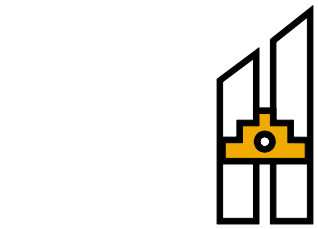
Can fruit and vegetables prevent Alzheimer's disease?

A systematic review of prospective cohort studies



HØGSKOLEN I BERGEN

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Mastergrad i kunnskapsbasert praksis i helsefag

Senter for kunnskapsbasert praksis

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Mastergradsstudium i kunnskapsbasert praksis i helsefag

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Kan frukt og grønnsaker forebygge Alzheimers sykdom?
En systematisk oversikt over prospektive kohortstudier

Tittel (norsk)

Can fruit and vegetables prevent Alzheimer's disease?
A systematic review of prospective cohort studies

Tittel (engelsk)

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Preface

When looking for a project for my master's thesis, the choice of doing a systematic review was quite obvious to me. By choosing a systematic review in the field of public health and nutrition I could combine my expertise as a nutritionist interested in biology, my position as a student wanting to learn more about research methodology, and as a journalist and information officer I could challenge my ability to find information in databases and research reports.

What triggered my interest in nutrition and Alzheimer's disease was an article from the Kame project reporting that intake of juices was inversely associated with the risk of Alzheimer's disease in old age. Was it false or true? The project of doing a systematic research on the intake of fruit and vegetables and Alzheimer's disease was a bigger challenge than I had imagined in advance. During the work I have indeed acquired new knowledge in research methodology, as well as on dementia and Alzheimer's disease.

My supervisor has been senior researcher Geir Smedslund at the Norwegian Knowledge Centre for Health Services in Oslo. In addition I have had two co-advisors – PhD student Lena Victoria Nordheim at the Centre for Evidence-Based Practice, Bergen University College, and senior researcher Anne Lise Brantsæter at the Norwegian Institute for Public Health. Thank you all for comments and support. Also thanks to colleagues for comments and questions demanding a clarification.

I will also thank my employer, The Norwegian Institute of Public Health, and my manager Gunhild Wøien for supporting my studies by giving me leave to go to Bergen for study weeks during the last three years, and for covering some of my expenses. Finally, I would like to thank family and friends for support during my three years as a master student in Bergen.

Oslo, May 2012

Hanna Hånes

Abstract

Background: The intake of fruits and vegetables has been reported to protect against the development of dementia. The objective of this systematic review was to assess the association between intake of fruits and vegetables and the risk of developing Alzheimer's disease (AD) in prospective cohort studies following participants for at least two years from 65 years of age.

Methods: I performed a systematic review of English-language studies published from 1980 through June 2011. Studies were identified from searches of MEDLINE, EMBASE, PsycINFO, AMED and CINAHL databases, all of the Cochrane databases, and Google Scholar using search terms for Alzheimer Disease, prospective cohort studies and fruits and vegetables. Methodological quality of included studies was assessed by risk of bias analyses. A meta-analysis using Review Manager 5 was performed.

Results: Three eligible prospective cohort studies published from 2006 onwards were identified. The studies had high or uncertain risks of bias. A meta-analysis indicated that a higher intake of fruits and vegetables was associated with a 29 % lower risk of AD (95% confidence intervals (CI) 0.59 to 0.86) compared with a lower intake. The studies included from 1589 to 8085 individuals (508 AD events in total) with an average follow-up from 3.48 to 6.3 years.

Conclusions: There is limited-suggestive evidence to say that a lower intake of fruits and vegetables is a cause of Alzheimer's disease from 65 years of age. Further analyses and studies are needed to determine if there is a causal relationship. More consistency in exposure assessment and adjustment models in further prospective cohort studies could facilitate dose-response analyses, increase control of bias and strengthen the documentation for national dietary advice. Further, systematic reviews with cognitive decline and total dementias as outcomes may supplement this review.

Keywords: Alzheimer disease, fruit, juice, meta-analysis, prospective cohort studies, systematic review, vegetables

Sammendrag

Bakgrunn: Studier tyder på at frukt og grønnsaker kan forebygge utvikling av demens. Målet med denne systematiske oversikten var å kritisk vurdere prospektive kohortstudier som har studert sammenhengen mellom inntak av frukt og grønnsaker og risikoen for utvikling av Alzheimers sykdom (AD) hos deltakerne som er fulgt i minst to år fra fylte 65 år.

Metode: En systematisk gjennomgang av engelskspråklige studier publisert fra 1980 til juni 2011 ble utført. Studiene ble identifisert ved søk i MEDLINE, EMBASE, PsycINFO, AMED og CINAHL, alle Cochrane-databasene og Google Scholar. Det ble benyttet søkeord for AD, prospektive kohortstudier, frukt og grønnsaker. Metodisk kvalitet på de inkluderte studiene ble vurdert ved å analysere risiko for systematiske skjevheter. En meta-analyse ble utført ved hjelp av Review Manager 5.

Resultater: Tre prospektive kohortstudier fylte inklusjonskriteriene. De ble publisert fra 2006 og framover og inkluderte fra 1589 til 8085 personer (508 tilfeller av AD totalt) med en gjennomsnittlig oppfølging fra 3,48 til 6,3 år. Meta-analysen tyder på at et høyere inntak av frukt og grønnsaker er assosiert med 29 prosent lavere risiko for Alzheimers sykdom (95 % konfidensintervall (KI) 0,59 til 0,86), sammenlignet med et lavere inntak. Studiene hadde høy eller usikker risiko for systematiske skjevheter.

Konklusjon: Det er en mulig årsakssammenheng mellom lavt inntak av frukt og grønnsaker og Alzheimers sykdom fra 65 års alder. Flere og bedre studier trengs for å avklare sammenhengen. I framtidige prospektive kohortstudier bør det være bedre konsistens når det gjelder måling av frukt- og grønnsaksinntak og justering for forvekslingsfaktorer. Det kan gjøre det mulig å utføre dose-respons-analyser, øke kontrollen av forvekslingsfaktorer og styrke dokumentasjonsgrunnlaget for allmenne kostholdsråd. Systematiske oversikter med kognitiv svikt og total demens som utfall kan supplere denne oversikten.

Nøkkelord: Alzheimers sykdom, frukt, juice, meta-analyse, prospektive kohortstudier, systematisk oversikt

Contents

ABBREVIATIONS

AD	Alzheimer's disease
APOE	Apolipoprotein E genotype
BMI	Body mass index
CASI	Cognitive Abilities Screening Instrument
CDR	Clinical Dementia Rating
CI	Confidence interval
CINAHL	Cumulative Index to Nursing and Allied Health Literature
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, version IV
DSM-III-R	Female
FFQ	Food frequency questionnaire
FV	Fruits and vegetables
HR	Hazard ratio
ICD Problems	International Statistical Classification of Diseases and Related Health
MeDi	Mediterranean diet
MMSE	Mini-mental state examination
NINDCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and related Disorders Association
WHICAP	Washington Heights-Inwood Columbia Aging Project
WHO	World Health Organization

1 Introduction/background

Treatment of dementia imposes a significant burden on patients, caregivers, and healthcare systems worldwide. In Norway alone there are 70 000 people with dementia today, this is expected to increase to 160 000 people in 2050 (Nasjonalt folkehelseinstitutt, 2010).

The prevalence of severe cognitive impairment is projected to quadruple from current levels to 81 million worldwide by 2040. It is estimated that there were about 10.1 million people with dementia in the whole of Europa in 2008.The total cost of dementia illnesses in Europe is estimated to 53.0 million Euro in northern Europe and 177 billion Euro in the whole of Europe, 22 000 Euro per demented per year (Wimo et al., 2011).

1.1 Alzheimer's disease and dementia

Dementia disorders are progressive and so far, incurable. The four main dementia disorders are Alzheimer's disease (AD), fronto-temporal dementia, vascular dementia and dementia with Lewy bodies (Cummings & Cole, 2002).

Alzheimer's disease (AD) is the main type and accounts for 60 % to 70 % of cases of dementia in elderly patients (Cummings & Cole, 2002).

Alzheimer's disease is named after the German medical doctor Alois Alzheimer (1864-1915). He treated a woman who developed dementia at the age of 51. In 1907 he described her syndrome which since has been known as Alzheimer's disease (AD) (NIH, 2010).

The onset of Alzheimer's disease is insidious, with a long preclinical period, and the course is gradual. See Figure 1, next page.

The early symptoms of AD are typically memory loss, deterioration of language, and visuospatial deficits. As the disease progresses, the patients experience increasing cognitive and functional decline (Cummings, 2004). AD is commonly diagnosed in adults age 65 and older. Early onset Alzheimer's disease has a lower incidence.

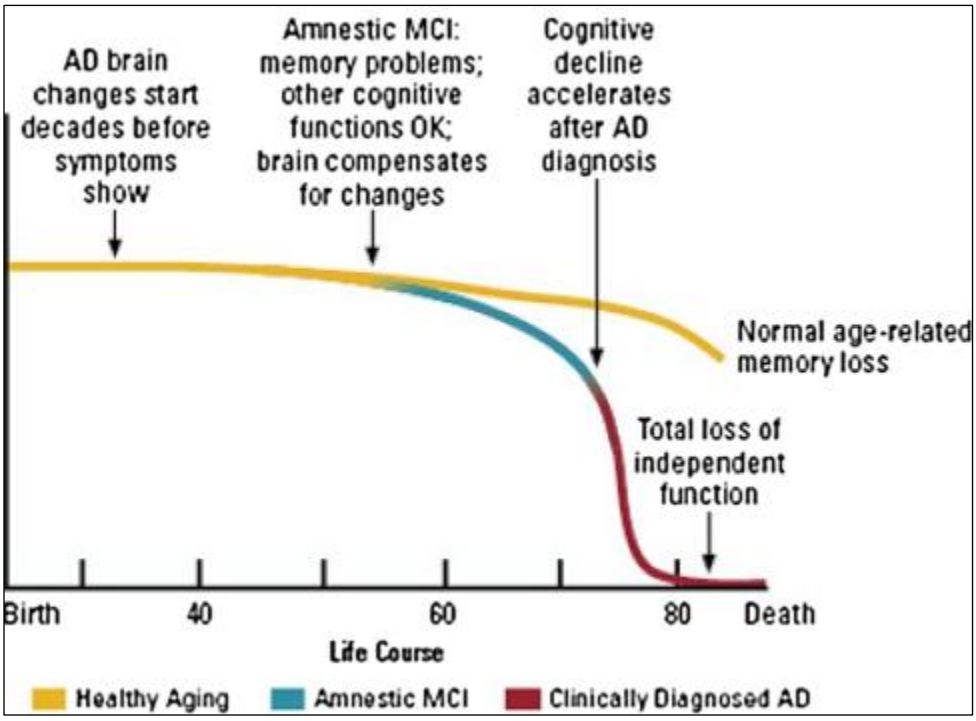


Figure 1. Change over time from healthy aging to Alzheimer's disease (AD).

Yellow-blue-red curve shows the course for people gradually moving to mild cognitive impairment and then possibly making the transition to AD. Yellow curve shows the course of people with normal age-related changes in cognition. Reprinted with permission from: National Institute on Aging, Alzheimer's Disease Education and Referral Center.

1.1.1 Diagnosis

AD is diagnosed by behavioral assessments, cognitive function tests and brain scans. Autopsy and microscopic examination of the brain are required for a definitive diagnosis. A sharp decline in functional ability, and to a lesser extent, declines in executive function seem to be important markers to predict conversion from cognitive decline to AD (Gomar et al., 2011). Standard diagnostic criteria for AD are developed by World Health Organization in International Classification of Diseases, 10th version (Organization, ICD-10) and by the American Psychiatric Association (APA, 2010).

Mild cognitive impairment (MCI) may be an early stadium of AD (Morris, 2010). Cognitive decline and MCI may be assessed by Clinical Dementia Rating (CDR), Functional Activities Questionnaire

(FAQ), and standardized tests for cognitive function such as Mini-Mental State Examination (MMSE), and tests of orientation and memory (Woodford & George, 2007).

No specific biomarker has been identified that predicts conversion from cognitive decline to AD or which qualitatively distinguishes AD from "normal" aging processes, but cerebrospinal fluid tests for pathological Aβ1-42 and total tau proteins help diagnosing the disease (NIH, 2010, p. 27-29 ; Lo et al., 2011).

1.1.2 Treatment

At present, pharmacological therapies are not able to halt progression or cure dementia, although some patients get short term symptomatic cognitive improvements (National Collaborating Centre for Mental Health, 2006 ; Socialstyrelsen, 2010).

1.2 Biology of Alzheimer's disease

AD patients lose neurons in their brain and develop brain atrophy, and this accounts for the cognitive and behavioral dysfunction which is observed clinically. The amyloid hypothesis is the leading mechanistic theory explaining the pathogenesis of Alzheimer's disease. Beta-amyloid is a normally secreted protein, but extracellular accumulation and deposition in the brain is thought to result in a pathological cascade with loss of neurons in the end (Tanzi & Bertram, 2005 ; Goedert & Spillantini, 2006 ; Hardy, 2006). The process is associated with oxidative injury, inflammatory activity, and acetylcholine deficits. Another characteristic lesion in the brain is intracellular neurofibrillary tangles made up of tau proteins.

Alzheimer's disease and cerebrovascular disease are related

Several studies have documented a close relationship between vascular disease and the risk of dementia and Alzheimer's disease (Kalaria, 2010 ; NIH 2010, p.77-79). Cerebrovascular disease increases the severity of Alzheimer's disease (Helzner et al., 2009 ; Scarmeas et al., 2006a ; Launer, Hughes & White, 2011).

An experimental real time study in mouse models by Garcia-Aloza et al (2011) demonstrates that experimental micro strokes can trigger accelerated amyloid deposition in the region surrounding the infarcted area during the first week after the infarction. This study indicates that occlusion of brain arterioles can trigger the onset of amyloid deposition. The study indicates that the most likely mechanism is an interference with amyloid clearance pathways.

The relationship between vascular disease and AD may be complex, but assuming a causal relationship between vascular dysfunction, occlusion of brain vessels and Alzheimer's disease, factors preventing vascular disease and brain vascular disease in particular, should be considered preventive factors for dementia and Alzheimer's disease as well.

1.3 Risk factors of Alzheimer's disease

1.3.1 Age, heritability, socio-economic and mental risk factors

Age is the main risk factor for Alzheimer's disease (Wittchen, Jacobi & Rehm, 2011). The heritability is high and the second greatest known risk factor is a positive family history (Gatz et al., 2006). For late onset AD a heritability of 70 percent is found in a study in US centers. Late onset AD is a highly polygenic disease (Wingo et al., 2012). The apo-lipoprotein ε4 polymorphism (ApoE ε4) is the most well established genetic risk factor, but 50 percent of the cases do not carry this genetic factor (Tanzi & Bertram, 2005).

The time of onset of symptoms varies by up to 18 years between monozygotic twins who eventually become concordant for the disease (Pedersen et al., 2004 ; Raiha et al., 1996 ; Brickell et al., 2007). This strengthens the assumption that modifiable life style factors might trigger the onset of the disease or influence the progression of the disease from cognitive decline to AD. Differences in brain β-amyloidosis cannot explain the dissociation between monozygotic twins, suggesting that modifiable risk factors may modulate the relationship between brain amyloidosis and neuro-degeneration (Scheinin et al., 2011).

Socio-economic risk factors are strongly related to the risk of dementia and AD (NIH, 2010; Plassman et al., 2010). This may be due to increased brain reserve, such as neuronal count, a greater capacity in compensatory mechanisms, more cognitive engagement, or a better lifestyle throughout life which help provide reserve against cognitive decline.

Depression and proneness to psychological distress may also serve as a risk factor for Alzheimer's disease, possibly through deleterious effects of stress on the brain. Wilson et al (2002) reported increased risk of AD onset among those with higher baseline levels of depressive symptoms, in a period of 7 years of follow-up. In another study the group reported that those with high distress proneness had twice the risk of developing AD compared to those low in distress proneness (Wilson et al., 2003).

1.3.2 Life style

Life style and other risk factors for vascular disease are associated with Alzheimer's disease (Reitz et al., 2010). These risk factors include midlife hypertension, diabetes, hypercholesterolemia (Helzner et al., 2009), plasma homocysteine (Tangney et al., 2011a), smoking, alcohol intake and sedentary life style (Scarmeas et al., 2011; Head et al., 2012). A recent systematic review concluded that physical activity is associated with a reduced the risk of dementia (Hamer & Chida, 2009).

Whether cardiovascular comorbidity increase the risk of incident AD is controversial (Scarmeas et al., 2006a ; Mielke et al., 2007), but cerebrovascular health may affect cognitive function and affect cognitive decline among individuals with prodromal AD and AD.

Obesity, particularly central obesity, and underweight in midlife is associated with an increased risk of AD and dementia in late-life (Anstey et al., 2011 ; Luchsinger et al., 2011). Low BMI and a faster decline in BMI in late life may be preclinical indicators of dementia (Hughes et al., 2009).

1.3.3 Diet

A Mediterranean dietary pattern and other dietary patterns rich in fruits and vegetables are associated with reduced risk for cognitive decline (Tangney et al., 2011b; Feart, Samieri & Rondeau et al., 2009; Scarmeas et al., 2009a) and reduced risk of AD in older age (Scarmeas et al., 2006b). A traditional Mediterranean diet is mainly plant-based and is rich in fruits and vegetables, nuts, pulses, whole grains, olive oil, and contains some fish and poultry, but small amounts of meat and hydrogenated trans fatty acids. Other dietary patterns similar to the traditional Mediterranean diet are also shown to be protective against development of AD (Guy et al, 2010).

Fatty acids or anti-oxidants

The hypotheses discussed to explain the association between AD and Mediterranean diet have mostly focused on these dietary patterns being rich in polyunsaturated omega-3 fatty acids and low in saturated fatty acids, and rich in antioxidants from fruits and vegetables, such as vitamin C and E, carotenoids and flavonoids (Barberger-Gateau et al., 2007; Devore et al., 2010; Féart, Samieri & Barberger-Gateau, 2010; Polidori et al., 2009).

