To clarify the relation between caffeine intake and dementia we conducted two studies with the following specific objectives:

1) To quantify the association between caffeine intake and cognitive decline in a cohort of Portuguese urban elderly subjects;

2) To review systematically and summarize the published studies addressing the effect of caffeine in cognitive decline and dementia, and to discuss the methodological heterogeneity of the available evidence.
6. References


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7.1. Manuscript 1

Caffeine intake is associated with a lower risk of cognitive decline: a cohort study from Portugal

Abstract
Alzheimer’s disease has emerged in recent decades as a major health problem and the role of lifestyles in the modulation of risk has been increasingly recognised. Recent epidemiological studies suggest a protective effect of caffeine intake in dementia. We aimed to quantify the association between caffeine dietary intake and cognitive decline, in a cohort of adults living in Porto. A cohort of 648 subjects aged ≥65 years was recruited between 1999-2003. Follow-up evaluation (2005-2008) was carried out on 58.2% of the eligible participants and 10.9% were deceased. Caffeine exposure in the year preceding baseline evaluation was assessed with a validated food frequency questionnaire. Cognitive evaluation consisted of baseline and follow-up Mini-Mental State Examination (MMSE). Cognitive decline was defined by a decrease ≥2 points in the MMSE score between evaluations. Relative risk (RR) and 95% confidence interval (95%CI) estimates adjusted for age, education, smoking, alcohol drinking, body mass index, hypertension and diabetes were computed using Poisson regression. Caffeine intake (>62 mg/day [3rd third] vs. <22 mg/day [1st third]) was associated with a lower risk of cognitive decline in women (RR=0.49, 95%CI 0.24-0.97), but not significantly in men (RR=0.62, 95%CI: 0.26-1.48). Our study confirms the negative association between caffeine and cognitive decline in women.

Key words: Caffeine; Dementia; Cohort Studies, Gender.
Introduction

Alzheimer's disease (AD) and other aging-related neurodegenerative disorders have emerged in the last decades as a major health problem in our society [1]. The efficacy of available treatments for these disorders is at present quite limited. The importance of identifying potential risk factors whose modulation might decrease the risk or attenuate the progression of these neurodegenerative disorders is thus obvious. The role of lifestyles in the prevention of AD has been emphasised [2], as it had been the case for Parkinson's disease, another neurodegenerative condition sharing similarities with AD [3].

Caffeine is widely consumed worldwide and acts as a nonspecific antagonist of adenosine receptors [4]. The blockade of A2A receptors has recently been demonstrated to limit the synaptotoxic effect of Aβ, a peptide that accumulates in the brain of AD patients [5]. Experimental studies conducted in animal models have also shown that adenosine A<sub>2A</sub> and metabotropic glutamate 5 receptors are co-located and that the former play a permissive role in mGlu5R receptor-mediated potentiation of NMDA effects in the hippocampus [6].

Epidemiological studies aiming to evaluate caffeine intake and the risk of Parkinson's disease have so far suggested a protective role for this substance, more pronounced in men than in women [3]. Few studies have tested a possible association between regular caffeine intake and the risk for Alzheimer's disease. A small case–control study suggested that caffeine exposure was significantly inversely associated with AD [7]. A prospective study also showed that regular consumption of coffee was associated with a reduced risk of AD after 5-year follow-up. The study evaluated both coffee and tea consumption but the total amount of caffeine intake was not considered and the association found might have been biased due to the exclusion of a large number of decedents [8]. Other epidemiological studies used a different approach, assessing whether caffeine consumption could influence cognitive decline, with conflicting results [9-11]. Recently, a large epidemiological prospective study (the Three City Study), enrolling about 7000 participants aged 65 and over, showed that women with high rates of caffeine consumption (over three units per day, which is equivalent to 300 mg of caffeine) had less decline in memory performance than women consuming one unit or less [12]. The protective effect of caffeine increased with age and was not observed in men. However, the incidence of AD was not decreased in caffeine consumers.
The aim of the present study is to assess, in a different elderly population, the association between caffeine intake and cognitive decline, and to further elucidate the gender dependency of its potential effect. The hypothesis that caffeine intake could decrease the risk of decline of at least 2 points in the score of a widely used cognitive test, the Mini-Mental State Examination (MMSE), was specifically tested in the participants of the EPIPorto study aged 65 and over.
Methods

Study population

This study was based on the evaluation of a cohort of adults living in Porto. The recruitment of the initial sample has been previously described [13]. Briefly, the recruitment of the cohort was conducted between 1999 and 2003 and comprised the evaluation of 2485 individuals, selected by random digit dialling having households as the sampling unit. When a household was selected, all residents were identified by age and gender, and one resident (aged 18 or more years) was randomly selected as the respondent, without replacement if there was a refusal. The participation rate was 70% [14]. A visit to the Department of Hygiene and Epidemiology of Porto Medical School was scheduled by telephone according to the participant’s convenience. A personal interview, using a structured questionnaire comprising data on socio-demographic, clinical, and lifestyle exposures, and a physical examination was performed by trained interviewers. From the whole cohort, 648 participants were aged 65 and over and 531 were selected for the present study, after exclusion of 62 cognitively impaired at baseline (the criteria used to define cognitive impairment is defined below), 32 for whom there was no baseline MMSE, and 23 for whom there was no information on caffeine intake.

Follow-up evaluation

The follow-up evaluation of the cohort took place between May 2005 and May 2008. The participants were scheduled to visit Porto Medical School and underwent a questionnaire and physical examination. Among the 531 eligible participants, 309 (59.2%) completed the follow-up evaluation (median follow-up: 48 months), 58 (10.9%) died before follow-up could be accomplished and there were 164 (30.9%) losses to follow-up.

Participants who died during the follow up period were more likely to be older and hypertensive (women), to have a lower BMI (men) and worse MMSE score (women). No statistically significant differences between the groups were found regarding education, diabetes, smoking, alcohol and caffeine consumption (Table 1).

Cognitive testing

The MMSE [15, 16] was used to assess global cognitive function at baseline and at follow-up. The MMSE, which includes questions on orientation, registration, attention and calculation, recall, language and visual construction, was originally designed for clinical
practice, but is now extensively used in epidemiological studies. Although it does not assess executive function, a major feature of cognitive decline [17], the MMSE is a reliable and valid test for cognitive impairment, has high test-retest reliability, and is a good indicator of clinically significant cognitive decline [18]. The cut-off values adjusted for education levels were used as proposed in other studies [19, 20]. The normative cut-off values of MMSE adjusted for education for the Portuguese population were used [16]. Subjects that had a MMSE score below cutoff at baseline were considered to be cognitively impaired and therefore excluded. Participants had to score above 15 if they were illiterate, above 22 if they had ≤ 11 years of education, and above 27 if they had > 11 years of education.

A decline of at least 2 points in the score of the MMSE from baseline to the follow-up visit was considered meaningful from a clinical point of view.

**Caffeine dietary intake**

Dietary habits in the 12 months preceding the baseline interview were evaluated using a semi-quantitative food frequency questionnaire (FFQ) comprising 82 food and beverage items or groups. It was designed according to Willett et al. [21], and was adapted by inclusion of a variety of typical Portuguese food items. For each FFQ item, subjects were asked the average frequency of consumption (nine possible responses ranging from never to six or more times per day) and the portion size usually consumed (based on a photograph manual with small, medium and large portion sizes). This information was used to estimate the average daily intake of each item by multiplying the usual frequency of intake per day by the average portion size of the corresponding item. Food Processor Plus®, version 5.0, was used to obtain estimates of caffeine dietary intake. The food items/groups of the FFQ from which caffeine could be obtained were: coffee (including all beverages containing coffee); tea (green and black); ice-tea; coke; chocolate (including candy bars and cocoa powder).

The FFQ was validated with four 7-day food records in 75 women and 71 men, and the reproducibility was evaluated through the comparison of two FFQ evaluations conducted in 72 men and 78 women with a one-year interval [22]. For caffeine, the Spearman correlation coefficients were 0.65 for validity and 0.68 for reproducibility.

*Socio-demographic, clinical and other behavioural factors*
Education was recorded as completed years of schooling and further categorized into the same three groups used for the normative MMSE cut-offs adjusted for education in the Portuguese population.

Blood pressure was measured on a single occasion by non-physician trained interviewers, using a mercury sphygmomanometer, taking phase I and V Korotkoff sounds as systolic and diastolic blood pressure, respectively, and following the recommendations of the American Heart Association [23]. Two measurements of blood pressure separated by at least 5 minutes were taken after a 10-minute rest. When the difference was larger than 5 mmHg for systolic or diastolic blood pressure a third measurement was taken and the mean of the 2 closest values was registered. Arterial hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or current antihypertensive drug therapy [24].

A 12-hour overnight fasting serum sample was obtained to assess glycaemia. Participants on anti-diabetic therapy and/or with fasting plasma glucose concentrations ≥126 mg/dL and/or diagnosed with diabetes by a health professional were considered to have diabetes mellitus [25].

Anthropometric measurements were obtained after an overnight fast, with the participant wearing light clothing and no footwear. Body weight was measured to the nearest 0.1 kg using a digital scale, and height was measured to the nearest centimetre in the standing position using a wall stadiometer. Body mass index (BMI) was calculated as weight (kg) divided by squared height (m$^2$), and further divided into the following categories [26]: obese (≥30 kg/m$^2$), overweight (25.0–29.9 kg/m$^2$), normal and underweight (<24.9 kg/m$^2$).

Regarding smoking habits, subjects were classified as smoker (one or more cigarettes per day, on average), occasional smoker (less than one cigarette per day, on average), ex-smoker (for more than six months) and never smoker, according to World Health Organization categories [27]. Regarding the consumption of alcoholic beverages, participants were classified as non-drinker, ex-drinker (for more than six months), occasional drinker (consumption of less than a drink per week, on average) and usual drinker (consumption of at least one drink per week, on average). For analysis, participants were further categorized in never- and ever-smokers and never- and ever-drinkers.

Statistical analysis
Data analysis was conducted in 309 subjects who at baseline were aged 65 or more and had a MMSE not compatible with cognitive impairment, and who were re-evaluated. Comparison of the baseline characteristics between subjects that were followed, died or were lost to follow up was done using the Chi-Square or the Kruskal-Wallis tests, as appropriate, to compare all groups.

The association between caffeine intake and the development of cognitive impairment was quantified through crude and age-, education-, diabetes-, smoking- and alcohol drinking-adjusted relative risks (RR) and respective 95% confidence intervals (95%CI) using Poisson regression. Data were analysed using STATA®, version 9.2.

Decline in cognitive performance was defined in the primary analysis as a decrease of at least two points on the MMSE from the baseline assessment to follow up [12]. Additional analyses were conducted using impairment in cognitive performance at follow-up (using normative cut-off values of MMSE adjusted for education for the Portuguese population) as the outcome.

To define categories of exposure to caffeine we used the estimated sample tertiles of dietary intake as cut-off points, and the average caffeine content in one espresso – 75 mg [28]. For stratified analyses according to age or smoking status we used the median caffeine intake (33 mg) as cut-off to maximize the power of the study for the comparisons in each group.

Separate analyses were conducted for men and women, as previous studies have shown gender-specific effects of caffeine on neurodegenerative diseases [3, 12]. The option for this stratified analysis and the small number of subjects presenting the outcomes of interest did not allow the choice of potential confounding factors to include in the multivariate models to be based on statistical criteria. We selected factors whose association with neurodegenerative disorders is established or under discussion, namely age [17], education [29, 30], smoking [31, 32], alcohol intake [33, 34], hypertension [35], diabetes [36], obesity [36, 37] and likely to be related to caffeine consumption (especially through coffee intake), even if this association is to a large extent culturally determined and varies with gender and across population settings [38, 39].

Ethics

This study was approved by a local Ethics Committee, and all participants gave written informed consent.
Results

Cognitive decline defined as $\Delta$MMSE≤-2 between baseline and follow-up

Caffeine dietary intake was associated with a lower risk of cognitive decline both in women, and in men (Table 2). In women this 50% reduction in the risk of cognitive decline was essentially unchanged after adjustment for potentially important confounding factors, and was also observed for a caffeine consumption equivalent to more than one espresso per day (average amount, 75 mg, Table 2). The inverse association between caffeine intake and cognitive decline was not statistically significant in men, although the magnitude of the association was similar to that found in women, and was attenuated when confounding was controlled (Table 2).

The negative association between caffeine intake and cognitive decline was apparent in both genders for participants aged below 75 years and for the older subjects (men <75 years: ≥33 mg caffeine/day vs. <33 mg caffeine/day, adjusted RR=0.68, 95% CI: 0.31-1.48; men ≥75 years: ≥33 mg caffeine/day vs. <33 mg caffeine/day, adjusted RR=0.66, 95% CI : 0.11-3.38; women <75 years: ≥33 mg caffeine/day vs. <33 mg caffeine/day, adjusted RR=0.66, 95% CI: 0.36-1.19; women ≥75 years: ≥33 mg caffeine/day vs. <33 mg caffeine/day, adjusted RR=0.69, 95% CI: 0.22-2.16).

The estimated RR obtained with stratified analysis for smoking habits suggests a stronger protective effect of caffeine in non-smoking men (non-smoking men: ≥33 mg/day vs. <33 mg/day, adjusted RR=0.30, 95% CI: 0.08-1.14; smoking men: ≥33 mg/day vs. <33 mg/day, adjusted RR=0.75, 95% CI: 0.32-1.78). Only 15 women were smokers, and stratified analysis according to smoking status was therefore not conducted in the female gender group.

Cognitive impairment defined as abnormal MMSE at follow-up

Among women, caffeine intake was associated with a decreased risk of cognitive impairment defined as a MMSE score below cut-off at follow-up, more strongly after adjustment for age, education, diabetes, hypertension, BMI, smoking and alcohol consumption. The results were similar when considering the traditional MMSE cut-off of 24 points [15] to define cognitive impairment, instead of the normative cut-off values of MMSE adjusted to education for the Portuguese population (data not shown).

Among men, a non-significant positive association was observed, but the reduced number of events had a dramatic effect on precision (Table 3).
Discussion

The present study shows a protective effect of caffeine intake on cognitive decline, as defined by the decrease in two or more points in the MMSE. In women, the results were concordant across different criteria to define levels of exposure to caffeine and when using two different endpoints, either the decrease in two or more points in the MMSE or the presence of a MMSE score below the cut-off for cognitive impairment. Among men the findings were similar, when considering the decrease in the MMSE score of two or more points, but the reduced precision of the RR estimates, due to the small number of cognitive impairment events, precluded more robust conclusions.

The results obtained are in agreement with several previously reports which suggested that caffeine reduces the risk of dementia or cognitive decline [7, 8, 11, 12, 40].

A case-control study, also conducted in Portugal, showed an inverse association between caffeine intake and dementia (OR adjusted for hypertension, diabetes, stroke, head trauma, smoking habits, alcohol consumption, education, family history of dementia and drugs: 0.40; 95%CI, 0.25-0.67). No distinction was made for gender-specific effects [7].

When investigating the association between dietary factors and neurodegenerative diseases, prospective designs may provide stronger evidence by overcoming important methodological flaws of case-control designs, especially regarding misclassification of the exposure.

The largest cohort study conducted so far had an average follow up of 3.5 years and aimed to evaluate cognitive performance in several domains. Regarding the decline in MMSE score, no significant association was found (>3 units of caffeine/day vs. <1 unit of caffeine/day, RR adjusted for age, education, baseline cognitive performance and center: 0.91; 95% CI, 0.73-1.14) and this was attributed to the limitations of MMSE to detect more subtle cognitive deterioration. However, a protective effect of caffeine was observed in women, both for verbal and visuospatial memory performances (>3 units of caffeine/day vs. <1 unit of caffeine/day: OR=0.67; 95% CI, 0.53-0.85, for verbal memory; OR = 0.82; 95% CI, 0.65-1.03, for visuospatial memory) [12].

A previously published large cohort study with a 5-year follow up period had shown that coffee consumption was associated with a lower risk of dementia (OR adjusted for age, sex and education: 0.69, 95%CI: 0.48-0.99). However, these results were not stratified by gender and the percentage of decedents was 16.1% of the eligible
participants. These were also demonstrated to be considerably different from the participants whom remained in the study, namely older, less educated and diagnosed with chronic diseases, which may reflect survival bias [8]. The cohort study with the longest follow-up (median: 28 years), with baseline exposures more likely to reflect the exposure experience of an inception cohort, showed no significant association between coffee consumption and cognitive impairment. Nevertheless, the ascertainment of cognitive status was performed only at the follow-up evaluation and by telephone interview [41].

The present study adds to previous research the consistency of the findings when using different categories of exposure to caffeine and definitions of cognitive impairment, based solely on the MMSE evaluation of cognitive status. Both the strength of the association and the apparent dose-response relationship, despite using only three categories of exposure, favor a causal relationship between caffeine and cognitive decline. Also, the assessment of exposure was done using the FFQ, which allowed the investigators to collect information on the vast majority of caffeine food sources available. Despite this, caffeine intake was assessed only at baseline and it is possible that participants may have changed their dietary habits throughout follow-up. Assuming that the putative neuroprotective role of caffeine was to be exerted during that period, misclassification might have occurred in these cases. It is reasonable to assume that individuals more likely to decrease coffee consumption are probably those more prone to chronic conditions and cognitive decline. This change in habits could therefore lead to an underestimation of the protective effect of caffeine. On the other hand, the strong risk reduction observed for relatively low dietary intakes of caffeine (corresponding to approximately one cup of coffee per day), may be explained, at least partially, by bias and uncontrolled confounding. Alternatively, low consumptions at baseline might be conveying information on the effect of higher intakes in the past that changed over time, especially in older individuals. Van Gelder et al reported an inverse J-shaped association between the number of cups of coffee consumed and cognitive decline, with the least decline for three cups a day [11]. These results also suggest a protective effect for moderate daily consumptions of coffee.

A consensus paper recently published suggested that a decrease of 3 or more points in MMSE for a period of six months could be used to define rapid cognitive decline in patients with mild to moderately severe AD [42], and it is reasonable to assume that it corresponds to a clinically meaningful deterioration also in subjects not cognitively impaired at baseline. This threshold, and even more so for higher thresholds, would only
detect the most serious conditions within the spectrum of severity of cognitive decline and thus be affected by a lower sensitivity. Previous studies on this topic defined cognitive decline as a variation of 2 or more points in the MMSE score over a 3-year period [12] or a variation in at least one point in MMSE over a 1-year period [43]. Given the median 48-month follow-up in our cohort we considered that a variation of MMSE score in one or more points would have a poor specificity and we adopted the same criterion followed by Ritchie et al [12].

According to the 2001 census [44] of the population, 63.4% of adults aged 65 years or older were women and 36.6% were men, while among the 648 participants in the cohort aged 65 years or older these proportions were 59.4% and 40.6%, respectively. Thus, men were slightly overrepresented in the study sample compared with the population, due to a higher participation rate among men than women, as previously reported [14]. When comparing men and women who did and did not accept to participate, there were no differences regarding marital status, education or occupation [14]. Thus, the study sample at baseline can be considered representative of the target population in what concerns these socio-demographic characteristics.

There is an important potential for bias in cohort designs, resulting from incomplete follow-up as well as selection criteria. The relatively large proportion of losses to follow-up in the present study (the analyses were restricted to 58.2% of the initial eligible group) could have contributed to biased conclusions. The comparison between participants for whom the follow-up evaluation was accomplished and those lost during this period shows minor differences regarding education, diabetes, smoking and alcohol drinking habits, as well as for caffeine intake and MMSE (among men). The non-differential drop-outs argue in favour of the validity of our RR estimates, if the relation between caffeine and cognitive decline among the non-participants is expected to be similar to the observed among the participants. However, a lower baseline MMSE score was observed in non-participant women and known risk factors for dementia such as hypertension or older age [17] were more frequent among subjects lost during follow-up (especially in women), possibly contributing for a higher risk of cognitive decline [17] and ultimately for the overestimation of the protective effect observed for caffeine, partially explaining the stronger associations observed among women. However, data on their cognitive performance evolution would have been invaluable to a proper understanding of the impact of follow-up losses on our results.
The observation that the participants deceased during follow-up were older and more likely to have been hypertensive or to have scored lower in the MMSE at baseline is also in accordance with methodological concerns regarding another potential source of bias common to other prospective evaluations of elderly subjects, named survival bias. In fact, exposure to caffeine in this cohort did not just begin at the time that follow up was initiated and its influence on the outcomes may have been exerted before the study period, leading to survival bias [45, 46]. Only an inception cohort, assuring that every individual would be observed from the beginning of exposure, would provide results free from this type of bias, which can hardly by achieved when assessing the effect of caffeine intake on conditions that tend to occur later in life. It is difficult to predict to what extent such bias may have affected our RR estimates due to the complex relation between caffeine intake and other exposures associated with higher mortality. For example, smoking is associated with coffee consumption and is a major determinant of early mortality [45]. Therefore subjects who were smokers and coffee drinkers would have been at a higher risk of cognitive decline, had they survived, but were underrepresented in our non-inception cohort, contributing to an overestimation of the negative association between caffeine intake and dementia. On the other hand, it is also important to note that caffeine half-life is decreased in smokers, contributing for a weaker protective effect under the same dietary exposures [47]. This is in keeping with our observation of a lower RR for the association between caffeine and cognitive decline among male non-smokers. Such an interaction could also contribute to explain an overall smaller beneficial effect of caffeine among men, more frequently smokers [48, 49], observed in our study as well as by Ritchie et al. [12].

Conversely, hypertension also accounts for early mortality. Even though most epidemiological evidence suggests that regular intake of caffeinated coffee does not increase the risk of hypertension [50], general public awareness makes hypertensive patients less likely to drink coffee [51]. For this reason, it is possible that individuals at a high risk of cognitive impairment and with lower coffee consumption habits are underrepresented in our cohort, thus contributing to an underestimation of an inverse relation between caffeine intake and cognitive decline.

The impact of survival bias tends to increase with the age of the cohort members at baseline [45]. In our study, however, the RR estimates were similar in participants aged below 75 years and in older subjects, arguing in favour of the validity of our estimates. Moreover, smoking was an exceptional habit in the women in this cohort, and typical of the higher social class categories [48]. Given the social and behavioural gender-related
particularities of our cohort, it is possible that the survival bias might have been more pronounced in men, with a more accurate estimate of risk in women, in what concerns the potential effects of this specific type of bias.