Another hypotheses is that increased contents of potassium and blood pressure lowering nutrients reduce the risk for AD (Sacks et al., 2001). The Dietary Approaches to Stop Hypertension (DASH) studies found that diets rich in vegetables (i.e. 8–10 servings a day) and low-fat dairy products can

lower blood pressure to an extent similar to that achieved with single hypotensive medications. Fruits and vegetables may also provide a preventive effect by replacing unhealthy foods.

Omega-3 fatty acids are major components of neuron membranes, and have vascular and anti-inflammatory properties which could explain a protective effect against dementia. Anti-oxidants are hypothesized to protect omega-3 fatty acids against peroxidation and thus to preserve brain neurons, or to interact with apo-lipoprotein E which plays a role in the cholesterol uptake and transport in the brain (NIH, 2010).

Other nutritive factors

Some investigators have studied possible associations between AD and folic acid, vitamin B_6 and B_{12}, choline and betaine, phytoestrogens, fiber, vitamin K and nitrate. A high level of total homocysteine (tHcy) is associated with an increased risk of dementia and AD. Supplements of folate, vitamin B_6 and B_{12} reduce tHcy levels. In some small clinical studies supplements have shown a positive effect on cognitive decline, but more studies are needed before any conclusion can be reached concerning benefit of B-vitamins and tHcy reduction (de Jager et al., 2011; Malouf & Grimley Evans, 2008). Green leafy vegetables, and fruits and berries like oranges and bananas are rich sources of folate.

Recent availability of food composition data (USDA Nutrient Data Laboratory, 2004) have motivated studies on choline and betaine as risk factors of chronic diseases. Choline, betaine and folate are interchangeable sources of one-carbon units and choline and betaine also reduces tHcy. Fruits and vegetables are poor sources of choline, but spinach is rich in betaine. In a population of middle-aged and elderly men and women in Norway plasma betaine was negatively associated with a western dietary pattern with a high loading of meat, pizza, sugar, and fat, whereas choline was not significantly associated with any identified dietary patterns (Konstantinova et al., 2008; Ueland, 2011).

Some studies including healthy or demented individuals reported improved performance on memory and learning tasks following supplementation with choline (Ueland, 2011, p.9). Experimental studies suggest that cognitive dysfunction in folate-deficient rats was not associated with plasma homocysteine or brain content of S-adenosylmethionine but related to depletion of phosphatidylcholine in the brain (Troen et al., 2006).

The integrity of the vascular system is essential for the optimal functioning of the brain (Kalaria, 2010). Production of nitric oxide NOx in the endothelial walls leads to vasodilation, decreased blood pressure, and support of vascular function. Dietary sources of nitrates and nitrites may have a role in vascular health by being metabolized to bioactive nitric oxide (Hord, Tang & Bryan, 2009). Health benefits of traditional Japanese diets may be partly be explained by nitrate content (Sobko et al., 2010).

Experimental studies on rodents indicate that vitamin K_1 (phylloquinone) is associated with cognitive deficits during aging (Carrié et al., 2011). Vitamin K is shown to accumulate in brain regions rich in white matter and participates in the modification of several proteins (Pedersen, Hjartåker & Anderssen, 2010). Green leafy vegetables like spinach, broccoli, brussel sprouts, and vegetable oils and spreads are the main dietary source of phylloquinone in western diets (Drevon, Henriksen & Sanderud et al., 2004). In eastern Japan pulses, fermented soybean products and algae are the main contributors to the total vitamin K intake (Kamao et al., 2007).

1.3.4 A life course perspective

Midlife environmental factors are related to the risk of AD, such as body weight and hypertension. Even intrauterine early life environment factors may be related to the risk in late life AD (NIH, 2010). Concerning diet as a potential protective factor, nutritive factors could play a role throughout life as well as in older age postponing the symptoms of the disease.

1.4 Evidence based public health

In public health, knowledge about causes of diseases should inform judgments about which interventions to implement. According to Brownson et al (2011, p. 6) public health evidence is usually the result of a complex cycle of observation, theory, and experiment. Three types of evidence should be considered for public health practice. Type 1 data are data on disease relationship and etiologic research, type 2 are evidence describing the relative impact of specific interventions, and type 3 data considers how an intervention should be implemented in a certain context.

This thesis concerns type 1 data, if the intake of fruits and vegetables play a role in the etiology of Alzheimer disease. To infer a causal relationship a set of criteria has been suggested: 1-consistency of studies in different settings, using various methods, 2-strength; the size of the effect estimate, 3-temporality; that cause precedes the effect in time, 4-dose-response relationship, 5-biological

plausibility, and 6-experimental evidence from human or animal trials (Rothman et al., 2008, p. 26 ; Brownson et al., 2011, p.41).

Several systems for grading the quality of evidence and assessing strength of recommendation exist. The GRADE system (Balshem et al., 2011) is a widely used method in the context of clinical work. According to GRADE the quality of evidence is graded in one of four levels – high, moderate, low or very low. Randomized controlled trials (RCT) of high quality are required for an initial rating of moderate and high quality of evidence.

In the field of nutrition research and chronic diseases RCTs are difficult or often impossible to accomplish, and evidence has to be based on observational and other research designs.

Another rating system is developed by the expert panel editing the report "Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global perspective". The panel graded evidence of causality in five levels (WCRF/AICR, 2007, p. 60-61), see table 1.

Table 1. Grading evidence of causality according to WCRF/AICR (2007, p. 60-1).

Convincing evidence	Requires all of the following: Evidence from more than one study type, from at least two independent cohort studies, no substantial unexplained heterogeneity between study types or populations, good quality studies, presence of a plausible biological gradient (dose response) in the association, and experimental evidence which is strong and plausible.
Probable evidence	Requires all of the following: Evidence from at least two independent cohort studies, or at least five case-control studies; no substantial unexplained heterogeneity between and within study types, good quality studies, and evidence for biological plausibility.
Limited –suggestive	All of the following generally required: Evidence from at least two independent cohort studies or at least five case-control studies. The direction of effect is generally consistent though some unexplained heterogeneity may be present. Evidence from biological plausibility.
Substantial effect on risk unlikely	Limited - no conclusion.
Limited – no conclusion	Evidence is so limited that no firm conclusion can be made.

Compared to GRADE, the WCRF/AICR panel grades cohort studies at a higher level of evidence. Cohort studies may be part of a "convincing" or "probable" level of evidence of causality if there are at least two independent cohort studies, there is no substantial unexplained heterogeneity between the studies or populations and they are good quality studies. Good quality means, according to WCRF/AICR, that one with confidence can exclude the possibility that the observed associations result from random or systematic error, including confounding, measurement error, and selection bias.

Limited evidence may be upgraded if there are a plausible biological gradient (dose response) in the association, a particularly large summary effect size after appropriate control for confounders or evidence from appropriate animal studies.

It may be reasonable to apply the WCRF/AICR grading system on nutrition research and AD, because both cancer and AD are diseases which have a long preclinical phase. Applying the WCRF/AICR grading system to the field of Alzheimer's disease means that a systematic review of observational cohort studies may be part of the basis for a public recommendation (or no recommendation) concerning the intake of fruits and vegetables to prevent AD (WCRF/AICR, 2007, p. 59).

1.5 Prospective cohort studies in nutrition research

A prospective cohort study follows over a period of time a predetermined sample of patients who have been exposed to some factor under investigation. Exposures, like the intake of fruits and vegetables and other life style factors, are determined during the initial interview and the participants are grouped together according to exposure (Rothman, 2002, p. 73). In this way systematic recall bias in exposure is eliminated when doing the dietary assessment at a point of time when the participants are cognitively healthy with normal memory. The period time at risk for disease occurs during the conduct of the study. There are repeated measures on the same subjects to detect the outcome(s) of interest. Cohort studies can be used to measure the incidence rates as well as the relative risk of outcome associated with exposure (Marko & Weil, 2010).

Retrospective (historic) cohort studies are graded at a lower level of evidence. These analyses are case-control studies comparing a sample of patients with a known outcome (cases) and a sample group without the outcome of interest (Marko & Weil, 2010; Rothman, 2002, p.70). For

retrospective studies there is an increased risk that important information may be missing or unavailable, and these studies are subject to recall bias (Rothman, 2002, p.70).

1.6 Assessment of intake of fruits and vegetables

Dietary intake varies from day to day and over the course of the life. There are interrelationships between food components, between foods in whole diets, and between diet, gender, education, marital status, age and behavioural characteristics such as physical activity and smoking (WCRF/AICR 2007, p.55 ; Kesse-Guyot et al., 2009). Smokers consume less fruits and vegetables than non-smokers. In studies from USA fat intake inversely correlates with vegetables and, particularly, fruit (WCRF/AICR 2007, p.82).

Methods for assessing food consumption are diet histories, 24 –hour dietary recalls, food frequency questionnaires (FFQ), and food diary or food record methods. Short-term-recall and diet-record methods are generally expensive, unrepresentative of usual intake, and inappropriate for assessment of past diet. Therefore FFQs are extensively used in nutrition research (Willett, 2008, p.590-1 ; Willett & Hu, 2006).

According to Willett (2008, p. 591) a FFQ is designed to measure a person's habitual dietary intake over a defined period of time, for example the preceding year, month or week. Typically a FFQ consists of a food list and a multiple choice section for the participant to report how often each food was eaten. If a portion size is specified, i.e. a glass of milk, the participant indicates how often he or she drinks a glass of milk. This technique is used for a semi-quantitative FFQ. A FFQ may include a list of images of food items and portion sizes. Most of the variation in intake of a food, however, is explained by frequency of use rather than differences in serving size.

The length of the food list of a FFQ varies, and several types of food lists are developed to suit different population groups and investigative focus.

Because of the measurement errors, a FFQ should be validated in the same sample or population as it is used to investigate dietary intakes. Comparison with a 24-hour recall or a 7-day weighed food record is often used. A close correlation in a validation study is not expected. The correlation between nutrients assessed by FFQ and the comparison methods have consistently varied between 0.4 and 0.7 when adjusted for total dietary intake (Willett p. 592). Correlation is attenuated if the list of food items in the FFQ does not cover a participant's habitual diet or the food record or 24-hour recall does not cover the same time period as the FFQ, and intake varies over time. One way of

improving measurement of diet in a longitudinal study is to repeat the dietary assessment over time. This will tend do attenuate random error and will also account for true changes in intake.

Both FFQ and other assessment methods may overestimate the intake of fruits and vegetables, because these foods are perceived as healthy and socially acceptable food. On the other hand, studies Norway indicate that a FFQ identifies high and low intakes of fruit, juice and vegetable more precisely than energy-yielding foods. This may indicate that under-reporting may be less common with food items considered healthy, than with energy-yielding food items (Brantsæter et al, 2008). Even if intake of fruits and vegetables is overestimated, a FFQ is able to rank individuals according to the intake (Kristjansdottir et al., 2006 ; Miller et al., 2008; Amanatidis, Mackerras & Simpson, 2001).

Application of biochemical indicators can provide a supplement to information about dietary intake, but for most dietary factors there have been no practical indicators or indicators affordable for large cohort projects (Willett 2008, p. 594). Plasma level of carotenoids and vitamin C are examples of biochemical indicators for intake of fruits and vegetables rich in these nutrients (Ferrari et al., 2005). Another example is urine level of flavonoids. This is an evolving area in nutrition research (Krogholm et al, 2012).

1.7 To measure a protective effect of foods in an observational study

To measure a protective effect of fruits and vegetables for AD we ideally should know the response curve. Presuming there is an effect, the response curve may be similar to the response curve seen for most nutrients; an inverted U-shaped association with optimum physiological function, which I have drawn in figure 2, inspired by Morris & Tangney (2011).

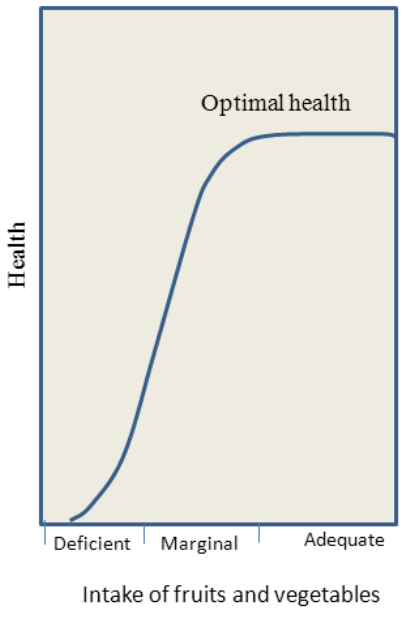


Figure 2. Relationship between level of nutrient status and health, transferred to a possible dose-response curve for intake of fruits and vegetables and health. For individuals who are already at optimal health, further intake would provide no additional benefit. Chart after Morris & Tangney (2011).

To observe an effect, the cut off for the low intake group has to be at a lower protective effect level than the high level of

intake group. In a cohort study an effect can only be observed if groups with sufficiently high and low intakes to estimate a protective effect are included and followed prospectively (Morris & Tangney, 2011).

1.8 Systematic review and meta-analyses of prospective cohort studies

By doing a systematic literature review all studies relevant for the research question can be found, appraised and interpreted. A systematic review of prospective cohort studies of observational design follows the general criteria for systematic reviews (Green et al., 2011; Liberati A et al., 2009):

- Pre-defined eligibility criteria
- An explicit, reproducible methodology
- A systematic literature search
- An assessment of the validity of the findings of included studies
- A systematic presentation, and synthesis, of the characteristics and findings

Cohort studies may need to be very large to have sufficient statistical power to provide evidence regarding the presence or absence of an association between an exposure and an outcome. By doing a meta-analysis the combined effect of several studies with the same design can be measured quantitatively. A meta-analysis provides a possibility to examine dose-response relationships and to identify heterogeneity between studies as well (WCRF/AICR, 2007, p.51).

1.9 Why it is important to do this review

Today there are no effective treatments for Alzheimer's disease. With increasing number of people expected to be affected by Alzheimer's disease in the future, preventive measures are of importance even for public policy makers and the economy of a society. Fruits and vegetables may be modifiable risk factors in prevention of AD. Bihan et al have shown that it is possible to stimulate people of all ages to increase the intake of these foods (2012) .

In the last two decades methods to measure diet applicable to epidemiologic studies have been developed, and their validity documented (Willett, 2008, p. 585-6). Several large prospective studies have been established that are providing data on food, dietary patterns and chronic diseases, among these studies are several cohort studies focusing on health in old age. Hence, a systematic literature review to investigate the association of the intake of fruits and vegetables with the risk of developing AD will be conducted.

1.10 Objectives

The aim of this thesis is to do a

- systematic literature review of prospective cohort studies on the association of the intake of fruits and vegetables with the development of Alzheimer's disease
- meta-analysis to provide a summary estimate of the effect of a higher intake of fruits and vegetables

The overall aim of the systematic literature review is to assess the scientific evidence of a potential association between fruits and vegetables intake and the development of AD.

2 Methods

2.1 Literature review

2.1.1 Inclusion and exclusion criteria

Table 2 next page gives an overview of the inclusion and exclusion criteria. A study design of prospective cohort studies was chosen because this design is less prone to recall and selection bias. As study population, non-demented participants with normal cognition, age 65 years or older and at least two years of follow-up was chosen. This will not exclude participants who are in a very early phase of pre-dementia, but this will exclude participants who have developed symptoms and changed their food habits as a consequence of cognitive changes.

2.1.2 Literature search

Search strategy

The literature search includes reports from 1980 onwards. This will encompass studies established at the time when a valid diagnosis measurement was taken up in different countries. A valid diagnosis measurement of AD was established in 1982 (APA, 2010). As this is a master's degree I chose to exclude studies with full text reports in other languages than English, as well as study results not yet published.

Based on the inclusion and exclusion criteria I generated lists of search terms (Appendix I). For this I got assistance from Wenche Jacobsen, a librarian experienced in systematic reviews within the field of public health. The search strategy was overlooked by one of my co-supervisors (LVN)[i]. I searched each of the following databases through the Ovid interface: Medline (R) in-process &

other non-indexed citations and MEDLINE (R) daily and MEDLINE (1980 to 2011 Week 25), EMBASE (1980 to 2011 Week 25), PsycINFO (1980 to June Week 4 2011) and Allied and Complementary Medicine (AMED, from 1980 to June 2011).

Search strategies for the four Ovid databases, as well as the search strategies for additional databases are presented in Appendix I.

Table 2. Inclusion and exclusion criteria.

Category	**Inclusion criteria**	**Exclusion criteria**
Study design	Original prospective cohort studies, comparing exposed to non-exposed.	Retrospective case-control studies, cross sectional studies, before-and-after study, interventional studies, non-human studies.
Study population	Aged 65 and older. Followed for at least two years. Normal cognition. Not institutionalized. No limits for ethnicity or cultural group.	Follow-up less than 2 years Institutionalized population. Population with cognitive decline, mild cognitive impairment (MCI) or dementia.
Risk factors/exposure	Fruits and vegetables, fruits alone, vegetables alone, subgroups of fruits or vegetables, juices. Fruits include berries. Individual dietary assessment at inclusion.	Studies reporting dietary patterns only. Studies without individual dietary assessment.
Outcome	Alzheimer's disease (AD). Diagnosis based on acceptable standard, such as the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Criteria (ADRDC).	Other dementias or MCI only. Studies not using validated measurement for AD.
Time period	Published 1980 and later.	Other time periods.
Publication language	English only.	Other languages.
Other criteria	Original research studies that provide sufficient detail regarding methods and outcome, able to be extracted from data presented in the papers.	Studies not reported in full text in English.

In addition to Ovid databases the following databases were searched on July 14, 2011: Cumulative Index to Nursing and Allied Health Literature (CINAHL) (1982—June 2011), and Google scholar (100 first references screened for eligibility). In Cochrane Database of Systematic Reviews I searched all of the Cochrane libraries in July 2011. These libraries include Cochrane reviews as well as Other reviews, Clinical trials, Methods studies, Technology assessments, and Economic evaluations. In addition I hand searched the Cochrane library by topic "Dementia and cognitive improvement" and by review group "Dementia and cognitive improvement group".

Records revealed in the additional searches were checked for duplicates. I did some additional searches in nutrition and neurology journals, as well as the database ISI Web of Science. These revealed no additional references and are not included in this systematic review.

Reference lists of relevant retrieved articles and relevant review articles were hand searched for further references.

As some cohort projects are running for years, publishing many reports, I contacted investigators of relevant cohort projects to check for additional and unpublished studies which might fulfill the inclusion criteria. These cohorts and investigators were identified by the literature search and by reading articles on cohort studies and reviews in full text.

2.1.3 Including and excluding studies

Using the pre-specified inclusion and exclusion criteria, titles and abstracts were examined for relevance. If the abstracts did not provide adequate detail about study design, I screened the methods section of full-text articles for relevance.

For eligibility I screened the articles in full text. Several of the full text articles had cognitive decline and/or total dementia as an outcome, only some had AD as an outcome as well. Concerning exposure I had to read the articles thoroughly as some of them were focusing on intake of anti-oxidants or dietary pattern like the traditional Mediterranean diet, which includes fruit and vegetables as a component. I only selected articles which presented separate analysis of FV intake.

To screen for the design of a prospective follow-up study I followed an algorithm from the Academy of Nutrition (Academy of Nutrition and Dietetics, 2012, ch. 2), modified for observational prospective studies with the selected exposure and outcome. The algorithm included the following: (i) group assignment is not manipulated by the researchers, (ii) no intervention is

provided, (iii) data about AD diagnosis or dementia are collected at more than one time point in the same subjects, (iv) comparison groups are defined according to dietary exposure and not according to AD already present, and then examining data about pre-existing intake of food items (case-control study), (v) subjects are enrolled in the study and followed forward through time with several data collection points, and data are not abstracted from existing longitudinal data sets or archival sources to answer new research questions (retrospective cohort design).

2.2 Data collection

To abstract key information from included studies I used a workingsheet template for observational studies in the field of nutrition, developed by the Academy of Nutrition and Dietetics, former American Dietetic Association (2011), and the manual (2012). The sheet is a tool to provide a description of what happened in the study; to describe features of the design, confounding factors considered and methods used to control for confounding, aspects of risk of bias and the results. For the workingsheet template, see appendix IV.

As a student and new to the field of systematic reviews I prepared for the work of extracting data by first reading the included reports thoroughly. For this I used a checklist for reporting observational studies made by the STROBE initiative, as well as an article from the same group explaining the checklist (Vandenbroucke et al., 2007; von Elm et al., 2007). This gave me more insight into the reporting from such studies and helped the following abstracting of information to the workingsheet, as well as working with checklists as part of the quality assessment.

Data items collected

I abstracted data on literature references, methodology, setting and population, exposure and outcome. Investigators were contacted if not enough information was given in the published reports.

For general methodological issues I sought data on invitation procedure, which is necessary to judge if group assignment is manipulated or not by investigators, of sample size and response rate to consider validity of effect size and confidence intervals. For characteristics of participants I collected data on mean age and other socio-demographic variables like ethnicity, gender and marital status, genotype (ApoE), health variables, and lifestyle variables which are important to consider confounders (alcohol intake, physical activity and smoking).

To assess the exposure to fruits and vegetables I collected data on method of assessment of dietary

intake, if measurements were repeated at follow-up, if the method was validated in the target population, and if exposure was adjusted for total energy intake.

To assess if cognitive health at the time of recruitment and at follow-up was objectively measured and assessed I sought data on screening procedure at baseline, measurement method for cognition and dementia diagnosis, follow-up time, losses to follow-up (attrition).

I collected data on methods for statistical analyses, non-adjusted and adjusted effect estimates according to different models in the included studies, variables that were entered into the multivariable models, 95 % confidence intervals, p-value, supplementary analyses done, i.e. to assess if losses to follow-up, follow-up time of cognitive status at baseline had any effects on outcome assessment.

To obtain enough information from each study I had to read several reports from the same studies. Still some information was difficult to find and in some cases I contacted investigators by e-mail to supplement the information from the published reports.

Data on funding were collected as well. If studies are sponsored by food industry, this may raise questions concerning bias.

The workingsheet was used to create a table for summary of study characteristics (Table 3) and an evidence overview table (Table 5).

2.3 Quality of included studies

The Cochrane Group draws a distinction between assessment of methodological quality and assessment of risk of bias (Higgins & Green, 2011). The former includes ethical issues and quality of reporting as well as risk of bias. A study may be performed to the highest possible standards and yet still have an important risk of bias. Therefore, in assessing the quality of the included studies I emphasized the risk of, i.e. the risk that the studies will overestimated or underestimated the true effect.

2.3.1 Quality criteria checklist

Quality criteria checklists are used to examine important details about the design of the study and its execution. Questions included in the checklists address scientific validity, including risk of bias, as well as applicability to practice.

For the checklists I decided to use the checklist for observational cohort studies from the Critical Appraisal Skills Programme (CASP, 2010), this is published in a Norwegian version as well (Kunnskapssenteret, 2010).

The CASP checklist (Appendix V) is not specific for the challenges of appraisal observational research in the field of nutrition. I therefore customized the checklist in the domain of confounding factors (as indicated by authors of the check list). For this I used the guideline from the Academy of Nutrition and Dietetics for primary research (2012, appendix 8), a checklist for sources of internal bias in longitudinal observational studies from Thompson et al (2011) and a checklist draft from the Nordic Council (Appendix VI). In addition I used these sources to make certain other relevant risks of bias were assessed, such as methods for assessment of mental health at the time of recruitment and follow-up, registered deaths and losses to follow-up, life style factors and measurement methods for co-variables.

The Cochrane collaboration strongly recommends that a review should be undertaken by more than one person independently (Green & Higgins, 2011), increasing the likelihood that errors are detected. This includes at least two persons completing the check list for each study independently, and afterwards discussing any discrepancies. As a master student I did this work alone, but discussed the rating of bias in the domain of exposure bias (intake of fruit and vegetables) with my co-supervisor ALB[ii]. To validate the judgments the work should be overlooked by one or two more persons before submitting the article draft for publication.

2.3.2 Risk of bias

To critically appraise each included study I used the customized check list and generated a risk of bias summary figure. The domains in this figure (Figure 4) follow the structure of the risk of bias tool developed by the Cochrane group for randomized trials (Higgins & Green, 2008). To construct the figure a list of requirements for a low risk of bias was created within seven domains of bias (Figure 4 with subtexts). All studies were checked using this list.

Particular care was given to assessment of confounding, as recommended by Cochrane Collaboration for the review of non-randomized studies trials (Reeves et al, 2011). A confounding factor has three properties (Rothman, 2002); it is associated with the disease (but is not an effect of the disease), is associated with the exposure (and imbalanced between exposure groups to be compared) and is not an effect of the exposure.

As outlined in the introduction/background section of this thesis several risk factors may act as confounders. Causal intermediates are not confounders, and as vascular disease may be considered a causal intermediate for AD, such disease is not considered a confounding factor.

Rothman (2002, ch. 4 and 5) writes that a study can be biased because of the way the subjects are selected, the way the study variables are measured, or some confounding factor that is not completely controlled (p. 94). Accordingly I paid special attention to the screening method for cognition (that study subjects are checked for early symptoms of cognitive decline or AD; selection bias), the measurement method for intake of fruit and vegetables, and known risk factors (confounding bias). Confounding factors may be controlled in the study design by stratification or in the data analysis by regression models.

In the domain of attrition I checked for number of drop-outs, reasons for drop-out and analysis of drop-out done by the investigators to check if drops outs were differently distributed between exposure groups. I also paid attention to analysis bias, i.e. grouping of variables and statistical method used to adjust for co-variables.

2.4 Summary measures and meta-analysis

AD is a time-to-event outcome for which hazard ratios is the most common summary measure. The hazard ratios and a 95 % confidence interval were presented in a summary table.

A meta-analysis was done computing a summary hazard ratio in a random-effects analyses. A random-effects analysis should be chosen (instead of a fixed-effects analysis) when the studies are estimating effects of different exposures, in this case different quantities of fruits and vegetables, or different subgroups of fruits and vegetables across studies (Deeks et al., 2011).

The Cochrane group (Reeves et al, 2011) recommends that a meta-analysis of non-randomized studies must consider how potential confounders were addressed, and consider the likelihood of increased heterogeneity resulting from residual confounding and from other biases that vary across studies. Following this, only studies with comparable adjustment models were planned for a meta-analysis. Review manager 5.1 (2011) was used for the computing.

2.5 Risk of bias across studies

Publication bias and selective reporting within studies were qualitatively assessed as there were too few studies to do a statistical analysis.

3 Results and discussion

3.1 Literature search and selection process

3.1.1 Results of literature search

The search in the four Ovid databases AMED, EMBASE, MEDLINE and PsychINFO revealed 1862 records. I total 1813 records were excluded based on titles and abstracts, 36 articles on 19 cohort studies were read in full text and the studies assessed for inclusion or exclusion. In addition I retrieved two more articles from searching reference lists. In the end I included three prospective cohort studies and excluded one because of a retrospective design.

I retrieved 13 review articles for screening and retrieved four review articles for full text reading. Three of the articles were prepared for a state-of-the-art-conference at the National Institutes of Health, USA (Daviglus et al., 2011; Williams et al., 2010; Plassman et al., 2010) and two were published by other investigator groups (Lee et al., 2010; Sofi et al., 2008).

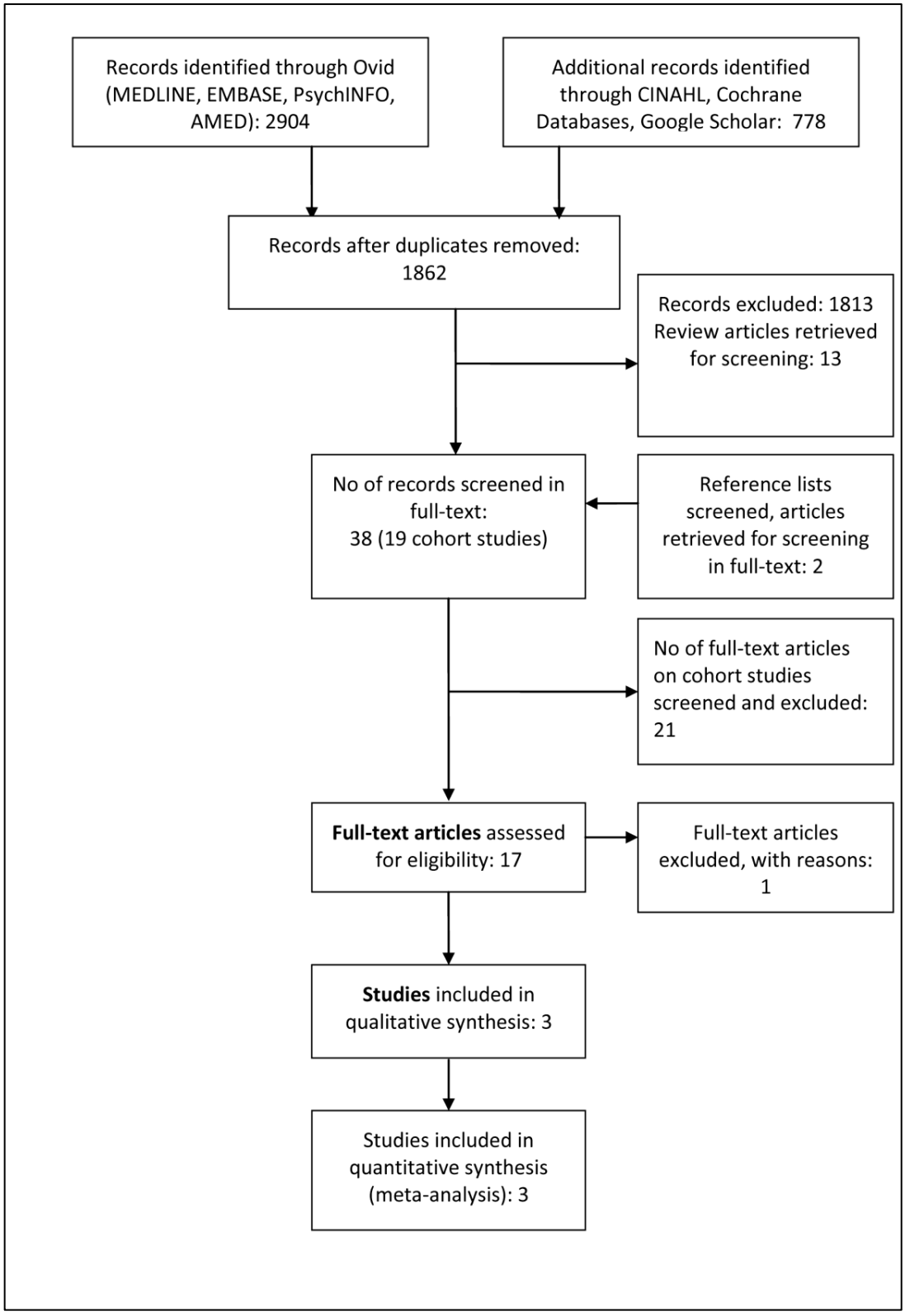
The retrieved systematic review articles did not reveal any additional relevant primary cohort studies except those already included in this systematic review.

A flow chart of the review process is displayed in Figure 3, next page.

3.1.2 Discussion of the literature search and selection of studies

Literature search

According to Cochrane (Cochrane Collaboration, 2010, p. 153) it is desirable for more than one person to repeat parts of the review process. Using at least two authors may reduce the possibility that relevant reports will be discarded. Most important is that the final selection of studies into the review is undertaken buy more than one author. In this case the screening process was done by myself as the only data extractor.



Figur 3. Literature search - flow chart.

The search identified a large amount of records because “fruit”, “vegetables” and similar words were used as text words in the literature search. Most of the records were commentaries and non-systematic reviews, and some were studies not eligible due to study design (cross-sectional or case-control studies), or because they investigated nutrients like anti-oxidants, dietary supplements or other food groups than fruits and vegetables, i.e. fish and dietary fat.

I could have repeated the literature search and selection process a second time, to validate my own work. I decided not to do this. The literature search revealed the same articles eligible for full-text assessment as listed in the preparation process completing the protocol. Further, I contacted investigators of relevant cohort projects, identified through the literature search, to ask if there were any reports from their cohorts which suited my research question, or if there were any upcoming reports in the near future.

Of those contacted, one investigator (M.C. Morris) submitted information on a probable future report from the CHAP cohort (Tangney et al., 2011b), see Appendix III. In addition I searched the reference lists of relevant review articles to check for any studies being missed in the literature search (National Institutes of Health, 2010; Plassman et al., 2010; Pasinetti & Eberstein, 2008; Carter et al., 2010; Feart, Samieri & Barberger-Gateau, 2010; Solfrizzi et al., 2011).

Selection of studies

The three studies included in this systematic literature search were the following: the Three City Study from France (Barberger-Gateau et al, 2007), the Kame project (Dai et al, 2006) from a population of Japanese immigrants in Seattle, USA, and the WHICAP study (Scarmeas et al, 2006b) which consists of two cohorts from a multiethnic society in New York, USA.

One study was excluded due to retrospective design (Hughes et al., 2010), see Appendix II.

In the protocol it was stated that to be included studies had to follow free living and not institutionalized subjects, and follow-up time should be at least two years. This was to lower the risk of selection bias. One of the studies included institutionalized subjects (Dai Q. et al., 2006), of whom 106 were included in the sample at baseline (Graves et al., 1996). As this was only 6.9% of the included participants and analyses were performed to adjust for cognitive score at inclusion, I decided to include this study.

The WHICAP study included some subjects with less follow-up than two years (Scarmeas et al., 2006b). As study level analyses confirmed that when both persons with mild cognitive impairment (CDR=0.5) and those followed for less than two years were excluded, the coefficients for the Mediterranean dietary pattern remained essentially unchanged and results were similar in adjusted models. I therefore included this study as well.

3.2 Results: Included studies

Table 3 next page shows the characteristics of the studies and table 5 a summary of evidence. The three included studies were conducted in different ethnicities and countries and among people of different ethnicity.

The *Three City study in France* (Barberger-Gateau et al., 2007) followed 8085 subjects for about four years in 1999-2004. The aim of this cohort study was to analyze the relationship between components rich in fat or antioxidants, and the risk of dementia or AD. The intake of fruits and vegetables were measured at baseline by a food frequency (FFQ) method as times per day or week of raw fruits, raw vegetables or cooked fruits and vegetables. In total 183 of 281 dementia cases (65%) were diagnosed as AD. The HR for daily consumption vs. no daily consumption of raw fruits, raw vegetables and cooked FV combined is 0.70 (CI95% 0.49 to 0.997) in a model adjusting for age and socio-demographic factors; gender, education, city, income and marital status. With further adjustments for possessing one or two apoE ε4 alleles, BMI and diabetes the adjusted estimate did not change considerably, but crossed the border of 95% significance.

Table 3. Characteristics of included studies.