Regarding confounding, we conducted multivariate analyses including a large number of potential confounders. Smoking and alcohol intake were dichotomised due to sample size limitations, but variables such as age, education and BMI were included in the models as continuous in an attempt to provide a finer adjustment to minimize residual confounding. We also conducted analyses including physical activity, consumption of fruit and vegetables, and previous history of stroke or ischaemic heart disease, and the results remained virtually unchanged (data not shown). Nevertheless, uncontrolled confounding may have contributed to the negative association between caffeine intake and cognitive decline observed in our study. In a recently published study, Smith et al. concluded that caffeine consumption was found to be associated with a reduced risk of depression [52]. Not having controlled for the potential confounding effect of depression may have contributed to overestimate the association in our study, as caffeine non-consumers are more likely to be depressed [12], and depressive symptoms may be responsible for poor performance in cognitive testing, namely in the MMSE [17]. We could not take into account the potential confounding effect of factors related to intellectual activity associated both with higher caffeine intake and to a lower probability of cognitive impairment. Adjustment for professional occupation (past or present) could provide some degree of confounding control at this level, and education may also be seen as a surrogate for this variable, but still it would have been important to classify participants according to the mental activity involved in their present or former line of work. The MMSE score at baseline may also be seen as a surrogate marker of intellectual activity and was further included in the models (data not shown) yielding virtually no changes in the RR estimates.

Gender differences have been suggested in the association between caffeine intake and Parkinson’s disease. Ascherio et al. reported a strong inverse association in men and a U-shaped relationship in women, with the lowest risk of Parkinson’s disease occurring at moderate intakes [53]. These authors described an interaction between the use of postmenopausal hormones and caffeine intake in the risk of Parkinson’s disease, with an increased risk among women on hormonal replacement therapy with a high caffeine intake [54, 55], although the reasons for this effect modification are not yet clear. In the present study the number of women under hormonal replacement therapy at baseline was too
small to allow the assessment of its potential to modify the association between caffeine intake and cognitive decline.

In conclusion, our findings do not rule out a negative association between caffeine intake and cognitive decline in men, and confirm the protective effect of caffeine in women. Several arguments discussed above favour the causal nature of this association. Despite this, potentially important confounding factors not accounted for could attenuate the magnitude of the observed association.

Acknowledgments
References


Table 1: Socio-demographic, clinical and behavioural characteristics of the cohort, according to gender.

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) *</td>
<td>70 (67-73)</td>
<td>75 (71-79)</td>
<td>73 (69-77)</td>
<td>&lt;0.001</td>
<td>71 (65-74.5)</td>
<td>72.5 (65.77)</td>
<td>71.5 (69-75.5)</td>
</tr>
<tr>
<td>Age (% ≥75 years)</td>
<td>17.7</td>
<td>50.0</td>
<td>35.6</td>
<td>&lt;0.001</td>
<td>25.0</td>
<td>43.8</td>
<td>33.3</td>
</tr>
<tr>
<td>Education (years) *</td>
<td>4 (3-8)</td>
<td>4 (3-4)</td>
<td>4 (3-6)</td>
<td>0.241</td>
<td>4 (4-9)</td>
<td>4 (4-9)</td>
<td>4 (4-9)</td>
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<tr>
<td>Education (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>10.5</td>
<td>11.5</td>
<td>12.5</td>
<td>0.962</td>
<td>8.0</td>
<td>0.0</td>
<td>3.3</td>
</tr>
<tr>
<td>&lt;12 years</td>
<td>77.9</td>
<td>80.8</td>
<td>78.9</td>
<td></td>
<td>81.2</td>
<td>81.2</td>
<td>80.0</td>
</tr>
<tr>
<td>≥12 years</td>
<td>11.6</td>
<td>7.7</td>
<td>10.6</td>
<td></td>
<td>18.0</td>
<td>18.8</td>
<td>18.7</td>
</tr>
<tr>
<td>Body mass index (Kg/m²) *</td>
<td>28.7 (26.2-31.3)</td>
<td>29.2 (25.5-32.7)</td>
<td>28.7 (25.3-31.6)</td>
<td>0.811</td>
<td>26.2 (23.6-29.0)</td>
<td>24.0 (21.2-28.0)</td>
<td>26.9 (24.0-30.2)</td>
</tr>
<tr>
<td>Body mass index (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤25.0 Kg/m²</td>
<td>17.3</td>
<td>20.8</td>
<td>20.2</td>
<td>0.702</td>
<td>34.9</td>
<td>56.2</td>
<td>35.0</td>
</tr>
<tr>
<td>25.0-29.9 Kg/m²</td>
<td>50.8</td>
<td>37.5</td>
<td>44.4</td>
<td></td>
<td>50.0</td>
<td>31.2</td>
<td>35.0</td>
</tr>
<tr>
<td>≥30.0 Kg/m²</td>
<td>31.8</td>
<td>41.7</td>
<td>35.4</td>
<td></td>
<td>15.1</td>
<td>12.5</td>
<td>30.0</td>
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<tr>
<td>Smoking (% ever smokers)</td>
<td>8.3</td>
<td>7.7</td>
<td>2.9</td>
<td>0.194</td>
<td>65.6</td>
<td>71.9</td>
<td>66.7</td>
</tr>
<tr>
<td>Alcohol drinking (% ever drinkers)</td>
<td>79.0</td>
<td>61.5</td>
<td>78.8</td>
<td>0.126</td>
<td>96.1</td>
<td>100.0</td>
<td>98.3</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>81.1</td>
<td>100.0</td>
<td>91.9</td>
<td>0.004</td>
<td>77.6</td>
<td>82.8</td>
<td>87.9</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>12.8</td>
<td>12.0</td>
<td>13.5</td>
<td>0.976</td>
<td>10.9</td>
<td>21.9</td>
<td>13.3</td>
</tr>
<tr>
<td>MMSE *</td>
<td>26 (26-29)</td>
<td>25.5 (24-26)</td>
<td>27 (25-28.5)</td>
<td>0.010</td>
<td>28 (27.5-29)</td>
<td>28.5 (27-29)</td>
<td>28 (27-29)</td>
</tr>
<tr>
<td>Caffeine intake (mg/day) *</td>
<td>32.2 (10.7-78.8)</td>
<td>31.5 (13.4-57.2)</td>
<td>31.6 (4.9-80.8)</td>
<td>0.554</td>
<td>33.2 (9.5-78.6)</td>
<td>52.2 (20.4-81.6)</td>
<td>33.4 (25.4-79.6)</td>
</tr>
</tbody>
</table>

* results are presented as median (percentile 25-percentile 75)
<table>
<thead>
<tr>
<th>Caffeine (mg)</th>
<th>Women</th>
<th>RR (95% CI)</th>
<th>Men</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person-months</td>
<td>Δ MMSE≤2</td>
<td>Crude</td>
<td>Adjusted</td>
<td>Crude</td>
</tr>
<tr>
<td>&lt;22</td>
<td>3192</td>
<td>28</td>
<td>1 [reference]</td>
<td>1 [reference]</td>
</tr>
<tr>
<td>22-62</td>
<td>3468</td>
<td>22</td>
<td>0.72 (0.41-1.26)</td>
<td>0.65 (0.37-1.17)</td>
</tr>
<tr>
<td>&gt;62</td>
<td>3096</td>
<td>14</td>
<td>0.51 (0.27-0.97)</td>
<td>0.49 (0.24-0.97)</td>
</tr>
<tr>
<td>Follow-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person-months</td>
<td>Δ MMSE≤2</td>
<td>Crude</td>
<td>Adjusted</td>
<td>Crude</td>
</tr>
<tr>
<td>&lt;75</td>
<td>7200</td>
<td>55</td>
<td>1 [reference]</td>
<td>1 [reference]</td>
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<tr>
<td>≥75</td>
<td>2556</td>
<td>9</td>
<td>0.46 (0.22-0.93)</td>
<td>0.47 (0.22-0.99)</td>
</tr>
</tbody>
</table>

RR = Relative risk; 95% CI = 95% Confidence Interval; MMSE = Mini-Mental State Examination; Δ = MMSE at follow-up - MMSE at baseline.

*a* 1st third; *b* 2nd third; *c* 3rd third; *d* average caffeine content in one espresso; *e* adjusted for age (continuous), education (continuous), diabetes, smoking (never/ever), and alcohol drinking (never/ever).
Table 3. Association between daily caffeine dietary intake and development of cognitive impairment*, according to gender.

<table>
<thead>
<tr>
<th>Caffeine (mg)</th>
<th>Women</th>
<th></th>
<th></th>
<th>Men</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Follow-up</td>
<td>RR (95% CI)</td>
<td></td>
<td>Follow-up</td>
<td>RR (95% CI)</td>
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<tr>
<td></td>
<td>Person-months</td>
<td>Cognitive impairment*</td>
<td>Crude</td>
<td>Adjusted</td>
<td>Person-months</td>
<td>Cognitive impairment*</td>
</tr>
<tr>
<td>22-62 b</td>
<td>3468</td>
<td>6</td>
<td>0.61 (0.21-1.59)</td>
<td>0.54 (0.19-1.59)</td>
<td>2292</td>
<td>-</td>
</tr>
<tr>
<td>&gt;62 c</td>
<td>3096</td>
<td>2</td>
<td>1.72 (0.04-1.06)</td>
<td>0.10 (0.01-0.81)</td>
<td>2400</td>
<td>3</td>
</tr>
<tr>
<td>Caffeine (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥75</td>
<td>2556</td>
<td>2</td>
<td>0.37 (0.08-1.64)</td>
<td>0.19 (0.02-1.48)</td>
<td>2160</td>
<td>3</td>
</tr>
</tbody>
</table>

RR – Relative risk; 95% CI – 95% Confidence Interval; MMSE – Mini-Mental State Examination

a 1st third; b 2nd third; c 3rd third; d average caffeine content in one espresso; e adjusted for age (continuous), education (continuous), body mass index (continuous), diabetes, hypertension, smoking (never/ever), and alcohol drinking (never/ever).

*The normative cut-off values of MMSE adjusted to the education for the Portuguese population were used [16]. Subjects were classified as cognitively impaired at follow-up when having a MMSE score below 16 if they were illiterate, 23 if they had ≤ 11 years of education, 28 if they had >11 years of education.
Caffeine intake and dementia: systematic review and meta-analysis

Abstract

A recent meta-analysis of 4 studies published up to January 2004 suggests a negative association between coffee consumption and Alzheimer's disease, despite important heterogeneity in methods and results. Several epidemiological studies on this issue were published since then, warranting an update of the insights on this topic.

We conducted a systematic review and meta-analysis of published studies quantifying the relation between caffeine intake and cognitive decline or dementia. Data sources searched included Medline, LILACS, Scopus, Web of Science and reference lists, up to September 2009. Cohort and case-control studies were included. Three independent reviewers selected the studies and extracted the data on to standardized forms.

Nine cohort and two case-control studies were included. Quantitative data synthesis of the most precise estimates from each study was accomplished through random effects meta-analysis. Heterogeneity was quantified using the $I^2$ statistic.

The outcomes of the studies considered for meta-analysis were Alzheimer's disease in four, dementia or cognitive impairment in two and cognitive decline in three studies.

The summary relative risk (RR) for the association between caffeine intake and different measures of cognitive impairment/decline was 0.84 [95% Confidence Interval (95%CI): 0.72-0.99; $I^2=42.6\%$]. When considering only the cohort studies the summary RR was 0.93 (95%CI: 0.83-1.04, $I^2=0.0\%$), and 0.77 (95%CI: 0.63-0.95, $I^2=34.7\%$) if the most influential study was excluded.

This systematic review and meta-analysis found a trend towards a protective effect of caffeine, but the large methodological heterogeneity across a still limited number of epidemiological studies precludes robust and definite statements on this topic.