Name of study, reference	Setting: Country, population, time-period for study	Mean age at inclusion (SD)	Intake of fruits and vegetables in sample	Cognitive screening at inclusion	Outcome: ▪AD assessment ▪Outcome measure
Three City Study (Barberger-Gateau P. et al., 2007)	France Electoral rolls of cities of Bordeaux, Dijon, Montpellier 1999/2000-2003/04	74.0 y (5.42)	About 50 % consumed fresh vegetables daily, 78% fresh fruits and 69% cooked vegetables and fruits.	▪ Examination by neurologist for all participants in cities Bordeaux, Montpellier, and for those screening positive for dementia in Dijon ▪ MMSE and Isaacs Set Test, cut off according to education level	▪ Independent panel ▪ NINDCDS-ADRDA
Kame Project (Dai Q. et al., 2006)	Seattle area, Washington, USA, population of Japanese origin 1992/94-2001	71.8 y Age in tertiles of juice intake: 71.7 (5.4) 71.6 (5.0) 72.1 (5.2)	Not reported by authors.	▪ Clinical evaluation ▪ CASI with cut off 86 pt. on 100 pt. scale (≥87pt.= no clinical evaluation)	▪ Consensus committee ▪ DSM-IV, INDCDS-ADRDA
WHICAP (Scarmeas et al., 2006b)	New York, USA, Multicultural population in Northern Manhattan, Medicare beneficiaries 1992- and 1999-	77.2 y (6.6)	Intake in low/middle/high MeDi tertile: Vegetables: 165/202/243 gm/day. Median intake=197 gm Fruits: 406/471/556 gm/day. Median= 472 gm.	▪ All participants clinically evaluated, neuropsychological battery ▪ CDR, images, neuropsychological tests	▪ Consensus diagnosis ▪ DSM-III-R, NINDCDS-ADRDA

Abbreviations: AD = Alzheimer's disease; CASI=Cognitive Abilities Screening Instrument; CDR=Clinical Dementia Rating; F=female; DSM= Diagnostic and Statistical Manual of mental Disorders, version IV (IV) or revised third edition (III-R); FFQ=food frequency questionnaire; Isaacs' Set test =test for cognition; MeDi=Mediterranean dietary pattern; NINDCDS-ADRDA=National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and related Disorders Association; MMSE=mini-mental state examination; SD=standard deviation; WHICAP=Washington Heights-Inwood Columbia Aging Project; y = years.

The *Kame Project* was studying memory and aging among Japanese immigrants in the Seattle area in USA. 1589 participants completed the dietary interview and were followed from 1992-94 through 2001. Among these 63 developed AD over an average of 6.3 years. HR for AD was 0.49 (95% CI 0.28 to 0.86) for drinking juices 3 or more times a week compared to less than once per week in a model adjusted for age and education. Further adjustments for gender, BMI, mental score at baseline, Apo E genotype, total energy intake, other dietary and lifestyle factors (fat intake, physical activity, smoking, alcohol, vitamin supplements) strengthened the association to 0.28 (95% CI 0.13 to 0.63) and to 0.24 (0.09-0.61) with adjustments for dietary intake of vitamin C, E and beta-carotene.

WHICAP in New York included a 1992- and a 1999 multiethnic cohort. 2258 subjects were followed for 4 years, of whom 262 of 294 demented subjects were diagnosed with AD. The aim of the study was to examine whether possible associations with a Mediterranean dietary pattern were driven by associations of particular food categories. As part of the analyses the association between the risk of AD and the intake of fruits and vegetables was analyzed. HR for a vegetable consumption above versus below median intake was 0.76 (95% CI 0.60 to 0.97). Median intake was 197 grams per day of vegetables and 472 grams of fruits. HR for the intake of fruits was stated as not significant in adjusted Cox models according to the report. Further the report states that adjusted association for the intake of vegetables with AD was not significant. Adjustments were done in one model only; age, energy intake, socio-demographic factors (cohort, gender, ethnicity, education), smoking, BMI and comorbidity. The effect of the intake of vegetables was reported as part of analyses of a dietary pattern and therefore the authors did not report any effect estimate for fruit, nor any adjusted effect estimates for fruit or vegetables (personal communication, N. Scarmeas).

Table 4 next page displays a summary of outcome in included studies.

Table 4. Summary of outcome in included studies. Observation period, age at final screening, exposure measured, adjustments, outcome.

Name of study, literature reference	Observation period (SD) AD/analyzed sample, sex	Exposure	Adjusted for covariates	Effect estimates, HR (95% CI)	Notes
Three City Study (Barberger-Gateau P. et al., 2007)	3.48 years (NA) (mean 3.65) 183/8085 (total dementia 281) F: 61.4%	Daily raw fruits+ raw vegetables + cooked FV vs. not daily (daily: n=2772)	▪ Model 1: age, gender, education, city, income, marital status ▪ Model 2: model 1+ApoE genotype (one or two ε4 allele) ▪ Model 3: model 2+ BMI, diabetes	▪ Model 1: HR 0.70 (0.49-0.997) P =0.048 ▪ Model 2: HR 0.72 (0.50-1.04), P=0.08 ▪ Model 3: HR 0.73 (0.50-1.05), P =0.09	Dietary pattern with lower consumption of fish, omega-3 rich oils and FV: HR 1.63 (95%CI 1.04-2.56) in model 2
Kame project (Dai Q. et al., 2006)	6.3 years (2.6) 63/1589 (total dementia not reported) F: 54.4%	FV juices times per wk: once, 1-2, >3	Age part of analytical model (age at entry, age at onset) ▪Model 1: (Age), education ▪Model 2: Model 1 + gender, phys act, BMI, CASI, olfaction gr, total E-intake, intake of fats, ApoE, smoking, alcohol, vit C, E, multi suppl.,tea, FV in model 2. ▪Model 3: Model 2 + dietary vit C, E, beta-carotene in model 3.	FV juices ≥3 times per wk vs. <once per wk (=ref=1) ▪Model 1: HR 0.49 (0.28-0.86), P for trend 0.01 ▪Model 2: HR 0.28 (0.13-0.63), P for trend <0.01 ▪Model 3: HR 0.24 (0.09-0.61), P for trend <0.01	Total intake of FV not reported

Table 4 Continued					
Name of study, literature reference	**Observation period (SD)** **AD/analyzed sample, sex**	**Exposure**	**Adjusted for covariates**	**Effect estimates, HR (95% CI)**	**Notes**
WHICAP (Scarmeas et al., 2006b)	4.0 years (3.0) (range 0.2-13.9), 262/2258 (total dementia 294) F: 68%	Fruits, vegetables Median daily intake, classed according to cutoffs for MeDi score tertiles Intake in low/middle/high MeDi tertile: Vegetables: 165/202/243 gm/day. Median intake=197 gm Fruits: 406/471/556 gm/day. Median intake = 472 gm.	Unadjusted and adjusted models Adjusted model: Cohort, age at intake, sex, ethnicity, education, ApoE, smoking, comorbidity index (Charlson), BMI, caloric intake	Unadjusted: HR 0.76 (0.60-0.97), P=0.030 for above median vegetable consumption vs. low. Adjusted: NS (effect estimate not reported by authors) HR for fruit NS (not reported)	HR for AD by MeDi score: Unadjusted: high vs. low 0.61 (95% CI 0.44-0.85) middle vs high 0.79 (0.60-1.04) P for trend 0.003 Adjusted: high vs. low 0.85 (0.63-1.16), middle vs. low 0.60 (0.42-0.87), P for trend 0.007 2 of 9 dietary components .of MeDi (V, alcohol) were associated with decreased risk for AD

3.3 Risk of bias in included studies

A summary of the risks of bias is given in Figure 4. In the following the risks of bias are summarized and discussed.

3.3.1 Risk of selection bias

For requirements concerning a low risk of selection bias, see Figure 4 with subtexts.

There were some differences in screening measurement in the three studies ((Mini-Mental State Examination (MMSE), Cognitive Abilities Screening Instrument (CASI)), but in all studies participants with a low cognitive score were examined by a neurologist to exclude subjects having dementia.

In Three City Study there were some differences in screening methods between cities. 12.5% of dementia cases could have been lost in city of Dijon, due to other screening procedures, and this may have led to underestimation of risk. But adjustments were done for city of living by adjustment model 1 (see Table 6). An analysis of participants at the 1st and 2nd follow-up separately, found very similar results in the association between food habits and dementia at 1st and 2nd follow up.

In the Kame project a subsample were living in institutions (109 persons), but supplementary analysis indicated that there were no differences in reporting of dietary intake among participants with high and low cognition score (above CASI cut off value) and short or long time of follow-up. For WHICAP associations between MeDi score (dietary pattern) and incident AD did not change when subjects with mild cognitive deficits at baseline and subjects with less than 2 years of follow-up were excluded from analysis. Supplementary analyses indicate that bias due to preclinical disease did not appear to be a likely explanation of the findings.

I judge the risk of selection bias for all three cohort studies to be low. See Figure 4, column 1.

3.3.2 Risk of bias by genotype

For requirements concerning a low risk of genotype bias, see Figure 4, column 2.
In all three studies there were analyses for apoE genotype, and I therefore consider the risk of ApoE-genotype bias as low in all three studies. See Figure 4, column 2.Because there are other genetic contributors to AD as well, we cannot exclude the possibility of other genotype bias, but these are not considered here.

3.3.3 Risk of bias by confounding

As most health-conscious individuals often have several healthy habits, I judge that there is a low risk of confounding bias if education level, physical activity and smoking are taken into consideration. For other confounding factors, see Figure 4 with subtexts.
Three City did not collect information on physical activity, but collected information on smoking habits. However adjustment for smoking is not discussed in the report. In the Kame project smoking was measured (never, former, present). Physical activity was measured as yes/no for regular physical activity, which is a rather inaccurate measure, and I therefore judge the risk of bias as uncertain.

WHICAP did not report adjustments for physical activity in the report which analyzed the effect of intake of FV, but states in another report that both MeDi dietary pattern and physical activity are independently associated with AD, and that the association weakened when adjusted for diet (Scarmeas et al., 2009b). Since WHICAP do not report adjusted effect estimates for any of these potential confounders, I consider the risk of bias as uncertain.
In addition there will always be a risk of residual confounding in observational studies. For instance these may be related to socio-demographic factors or healthy life-style factors which are not accounted for by analyses. For summary of confounding factors, se column 3, Figure 4.

3.3.4 Risk of exposure bias

Risk of exposure bias was discussed with ALB[ii], and we agreed on the following requirements for a low risk of bias in measuring intake of fruits and vegetables: a) a FFQ covering the past week or month to capture usual intake, and b) use a FFQ which include several food items, and which does not focus on fruits and vegetables in particular. Additional factors to consider according to Willett (2008, p.586-97): Adjusted to c) energy-intake, d) repeated at follow-up and e) validated. See Figure 4, column 4 with subtexts .

Adding a biologic measurement would be a supplement to the validation, but such validation methods are still not extensively used in cohort studies due to expenses and availability.

In the Three City Study a short FFQ covering all food groups in the habitual diet was used. A report from the same sample indicates that the dietary pattern is quite stable in the population (Larrieu et al., 2004). A validation of the FFQ is done in the city of Bordeaux, and this indicates that FV intake data did not change when adjusted for energy intake. The dietary interview is not repeated, but

taken together I consider the risk of exposure bias as low for the Three City Study.

In the Kame project a FFQ covering fruits and vegetables is used, but the full list is not published (Rice et al., 2001). Information on types of juice was not collected. The dietary intake of beta-carotene and vitamin C, however, was reported. The two nutrients are abundant in some fruits and vegetables. The intake of beta-carotene was almost the same in the three tertiles of juice intake (from low to high: 3.6, 3.5, 3.7 grams/day). The dietary intake of vitamin C by tertile was 91, 104 and 161 grams per day. A glass of orange juice may account for the difference, or it may indicate that the group with the highest tertile of juice intake also had a higher intake of whole fruits and vegetables than the two lower tertiles. The FFQ has been validated among five ethnic groups including Japanese, but I found no information on validation of the intake of fruit and vegetables in particular, see Table 5. Taken together I consider the risk of exposure bias for fruit and vegetable juices as low, but it certainly could be discussed if the intake of juices can be used as a surrogate for FV intake. This is discussed in 3.3.6 (analyses/reporting bias).

The WHICAP study has calculated the intake of fruits and vegetables in grams per day. The FFQ method is validated in the population and used at follow-ups as well as at baseline screening. MeDi score was stable over 7 years for subjects with several follow-ups, indicating a stable diet. I judge the risk of exposure bias as low for the WHICAP study.

It could be discussed if all three studies should be rated as “uncertain” in the exposure domain, because there were no biological measurements. The judgment of exposure bias may change in future studies supplementing FFQ with biological measurements.

Table 5. Description of the dietary assessment methods, time period covered and exposure in the included studies.

Name of study, reference	FFQ method, and if assessment repeated	Time period covered	Validation of FFQ	Exposure of fruits and vegetables (FV)	Range of exposure
Three City Study (Barberger-Gateau P. et al., 2007)	▪Face-to-face interview ▪Short FFQ (3 FV items + 9 other items) ▪Not repeated	Habitual intake	Validated in the city of Bordeaux, 24 h recall, 2 years after baseline examination. No association found between energy-intake and dementia 2 years later.	Raw fruits, Raw vegetables, cooked FV, each recorded in 6 categories. Further reduced into frequent consumption = raw fruit+ raw vegetables +cooked FV daily	daily 4-6 per wk 2-3 per wk 1 per wk <1 per wk never
Kame Project (Dai Q. et al., 2006)	▪ Self-administered, English or Japanese ** ▪ 33 item FFQ with images of portion sizes,. Eating preferences (Asian, western). ▪ Not repeated	During past week or another usual, recent week	Validated among 5 ethnic groups by 4-week food records throughout 1 year. Intraclass correlation coefficients for Japanese men was 0.77 for beta-carotene and 0.42 for vitamin C; women 0.42 and 0.42.	Fruit and vegetable juices in 8 frequency categories and 3 portion sizes Further reduced into <once per week 1-2times per week ≥3times per week	8 fequency options 3 portion sizes
WHICAP (Scarmeas et al., 2006b)	▪ administered by trained interviewer by telephone, in English or Spanish ▪ Willett's 61-items semi-quantitative FFQ* ▪ Repeated	Consumption of fruit and vegetables past year	Validated in same sample by two 7-day food records, and reliability by two 3-month frequency assessments of various components of the FFQ. Intraclass correlations: vitamin A 0.32, vitamin C 0.57, vitamin E 0.45. 86% of vitamin A was carotenes [58].	▪ Exposure includes subgroups of fruit, vegetables ▪ Calculated weight of daily intake in tertiles of MeDi dietary pattern, by 25^{th} and 75^{th} percentile, and median intake ▪ FV calorie-adjusted to 2000/2500 kcal/day for women/men	Range of exposure not reported by authors

* (Willett et al, 1985) Copyright, Harvard University, Channing Laboratories **FFQ unpublished, not reported by authors in detail.
Abbreviation: FFQ=food frequency questionnaire; FV=fruit and vegetables; MeDi =Mediterranean Diet (Trichopolou, 2003); wk=week.

3.3.5 Risk of outcome bias

I all studies there were diagnostic committees using standard tools, see Figure 4 and subtexts, column 5. There are always a risk for some variation between baseline interviewers and clinicians, but I judge the risk of outcome bias within the studies as low.

For WHICAP it was stated that the diagnostic committee was blinded to the dietary data, for the other studies this issue is not discussed. I consider the risk of bias as low for all studies, because blinding is not a big issue in cohort projects studying many aspects of aging and cognitive decline.

3.3.6 Risk of attrition bias (drop-outs) and bias due to deaths

If participants with dementia or cognitive impairment were selectively lost to follow-up, the incidence rates calculated in the exposed and non-exposed groups would be difficult to interpret. According to Rothman (2002, p. 71) tracing subjects is a major component of the expense of prospective cohort studies. The validity of the study is threatened if a large proportion of the participants are lost to follow-up. If fewer than 60 % of subjects are traced, the study is generally regarded with skepticism. Even follow-up of 70- 80 % or more can be too low if the subjects lost to follow-up are lost for reasons related to both exposure and the disease. Hence, for a low risk of bias in this domain less than 20 percent lost to follow up is required, see Figure 4 with subtexts, column 6.

In the Three City Study drop outs were 2.1 years older and had lower income and education, a poorer diet and more often diabetes. The reasons for drop-out are associated with dementia, but were of minor magnitude. According to the authors some cases of dementia may have been missed among those not followed-up, and this could have led to an underestimation of the association between food habits and dementia because of competing risk of illness and death. I therefore consider the risk low/uncertain.

In the Kame project loss to death and follow-up is not stated in the report on intake of juices (Dai et al., 2006), and I consider the risk as uncertain.

In WHICAP losses to follow-up were 18% and in addition 3.5% died within 1.5 years from baseline assessment. The MeDi scores (dietary pattern) did not differ between participants and drop-outs, and analysis indicate that it is unlikely that the results could be explained by losses to follow-up. Taken together I consider the risk of attrition bias as low, see Figure 4 column 6.

3.3.7 Risk of bias in statistical analyses and by selective reporting

All included studies are reporting hazard ratios with 95% confidence intervals (CI), which is regarded as a standard measure. But one study do not report age-adjusted effect-estimates and there are some uncertainty concerning choice of exposure groups and adjustment models. For requirements for a low risk of bias, see Figure 4 with subtexts, column 7.

The Three City study (yes/no for daily intake of raw F,V, and cooked FV) and WHICAP (below/above median intake) are both using binary exposure variables for the analyses. Binary categories at these levels of intake may not be sufficient to demonstrate an effect

In the Kame project only effect estimate for juices are presented, and there is no discussion concerning total intake of fruit and vegetables. All taken together I judge the risk of bias in analyses as uncertain. See also chapter 3.4 about dose-response effect.

WHICAP did not report effect estimates adjusted for age separately; instead this was included in a combined adjustment model including age, gender and marital status (see Table 6). This may have led to risk of bias in effect estimates, since age is the main known risk factor for AD.

There may also be a risk of selective reporting within studies. Due to this risk, Peters and Mengersen recommend that for a meta-analysis both unadjusted and adjusted study estimates should be extracted (2008). According to Tuma investigators analyze the data and then decide what to publish, focusing on the statistically significant results – without mentioning other factors that were tested but that failed to show significant association (Tuma, 2007). The studies included in this review are focusing mostly on a Mediterranean diet, anti-oxidants, and fruits, vegetables and juices as sources of anti-oxidants. Other potential beneficial nutrients or the overall benefit of fruits and/or vegetables are not a main issue. This may have affected the analyses and reporting from included studies.

3.3.8 Summary of risk of bias in included studies

Assessment of quality revealed some heterogeneity between the included studies. Figure 4 displays a summary of the risk of bias.

Figure 4. Summary of risk of bias.

Study	1-Selection	2-ApoE genotype	3-Confounding factors	4-Food exposure assessment	5-Outcome assessment	6-Loss to follow-up	7-Analytical
Three City Study	Low	Low	Uncertain *	Low *	Low	Low /Uncertain*	Low*
Kame cohort	Low	Low	Uncertain **	Low *,**	Low	Uncertain**	Uncertain **
WHICAP	Low	Low	Uncertain*	Low *	Low	Low	High risk*,***

1. Low risk=a) objective measurement used for cognitive screening, b) validated method, c) same procedure for all participants or adjustments if different procedurea. Other factors to be considered: d) supplementary analyses for cognitive status at baseline, e) adjustments for cases detected early during follow-up period.

2. Low risk = analyses for genotype ApoE performed.

3. Low risk= relevant confounding factors measured and included in analyses. Objective methods for measuring. Risk factors to be adjusted for rating a low risk of bias: education, physical activity, smoking. Other: gender, BMI, alcohol intake. *Data on exercise not available or not included in analysis. ** Dichotomous measure for exercise.

4. Low risk= a) a FFQ covering the past week or month to capture usual intake, and b) use a FFQ which include several food items, and which does not focus on fruits and vegetables in particular. Other factors to be considered: c) adjusted for total energy intake, d) repeated measurements at follow-up, e) validated. *No biologic measurement, **Intake of juices.

5. Low risk=a) DSM, NINCDS-ADRDA measurement used, b) committee with several persons including neurologist assessing diagnosis, c) no differences in screening method within sample which are not discussed or adjusted for, d)committee blinded to diet or blinding is no issue..

6. Low risk= a) <20 percent or b) analyses showing results unlikely to be affected by losses to f-up. * Some risk of underestimation due to deaths and losses to follow-up, rated as low risk. **Loss to follow-up not reported by authors.

7. Low risk=a) appropriate methods for analysis used, b) age-adjustment, c) it is clear which type of dementia is included in risk analysis, d) appropriate grouping of FV intake. * d) binary exposure levels used for effect estimate, ** d) not adjusted for total intake of FV, *** age-adjusted effect estimate not reported.

3.3.9 Risk of publication bias

Since the number of studied is limited in this review, a funnel plot or statistical analyses of reporting bias is not appropriate. There may be publication bias if investigators in other cohorts than those included in this review did not report their results. Because I contacted relevant groups of investigators, the risk of reporting bias was reduced. Not all answered my request and there may still be a residual risk.

3.4 Dose-response effects

A dose-response effect adds weight to the assumption that there is a true association between the intake of food and a disease (WCRF/AICR, 2007, p. 52). Some indications of a dose-response effect were found in all three included studies, but only one study reported dose-response analyses.

A certain dose-response-effect is found in analyses of dietary patterns in the Three City Study, but there were no dose-response analyses for vegetables alone or fruit and vegetables combined (Barberger-Gateau, 2007). A follow-up of subjects in the city of Montpellier (one of the cities in the Three City Study) for a median of 7.3 years shows that fewer than two FV servings a day was associated with a 26 % higher risk of mild cognitive impairment or dementia. Mild cognitive impairment was treated as a prodrome of dementia in the analyses, as only 7.6% of those who developed full dementia did not pass through a phase of mild cognitive impairment. In this study, AD was not specified as an outcome (Ritchie et al., 2010).

The Kame Project presents trend analyses and fully adjusted hazard ratios for several levels of intake of juices, and these adjusted models supports a dose-response relationship for juices (p for trend 0.01, <0.01 and <0.01 in adjustment models 1-3).

As for the WHICAP study a dose-response effect was seen for a dietary pattern including vegetables and subgroups of vegetables, but there were no analyses for vegetables of fruit alone (Scarmeas et al, 2006b; Gu et al, 2010).

3.5 Quantitative analyses

A meta-analysis was performed combining effect estimates from all three studies. Table 6 (next page) presents an overview of the adjustment models used in the three studies. The least adjusted models were chosen for a meta-analysis. Green colour indicates the models used for the meta-analysis.

The meta-analysis indicated that a higher intake of fruit and vegetables was associated with a reduced risk of AD compared with a lower intake (HR, 0.71 (CI, 0.59 to 0.86)). The heterogeneity between studies was low (Figure 5).

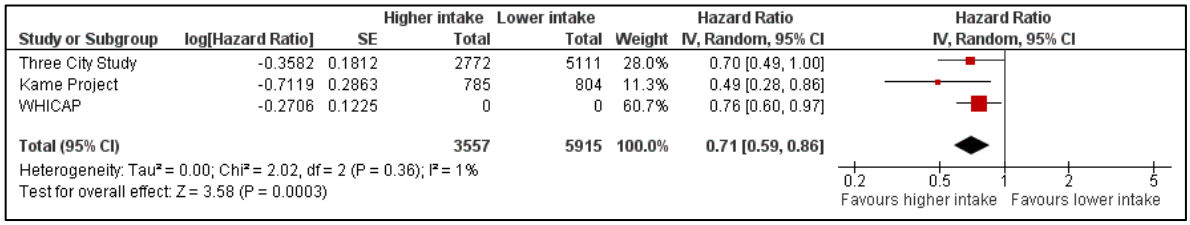


Figure 5. Forest plot of a meta-analysis of the included studies.
Exposures measured: Three city study (Barberger-Gateau et al, 2007): fruit and vegetables combined. Kame cohort (Dai et al, 2007): juices. WHICAP study (Scarmeas et al, 2006b): vegetables. Three City study and Kame cohort adjusted for socio-demographic variables, WHICAP no adjustments. Total number of participants in the graph does not include the WHICAP study (2258 subjects). Random-effects analyses by Review Manager 5.1.

As seen in the forest plot, the estimate from Kame project had a greater risk for random error, demonstrated by the broadest confidence interval. This is to be expected as this was the smallest study. The WHICAP study and the Three City study showed more narrow confidence intervals. The effect estimate from the Three City Study was not significant in further adjusted models. WHICAP reported that the confidence interval crossed 1.0 if adjusted by all relevant covariates, including age.

Table 6. Adjustment models in included studies, with green color indicating adjustment models included in the meta-analysis.

Cohort (exposure)	**Crude model**	**Age, income / education**	**Gender**	**Marital status**	**ApoE genotype**	**Life style factors and cognitive screening**	**Diabetes, hypertension, vascular disease**
Three city Study (FV)		Yes (part of model 1)	Yes (part of model 1)	Yes (part of model 1)	Adjusted in model 2 (= model 1 + ApoE genotype)	Adjusted in model 3 (= model 2 + BMI+diabetes)	Adjusted in model 3 (= model 2 +BMI+diabetes)
Kame project (Juices)		Yes (model 1)	Included in model 2		Included in model 2	Model 2: +Physical activity, BMI, E-intake, fat intake, smoking, alcohol, vitamin supplements, tea, CASI, olfactory group Model 3: +dietary vitamins C, E, beta carotene	No adjustments
WHICAP, New York (V)		included in adjusted model	included in adjusted models		included in adjusted model	Smoking, BMI, energy intake in adjusted model	Comorbidity index in adjusted model, no adjustments for vascular variables (considered mediators)

Abbreviations: ApoE=genotype ApoE4, BMI = body mass index, FV = fruit and vegetables, olfactory group = test for cognition, V= vegetables

3.6 Generalizability of the studies

A summary of the relevant factors concerning generalizability are shown in table 7.

The three studies are including participants from different ethnicities and countries and at relevant age ranges, this strengthens the generalizability. On the other hand the relation between AD and total dementia differs between the studies. In WHICAP 262 of 294 subjects diagnosed with dementia had AD (89%), in Three City the percentage was 65% and in the Kame project 55%. Incidence of AD varies considerably between countries, and diagnostic assessment may be one reason for this. This may in part be due to different risk of AD in the cohort due to genetic or congenital factors, or the difference may be caused by other risk factors, or differences in diagnostic procedures. The difference in AD/total dementia is difficult to interpret on behalf of these three studies. For generalizability one has to take into account the dietary patterns of the cohorts and ranges of intake as well.

Tabell 7. Characteristics of the sample in included studies

External bias	**Three City Study, France**	**Kame cohort, Washington, USA**	**WHICAP, New York, USA**
Population	The participants belong to a higher socio-economic level	Japanese immigrants, mostly well educated, higher socio-economic level	Medicare population, multiethnic, lower educated population
Age of participants at baseline, range (mean)	69-80 years (74.0)	66-84 years (71.8)	(77.2 years)
AD/total dementia	Lower frequency of cognitive disorders than in general population in France 65% AD of total dementia in sample	55% AD of total dementia in sample*	89% AD of total dementia in sample
Baseline diet: intake of vegetables/fruit	High intake of FV: 50% eat raw vegetables daily, 70% cooked	Not reported by authors	High intake of fruit, probably medium intake of vegetables

Abbreviations: AD=Alzheimers Disease, FV= fruits and vegetables, WHICAP=Washington Heights and - Inwood Columbia Aging Project. * Calculated from Hughes et al, 2009.

4. Summarizing discussion

In the present systematic review, I included prospective cohort studies investigating the association between the intake of fruits and vegetables and Alzheimer's disease from 65 years of age. This review includes three studies which show a negative association between the intake of fruits and vegetables, vegetables alone, or juices and the risk of Alzheimer's disease after 65 years of age. In fully adjusted models, however, two of the studies were of borderline significance. The included studies all have high or uncertain risks of bias.

Three City Study (Barberger-Gareau et al, 2007) did not report level of physical activity and the Kame project was measuring physical activity with a yes/no for regular activity, which probably is a measure subject to great random error (Dai et al., 2006). If a high intake of fruits and vegetables correlates with a high level of physical activity, this may have attenuated the reported associations between a high FV intake and risk of AD. The WHICAP study (Scarmeas et al, 2006b) did not present adjustment for age, which is the main risk factor for AD.

Physical activity, particularly activity on a higher level, is associated with a lower risk of cognitive decline and AD (Hamer & Chida, 2009 ; Middleton et al., 2007; Sofi et al., 2011; Scarmeas et al., 2009b). In addition there is always a risk of residual confounding in observational studies even if minimized at the stage of analyses (Reeves, van Binsbergen & van Weel, 2005). For example such residual confounding may be caused by health behaviors in the high income and education groups in the Three City Study or by factors connected to Japanese culture or diet in the Kame cohort which are not being completely adjusted for. Socioeconomic factors are strongly associated with diet as well as with dementia and AD (Plassman et al., 2010; Larrieu et al., 2004).

Moreover, measurement and reporting of intake of fruits and vegetables differ between the studies. There will be random errors if intakes are self-reported and not validated by validation studies, repeated measurements or biological analyses (Michels et al., 2005). The WHICAP study used an extensive semi-quantitative food frequency questionnaire which was repeated at follow-up, while the Three City Study and Kame project used shorter lists, thereby giving less extensive information on the fruit and vegetable intake group, the quantity of intake as well as a greater risk for measurement errors. This may be reflected by the more narrow confidence intervals in the WHICAP study. The Kame project did not report the complete intake of different food items in the juice intake categories, and the intake of foods associated with juices may be overlooked.

The variety of measurement methods and reporting may partially reflect that the studies were

planned and reported at a time when the theory of antioxidants as a causal factor for chronic diseases was extensively discussed and before a more standardized reporting of observational studies took place (Vandenbroucke et al., 2007).

According to Willett (2008, p. 582) realistic relative risks in most studies of diet and disease are likely to be modest, within the range of 0.5 to 2.0. This is because of the limited range of variation in diet within most populations and error in measuring intake. The included studies show a reduced risk of 30-51% and the meta-analysis a reduced risk of 29 percent, i.e. a relative risk of 1.4 for a lower intake of fruits and vegetables compared to a higher intake. If over-reporting of fruits and vegetables was more common among "less healthy" than healthy participants, there was a risk of systematic error in all three studies, attenuating the estimates.

An effect of a food item or a nutritional factor can only be observed if groups with sufficiently high and low intakes to estimate a protective effect are included in the sample and if the intakes are sufficiently grouped (Morris & Tangney, 2011). The WHICAP study in New York found an effect of vegetables. That the association is no longer significant when adjusting for covariates may be explained by too small differences in intake between those above and below median intake. The Kame project found the strongest effect estimate. This may partly be explained by this study comparing high and low intakes (at least 3 times per week versus less often than once per week) and not binary variables.

The WHICAP study indicated that there is an association for vegetables only, even if this association is weak. An association for vegetables, but not fruits, is supported by another study from the WHICAP cohorts, which found a dietary pattern associated with reduced risk of AD (Gu et al., 2010). This dietary pattern was characterized by higher intakes of several foods, among these are higher intakes of cruciferous vegetables and dark and green leafy vegetables. The Three city study reported age-adjusted analyses for fruits and vegetables separately in addition to their main findings. These indicates a stronger dose-response relationship for raw vegetables than for raw fruits, but these figures are not discussed by authors and no p-value for trend is reported (Barberger-Gateau et al., 2007, Table 1).

4.1 Comparison with other studies

Primary follow-up studies of other observational designs and outcomes in the field of cognition and dementia among the elderly support an association between fruits and vegetables and AD or preclinical AD/cognitive decline. A nested retrospective case-control follow up study from Sweden

found an association between a higher intake of fruit and vegetables and odds ratio for AD (Hughes et al., 2010). For this study the intake of fruit and vegetables, assessed by a single question in a FFQ, 30 years earlier was associated with a lower risk of AD after the age of 65. There were no data on the intake on fruits and vegetables in later life and hence a great risk of random error and risk of bias concerning exposition to intake of FV in older age groups.

Also in the Whitehall II study which was conducted among office workers in England, the consumption of fresh FV was assessed by a single question. Subjects who reported consuming less than 2 servings FV a day had nearly 2.5 times the risk of poor cognition over 17 years of follow-up, at a mean age of 61 years. FV intake was assessed every two years and there was a cumulative risk associated with low FV intake over time (Sabia et al., 2009).

In 2012 results from the Chinese Longitudinal Health Longevity Study were published showing that Chinese illiterate elderly who always included vegetables and legumes in their diet had a reduced odds ratio for cognitive decline over 3 years of follow-up (Chen, Huang & Cheng, 2012). The adjusted odds ratios in multivariate logistic regression analysis were 0.66 (95% CI 0.58, 0.75) for always eating vegetables and 0.78 (95% CI 0.64, 0.96) for legumes. The mean age of the participants was 83 years. As this was a study among illiterate subjects, the study may be less subject to residual confounding than studies recruiting mostly well-educated subjects.

The Doetinchem Cohort Study from the Netherlands showed an association between some subgroups of vegetables and some, but not all, measurements for cognitive decline after 5 years of follow up of 43-70 years olds (Nooyens et al., 2011). The associations did not apply for fruits and for total intake of fruits and vegetables. Further the SU.VI.MAX 2 study from France found a differential effect of vegetables, fruits and the fruits and vegetables combined on memory and executive function (Péneau et al., 2011). A cross- sectional study of 70 year olds in UK does not support a causal role for fruits and flavonoids in the prevention of cognitive decline among 70 year olds (Butchart et al., 2011).

4.2 Biological mechanisms

So far, most human studies on diet and AD have focused on antioxidants and anti-inflammatory substances as possible protective factors. However, clinical trials with anti-oxidant vitamins C and E have found no protective effect on AD (Isaac et al, 2008; Bootby et al, 2005). Recently a clinical trial raised caution about faster cognitive decline after a 16 week trial of combined antioxidant intake (Galasko et al., 2012). If fruits and vegetables are truly protective, these studies indicate that

protection might not be caused by antioxidants.

Epidemiologic studies have shown that a high intake of fruits and vegetables is negatively associated with vascular disease. (He et al., 2007; He, Nowson & MacGregor, 2006). Vascular disease may be part of the causal pathway of AD, and it may be difficult to tease apart general vascular dietary risk factors, vascular dietary risk factors of particular importance for brain vasculature, and potential non-vascular dietary risk factors for AD.

Several dietary risk factors may act as both vascular risk factors and as risk factors for AD. In the brain fruits and vegetables may prevent occlusion of brain arterioles and brain vessel dysfunction through mechanisms other than anti-oxidative and anti-inflammatory. Nitrates from green leaves, red beet and other vegetables may increase NO production in the vessel walls and thus counteract vasodilatary dysfunction and occlusion of arterioles (Tang, Jiang & Bryan, 2011). An increased level of homocysteine is found to be a predictor of cognitive decline in AD (Oulhaj et al., 2010; Smith et al, 2010). Folate, which is especially abundant in spinach, but also found in fruits and other vegetables, may contribute to a reduced homocysteine level. Further potassium, vitamin K and other nutrients and bioactive factors abundant in fruits and vegetables may play a role in counteracting neuro-degeneration (Presse et al, 2008).

There is also the possibility that two or more dietary factors act in concert. Nutrients associated with the development of disease may be distributed in several foods included in the diet, for example in vegetables as well as in other vegetarian foods and seafood. Prospective cohort studies have found an association with dietary patterns of Mediterranean type, which are rich in several vegetarian foods, as well as low in saturated and trans fatty acids. These studies include WHICAP, Three City Study, a prospective cohort study on cognitive decline (Tangney et al., 2011b) and a cross-sectional study in Hordaland, Norway (Nurk et al., 2010).

A cross-sectional study on biological nutrient markers found associations between three patterns of biological markers and cognitive function. A favorable pattern was characterized by vitamins B, C, D and E, another by level of marine omega-3 fatty acids in plasma, and a third unfavorable pattern was characterized by higher levels of trans fatty acids (Bowman et al., 2012). These nutrients may be markers of a dietary pattern or of the intake of unknown causative nutrition factors, rather than being protective factors by themselves.

If there is a true causal relationship between intake of fruits and vegetables and AD, these foods may exert an effect early or late in life. For some diseases diet may be important during childhood,

as observed in a study from England and later reproduced in other populations (Barker et al., 1989). For other diseases intake near the time before diagnosis may be important to inhibit the development of the disease. Diets of individuals are rather consistent over years (Willett, 2008, p. 588), and hence prospective cohort studies may provide data to resolve the issue on diet and period of time for which diet may play a role in the development of AD. Even if a low intake of fruit and vegetables, and of vegetables in particular as indicated by one of the studies in this review, is exerting a modest relative risk of developing cognitive impairment and AD, this could be important from a perspective of public health (Ritchie et al., 2010).