Key words: Caffeine; Alzheimer disease; Dementia; Meta-analysis.
Introduction

Dementia is a syndrome caused by a range of illnesses, including Alzheimer’s disease (AD), which is the most prevalent form, vascular, fronto-temporal lobe, Lewy body and other types of dementia [1]. The age-standardized Disability Adjusted Life Years (DALYs) for dementia in 2004 was 260/100,000 in the USA and 350/100,000 in Europe [2]. According to the World Health Organization [2], AD and other dementias are the fourth most important cause of DALYs in high-income countries, corresponding to 3.6% of the total DALYs, a number that is expected to grow up to 5.6% in 2030 [3]. The growing burden of AD and other types of dementia have highlighted the importance of research in this field [4], and in recent years there has been a virtual explosion of information concerning the epidemiology, diagnosis, neuropathology and pathophysiology of dementing disorders.

At present, the efficacy of the pharmacological treatments available is limited and thus, one of the aims of clinical research should be the identification of modifiable risk factors [5] and their interaction with genetic susceptibility markers [6]. Lower education is a recognized risk factor for dementia [7] and it has been suggested that diabetes mellitus, insulin resistance, high cholesterol, hypertension, reduced exercise and obesity are also associated with AD [1]. Smoking may also be a risk factor for dementia and AD [8], especially among the APOE ε4 carriers [6]. The potential effect of diet on the prevention of dementia [9] and cognitive decline further extends this research field, and caffeine intake has been receiving growing attention.

Caffeine is a widely available and consumed substance [10], and it displays affinities for several kinds of receptors present in the synaptic membranes and also for cytoplasmic phosphodiesterases, enabling the modification of synaptic mechanisms [10]. It acts as a nonselective antagonist of adenosine receptors and the blockade of A2A receptors has recently been demonstrated to limit the synaptotoxic effect of Aβ [11]. Experimental studies in animal models have also shown that adenosine A2A and metabotropic glutamate 5 receptors are co-located and that the former play a permissive role in mGlu5R receptor-mediated potentiation of N-methyl D-aspartate (NMDA) effects in the hippocampus [12].

Several epidemiological studies have shown an inverse association between coffee drinking and idiopathic Parkinson’s disease, another important neurodegenerative condition, particularly in men [13]. The plausible biological mechanisms evoked suggest
that caffeine may attenuate the loss of striatal dopamine and dopamine transporter binding sites [14].

A recent meta-analysis [15] of 4 studies published up to January 2004 suggests a negative association between coffee consumption and Alzheimer’s disease, despite important heterogeneity in methods and results. Several epidemiological studies on this issue were published since then, warranting an update of the insights on this topic.

We aimed to review systematically and summarize the published studies addressing the effect of caffeine in cognitive decline and dementia, and to discuss the methodological heterogeneity of the available evidence.
Material and Methods

Search Strategy

Potential eligible studies were identified through an electronic search of the databases Medline, LILACS (Latin America and Caribbean), Scopus and Web of Science, and by extensive searching using cross references from original articles and reviews. Electronic databases were searched from inception to September 2009. The search used the following terms to identify the risk exposure (coffee OR caffeine) combined with terms to identify the outcomes of interest: dementia OR Alzheimer OR [(Alzheimer* OR vascul* OR cerebrovascular OR cereb*) AND (dement* OR deteriorat* OR insufficien*)] OR [(cognit* OR memory* OR mental*) AND (declin* OR impair* OR los* OR deteriorat*)]. When applicable, we searched all terms as indexed and as free text terms to increase sensitivity. A search filter was developed (Cohort Studies OR Case Control Studies OR Prospective Studies OR Follow-Up Studies OR Cross-Sectional Studies OR Retrospective studies OR Epidemiological OR Incidence OR Risk Factors OR Risk Assessment OR Risk Reduction OR Relative Risk OR Behavior Regression Analysis OR Multivariate Analysis OR Proportional Hazards Models) and applied to the search results in order to retrieve epidemiological studies. There were no language restrictions on searching. We screened titles, keywords, and abstracts of the citations downloaded from the electronic searches and obtained full copies of potentially suitable reports for further assessment. We considered studies published as a full paper or abstract as long as relevant data could be extracted.

Selection Criteria

We included studies with a cohort, case-control and cross-sectional design that addressed the relation between caffeine consumption, through coffee and/or tea intake regardless of assessment of other dietary sources of caffeine, and different forms of dementia, cognitive impairment or cognitive decline (all diagnostic criteria were considered).

Cross-sectional studies or analyses relying in retrospective assessment of exposure were excluded when cognitively impaired subjects were the informants for estimation of their own caffeine intake. No studies were excluded a priori for weakness of
design or data quality. However, to be included in the meta-analysis, studies had to provide or allow the calculation of the relative risk (RR), or the odds ratio (OR) in case-control studies, and the respective variance estimates.

An additional cohort study being published in the current issue of the Journal of Alzheimer's Disease was also considered in this review [16].

**Data extraction**

The selected articles were reviewed independently by three researchers (JC, JS and CS) and data were extracted using a predefined form. Discrepancies in the evaluation of the articles were resolved by consensus, involving a fourth researcher (NL).

From each study we collected information on: year of publication; country of origin and population evaluated (general description, number, age and gender of the participants); type of study (cohort, nested case-control, case-cohort, population- or hospital-based case-control); exposure assessment (instruments, period of exposure and informants); outcomes and criteria for outcome definition; control for confounding; data on the relation between caffeine exposure and the outcomes. From cohort studies we also extracted information regarding the length and completeness of follow-up.

In the report by Broe et al [17] one of the comparisons presented had ever consumers as the reference category and we computed the OR using never consumers as the reference class, as the distribution of matched pairs across exposure categories was provided.

When a study provided results with different degrees of adjustment for confounders, the estimates adjusted for the largest number of possible confounding variables were selected. Sex-specific results were extracted whenever available.

The authors from the studies included in the present systematic review were not contacted to retrieve additional data.

**Data synthesis and meta-analysis**

Each study is summarized in table 1 and figure 2. The results from studies not providing RR estimates are described in the text. Data synthesis is further accomplished through meta-analysis (figure 3) and visual inspection of scatter plots representing the log
Relative Risk (RR) estimates from each study according to the distribution of methodological characteristics with potential impact on the heterogeneity of results (figure 5).

Studies were grouped by the outcome addressed. Dementia and Alzheimer’s disease were defined according to the existing clinical criteria (DSM-IV and/or NINCDS-ADRDA). Cognitive decline was considered when studies quantified the difference in score performance using neuropsychological instruments in two distinct occasions, regardless of the cutoff values. An abnormal score in at least one of the tests, at any time, was defined as cognitive impairment.

The forest plot corresponding to figure 2 represents the RR estimates provided in each study for the association between caffeine intake and dementia. Several estimates from the same study may be provided, referring to different exposures (e.g. coffee consumption or caffeine intake estimated through a food frequency questionnaire) and levels of exposure, different outcomes (e.g. dementia and cognitive impairment) and different criteria for definition of the same outcome (e.g. variation in Mini Mental State Examination – MMSE – or in Benton Visual Retention Test scores to define cognitive decline).

Quantitative data synthesis was accomplished through random effects meta-analysis (DerSimonian and Laird method), conducted with STATA®, version 9.2. Relative Risks (cumulative incidence ratios or incidence density ratios) and ORs were treated the same and are referred to as RR [18]. Summary estimates for exposure to caffeine were computed considering the individual RR estimates corresponding to coffee, coffee and tea or overall caffeine intake, as available from each article, under the assumption that coffee is the main contributor for caffeine intake [19, 20].

Since more than one RR estimate were available from several studies and the small number of independent investigations precluded meaningful subgroup or trend estimation analyses, only the most precise measures of association were used from each report (except for sex-specific estimates, which were considered separately as if obtained from different studies). These criteria were applied to the selection of a single estimate per study when RR estimates were provided for different categories of exposure. The reports by Eskelinen et al [21], Laitala et al [20] and Santos et al [16] provided RR estimates for different outcomes and only the results for the outcome with the most precise estimates were considered. Laitala et al [20] defined the same outcome using different criteria, and only the criterion with the most precise estimates was considered. Ritchie et al [22]
addressed the effect of caffeine on cognitive decline relying on three different methods for assessment of cognitive performance, and the estimates corresponding to variation in the MMSE score were selected to allow a more meaningful comparison with the other studies assessing the risk of cognitive decline [16, 19].

Heterogeneity was quantified using the $I^2$ statistic [23]. Sensitivity analyses were conducted excluding highly influential studies or those not providing RR estimates adjusted for potentially important confounding factors in addition to age and sex. The report by Tyas et al. [24] refers to a subsample of study published by Lindsay et al. [25] and was also excluded in sensitivity analysis.

Publication and publication-related biases were examined through visual inspection of the funnel plot (figure 4). The Begg adjusted rank correlation test [26], and the Egger's regression asymmetry test [27] were used for further assessment of these biases through hypothesis testing.

Figure 5 includes scatter plots aiming to further explore the reasons for heterogeneity of results within and across cohort studies. Only results from studies providing RR estimates for at least two categories of exposure compared with the referent were plotted. In each study, when more than one criterion was used to define the same outcome, only the results corresponding to the criterion yielding the more precise estimates were selected. All the RR estimates corresponding to each outcome addressed in the eligible studies were represented. In figure 5a the log RR is represented across levels of caffeine intake. The exposures for which RR estimates were computed were assumed to correspond to the midpoint of the index category range subtracted by the midpoint of the reference category range. For this purpose, we assumed that the open-ended upper category had the amplitude of the preceding stratum.

Figure 5 (b to d) addresses the potential for selection and information bias in each study, using the participants' age at baseline, the duration of follow-up and its completeness as surrogate markers for the latter effects. The pattern of association between these methodological characteristics and the RR estimates from each study may contribute to understand the heterogeneity between studies.
Results

Eleven studies fulfilled the inclusion criteria for systematic review [16, 17, 19-22, 24, 25, 28-30]. The main characteristics of the studies and results on the relation between caffeine intake and cognitive impairment are summarized in table 1 and figure 2.

The publication year ranged from 1989 to 2009. The studies were conducted in Europe (three in Finland [20, 29, 30], two in Portugal [16, 28], one in France [22], one multicentric study conducted in Italian, Finish and Dutch populations [21]), one in Australia [17], two in Canada [24, 25] and one in China [19]. Two were case-control [17, 28] and nine were cohort studies [16, 19-22, 24, 25, 30]. The age of the youngest participant at the time of baseline evaluation ranged from 37 to 68 years. The results were stratified according to gender in three of the studies [16, 19, 22] and one was conducted on a male cohort [30].

Different sources of caffeine were accounted for in the reports reviewed. Most studies evaluated coffee and tea intake, assessing their effects separately [17, 19, 24, 25] or considering the consumption of both beverages [22]. Two studies extended exposure assessment to all caffeinated beverages [16, 28] and products containing chocolate [16]. van Gelder et al. and Laitala et al. only evaluated coffee consumption [20, 30]. Different categories of exposure were considered, ranging from less than one to 8 units equivalent to coffee cups/day. Also, the reference categories included different proportions of non-caffeine consumers and consumers of different amount of caffeine (table 1).

In one study, the outcome was measured as a continuous variable according to the scores obtained in several neuropsychological tests, and a correlation coefficient was the only measure of association reported [29]. van Gelder et al. presented results on the decline in cognitive performance (variation in MMSE scores as a quantitative variable) according to coffee intake [30].