On the other hand, there is a potential for reverse causation to explain the findings, meaning that those who have a low intake of FV have decreased their intake as a cause of early cognitive changes. If this is the case, measurement of dietary intake should be done before 65 years of age.

4.3 Grading of evidence

The evidence of low intake of fruits and vegetables being a cause of AD can be graded as "limited-suggestive", according to WCRF/AACR criteria (2007). This grade of evidence requires evidence from at least two cohort studies, generally consistent direction of effect though some unexplained heterogeneity may be present and lastly, evidence for biological plausibility.

To upgrade this evidence to "plausible evidence", evidence from two good cohort studies are required, meaning that one with confidence can exclude the possibility that observed associations result from random or systematic error. Alternatively an upgrading can be done on the basis of a dose-response effect in the association, or a particularly large summary effect size after appropriate control for confounders. As this systematic review shows, there is a lack of dose-response analyses in two of the included studies, as well as uncertain or high risk of bias by confounding. Therefore an upgrading of evidence is not appropriate at present.

4.4 Limitations of this review

The main limitations of this study are the quantity and the quality of the primary evidence. I found only three prospective cohort studies. The studies reported different categories for exposures, and they also varied in the degree of adjustment for confounders in their risk estimates. These variations could lead to potential bias in the combined estimate.

Publication bias and selective reporting are also potential limitations but are difficult to assess. All included studies had a main focus on antioxidants or dietary pattern, and this may have affected the reported analyses as well as the reporting.

In this review there may also be a possibility of incomplete retrieval of studies as only studies in English language are included. Further the literature search, extraction of data and analyses were done by one person only.

The strength of the review is an extensive literature search and inclusion of prospective cohort studies with low risk of recall and selection bias, covering populations in different countries, and of different ethnicity, socio-economic level and with different dietary patterns.

5.0 Conclusions

The number of studies in this systematic review is limited, and exposure measurement and adjustments models are inconsistent. However, there is a consistent direction of effect of a high intake of fruits and vegetables being a protective factor against AD from 65 years of age. According to WCRF/AICR criteria there are limited-suggestive evidence of fruits and vegetables being a protective factor. This is, however, insufficient to justify recommendations designed to reduce the incidence of AD.

More consistency in exposure assessment and adjustment models in further prospective cohort studies could facilitate dose-response analyses, reduce residual confounding and increase quality of evidence. To further explore the relationship between fruit and vegetables and AD, this review could be supplemented by a review on cognitive decline as an outcome. A broader systematic review may give possibilities for sub-analyses of different exposures and outcomes; fruits, vegetables, juices, and fruits and vegetables combined for the risk of cognitive decline/preclinical AD and AD.

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APPENDIX

Article draft for BMC Public Health:

Intake of fruit and vegetables and risk of Alzheimer's disease: a systematic review and meta-analysis of prospective cohort studies

Intake of fruit and vegetables and risk of Alzheimer's disease: a systematic review and meta-analysis of prospective cohort studies

Article draft for BMC Public Health

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Abstract

Background

The intake of fruit and vegetables has been reported to be protective against the development of cognitive decline and dementia. The objective of this systematic review was to assess is there is a relationship between intake of fruit and vegetables and the risk of developing Alzheimer's disease (AD) in prospective cohort studies following participants for at least two years from 65 years of age.

Methods

A systematic review of English-language studies published from 1980 through June 2011 was performed. Studies were identified from searches of MEDLINE, EMBASE, PsycINFO, AMED and CINAHL databases, all of the Cochrane databases, and Google Scholar using search terms for Alzheimer Disease, prospective cohort studies and fruit and vegetables. We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (PRISMA) to guide this review. The methodological quality of included studies was assessed according to World Cancer Research Fund criteria, and a meta-analysis using Review Manager 5 was performed.

Results

Three studies met the inclusion criteria. A meta-analysis indicated that a higher intake of fruit and vegetables was associated with a 29 % lower risk of AD (95% confidence intervals (CI) 0.59 to 0.86) compared with a lower intake. The analysis combined studies with some differences in exposure; vegetables, fruit and vegetables combined, and juices in

unadjusted and minimally adjusted models. The studies included from 1589 to 8085 individuals (508 AD events in total) with an average follow-up from 3.48 to 6.3 years.

Conclusions

There is limited-suggestive evidence to say that a lower intake of fruit and vegetables is a cause of Alzheimer's disease from 65 years of age. There is a need for more prospective cohort studies which aim at more consistency in exposure assessment and adjustment models to decrease risk of confounding and facilitate dose-response analyses.
Keywords: Alzheimer disease, fruit, juice, meta-analysis, prospective cohort studies, systematic review, vegetables

Background

Treatment of dementia imposes a significant burden on patients, caregivers, and healthcare systems worldwide. The early symptoms of Alzheimer's disease (AD) are typically memory loss, deterioration of language, and in later stages of the illness the patients experience increasing cognitive and functional decline [1]. AD is commonly diagnosed in adults age 65 and older.

The prevalence of severe cognitive impairment is projected to quadruple from current levels to 81 million worldwide by 2040. It is estimated that there were about 10.1 million people with dementia in the whole of Europe in 2008.The total cost of dementia illnesses is estimated to 53.0 billion Euro in northern Europe and 177 billion Euro in the whole of Europe, 22 000 Euro per demented per year [2].

Dementia disorders are progressive and so far, incurable. Of the four most prevalent dementia disorders, AD is the main type and accounts for 60 % to 70 % of cases of dementia in elderly patients [3].

Age is the main risk factor for AD [4]. The second greatest known risk factor is a positive family history. The apo-lipoprotein ε4 polymorphism (ApoE ε4) is the most well established genetic risk factor, but 50 percent of the cases do not carry this genetic factor [5].

The time of onset of symptoms varies by up to 18 years between monozygotic twins who eventually become concordant for the disease [6-8]. This strengthens the assumption that modifiable life style factors might trigger the onset of the disease or influence the progression of the disease from cognitive decline to AD.

Socio-economic risk factors are related to the risk of dementia and AD [9, 10]. This may be due to increased brain reserve, such as neuronal count, a greater capacity in compensatory mechanisms or a better lifestyle throughout life which help provide reserve against cognitive decline. Low physical activity and smoking seem to increase the risk. Dietary patterns rich in fruit and vegetables and other vegetarian foods are shown to be associated with reduced risk for cognitive decline and reduced risk of AD in older age [11]. A preventive effect may be caused by substances in fruit and vegetables which have an anti-oxidative, immunological, vascular, neuroprotective, or combined effect. The hypothesis for this study was that fruit and vegetables may prevent or postpone the onset of AD after 65 years of age. Prospective cohort studies may provide evidence of dietary risk factors for diseases with a long pre-clinical phase. Hence, the purpose of this study was to conduct a systematic review and meta-analysis of prospective cohort studies to investigate if the intake of fruit and vegetables was associated with AD after 65 years of age.

Methods

Data sources

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (PRISMA) to guide this review [12-13]. The following databases were searched through the Ovid interface: MEDLINE ® in-process & other non-indexed citations and MEDLINE ® daily and MEDLINE (1980 to 2011 Week 25), EMBASE (1980 to 2011 Week 25), PsycINFO (1980 to June Week 4 2011) and Allied and Complementary Medicine (AMED, from inception to June 2011).

All searches were limited to English language and humans. The search was restricted to articles published from 1980 onwards, because the diagnostic criteria for AD were first published about 1980. In addition the following databases were searched: Cumulative Index to Nursing and Allied Health Literature (CINAHL) (1982—June 2011), the Cochrane Library databases (from inception to issue 1, 2011), and Google Scholar (June 2011, 100 first references screened for eligibility).

As some cohort projects are running for years, publishing many reports, investigators of relevant cohort projects were contacted to check for additional and unpublished studies which might fulfill the inclusion criteria. These investigators and cohorts were identified by reading eligible articles on cohort studies and reviews in full text.

All articles meeting inclusion criteria were checked for additional studies.

Inclusion and exclusion criteria

To be included, studies had to have a prospective cohort design and investigate the association between the intake of fruit and vegetables and the incidence of AD. Retrospective studies, case-control and cross sectional studies were not to be included. The

study population had to be aged 65 years or older, with normal cognition at inclusion and to be followed for at least two years. Exposure had to be individually assessed and report intake of fruit and vegetables combined or separately, or as subgroups. Outcome had to be reported as a standard assessment of AD, and eligible studies had to report estimates of relative risk in sufficient detail (hazard ratio or risk ratio with 95 percent confidence intervals).

Search strategy and study selection

Search terms for Medline are listed in Table 1. Similar search terms were used for the other databases. The search terms were developed by reviewer HH in cooperation with a trained librarian and overlooked another trained librarian (EVN)[1].

Table 1. Search strategy

One reviewer (HH) performed the searches. After removing of duplicates, titles and abstracts all records were screened for eligibility criteria. All studies potentially relevant for inclusion were retrieved in full text and assessed according to the inclusion and exclusion criteria.

Data collection process

One investigator (HH) extracted the data. All studies meeting inclusion criteria were assessed and tabulated. Three investigator groups were contacted to ask for additional information. For all studies several reports had to be read to find information on study methodology.

[1] Lena Victoria Nordheim, Bergen University College

Data items

Data were extracted using a piloted form from the Academy of Nutrition and Dietetics [14-15]. This included (1) first author, year of publication, (2) methodological issues (year of data collection, recruitment method, sample size, follow-up and methods for statistical analyses, assessment methods for dietary intake, methods for cognitive screening and dementia diagnostics), (3) setting and characteristics of participants (age, gender, ethnicity, education and income/socio-demographic characteristics, genotype, cognitive status, somatic health, lifestyle variables), (4) exposure (dietary variables), (5) outcome (AD, AD as percentage of total dementia, length of follow-up, drop-out, authors conclusion).

Risk of bias

Risk of bias was assessed by using a check list from the Critical Appraisal Skills Programme [16]. The domain of exposure in the check list was customized to appraise a prospective study in the field of nutrition. On the basis of the bias tool of the Cochrane Collaboration [17], a checklist for sources of internal bias in longitudinal observation studies from Thompson et al [18], and a checklist draft from the Nordic Council of Ministers [19], these design aspects were considered likely to introduce bias: (1) selecting and screening participants, (2) genotype, (3) confounding factors (education or socio-economic level, lifestyle), (4) exposure - assessment of food intake, (5) assessment of outcome, (6) loss to follow-up, and (7) analyses (grouping of variables and statistical method used to adjust for co-variables). A risk of bias figure with these domains, resembling the structure of the risk of bias tool developed by the Cochrane Collaboration [17] was generated, and each study was assessed according to this. One reviewer (HH) developed the figure (Figure 2) and assessed the risks. The risk of exposition bias was then

discussed with an experienced nutritionist in the field of epidemiology (ALB)[ii] and adjusted. Assessment of publication bias and generalizability of the review were done qualitatively.

Synthesis of results

Hazard ratio for AD was the only measure of outcome. Outcome data were summarized in a table for qualitative analysis and an overall assessment discussed according to the WCRF/AICR criteria [20]. A meta-analysis was performed using Review Manager 5 from the Cochrane Collaboration [21], using a random effects model to calculate summary hazard ratio (HR) and 95% confidence interval (CI) for higher versus lower intake of FV. Studies with comparable adjustment models were included in the meta-analysis.

Results

Study selection

The search identified 1862 articles. Because “fruit”, “vegetables” and similar words were used as text words in the literature search, the search identified a large amount of commentaries and non-systematic reviews, as well as studies which were not eligible due to study design (cross-sectional or case-control studies), or because they investigated nutrients like anti-oxidants, dietary supplements or other food groups than fruit and vegetables, i.e. fish and dietary fat.

Three studies were identified which fitted the inclusion criteria [22-24]. One study was excluded due to a retrospective cohort design [25]. Figure 1 shows the selection process.

Figure 1 Flowchart of search results

Characteristics of included studies

The Three City study in France [22] was following subjects recruited from electoral rolls in three cities. The Kame Project [23] was a study among Japanese immigrants in the Seattle area in USA. WHICAP [24] was a study of two multiethnic cohorts in New York, USA; the 1992 and 1999 cohorts. The three studies included from 1589 to 8085 participants and mean study length ranged from 3.5 to 6.3 years.

Characteristics of included studies are shown in Table 2.

Table 2 Characteristics of included studies

Exposure

Diet was assessed at baseline, measurement methods and analyses for the intake of fruit and vegetables differed between the studies. An overview of dietary assessment methods and validation methods is given in Table 3. None of the validations referred to by the authors is focusing on validation of the intake of fruit and vegetables. On the other hand, studies indicate that a FFQ identifies high and low intake of fruit, vegetables and juices more precisely than energy-yielding foods, probably because food habits concerning fruit and vegetables are easy to remember [26].

Table 3. Description of the dietary assessment methods, time period covered, exposure of fruit and vegetables and range in the included studies

The Three City Study [22] in France used a short food frequency questionnaire (FFQ) to assess the dietary intake. The FFQ included three items of fruit and vegetables; frequency of intake of raw vegetables, raw fruit and cooked vegetables and fruit (i.e. soups etc). The exposure was reported as daily intake of these three items combined.

The Kame project [23] assessed dietary intake by a semi-quantitative FFQ including images for portion estimation. Intake of fruit and vegetable juices (FVjuices) were reported in tertiles (<1 time per week, 1-2 times per week, ≥3 times per week).

The WHICAP study [24] assessed habitual average diet using Willetts 61-item semi-quantitative FFQ, which was used to calculate the median intake of fruit and vegetables in grams per day. As the only of the three included studies WHICAP repeated the dietary assessment every 1,5 year at follow-ups. WHICAP also reported adjustments of intake to total energy intake.

None of the studies controlled the intake with nutritional biomarkers such as serum measurement of vitamin C.

Assessment of the AD outcome

All studies screened participants for cognition at inclusion. Diagnosis of dementia or AD was measured by standard methods in all included studies.

Assessment of association between fruit and vegetable intake and AD

Effect measures in all studies were reported as hazard ratio (HR). Adjustments of effects were done according to different adjustment models in the Three City Study and in the Kame Project, see Table 4. The WHICAP, on the other hand, reported the effect of the intake of vegetables as part of the analyses of a dietary pattern (personal communication, N.

Scarmeas) and therefore the authors did not report any effect estimate for fruit (reported as not significant), nor any adjusted effect estimates for fruit or vegetables.

Table 4. Summary of results in included studies

Risk of bias

Risk of bias within each study was rated according to a risk of bias tool. The overall result is displayed in figure 2.

Figure 2. Risk of bias in included studies

None of the studies was rated as having a low risk of bias in all domains. The Three City Study and the Kame Project had uncertain risk of bias in the domain of confounding factors, due to physical activity not being reported, or reported by a single question used as a binary variable. The Kame project reported on FVjuices, but did not report effect estimates adjusted for the intake of fruit, vegetables or total intake of fruit and vegetables. As WHICAP did not report adjustment for age, there is a risk of bias, see column 7 in the risk of bias figure. Results of individual studies

Table 4 shows the main effect estimates from each study. In the French Three City Study [22] the hazard ratio for daily consumption vs. no daily consumption of raw fruit, raw vegetables and cooked FV combined is 0.70 (CI 95% 0.49 to 0.997) when adjusted for age and socio-demographic factors; gender, education, city, income and marital status. Further

adjustments for genotype (ApoE-alleles), BMI and diabetes did not change effect estimates substantially.

In the Kame Project [23] it was reported a hazard ratio of 0.49 (95% CI 0.28 to 0.86) for drinking juices 3 or more times a week compared to less than once per week when adjusted for age and education. Further adjustments for gender, BMI, mental score at baseline, Apo E genotype, total energy intake, fat intake, physical activity, smoking, alcohol and vitamin supplements reduced the inverse association to 0.28 (95% CI 0.13 to 0.63) and further to 0.24 (0.09 to 0.61) with adjustments for dietary intake of vitamin C, E and beta-carotene. A dose-response effect was demonstrated, and a significant trend was seen in all adjusted models.

In WHICAP [24] the association between risk of AD and intake of fruit and vegetables were analyzed as part of the analyses of a dietary pattern. The hazard ratio for a vegetable consumption above median intake vs. below median was 0.76 (95% CI 0.60 to 0.97). An adjusted association was not significant and is not reported by the authors because intake of fruit and vegetables was not a main focus in this study. Median intake of vegetables was 197 grams per day. The hazard ratio for intake of fruit (median 472 grams) was not significant and not reported.

Synthesis of results

As shown in table 4 all three studies presented effect estimates favoring a high intake, and with 95% confidence intervals below 1.0 in unadjusted or models adjusted for socio-demographic factors only. As expected the smallest study (Kame project) had a greater risk for random error demonstrated by the broadest confidence interval. The WHICAP study and the Three City study had narrower confidence intervals. WHICAP reported that the

confidence interval was crossing 1.0 if adjusted by all relevant covariates, including age. The effect estimates from the Three City Study were not significant in further adjusted models.

A meta-analysis of the included studies gives a combined estimate of 0.71 (0.59 to 0.86) favouring a high intake with low heterogeneity between studies.

Figure 3 - Meta-analysis of included studies

Grading of evidence

The evidence of low intake of fruit and vegetables being a cause of AD can be graded as "limited-suggestive", according to WCRF/AACR criteria [20]. This grade of evidence requires evidence from at least two cohort studies, generally consistent direction of effect though some unexplained heterogeneity may be present and lastly, and evidence for biological plausibility. To upgrade this evidence to "plausible evidence", evidence from two good cohort studies are required, meaning that one with confidence can exclude the possibility that observed associations result from random or systematic error. The included studies do not satisfy this requirement.

Discussion

In the present systematic review, we systematically reviewed prospective cohort studies investigating the association between the intake of fruit and vegetables and Alzheimer's disease from 65 years of age. This review includes three studies which show a consistent inverse association between the intake of fruit and vegetables, vegetables alone, or juices

and the risk of Alzheimer's disease after 65 years of age. In fully adjusted models, however, two of the studies were no longer significant.