RR estimates were computed in nine studies [16, 17, 19-22, 24, 25, 28] (figure 2). Five had Alzheimer's disease as the outcome [17, 21, 24, 25, 28], defined by the NINCDS/ADRDA criteria. Of these, only one [21] presented more than one RR estimate to different types of caffeine-containing beverages for the same reference category. Maia et al. published a case-control study in which exposure was assessed twenty-years before outcome definition [28]; the work by Broe et al. was also a case-control [17], but did not specify the period to which exposure assessment referred, and the remaining three studies refer to cohort designs [21, 24, 25]. The risk of dementia (as defined by the
authors) or cognitive impairment was assessed in 3 studies. Laitala et al defined the outcomes according to the scores obtained on two sets of cognitive tests designed to be applied by telephone interview [20]. Two other studies used the DSM-IV clinical criteria [21, 22].

The MMSE was used in two longitudinal studies designed to assess cognitive decline and impairment over a 5 and 1.5-year period, in Portuguese [16] and Chinese [19] populations, respectively. Ritchie et al had used the MMSE as well as other complementary neuropsychological tests, to define decline in cognitive performance [22].

The summary RR for the association between caffeine intake and different measures of cognitive impairment/decline was 0.84 (95% Confidence Interval: 0.72-0.99), with moderate heterogeneity (I²=42.6%). The visual inspection of the forest plot shows no evidence of publication bias, as the results from individual studies scatter around the overall summary RR estimate depicting a funnel-like shape with no meaningful asymmetry. The Egger’s regression asymmetry test (p=0.277) and the Begg adjusted rank correlation test (p=0.784) provide further support to the hypothesis of no publication bias.

The two case-control studies provided the strongest negative associations [17, 28], corresponding to a summary RR of 0.49 (95%CI: 0.308-0.791; I²=33.0%). When considering only the nine cohort studies the summary RR was 0.93 (95%CI: 0.83-1.04) and results were homogeneous (I²=0.0%). Among the cohort designs, the study by Ritchie et al [22] is highly influential, with a weight of 60% in the overall RR estimate. When the more influential study is excluded, summary RR for all studies was 0.77 (95%CI: 0.63-0.95, I²=34.7%) and the summary RR for cohort studies was 0.85 (95%CI: 0.71-1.01, I²=0.0%) The sample evaluated by Tyas et al [24] partially overlapped with the larger one studied by Lindsay et al [25], but had a small weight in the overall estimates and its exclusion did not change the overall conclusions meaningfully.

The summary RR was 0.83 (95%CI: 0.32-2.15, I²=40.5%) for Alzheimer’s disease, although it combines estimates from 2 case-control and 2 cohort studies, and 0.98 (95%CI: 0.87-1.11, I²=40.5%) for cognitive decline, but results are driven predominantly a single large study [22].

The studies by Lammi et al [29] and van Gelder et al [30] did not provide RR estimates for the association between caffeine and dementia. The latter suggests a protective effect, although only a correlation coefficient was used to quantify the association, and the former a protective effect with a J-shaped pattern.
The studies considered for meta-analysis provided estimates adjusted for different potential confounding factors and for a varying number of variables, but there was no clear pattern regarding the degree of confounding control and the RR estimates. The summary RR was 0.80 (95%CI: 0.59-1.08, I²=51.6%) when considering only 5 studies (7 RR estimates) providing RRs adjusted for both smoking and hypertension, and 0.90 (0.76-1.06) for the remaining 4 studies (5 RR estimates).

Regarding the relation between levels of exposure to caffeine and the RR estimates (Figure 5a), results were more heterogeneous for lower intakes, albeit the preponderant role of the study published by Ritchie et al [22] in this particular analysis, but were also more likely to be inversely associated with the outcomes.

Survival bias is likely to occur in non-inception cohorts, and is expectedly stronger in older cohorts [31]. The extent to which a complete follow-up is not achieved also reflects selection bias, in addition to the latter. Changes in caffeine consumption habits with time and with the development of cognitive impairment may contribute to information bias, especially when follow-up periods are longer. Cohort studies including younger participants, having longer or more complete follow-ups yielded more homogeneous results and the putative protective role of caffeine was more evident in those enrolling older individuals, when follow-up was more complete or shorter (Figures 5b, 5c and 5d). However, no linear relation or other consistent pattern was observed between log RR estimates and age of the cohort participants, follow-up duration or completeness of follow-up.
Discussion

This systematic review and meta-analysis updates a previous quantitative synthesis based in a small number of studies and provides a summary of the best available evidence on this topic. The large methodological heterogeneity across a still limited number of reports is probably the most remarkable observation in our study, which precludes robust and definite statements on a possible negative association between caffeine intake and dementia.

The conclusions reached by systematic reviews and meta-analyses depend, among other factors, on the comprehensiveness of the search strategy and on the criteria for study inclusion and selection of data for quantitative synthesis. These issues have implications in the validity of our findings and deserve further discussion.

The number of studies available for review was small, namely when compared to the 43 reported included in a recent meta-analysis on smoking and Alzheimer’s disease [32]. However, studies on the etiologic role of caffeine intake are less frequent than those focusing on the effects of tobacco, as shown in two meta-analyses addressing the association between coffee drinking, cigarette smoking and Parkinson’s disease, with 13 and 48 individual studies, respectively [33]. Furthermore, there was no evidence of publication bias.

Two studies [29, 30] did not provide RR estimates for the association between caffeine and dementia, both suggesting a protective effect, but no changes in the conclusions of the present review and meta-analysis could be expected if these studies could be included, given the large heterogeneity observed across the investigations. Two reports providing only a cross-sectional assessment of the association between caffeine intake and dementia [34, 35] were not included in the review since the RR estimates were likely to be biased because in participants cognitively impaired the exposure information was obtained by self-report. One of these studies concluded that caffeine intake was associated with a better cognitive performance in tests evaluating reaction time, incidental verbal memory and visuo-spatial reasoning [34]. The study by Johnson-Kozlow et al suggested a relation between higher current caffeine consumption and a better performance in two cognitive tests (Short Term Recall and Blessed Items), in women [35].

The number of studies on this topic was small and heterogeneous regarding the methodological options. Four different outcomes were investigated in eleven reports (Alzheimer’s disease, dementia, cognitive impairment and cognitive decline), and with the
exception of Alzheimer's disease, the instruments and criteria for outcome definition in each report were also heterogeneous. The food sources and chosen categories of exposure also differed widely across studies. Under these circumstances, and taking into account that the same study frequently addresses more than one outcome and presents different definitions of exposure, the criteria used for selection of a RR single estimate from each study is an important determinant of the validity of our conclusions. To select independent observations to be combined in meta-analysis we opted for the more precise estimates from each study, regardless of the corresponding sources and levels of exposure and outcomes assessed. The precision of the individual RR estimates is not dependent on the direction of the association and with this criterion the selection of the exposures corresponding to the largest number of participants is the most likely. However, if the categories of exposure in each individual study are defined to include a similar number of participants per group this criterion leads to the selection of the estimates reflecting the weakest associations, which may have contributed to a slight underestimation of the summary RR. On the other hand, the precision of the summary estimates is reduced by considering only part of the overall sample from each study in the meta-analysis. Sensitivity analysis extends the assessment of the robustness of the summary estimates across different inclusion criteria, as well as the discussion of the methodological heterogeneity.

Two case-control studies assessed exposure through surrogate informants, and were therefore eligible for our review, however, misclassification of exposure as well as information bias weaken this methodological approach, especially when a long-term recall of exposure is required. Furthermore, in the case-control study conducted by Broe et al [17], the recall period was not provided and only the potential confounding effects of age and gender were accounted for, which preclude a sound discussion of the extent to which recall bias and uncontrolled confounding could have influenced validity of the OR estimates. The exclusion of the case-control studies in sensitivity analysis allowed a meta-analysis of homogeneous results.

Even though Tyas et al [24] and Lindsay et al [25] partly share their cohorts of participants, there is a stronger protective effect described for tea in the first case, in a predominantly rural setting, whereas in the second case coffee is the substance associated with a lesser cognitive decline. This may be related to other habits associated with the predominant caffeine intake source; in the study by Lindsay et al [25], the population was essentially urban and this difference may partly account for the results.
Nevertheless, the wide categories of exposure used in analysis (daily regular consumption vs. irregular consumption) precluded a finer assessment of the relation between caffeine intake and Alzheimer's disease. A Chinese cohort study evaluating coffee and tea separately and adjusting mutually for these two sources of caffeine showed that total tea intake was significantly associated with a lower prevalence of cognitive impairment although no protective effect was observed for coffee consumption [19]. In this population, coffee intake was the most commonly consumed caffeine containing beverage, despite the high proportion of drinkers of different types of tea. The results could be due to the fact that other components rather than caffeine may also exert a role on cognitive decline, but in addition to this Chinese study [19], only Tyas et al [24] showed associations in different directions for coffee and tea.

Among cohort investigations the summary RR estimate is driven predominantly by the study conducted by Ritchie et al [22], which showed no significant association between decline in MMSE score and caffeine intake. However, a protective effect of caffeine was observed in women, both for verbal and visuospatial memory performances (>3 units of caffeine/day vs. <1 unit of caffeine/day: OR=0.67; 95%CI, 0.53-0.85, for verbal memory; OR = 0.82; 95%CI, 0.65-1.03, for visuospatial memory). If these estimates had been selected for our meta-analysis a stronger protective effect had been achieved. The remaining cohort studies have similar weights in meta-analysis and the exclusion of the highly influential study by Ritchie et al yields a stronger risk reduction associated with caffeine intake.

The most recently published cohort study, conducted by Laitala et al [20], relied on two validated neuropsychological tests (Telephone Interview for Cognitive Status – TICS – and Telephone Assessment for Dementia - TELE) to classify participants in relation to dementia or cognitive impairment, but none of the widely adopted clinical criteria were used, and no significant protective effect of caffeine was observed. However, the RR estimate selected for meta-analysis according to previously established criteria was one of the lowest. If other estimates had been selected the negative association suggested by the summary RR estimate would have been slightly weaker. The studies by Eskelinen et al [21] and Santos et al [16] provide RR estimates for different outcomes, but no substantial changes in the summary estimate would have been observed if other individual RR estimates had been selected from each study, except if the levels of exposure considered were different. The differences in the relation between caffeine
intake and cognitive function observed in these studies according to the levels of exposure depict one of the sources of heterogeneity across cohort studies.

The small number of heterogeneous studies precluded a formal trend estimation analysis, but figure 5a allows a systematic approach to the discussion of the dose-response relationship. The results from studies providing RR estimates for different levels of exposure suggest a stronger protective effect for lower consumptions, despite the limited number of studies and the large weight of the study conducted by Ritchie et al do not allow stronger inferences on this issue. The cohort study conducted by van Gelder et al was not included in meta-analysis because no RR estimates are provided, but reported an inverse J-shaped association between the number of cups of coffee consumed and cognitive decline, with the least decline for three cups a day [30]. The case-control study by Broe et al [17] provided RR estimates for consumers of more than 4 cups/day vs. consumers of 4 or less cups/day and allowed the computation of estimates for ever vs. never consumers, showing a protective effect for coffee intake, but a deleterious effect for the consumption of large amounts. A J-shaped relation may therefore be hypothesized, but uncontrolled or residual confounding are also likely explanations for such pattern.