The effect estimates in the included studies may have been biased by confounders. In the WHICAP no adjustment for age was performed, and this may have strengthened the effect measure. The Three City and WHICAP studies did not report level of physical activity and the Kame Project measured physical activity with a yes/no for regular activity. If physical activity correlates both with a high intake of fruit and vegetables and AD, this may have confounded the associations. Physical activity, particularly activity on a higher level, is associated with a lower risk of cognitive decline and AD [27-30]. The Three City Study collected data on smoking, but did not refer to these data in their adjustment models. In addition there is always a risk of residual confounding in observational studies even if minimized at the stage of analyses [31]. For example such residual confounding may be caused by health behaviors not being completely adjusted for.

Moreover, measurement and reporting of intake of fruit and vegetables differ between the studies. The Kame project [23] did not report the complete intake of food items in the FV categories, and it is possible that a high intake of juices is reflecting a healthy dietary pattern or other healthy behaviours. The variety in measuring and reporting may partially reflect that the studies were planned and reported at a time when the theory of antioxidants as a causal factor for chronic diseases was extensively discussed and before a more standardized reporting of observational studies took place [32].

An effect of a food item or a nutritional factor can only be observed if groups with sufficiently high and low intakes to estimate a protective effect are included in the sample, and if the intakes are sufficiently grouped [33]. The WHICAP study in New York found an effect of vegetables. That the association is no longer significant when adjusting for

covariates may be explained by too small differences in intake between those above and below median intake. The Kame project found the strongest effect estimate. This may be explained by this study comparing high and low intakes (at least 3 times per week versus less often than once per week) and not binary variables.

An association for vegetables, but not fruit, is supported by another study from the WHICAP cohort which found a dietary pattern associated with reduced risk of AD [11]. One of several characteristics of this dietary pattern was a higher intake of cruciferous vegetables and dark and green leafy vegetables. The WHICAP also found an indication of a dose-response effect for the MeDi dietary pattern [24].

If there is a causal relationship between FV intake and AD, it is not clear in which time window these foods may exert an effect. For some diseases diet may be important during childhood, for other diseases intake in mid-life or near the time before diagnosis may be important to delay the development of the disease. Further, AD is a disease developing over years, and in this context a follow-up period of 3.5 to 6.3 years may be too short.

There is a potential for reverse causation to explain the findings, meaning that those who have a low intake of FV have decreased their intake as a cause of early cognitive changes. If this is the case, measurement of dietary intake should be done before 65 years of age.

The associations observed in these cohort studies may be explained by unknown differences between exposure groups not being adjusted for. The evidence is preliminary and the findings need to be confirmed. Intake of fruit and vegetables is not a factor which is easily amenable to randomization and clinical trials. Rigorous observational studies are required to confirm the findings. Diets of individuals are rather consistent over years [34, p. 588], and hence prospective cohort studies may provide data to resolve the issue on diet and period of time for which diet may play a role in the development of AD. If intake of

fruit and vegetables have even a modest preventive effect on the development of cognitive impairment and AD this would be important from a perspective of public health [35].

Comparison with other studies

An evidence report on prevention of AD and cognitive decline was commissioned in preparation for a State-of-the-Science Conference on Alzheimer's disease in April 2010 [36]. In this report Williams et al reviewed two studies on fruit and vegetables; the Kame prospective cohort study and a nested retrospective case-control follow up study from Sweden [37]. Williams et al states that the preliminary evidence on fruit and vegetables preventing AD needs to be confirmed. This systematic review includes two more studies, and is consistent with one recent study from the Chinese Longitudinal Health Longevity Study which showed that Chinese illiterate elderly who stated that they always included vegetables and legumes in their diet had a reduced odds ratio for cognitive decline over 3 years of follow-up [38]. On the other hand, the Doetinchem Cohort Study from the Netherlands showed an association between some subgroups of vegetables and some, but not all, measurements for cognitive decline after 5 years of follow up of 43-70 years olds [39]. The associations did not apply for fruit and for total intake of fruit and vegetables. Further, the SU.VI.MAX 2 study from France found a differential effect of vegetables, fruit and the fruit and vegetables combined on memory and executive function [40]. A cross sectional study of 70 year olds in UK did not support a causal role for fruit and flavonoids in the prevention of cognitive decline among 70 year olds [41].

So far most studies on dietary intake of fruit and vegetable in the prevention of dementia have focused on antioxidants and anti-inflammatory substances as possible protective factors. However, clinical trials with vitamin C and E have found no consistent protective

effect on AD [42-43]. Recently a clinical trial raised caution about faster cognitive decline after a 16 week trial of combined antioxidant intake [44].

Biological mechanisms

Epidemiologic studies have shown that a high intake of fruit and vegetables is negatively associated with vascular disease [45-46]. Vascular disease may be part of the causal pathway of AD [47-48]. In the brain fruit and vegetables may prevent occlusion of brain arterioles and brain vessel dysfunction through mechanisms other than anti-oxidative and anti-inflammatory [49-50]. There is a possibility that two or more dietary factors act in concert. Prospective cohort studies have found an association between dietary patterns rich in several vegetarian foods and cognitive decline and dementia. These include WHICAP [11, 24], Three City Study [51], a prospective cohort study on cognitive decline in USA [52] and a cross-sectional study in Hordaland, Norway [53].

Limitations and strengths of the review

The main limitations of this study are the quantity and the quality of the primary evidence. We found only three prospective cohort studies. All included studies had a main focus on antioxidants or dietary pattern, and this may have affected the analyses. The studies reported different categories for exposures, and they also varied in the degree of adjustment for confounders in their risk estimates. These variations could lead to potential bias in the combined estimate.

In this review there may also be a possibility of incomplete retrieval of studies as only studies in English language are included. Further the literature search, extraction of data and analyses were done by one person only. The main strength of this review is that it is

based on prospective cohort studies, and the findings are unlikely to be explained by recall bias and selection bias.

Conclusions

In conclusion, this systematic review shows that there is limited-suggestive evidence from prospective cohort studies to conclude that a higher intake of fruit and vegetables is associated with a decreased risk of AD from 65 years of age. The result cannot be used to infer direct causal relationship between AD and intake of fruit and vegetables, and a dose necessary for risk reduction remains to be defined. Further analyses and prospective cohort studies are needed to determine if there is a causal relationship and not mere associations between the intake of fruit and vegetables and AD. These studies should aim at using consistent adjustment models, differentiate between intake of fruit and vegetables and subgroups of vegetables, focus on other aspects of FV than anti-oxidants and to analyze dose-response effects.

Conflicts of interest

We declare that we have no conflicts of interest.

Abbreviations

AD= Alzheimer's disease; APOE= Genotype of apolipoprotein E; CASI=Cognitive Abilities Screening Instrument; CDR=Clinical Dementia Rating; CI=Confidence interval; DSM= Diagnostic and Statistical Manual of Mental Disorders, version IV (IV) or revised third edition (III-R); E-intake=energy intake; F=female; FFQ= Food frequency questionnaire; FV= Fruit and vegetables; FVjuices = fruit and vegetable juices; HR=

Hazard ratio; ICD= International Statistical Classification of Diseases and Related Health Problems; MeDi=Me3diterranean dietary pattern; NINDCDS-ADRDA=National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and related Disorders Association; MMSE=mini-mental state examination; SD=standard deviation; WHICAP=Washington Heights-Inwood Columbia Aging Project; y=year;

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Authors' contributions

<To be revised before submitting for publication>

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Figures

Figure 1 - Search flow chart

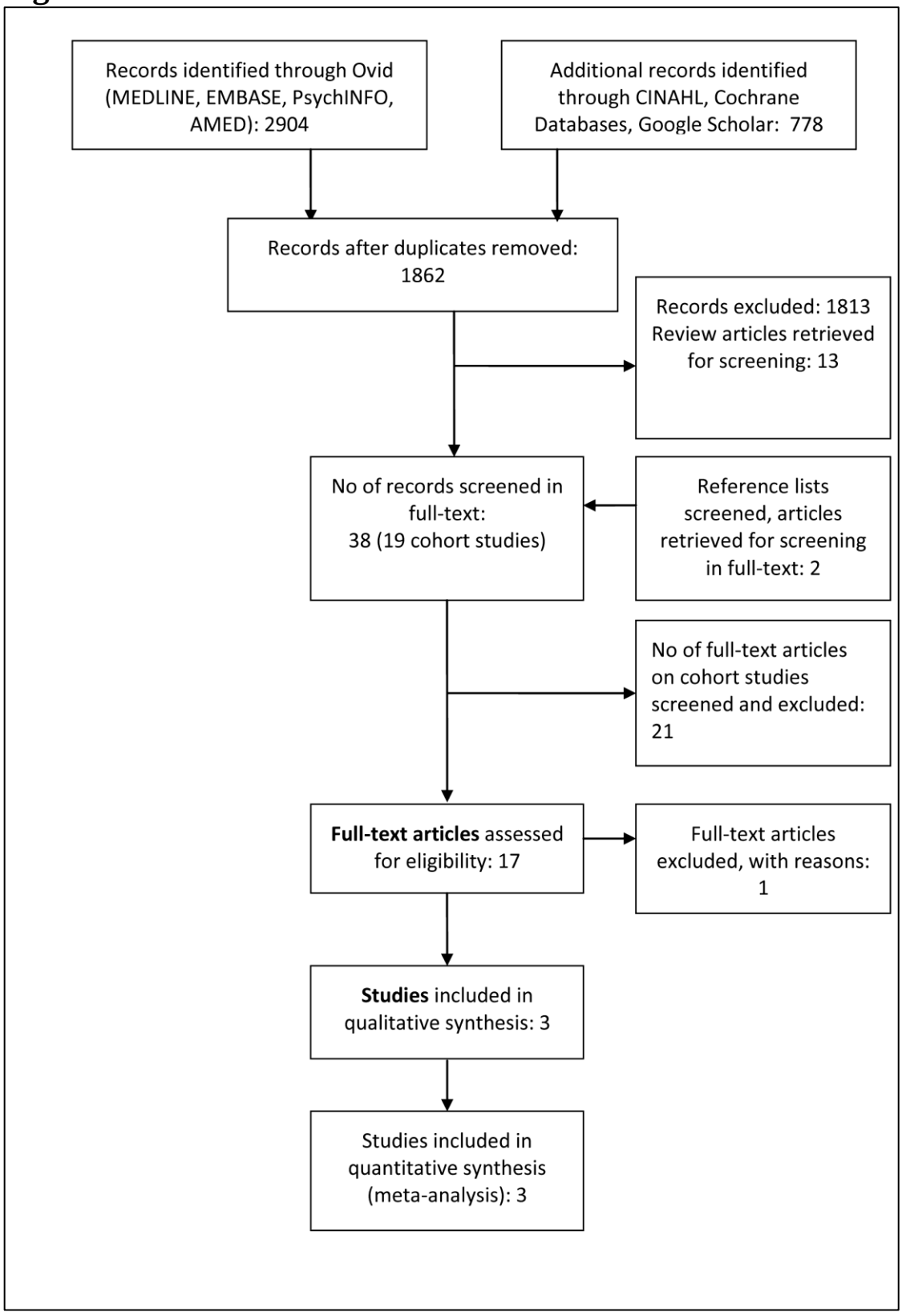


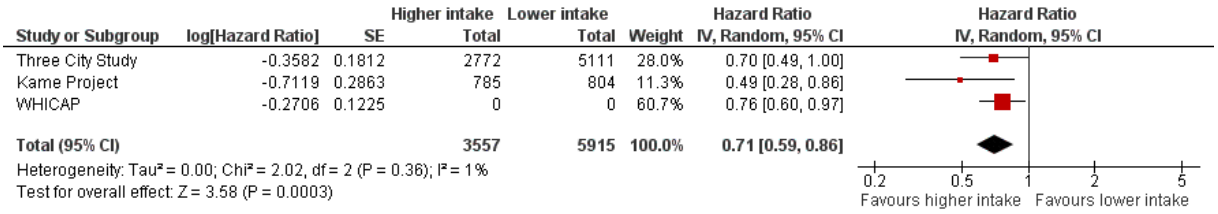
Figure 2 - Risk of bias in included studies

Study	1-Selection	2-ApoE genotype	3-Confounding factors	4-Food exposure assessment	5-Outcome assessment	6-Loss to follow-up	7-Analytical
Three City Study	Low	Low	Uncertain *	Low	Low	Low /Uncertain*	Low*
Kame cohort	Low	Low	Uncertain **	Low *	Low	Uncertain**	Uncertain **
WHICAP	Low	Low	Uncertain*	Low	Low	Low	High risk *, ***

3* Data on exercise not available or not included in analysis. ** Exercise assessed by one single question. 4* For intake of juices. 6* Some risk of underestimation due to deaths and losses to follow-up, rated as low risk. **Loss to follow-up not reported by authors. 7* Binary exposure variables, ** not adjusted for total intake of fruit and vegetables, *** age-adjusted effect estimate not collected or not reported.

Abbreviations: WHICAP = Washington Heights- Inwood Columbia Study Aging Project; ApoE = ApoE genotype.

Figure 3 - Meta-analysis of included studies



Forest plot with meta-analysis of higher vs. lower intake of fruit and vegetables. Hazard ratio for Alzheimer's disease 0.71 (95% confidence interval (CI) 0.59 to 0.86). Exposures: raw and cooked fruit and vegetables in Three City study [22], fruit and vegetables juice in the Kame cohort [23], and vegetables in the WHICAP study [24]. The Kame Project and Three City studies are adjusted for socio-demographic variables (model 1), and WHICAP is unadjusted. Total number of participants in the graph does not include the WHICAP study (2258 subjects in total). Random effects analyses by Review Manager 5.1 [21].

Tables

Table 1 - Search strategy

Search Strategy in Medline.

1	epidemiologic studies/ or cohort studies/ or longitudinal studies/ or follow-up studies/ or prospective studies/
2	epidemiolog$.tw.
3	cohort$.tw.
4	longitudinal.tw.
5	follow-up.tw.
6	observational.tw.
7	prospective.tw.
8	epidemiologic method$.tw.
9	incidence stud$.tw.
10	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9
11	Diet/
12	food/ or fruit/ or vegetables/
13	fruit$1.tw.
14	juice$.tw.
15	vegetable$.tw.
16	mediterranean.tw.
17	diet$.tw.
18	dietary pattern$.tw.
19	lifestyle.tw.
20	antioxida$.tw.
21	food pattern$.tw.
22	11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21
23	dementia/ or Alzheimer disease/
24	dementia$.tw
25	Alzheimer$.tw
26	preclinical AD.tw
27	(dementia adj2 risk).tw
28	(Alzheimer$ adj2 risk).tw
29	23 or 24 or 25 or 26 or 27 or 28
30	10 and 22 and 29

Table 2 - Characteristics of included studies

Name of study, reference	**Setting: Country, population, time-period for study**	**Mean age at inclusion (SD)**	**Intake of fruit and vegetables in sample**	**Cognitive screening at inclusion**	**Outcome:** ▪**AD assessment** ▪O**utcome measure**
Three City Study [22]	France Electoral rolls of cities of Bordeaux, Dijon, Montpellier 1999/2000-2003/04	74.0 y (5.42)	About 50 % consumed fresh vegetables daily, 78% fresh fruit and 69% cooked vegetables and fruit [54].	▪ Examination by neurologist for all participants in cities Bordeaux, Montpellier, and for those screening positive for dementia in Dijon ▪ MMSE and Isaacs Set Test, cut off according to education level	▪ Independent panel ▪ NINDCDS-ADRDA
Kame Project [23]	Seattle area, Washington, USA, population of Japanese origin 1992/94-2001	71.8 y Age in tertiles of juice intake: 71.7 (5.4) 71.6 (5.0) 72.1 (5.2)	Not reported by authors.	▪ Clinical evaluation ▪ CASI with cut off 86 pt. on 100 pt. scale (≥87pt.= no clinical evaluation)	▪ Consensus committee ▪ DSM-IV, INDCDS-ADRDA
WHICAP [24]	New York, USA, Multicultural population in Northern Manhattan, Medicare beneficiaries 1992- and 1999-	77.2 y (6.6)	Intake in low/middle/high MeDi tertile: Vegetables: 165/202/243 gm/day. Median intake197 gm Fruit: 406/471/556 gm/day. Median intake 472 gm.	▪ All participants clinically evaluated, neuropsychological battery ▪ CDR, images, neuropsychological tests	▪ Consensus diagnosis ▪ DSM-III-R, NINDCDS-ADRDA

Abbreviations: AD = Alzheimer's disease; CASI=Cognitive Abilities Screening Instrument; CDR=Clinical Dementia Rating; F=female; DSM= Diagnostic and Statistical Manual of mental Disorders, version IV (IV) or revised third edition (III-R); FFQ=food frequency questionnaire; Isaacs' Set test =test for cognition; MeDi=Me3diterranean dietary pattern; NINDCDS-ADRDA=National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and related Disorders Association; MMSE=mini-mental state examination; SD=standard deviation; WHICAP=Washington Heights-Inwood Columbia Aging Project; y = years;

Table 3 - Description of the dietary assessment methods, time period covered, exposure of fruit and vegetables and range in the included studies

Name of study, reference	FFQ method, and if assessment repeated at follow-up	Time period covered	Validation of FFQ	Exposure of fruit and vegetables (FV)	Range of exposure
Three City Study [22]	▪Face-to-face interview ▪Short FFQ (3 FV items and 9 other items/food groups) ▪Not repeated	Habitual intake	Validated in the city of Bordeaux, 24 h recall, 2 years after baseline examination. No association found between energy-intake and dementia 2 years later.	Raw fruit, Raw vegetables, cooked FV, each recorded in 6 categories,Further reduced into frequent consumption = raw fruit+ raw vegetables +cooked FV daily	daily 4-6 per wk 2-3 per wk 1 per wk <1 per wk never
Kame Project [23]	▪ Self-administered, English or Japanese version** ▪ FFQ with images showing portion sizes, 33 uitems Eating preferences (Asian, western). ▪ Not repeated	During past week or another usual, recent week	Validated among 5 ethnic groups by 4-week food records throughout 1 year. Intraclass correlation coefficients for Japanese men was 0.77 for beta-carotene and 0.42 for vitamin C; women 0.42 and 0.42.	▪ Fruit and vegetable juices in 8 frequency categories and 3 portion sizes** Further reduced into <once per week 1-2times per week ≥3times per week	8 frequency options 3 portion sizes
WHICAP [24]	▪ Administered by trained interviewer by telephone, in English or Spanish ▪ Willett's 61-items semi-quantitative FFQ* ▪ Repeated	Consumption of fruit and vegetables past year	Validated in subsample of 78 individuals using two 7-day food records. Intraclass correlations: vitamin A 0.32, vitamin C 0.57, vitamin E 0.45. 86% of vitamin A was carotenes [55].	▪ Exposure not reported in detail, includes subgroups of fruit, vegetables ▪ Calculated weight of daily intake in tertiles of MeDi dietary pattern, by 25th and 75th percentile, and median intake ▪ FV values calorie- adjusted to 2500 kcal/day for men, 2000 kcal/day women	Range of exposure not reported by authors

[56] Copyright by Harvard University, Channing Laboratories. ****** FFQ unpublished, not reported by authors in detail [57]

Abbreviation: FFQ=food frequency questionnaire; FV=fruit and vegetables; MeDi =Mediterranean Diet (Trichopolou, 2003); wk=week.