Cohort designs are less prone to information bias, but also have potential limitations related to survival bias due to the enrollment of non-inception cohorts, and selection bias resulting from incomplete follow-up. Studies less likely to suffer from these biases tended to show weaker negative or even positive associations between caffeine intake and dementia. On the other hand, information bias may also be a concern, especially in studies with longer follow-up, as caffeine intake habits may decrease with time as cognitive impairment occurs, and a protective effect of caffeine was more likely in studies with shorter follow-up.

None of the studies assessed the relation between duration of exposure to caffeine and dementia or cognitive decline, and the timing of exposure evaluation is also a source of heterogeneity across studies. The change in caffeine intake habits over time is difficult to disentangle from the choice of the most appropriate moment to assess exposure, from a biological plausibility point of view. Because there are no definite data as to when caffeine may start exerting its putative role on neurodegeneration, the lag between caffeine exposure and its potential effects on cognitive performance cannot be accurately determined for the time being.

The evaluation of non-inception cohorts is of particular concern in longitudinal studies recruiting older individuals. Because participants suffering from chronic illnesses
are more likely to die before being recruited, this will probably lead to an under-
representation of individuals at a high risk of cognitive impairment in the cohort. 
Depending on their caffeine consumption habits and coexisting dementia risk factors, this 
could lead to biased estimates of risk. Hypertension and smoking are especially important 
in this discussion; the relation between hypertension and coffee is complex, but it is well 
recognized that hypertensive patients are less likely to drink coffee due to public 
perception issues [36]. Because hypertension is a well known risk factor for dementia, the 
exclusion of such individuals could lead to an underestimation of the protective effect of 
caffeine in cognitive decline. On the other hand, smokers tend to drink more coffee, are 
more prone to suffer from chronic illnesses and have a higher mortality rate [32]. 
Assuming that less fit individuals are more likely to become cognitively impaired, 
excluding them from study cohorts could lead to an overestimation of the protective effect 
exerted by caffeine.

The knowledge of caffeine pharmacokinetics is important when discussing its 
potential neuroprotective role. Caffeine’s half-life is reduced in smokers [37], which may 
contribute to a weaker protective effect in tobacco consumers, under the same caffeine 
exposures. As smoking is associated with coffee consumption, the latter effect is more 
likely in men, among whom the prevalence of tobacco consumption tends to be higher 
[38], in accordance with the observation of a stronger protective effect of caffeine in 
women by some authors [16, 19, 22]. Hormonal replacement therapy (HRT) could also 
interfere with caffeine metabolism and constitute another possible explanation for 
differences between men and post-menopausal women. Studies conducted by Ascherio 
et al addressing caffeine intake and Parkinson’s disease have shown an increasing risk in 
women on HRT and with higher caffeine intake habits [13]. Regarding cognitive 
deterioration, Ritchie et al found no interaction between caffeine and HRT [22], but there 
was no additional data on this issue in the remaining articles reviewed.

Two studies analyzed the potential effect of APOE polymorphism ε4 gene in the 
association between caffeine and dementia. The results obtained through stratification 
according to the APOE ε4 gene carrier status by Eskelinen et al suggest a stronger 
protective effect in APOE ε4 gene carriers (carriers: moderate consumption vs. low 
consumption: OR=0.32, 95%CI 0.11-0.92; non-carriers: moderate consumption vs. low 
consumption: OR=0.44, 95%CI 0.12-1.55) [21]. In the study by Ng et al, the negative 
association between tea consumption and cognitive decline in the category defined as 
“high intake” did not change meaningfully after adjustment for APOE ε4 gene carrier
status (OR=0.57 95%CI 0.32-1.03 after adjustment vs. OR=0.62 95%CI 0.36-1.08 before adjustment) [19].

Caffeine consumption is significantly associated with a wide range of variables also known as being related to cognitive decline [22]. Vascular risk factors, age, education and depressive symptoms are among the ones generally agreed upon. It is thus relevant to account for these variables as potential confounding factors, which was accomplished in most studies [16, 19-21], although to different extents. Uncontrolled or residual confounding and effect modification not accounted for in the majority of studies reviewed may contribute to explain the heterogeneous results, although no formal assessment of these effects is possible due to the small number of highly heterogeneous reports.

Finally, a problem inherent to all dementia research designs is the accuracy of diagnosis. All the studies reviewed rely either on a clinical diagnosis or on neuropsychological test scores. Because the definite diagnosis of dementia requires pathological examination, misclassification is likely to have contributed to biased results [39]. However, the specificity and sensitivity of clinical diagnosis are poorly known and it is recognized that they differ according to the type of dementia (e.g. vascular, Alzheimer's disease) and the criteria used [40, 41]. This reaffirms the need for internationally consensual criteria for clinical diagnosis of dementia in epidemiological research.

In conclusion, further prospective studies evaluating the association between caffeine consumption and cognitive decline are needed. Addressing the potential bias and confounding sources described above is essential. Setting consensual criteria for the definition of outcome as well as creating defined categories and types of exposure might be useful in conducting meta-analyses and increase statistical power for the detection of an association between caffeine and cognitive impairment or dementia.
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<table>
<thead>
<tr>
<th>Country</th>
<th>First author</th>
<th>Type of study</th>
<th>Sample characteristics</th>
<th>Outcome assessment</th>
<th>Definition of cognitive impairment/dementia</th>
<th>Evaluation of exposure</th>
<th>Results</th>
<th>Control of confounding</th>
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<tbody>
<tr>
<td>UK</td>
<td>Lammi</td>
<td>Cohort</td>
<td>Community-dwelling men from regions in the east and west of Finland. Follow-up (mean): 10 yrs. Final study population: 716 Men Age range: 65-84 yrs. Completeness of follow-up: NS.</td>
<td>Cognitive assessment:</td>
<td>Mental disability was defined by a sum score of MSQ≥4</td>
<td>Two moments: 1. At the time of cognitive assessment 2. Ten years previously</td>
<td>&quot;Coffee consumption showed a positive correlation with mental disability measured by MSQ&quot; (not further specified)</td>
<td>NS</td>
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<td>Finland</td>
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<td>Australia</td>
<td>Broe</td>
<td>Case-Control</td>
<td>Cases were referred from GP and matched (sex and age) population based controls. Cases/Controls: 170 (64 Men, 106 Women)/170 Age range: 52-96 yrs.</td>
<td>NINCDS-ADRDA criteria for probable or possible AD</td>
<td>2-hour interview designed to assess lifestyle habits (Risk Factor Interview)</td>
<td>Coffee consumption: Ever vs. never, OR=0.65 (95%CI: 0.38-1.19)</td>
<td>Coffee, Tea</td>
<td>Age and sex (matching)</td>
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<tr>
<td>Canada</td>
<td>Tyas</td>
<td>Cohort</td>
<td>Participants randomly sampled from the province of Manitoba (health insurance plan list) = approximately 1/4 also participated in the CSHTA cohort (Lindsay et al, 2002). Baseline population: 1039 Age range: ≥65 yrs. Follow-up: 5 yrs Final study population: 694 (261 Men and 433 Women) Mean age (SD): 74 yrs (5.8) Completeness of follow-up: 66.7%</td>
<td>Two-phase assessment: 1. Screening with the 3MS test (score &lt;75/100; participants selected for further evaluation) 2. Diagnosis of possible/probable AD using the NINCDS-ADRDA criteria</td>
<td>Risk factor questionnaire (interview and e-mailed)</td>
<td>Regular (nearly every day) vs. non-regular consumption: At baseline</td>
<td>Coffee and Tea</td>
<td>Age, sex, education</td>
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<tr>
<td>Lindsay</td>
<td>Cohort</td>
<td>Three-step protocol (screening, clinical phase, differential diagnostic phase)</td>
<td>Self-administered questionnaire including quantitative assessment of coffee and tea consumption</td>
<td>Daily vs. not daily consumption:</td>
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<tr>
<td>Canada</td>
<td>Population-based random samples</td>
<td>Individuals scoring &lt;70/100 3MS addressed for further evaluation</td>
<td>At baseline</td>
<td>Age, sex, education</td>
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<td>2002</td>
<td>Baseline population: 5897</td>
<td>Diagnosis of dementia based on DSM-IV criteria and the probable and possible AD, vascular dementia and cognitive impairment on the NINCDS/ADRDA criteria</td>
<td>Coffee, OR=0.89 (95%CI: 0.50-0.96)</td>
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<td>Follow-up: 5 yrs</td>
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<td>Diagnosis Categories: No cognitive impairment; Cognitive impairment: No dementia; Probable AD; Possible AD; Vascular Dementia; Other Specific Dementia; No classified Dementia</td>
<td>NS</td>
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<td>Final study sample:</td>
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<td>Tea, OR= 1.12 (95%CI: 0.78-1.81)</td>
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<td>4615 (1718 men, 2370 women)</td>
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<td>Coffee, tea</td>
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<td>Mean age: Dementia or AD, 81 yrs; subjects without the outcomes under study, 72.9 yrs</td>
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<tr>
<td>Completeness of follow-up: 78.2%</td>
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<p>| Maia        | Case-Control | Clinical history, neurological examination, neuropsychological testing (including MMSE), brain computerized axial tomography or magnetic resonance imaging | Type of questionnaire NS; self-reporting in controls and proxy information in cases | 'Exposed' vs. 'not exposed'; OR=0.40 (95%CI: 0.24-0.65) | OR estimates adjusted for: gender (matching), age (matching), hypertension, diabetes, stroke, head trauma, smoking habits, alcohol consumption, non-steroid anti-inflammatory drug use, vitamin E, gastric disorder, heart disease, education and family history of AD |
| Portugal    | Cases were selected at the Dementia Outpatient Clinic – Hospital Santa Maria | Probable AD was defined according to the NINCDS/ADRDA criteria | 20 yrs before the diagnosis of AD | Average daily caffeine intake (mg) in the 20 yrs before AD (mean[SD]): cases, 73.9 (97.8); controls, 198.7 [35.7] | |
| 2002        | Controls were people accompanying patients, matched for gender and age | Accuracy perceptions assessment (score &gt;4-5), cross checking with 3rd person | Average daily caffeine intake (mg) from 25 yrs old to 20 yrs before AD: cases, 89.6 [94.1]; controls, 194.6 [137.2] | Average daily caffeine after AD: cases, 36.3 [64.1]; controls, 177.1 [123.7] | |</p>
<table>
<thead>
<tr>
<th>First author</th>
<th>Type of study</th>
<th>Country</th>
<th>Sample characteristics</th>
<th>Definition of cognitive impairment/dementia</th>
<th>Evaluation of exposure</th>
<th>Results</th>
<th>Control of confounding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ritchie</td>
<td>Cohort</td>
<td>France</td>
<td>Community-dwelling persons selected from the electoral rolls of three French cities</td>
<td>Cognitive examination: MMSE Benton Visual Retention Test Isacs Set Test All individuals further evaluated by a neurologist in Montpellier and Bordeaux; only persons suspected of having cognitive deficit in Dijon DSM-IV criteria used to diagnose prevalent cases of dementia Decline in cognitive performance: Decrease from baseline of at least 6 points on the Isacs Test, or at least 2 points on the Benton Test and the MMSE</td>
<td>Standardized interview administered by research collaborators (units/day)</td>
<td>1-2 vs. 0 coffee/tea units&lt;sup&gt;1&lt;/sup&gt; Δ Isacs ≤ 6 points: women, OR=0.83 (95%CI: 0.70-1.04); men, OR=1.08 (95%CI: 0.70-1.04) Δ Benton ≤ 2 points: women, OR=0.93 (95%CI: 0.83-1.22); men, OR=1.11 (95%CI: 0.88-1.40) Δ MMSE ≤ 2 points: women, OR=0.89 (95%CI: 0.73-1.08); men, OR=1.00 (95%CI: 0.79-1.27) &gt;3 vs. 0 coffee/tea units&lt;sup&gt;1&lt;/sup&gt; Δ Isacs ≤ 6 points: women, OR=0.87 (95%CI: 0.53-0.95); men, OR=1.16 (95%CI: 0.77-1.59) Δ Benton ≤ 2 points: women, OR=0.82 (95%CI: 0.82-1.03); men, OR=0.92 (95%CI: 0.68-1.23) Δ MMSE ≤ 2 points: women, OR=0.91 (95%CI: 0.73-1.14); men, OR=1.19 (95%CI: 0.89-1.59)</td>
<td>Benton and Isaacs tests (women): Age, education, baseline cognitive performance, centre Hypertension, smoking, alcohol consumption, diabetes, BMI, depression and cardiovascular disease Benton and Isaacs tests (men): Age, education, baseline cognitive performance and centre MMSE (both genders): Age, education, baseline cognitive performance and centre</td>
</tr>
</tbody>
</table>
Table 1. Main characteristics of the studies included in the systematic review (continued).

<table>
<thead>
<tr>
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<th>Results</th>
<th>Control of confounding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Gelder</td>
<td>Cohort</td>
<td>MMSE was used to assess global cognitive function</td>
<td>Finland and Italy: standardized self-administered questionnaire (cups of coffee/day) 0 cups/day, Δ MMSE= -2.6 points; Netherlands: information collected in a dietary survey in which a cross-check dietary history method was used (past 2-4 weeks).</td>
<td>Coffee consumption: 2 cups/day, Δ MMSE= -1.3 points;</td>
<td>Age, education, country, cigarette smoking, alcohol use and physical exercise</td>
</tr>
<tr>
<td>2007</td>
<td>Participants from the Seven Countries: Δ MMSE score was the outcome measured</td>
<td>Baseline Population: NS</td>
<td>At baseline</td>
<td>Coffee</td>
<td></td>
</tr>
<tr>
<td>Finland, Italy and the Netherlands</td>
<td>Cohort (population-based participants from Finland, Italy and the Netherlands)</td>
<td>Follow-up: 10 yrs</td>
<td></td>
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<tr>
<td></td>
<td>Final study population: 676 men</td>
<td>Mean age (SD): 76.1 yrs (4.15)</td>
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<tr>
<td></td>
<td>Completeness of follow-up: NS</td>
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<tr>
<td>Ng</td>
<td>Cohort</td>
<td>Cognitive Decline: Defined by Δ MMSE ≥ 1 point</td>
<td>NS questionnaire</td>
<td>Coffee consumption: Occasionally vs. rarely: women, OR=0.79 (95%CI: 0.55-1.11); men, OR=1.13 (95%CI: 0.49-2.61); ≤1 cup/day vs. rarely: women, OR=1.03 (95%CI: 0.70-1.51); men, OR=1.31 (95%CI: 0.71-2.45)</td>
<td>Age, sex, education, BMI, depression, ApoE carrier status, hypertension, diabetes, cardiovascular diseases, smoking, alcohol consumption, baseline MMSE score, physical activity, food habits</td>
</tr>
<tr>
<td>2008</td>
<td>Community-living Chinese adults from the Singapore Longitudinal Ageing Studies cohort</td>
<td>Cognitive Impairment: MMSE total score ≤23</td>
<td>At baseline</td>
<td>Coffee, tea</td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>Studies cohort</td>
<td></td>
<td></td>
<td>Black or oolong tea consumption: Occasionally vs. rarely: women, OR=0.67 (95%CI: 0.45-1.01); men, OR=0.18 (95%CI: 0.07-0.49); ≤1 cup/day vs. rarely: women, OR=0.38 (95%CI: 0.23-0.63); men, OR=0.60 (95%CI: 0.22-1.25)</td>
<td></td>
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</tbody>
</table>
Table 1. Main characteristics of the studies included in the systematic review (continued).

<table>
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<tr>
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<th>Evaluation of exposure</th>
<th>Results</th>
<th>Control of confounding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eskelinen</td>
<td>Cohort</td>
<td>Definition of cognitive impairment/dementia</td>
<td>Survey questionnaire including quantitative assessment of coffee and tea AD consumption</td>
<td>Coffee consumption: 3.5 vs. 0.2 cups, OR=0.42 (95%CI: 0.12-1.46)</td>
<td>Dementia and AD: age, sex, education, follow-up time, community of residence, midlife smoking, systolic blood pressure, serum total cholesterol, BMI, physical activity, ApoE e4, late-life MCI/stroke/diabetes/Beck depressive scale</td>
</tr>
<tr>
<td>2009</td>
<td>Participants randomly selected from survivors of population-based random samples</td>
<td>Three-step protocol (screening, clinical phase, differential diagnostic phase)</td>
<td>At baseline</td>
<td>&gt;5 vs. 0.2 cups, OR=1.91 (95%CI: 0.33-10.9)</td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>Baseline population: 2000</td>
<td>Individuals scoring ≤24 MMSE were addressed for further diagnostic examination</td>
<td>NS</td>
<td>(95%CI: 0.33-3.09)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean age (SD): 50.4 yrs (6)</td>
<td>Diagnosis of dementia based on DSM-IV criteria and the probable and possible AD on the NINCDS/ADRDA criteria</td>
<td>Participants for whom follow-up evaluation was not accomplished were classified as having dementia based on clinical records</td>
<td>Coffee, tea</td>
<td>Dementia: 3.5 vs. 0.2 cups, OR=0.30 (95%CI: 0.10-0.93), &gt;5 vs. 0-2 cups, OR=0.03 (95%CI: 0.32-2.15)</td>
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<tr>
<td></td>
<td>Follow-up (mean): 21 yrs</td>
<td></td>
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<td>Tea consumption:</td>
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<td></td>
<td>Final study population: 1409 (534 men, 875 women)</td>
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<td>AD:</td>
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<td></td>
<td>Mean age (SD): 71 yrs (4)</td>
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<td>≥1 cup/day vs. non drinking, OR=0.91 (95%CI: 0.48-1.71)</td>
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<tr>
<td></td>
<td>Completeness of follow up: 71%</td>
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<tr>
<td>Santos</td>
<td>Cohort</td>
<td>Cognitive decline: Defined by Δ MMSE ≥ 2 points</td>
<td>FFQ</td>
<td>Caffeine consumption:</td>
<td>Age, education, BMI, hypertension, diabetes, smoking, alcohol consumption</td>
</tr>
<tr>
<td>2008</td>
<td>Community-dwelling Portuguese participants</td>
<td>Cognitive impairment: MMSE total score below cut-off values adjusted for education, for the Portuguese population</td>
<td>At baseline</td>
<td>Cognitive Decline: 22-62 vs. &lt;22 mg/day women, RR=0.54 (95%CI: 0.19-1.59)</td>
<td></td>
</tr>
<tr>
<td>Portugal</td>
<td>Baseline Population: 531 (220 Men, 311 Women)</td>
<td>Age range: ≥65 yrs</td>
<td>Validated</td>
<td>&gt;62 vs. &lt;22 mg/day women, RR=0.10 (95%CI: 0.01-0.81); men, RR=1.53 (95%CI: 0.21-11.04)</td>
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<tr>
<td></td>
<td>Follow-up (median): 4 years</td>
<td></td>
<td>Coffee, tea, soft drinks and chocolate products</td>
<td>Cognitive Decline: 22-62 vs. &lt;22 mg/day women, RR=0.54 (95%CI: 0.19-1.59)</td>
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<tr>
<td></td>
<td>Final Study Sample: 306</td>
<td></td>
<td></td>
<td>&gt;62 vs. &lt;22 mg/day women, RR=0.10 (95%CI: 0.01-0.81); men, RR=1.53 (95%CI: 0.21-10.94)</td>
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<tr>
<td></td>
<td>Completeness of follow up: 58.2%</td>
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</table>
Table 1. Main characteristics of the studies included in the systematic review (continued).

<table>
<thead>
<tr>
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<th>Evaluation of exposure</th>
<th>Results</th>
<th>Control of confounding</th>
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</thead>
<tbody>
<tr>
<td>Laitala</td>
<td>Cohort</td>
<td>Telephone interview questionnaire, assessing TELE and TICs score¹.</td>
<td>At baseline</td>
<td>Coffee consumption: Dementia (TELE &lt; 16): 3.5-6 vs. 0-3 cups/day, OR=0.91 (95% CI: 0.52-1.59); &gt;8 vs. 0-3 cups/day, OR=1.64 (95% CI: 0.86-4.38)</td>
<td>Education, age at interview, sex, alcohol and tobacco consumption, hypertension, hypercholesterolemia, diabetes, BMI, cardiovascular disease</td>
</tr>
<tr>
<td>2009 Finland</td>
<td>Participants from the Finnish Twin Cohort Study (selected from the National Twin Registry)</td>
<td>Demented (score: TELE &lt;16, TICs &lt;22.5)</td>
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<tr>
<td>Baseline Population: 3310</td>
<td>Cognitively impaired (score: TELE 16-17.5, TICs 22.5-26.5) Healthy (score: TELE &gt;17.2, TICs &gt;26.5)</td>
<td>Postal questionnaire (daily coffee consumption)</td>
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<td>Follow-up (median): 28 yrs</td>
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<td>Final study population: 2609 (1355 men, 1251 women)</td>
<td>Mean age (SD): 74.4 yrs (5.27)</td>
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<td>Completeness of follow-up: 79%</td>
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MSQ – Mental Status Questionnaire; NS – Not specified; GP – General Practitioner; SD – Standard Deviation; NINCDS-ADRDA – National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association Criteria for Alzheimer’s Disease; 3MS – Modified Mini Mental Status Examination; * - computed by the authors of this systematic review using the information provided in the original report; DSM-IV – Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; FFQ – Food Frequency Questionnaire; MMSE – Mini Mental State Examination; Benton – Benton Visual Retention Test; Isaacs – Isaacs Set Test; ¹ one unit=100 mg of caffeine; TELE – Telephone assessment for dementia; TICs – Telephone Interview for Cognitive Status
Figure 1. Systematic review flow-chart.

264 publications identified from electronic databases search

132 excluded (non-relevant):
5 review articles
127 non-epidemiological studies

5 articles

6 articles identified through reference lists search

11 articles included in the systematic review
Figure 2. Relative risk estimates for the association between caffeine and different forms of dementia/cognitive decline, according to sources of caffeine intake and levels of exposure.

* regular consumption stands for 'nearly every day'; † daily consumption

a – TELE < 18; b – TICS < 22.5; c – 10 ≤ TELE ≤ 17.5; d – 22.5 ≤ TICS ≤ 26.5; e – MMSE; f – Δ MMSE ≤ -2; g – Δ Benton ≤ -2; h – Δ Isaacs ≤ -6; i – Δ MMSE ≤ -1; M – Male; F – Female; C – coffee; T – tea; CT – coffee and tea; CB – caffeinated beverages; CB+Choc. – caffeinated beverages and chocolate.
Figure 3. Meta-analysis for the association between caffeine and different forms of dementia/cognitive decline, including the most precise RR estimates from each individual study.

ID – Identification; RR – Relative Risk; M – Male; F – Female
Figure 5. Relation between caffeine intake, participants’ age at baseline, completeness and duration of follow-up and log Relative Risk estimates for the relation between caffeine intake (including only the studies presenting RR estimates for more than one category of exposure) and different measures of cognitive performance*

AD – Alzheimer’s disease; D – Dementia; CI – Cognitive impairment; CD – Cognitive decline
* the areas of the symbols are proportional to the precision of the estimates, reflecting their relative weight when summary estimates are computed
8. Summary and Conclusions

Dementia is the most common form of neurodegenerative condition and is increasingly frequent as world population ages. It is a leading cause of death and is responsible for considerable morbidity, expressed in the high levels of functional dependence and need for burdensome interventions characteristic of late stages of dementia. This has profound social and economical consequences which justify a multidisciplinary approach regarding research and social interventions, aiming to minimize the impact of dementia.

The fact that there are at present no disease-modifying treatments justifies an investment in the identification of modifiable risk factors that may be contemplated in prevention strategies. The association between exposure to environmental exposures and dementia has received considerable attention. Among these, dietary habits are increasingly investigated, as occurs for most chronic illnesses. Caffeine is a widely available substance, being present in coffee, tea, soft drinks, chocolate and dietary supplements and consistent epidemiological evidence suggests that exposure to this substance is protective for Parkinson's disease. Caffeine has the potential to induce changes in adenosine-dependent modulating mechanisms, which have been described to influence synaptic plasticity; in addition, it participates in non-adenosine dependent mechanisms that may modulate synaptic and molecular events involved in cognition. This makes caffeine a potentially interesting target for research on preventive strategies to neurodegenerative conditions, namely dementia.

Two investigations were conducted, with the following specific objectives:

- To quantify the association between caffeine intake and cognitive decline in a cohort of Portuguese urban elderly subjects (manuscript 1).
- To review systematically and summarize the published studies addressing the effect of caffeine in cognitive decline and dementia, and to discuss the methodological heterogeneity of the available evidence (manuscript 2).
Manuscript 1: Caffeine intake is associated with a lower risk of cognitive decline: a cohort study from Portugal

A cohort of 648 subjects aged ≥65 years was recruited between 1999-2003. Follow-up evaluation (2005-2008) was carried out on 58.2% of the eligible participants and 10.9% were deceased. Caffeine exposure in the year preceding baseline evaluation was assessed with a validated food frequency questionnaire. Cognitive evaluation consisted of baseline and follow-up Mini-Mental State Examination (MMSE). Cognitive decline was defined by a decrease ≥2 points in the MMSE score between evaluations. Relative risk (RR) and 95% confidence interval (95%CI) estimates adjusted for age, education, smoking, alcohol drinking, body mass index, hypertension and diabetes were computed using Poisson regression.

The present study confirms a protective effect of caffeine intake on cognitive decline, in women. Results in this group were concordant across different criteria to define levels of exposure to caffeine and when using two different endpoints: the decrease in two or more points in the MMSE ([3rd third vs 1st third]: RR=0.49, 95%CI 0.24-0.97; [≥75 mg/day vs <75 mg/day]: RR=0.47, 95%CI 0.22-0.93) and the presence of a MMSE score below the cutoff for cognitive impairment ([3rd third vs 1st third]: RR=0.10, 95%CI 0.01-0.81; [≥75 mg/day vs <75 mg/day]: RR=0.19, 95%CI 0.02-1.48). Among men, the findings were similar when considering the decrease in the MMSE score of two or more points ([3rd third vs 1st third]: RR=0.65, 95%CI 0.27-1.54), but the reduced precision of the RR estimates, due to the small number of cognitive impairment events, precluded more robust conclusions.

Manuscript 2: Caffeine intake and dementia: systematic review and meta-analysis

We reviewed studies quantifying the relation between caffeine intake and cognitive decline or dementia published up to September 2009. Published reports were identified through Medline, LILACS, Scopus, Web of Science and reference lists searches. Three independent
reviewers selected the studies and extracted the data on to standardized forms. Nine cohort and two case-control studies were included. Quantitative data synthesis of the most precise estimates from each study was accomplished through random effects meta-analysis. Heterogeneity was quantified using the $I^2$ statistic.

The outcomes of the studies considered for meta-analysis were Alzheimer’s disease in four, dementia or cognitive impairment in two and cognitive decline in three studies.

The summary relative risk (RR) for the association between caffeine intake and different measures of cognitive impairment/decline was 0.84 (95%CI 0.72-0.99; $I^2$=42.6%). When considering only the cohort studies the summary RR was 0.93 (95%CI: 0.83-1.04, $I^2$=0.0%), and 0.77 (95%CI: 0.63-0.95, $I^2$=34.7%) if the most influential study was excluded.

Conclusions:

- Regarding the association between caffeine intake and cognitive decline in a cohort of Portuguese urban elderly subjects, the results do not rule out a negative association between caffeine intake and cognitive decline in men, and confirm the protective effect of caffeine in women. However, the large associations observed may be partially explained by potentially important confounding factors not accounted in the analysis.

- The systematic review and meta-analysis of studies on the association between caffeine intake and dementia/cognitive decline found a trend towards a protective effect of caffeine, but the large methodological heterogeneity across a still limited number of epidemiological studies is probably the most remarkable observation of this investigation. Further prospective studies conducted on more valid and homogeneous methodological basis are needed before robust and definite conclusions can be drawn.
9. Sumário e Conclusões

A demência é a entidade nosológica neurodegenerativa mais comum e a sua frequência tende a aumentar, refletindo a tendência mundial de envelhecimento da população. É uma das principais causas de morte e é responsável por uma morbidade expressiva, que se traduz em níveis elevados de dependência de terceiros, institucionalização e por vezes intervenções invasivas nos estádios mais avançados da doença. Esta situação tem importantes consequências socioeconómicas, que justificam uma abordagem multidisciplinar no que diz respeito à investigação e implementação de intervenções sociais que tenham como objectivo minimizar o impacto da demência.

Uma vez que não existem até à data tratamentos modificadores do curso da doença, justifica-se um investimento na identificação de factores de risco modificáveis que possam ser alvo de estratégias de prevenção. A associação entre a exposição a factores ambientais e o desenvolvimento de demência tem vindo a ser alvo de estudos recentes. A cafeína é uma substância amplamente utilizada e presente predominantemente em café, chá, chocolate e suplementos alimentares e diversos estudos epidemiológicos demonstraram um efeito protector em relação ao desenvolvimento da doença de Parkinson. A cafeína induz alterações nos mecanismos de neurotransmissão dependentes da adenosina, que estão envolvidos em fenómenos de plasticidade sináptica, apresentando igualmente a capacidade de modular mecanismos moleculares não dependentes da adenosina, mas igualmente envolvidos na cognição. Estes dados tornam a cafeína numa substância potencialmente interessante em investigações conducentes ao desenvolvimento de estratégias de prevenção de patologias neurodegenerativas, nomeadamente a demência.

Foram realizadas duas investigações com os seguintes objectivos específicos:

- Quantificar a associação entre o consumo de cafeína e a deterioração cognitiva, numa coorte urbana de indivíduos Portugueses com mais de 65 anos de idade (manuscrito 1).
- Rever de forma sistemática os estudos publicados que avaliem o efeito da cafeína na deterioração cognitiva e/ou demência, discutindo questões de heterogeneidade metodológica (manuscrito 2).
Manuscrito 1: Caffeine intake is associated with a lower risk of cognitive decline: a cohort study from Portugal

Foi avaliada uma coorte de 648 indivíduos com idade ≥65 anos, constituída entre 1999-2003. Cerca de 58.2% dos participantes foram reavaliados no seguimento, que decorreu entre 2005 e 2008; 10.9% dos indivíduos morreram durante este período. A avaliação da exposição à cafeína no ano que precedeu o momento da primeira avaliação foi efectuada através do recurso a um questionário validado de frequência alimentar. A avaliação cognitiva consistiu na administração do teste de MMSE nos dois momentos. O declínio cognitivo foi definido como uma diminuição de ≥2 pontos na escala MMSE, entre avaliações. Foram calculados os Riscos Relativos (RR) e respectivos intervalos de confiança a 95% (IC95%) ajustados para a idade, educação, hábitos tabágicos e etílicos, índice de massa corporal, hipertensão e diabetes, por regressão de Poisson.

O presente estudo confirma um efeito protector da cafeína em relação ao declínio cognitivo, nas mulheres. Os resultados neste grupo de participantes foram concordantes para os diferentes critérios usados para definir categorias de exposição e para os dois eventos escolhidos: o decréscimo de 2 ou mais pontos na classificação obtida no MMSE ([3° terço vs 1° terço]: RR=0.49, 95%IC 0.24-0.97; [≥75 mg/dia vs <75 mg/dia]: RR=0.47, 95%IC 0.22-0.93) e a deterioração cognitiva definida como uma potuação considerada abaixo do normal definido para o grau de escolaridade ([3° terço vs 1° terço]: RR=0.10, 95%IC 0.01-0.81; [≥75 mg/dia vs <75 mg/dia]: RR=0.19, 95%IC 0.02-1.48). Em relação aos homens, os resultados foram semelhantes quanto ao decréscimo de 2 ou mais pontos na classificação obtida no MMSE ([3° terço vs 1° terço]: RR=0.65, 95%IC 0.27-1.54), embora a precisão da estimativa seja baixa devido ao reduzido número de eventos observados.

Manuscrito 2: Caffeine intake and dementia: systematic review and meta-analysis

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Foram revistos os artigos publicados até Setembro de 2009, em que era quantificada a relação entre o consumo de cafeína e a deterioração cognitiva ou demência. Estes estudos foram identificados através de pesquisas nas bases Medline, LILACS, Scopus, Web of Science e listas de referências bibliográficas constantes nos artigos selecionados. Os dados foram extraídos por 3 investigadores, de forma independente. Nove estudos de coorte e dois de caso-controlo foram incluídos.

Foi efectuada a meta-análise das estimativas mais precisas de cada estudo, através de um modelo de efeitos aleatórios. A heterogeneidade foi quantificada utilizando a estatística $I^2$.

Os outcomes definidos e considerados para a meta-análise foram a Demência de Alzheimer em 4, demência ou deterioração cognitiva em 2 e deterioração cognitiva em 3 dos estudos.

O Risco Relativo (RR) para a associação entre o consumo de cafeína e as diferentes formas de avaliar o declínio cognitivo ou demência foi de 0.84 [intervalo de confiança a 95% (IC95%): 0.72-0.99; $I^2=42.6\%$]. Quando considerados apenas os estudos de coorte, o RR foi de 0.93 (IC95%: 0.83-1.04, $I^2=0.0\%$), e 0.77 (IC95%: 0.63-0.95, $I^2=34.7\%$), após a exclusão do estudo com mais peso no resultado final.

Conclusões:

- Os resultados da avaliação de uma coorte de indivíduos portugueses com mais de 65 anos não excluem uma associação negativa entre o consumo de cafeína e a deterioração cognitiva nos homens, e confirmam um efeito protector nas mulheres. Contudo, a elevada magnitude das associações observadas poderá ser parcialmente explicada pelo efeito de potenciais factores de confusão não considerados na análise.

- A revisão sistemática e meta-análise dos estudos publicados sobre a associação entre o consumo de cafeína e o desenvolvimento de deterioração cognitiva e/ou demência sugere um efeito protector desta substância em relação ao desempenho
cognitivo. No entanto, a heterogeneidade metodológica e o reduzido número de trabalhos publicados constituem a observação mais marcante desta revisão. Serão necessários mais estudos metodologicamente válidos e homogêneos para que possam ser retiradas conclusões definitivas acerca deste tema.