Table 4 - Summary of results in included studies. Observation period, age at final screening, exposure measured, adjustments, outcome

Name of study, literature reference	**Observation period (SD)** **AD/analyzed sample, sex**	**Exposure**	**Adjusted for covariates**	**Effect estimates, HR (95% CI)**	**Notes**
Three City Study [22]	3.48 years (NA) (mean 3.65) 183/8085 (total dementia 281) F: 61.4%	Daily raw fruit+ raw vegetables + cooked FV vs. not daily (daily: n=2772)	▪ Model 1: age, gender, education, city, income, marital status ▪ Model 2: model 1+ApoE genotype (one or two ε4 allele) ▪ Model 3: model 2+ BMI, diabetes	▪ Model 1: HR 0.70 (0.49 to 0.997) P =0.048 ▪ Model 2: HR 0.72 (0.50 to 1.04), P=0.08 ▪ Model 3: HR 0.73 (0.50 to1.05), P =0.09	Dietary pattern with lower consumption of fish, omega-3 rich oils and FV: HR 1.63 (95%CI 1.04 to 2.56) in model 2
Kame project [23]	6.3 years (2.6) 63/1589 (total dementia not reported) F: 54.4%	FV juices times per wk: once, 1-2, >3 (≥3 per week: n=785)	Age part of analytical model (age at entry, age at onset) ▪Model 1: (Age), education ▪Model 2: Model 1 + gender, phys act, BMI, CASI, olfaction gr, total E-intake, intake of fats, ApoE, smoking, alcohol, vit C, E, multi suppl.,tea, FV in model 2. ▪Model 3: Model 2 + dietary vit C, E, beta-carotene in model 3.	FV juices ≥3 times per wk vs. <once per wk (=ref=1) ▪Model 1: HR 0.49 (0.28 to 0.86), P for trend 0.01 ▪Model 2: HR 0.28 (0.13 to 0.63), P for trend <0.01 ▪Model 3: HR 0.24 (0.09 to 0.61), P for trend <0.01	Total intake of FV not reported

Table 4 Continued

Name of study, literature reference	Observation period (SD) AD/analyzed sample, sex	Exposure	Adjusted for covariates	Effect estimates, HR (95% CI)	Notes
WHICAP [24]	4.0 years (3.0) (range 0.2-13.9), 262/2258 (total dementia 294) F: 68%	Fruit, vegetables Median daily intake, classed according to cutoffs for MeDi score tertiles Intake in low/middle/high MeDi tertile: Vegetables: 165/202/243 gm/day. Median intake=197 gm Fruit: 406/471/556 gm/day. Median intake = 472 gm.	Unadjusted and adjusted models Adjusted model: Cohort, age at intake, sex, ethnicity, education, ApoE, smoking, comorbidity index (Charlson), BMI, caloric intake	Unadjusted: HR 0.76 (0.60 to 0.97), P=0.030 for above median vegetable consumption vs. low. Adjusted: NS (effect estimate not reported by authors) HR for fruit NS (not reported)	HR for AD by MeDi score: Unadjusted: high vs. low 0.61 (95% CI 0.44 to 0.85) middle vs high 0.79 (0.60 to 1.04) P for trend 0.003 Adjusted: high vs. low 0.85 (0.63to 1.16), middle vs. low 0.60 (0.42 to 0.87), P for trend 0.007 2 of 9 dietary components .of MeDi (V, alcohol) were associated with decreased risk for AD

Number of subjects in exposure groups calculated from Table 4 (22) and Table 1 (23).

Abbreviations: AD = Alzheimer's disease; ApoE=apolipoprotein E genotype; BMI=body mass index; CI=confidence interval; E-intake=energy intake; F=female; FV=fruit and vegetables; gm=grams; HR = hazard ratio; MeDi =Mediterranean Diet (Trichopolou, 2003); NA=not available; NS=not significant; SD=standard deviation; V=vegetables; WHICAP=Washington Heights-Inwood Columbia Aging Project; wk=week.

APPENDIX I

Literature search

Search Ovid in databases: AMED, EMBASE, MEDLINE, PsychINFO. Date: 1 July 2011
Database: AMED (Allied and Complementary Medicine) <1985 to June 2011>, Embase <1980 to 2011 Week 25>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1948 to Present>, PsycINFO <1967 to June Week 4 2011>
Search Strategy:

1 exp epidemiology/ or epidemiologic methods/ or epidemiologic studies/ (1491271)
2 exp cohort analysis/ or cohort studies/ or longitudinal studies/ or longitudinal study/ (1255894)
3 follow up studies/ or follow up/ or prospective studies/ or prospective study/ or risk factors/ (2151416)
4 epidemiolog$.tw. (491166)
5 cohort$.tw. (435622)
6 longitudinal.tw. (279353)
7 follow-up.tw. (1132584)
8 observational.tw. (122685)
9 prospective.tw. (630410)
10 epidemiologic method$.tw. (1483)
11 incidence stud$.tw. (2375)
12 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 (5052581)
13 exp Diet/ or food/ or nutrition/ or food habits/ or nutrition surveys/ (467956)
14 exp fruit/ or exp vegetables/ (250366)
15 diet mediterranean/ or diet vegetarian/ (6894)
16 fruit$1.tw. (83529)
17 juice$.tw. (39282)
18 vegetable$.tw. (55543)
19 mediterranean.tw. (33416)
20 nutri$.tw. (453632)
21 diet$.tw. (699243)
22 food$.tw. (516443)
23 antioxidant$.tw. (182100)
24 lifestyle.tw. (86957)
25 (dietary pattern$ or food habit$ or food pattern$).tw. (9374)
26 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 23 or 24 or 25 (1738489)
27 dementia/ or Alzheimer disease/ or alzheimers disease/ (252486)
28 dementia$.tw. (155090)
29 Alzheimer$.tw. (185641)
30 preclinical AD.tw. (411)
31 (dementia adj2 risk).tw. (3564)
32 (Alzheimer$ adj2 risk).tw. (3591)
33 27 or 28 or 29 or 30 or 31 or 32 (327236)
34 12 and 26 and 33 (3583)
35 limit 34 to english language (3285)
36 limit 35 to yr="1980 -Current" (3284)
37 limit 36 to humans [Limit not valid in AMED,PsycINFO; records were retained] (2904)
38 remove duplicates from 37 (**1862)**

CINAHL

Search History 14-7-2011

Interface - EBSCOhost

Search ID#	Search Terms	Result
S45	S10 and S37 and S44	(634)
S44	S38 or S39 or S40 or S41 or S42 or S43	(31027)
S43	TX "risk of Alzheimer*"	(252)
S42	TX "risk of dementia"	(327)
S41	TX Alzheimer*	(15598)
S40	TX dementia	(22239)
S39	(MH "Alzheimer's Disease")	(12031)
S38	(MH "Dementia")	(14856)
S37	S11 or S12 or S13 or S14 or S15 or S16 or S17 or S18 or S19 or S20 or S21 or S22 or S23 or S24 or S25 or S26 or S27 or S28 or S29 or S30 or S31 or S32 or S33 or S34 or S35 or S36	(170450)
S36	(MH "Nutritional Status")	(3942)
S35	(MH "Nutritional Assessment")	(7540)

S34	TX nutri*	(101592)
S33	(MH "Nutrition")	(9798)
S32	TX mediterranean	(3350)
S31	(MH "Mediterranean Diet")	(693)
S30	TX ("dietary pattern?") or ("food pattern?") or ("food habit?")""	(184)
S29	TX lifestyle	(12389)
S28	(MH "Life Style")	(9310)
S27	TX flavonoid*	(1432)
S26	TX polyphenol*	(773)
S25	TX antioxidant*	(7610)
S24	(MH "Polyphenols")	(313)
S23	(MH "Antioxidants")	(5519)
S22	TX vegetable*	(6256)
S21	(MH "Vegetables")	(3829)
S20	TX citrus	(414)

S19	TX juice*	(1844)
S18	TX fruit? OR TX fruit	(7479)
S17	(MH "Citrus")	(229)
S16	(MH "Fruit Juices")	(451)
S15	(MH "Fruit")	(3831)
S14	TX food*	(61232)
S13	(MH "Food and Beverages")	(51)
S12	TX diet	(50622)
S11	(MH "Diet")	(18070)
S10	S1 or S2 or S3 or S4 or S5 or S6 or S7 or S8 or S9	(344338)
S9	TX incidence	(48956)
S8	TX prospective	(136018)
S7	TX observational	(17557)
S6	TX follow-up	(55739)
S5	TX longitudinal	(18546)

S4	TX epidemiolog*	(168835)
S3	TX cohort*	(35834)
S2	(MH "Prospective Studies")	(118576)
S1	(MH "Epidemiology")	(1981)

Cochrane all databases

Search July 14 2011

Current Search History

ID	Search	Hits	Edit	Delete
#1	MeSH descriptor **Epidemiologic Studies** explode all trees	97436	edit	delete
#2	MeSH Alzheimer Disease	2252	edit	delete
#3	MeSH descriptor **Food and Beverages** explode all trees	18863	edit	delete
#4	(#1 AND #2 AND #3)	8	edit	delete
#5	MeSH descriptor **Diet, Mediterranean** explode all trees	104	edit	delete
#6	(MeSH Diet)	15982	edit	delete
#7	(#1 AND #2 AND (#5 OR #6))	5	edit	delete
#8	(#2 AND (#3 OR #5 OR #6))	63	edit	delete

Save Search Strategy | Clear History

There are **18** results out of **6720 records** for: "(**#2** AND (**#3** OR **#5** OR **#6**)) in **Cochrane Database of Systematic Reviews**"

APPENDIX II

Excluded studies

Reference	Reason for exclusion
Hughes T.F., Andel R., Small B.J., Borenstein A.R., Mortimer J.A., Wolk A., Johansson B., Fratiglioni L., Pedersen N.L. & Gatz M. (2010) Midlife Fruit and Vegetable Consumption and Risk of Dementia in Later Life in Swedish Twins. *Am J Geriatr Psychiatry,* 18 (5), s. 413-420.	Retrospective cohort design.

APPENDIX III

Ongoing studies

Study name	Chicago Health and Aging Project (CHAP)
Methods	Prospective cohort study
Participants	3700 participants, aged 65 years and older
Exposition	Fruit/vegetables, in quintiles
Outcomes	AD
Starting date	Ongoing, waiting for additional data.
Contact information	Martha Clare Morris, e-mail: Martha_Morris@rush.edu
Notes	Report published in 2006 on intake of fruit and vegetables, including sub-groups. High intake of vegetables, but not fruit, was associated with age-related cognitive decline after 6 years of follow-up. Morris, M. C., Evans, D. A., Tangney, C. C., Bienias, J. L. & Wilson, R. S. (2006) Associations of vegetable and fruit consumption with age-related cognitive change. Neurology, 67, p. 1370-1376.

APPENDIX IV

Worksheet template

Template from Academy of nutrition and Dietetics
(former: American Dietetic Organization)
Downloaded from: http://www.adaevidencelibrary.com/category.cfm?cid=7&cat=0
(ADA, 2011)

Academy of Nutrition and Dietetics
Evidence Analysis Library® Worksheet Template and
Quality Criteria Checklist: Primary Research

Academy of Nutrition and Dietetics

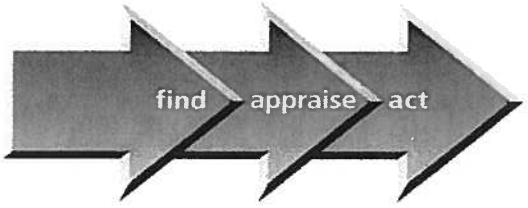
Citation	
Study Design	
Class	
Quality Rating	☐ + (Positive) ☐ - (Negative) ☐ ⊘ (Neutral)
Research Purpose	
Inclusion Criteria	
Exclusion Criteria	
Description of Study Protocol	Recruitment: Design: Blinding used (if applicable): Intervention (if applicable): Statistical Analysis:
Data Collection Summary	Timing of Measurements: Dependent Variables: Independent Variables: Control Variables:
Description of Actual Data Sample	Initial: (Males Females) Attrition (final N): Age: Ethnicity: Other relevant demographics: Anthropometrics: Location:
Summary of Results	Key Findings: Other Findings:
Author Conclusion	
Reviewer Comments	
Funding Source	

APPENDIX V

CASP checklist

(CASP, 2010)

CRITICAL APPRAISAL SKILLS PROGRAMME
Making sense of evidence about clinical effectiveness



12 questions to help you make sense of cohort study

General comments

- Three broad issues need to be considered when appraising a cohort study.

  Are the results of the study valid?

  What are the results?

  Will the results help locally?

The 12 questions on the following pages are designed to help you think about these issues systematically.

- The first two questions are screening questions and can be answered quickly. If the answer to those two is "yes", it is worth proceeding with the remaining questions.

- There is a fair degree of overlap between several of the questions.

- You are asked to record a "yes", "no" or "can't tell" to most of the questions.

- A number of italicised hints are given after each question. These are designed to remind you why the question is important. There will not be time in the small groups to answer them all in detail!.

A/ Are the results of the study valid?

Screening Questions

1 Did the study address a clearly focused issue? *HINT: A question can be focused in terms of?* • *the population studied* • *the risk factors studied* • *the outcomes considered* • *is it clear whether the study tried to detect a beneficial or harmful effect?*	**Yes** ❑	**Can't tell** ❑	**No** ❑
2 Did the authors use an appropriate method to answer their question? *HINT: Consider* • *is a cohort study a good way of answering the question under the circumstances?* • *did it address the study question?*	**Yes** ❑	**Can't tell** ❑	**No** ❑

Is it worth continuing?

Detailed Questions

3 Was the cohort recruited in an acceptable way? *HINT: We are looking for selection bias which might compromise the generalisability of the findings:* • *Was the cohort representative of a defined population?* • *Was there something special about the cohort?* • *Was everybody included who should have been included?*	**Yes** ❑	**Can't tell** ❑	**No** ❑

4 Was the exposure accurately measured to minimize bias? *HINT: We are looking for measurement or classification bias:* • *Did they use subjective or objective measurements?* • *Do the measures truly reflect what you want them to (have they been validated)?* • *Were all the subjects classified into exposure groups using the same procedure?*	**Yes** ❑	**Can't tell** ❑	**No** ❑
5. Was the outcome accurately measured to minimize bias? HINT: We are looking for measurement or classification bias: • *Did they use subjective or objective measurements?* • *Do the measures truly reflect what you want them to (have they been validated)?* • *Has a reliable system been established for detecting all the cases (for measuring disease occurrence)?* • *Were the measurement methods similar in the different groups?* • *Were the subjects and/or the outcome assessor blinded to exposure (does this matter)?*	**Yes** ❑	**Can't tell** ❑	**No** ❑
6 A, Have the authors identified all important confounding factors? **List the ones you think might be important, that the author missed.** **B. Have they taken account of the confounding factors in the design and/or analysis?** HINT: • *Look for restriction in design, and techniques eg modelling, stratified-, regression-, or sensitivity analysis to correct, control or adjust for confounding factors*	**Yes** ❑ **Yes** ❑ **List:**	**Can't tell** ❑ **Can't tell** ❑	**No** ❑ **No** ❑

7. A. Was the follow up of subjects complete enough?	**Yes** ❑	**Can't tell** ❑	**No** ❑
B. Was the follow up of subjects long enough?	**Yes** ❑	**Can't tell** ❑	**No** ❑
HINT: • *The good or bad effects should have had long enough to reveal themselves* • *The persons that are lost to follow-up may have different outcomes than those available for assessment* • *In an open or dynamic cohort, was there anything special about the outcome of the people leaving, or the exposure of the people entering the cohort?*			

B/ What are the results?

8 What are the results of this study? *HINT:* • *What are the bottom line results?* • *Have they reported the rate or the proportion between the exposed/unexposed, the ratio/the rate difference?* • *How strong is the association between exposure and outcome (RR,)?* • *What is the absolute risk reduction (ARR)?*			
9. How precise are the results? *HINT:* • *Size of the confidence intervals*			
10. Do you believe the results? HINT: • *Big effect is hard to ignore!* • *Can it be due to bias, chance or confounding?* • *Are the design and methods of this study sufficiently flawed to make the results unreliable?* • *Consider Bradford Hills criteria (eg time sequence, dose-response gradient, biological plausibility, consistency).*	**Yes** ❑	**Can't tell** ❑	**No** ❑

C/ Will the results help me locally?

11. Can the results be applied to the local population? *HINT: Consider whether* • *The subjects covered in the study could be sufficiently different from your population to cause concern* • *Your local setting is likely to differ much from that of the study* • *Can you quantify the local benefits and harms?*	**Yes** ❑	**Can't tell** ❑	**No** ❑
12. Do the results of this study fit with other available evidence?	**Yes** ❑	**Can't tell** ❑	**No** ❑

One observational study rarely provides sufficiently robust evidence to recommend changes to clinical practice or within health policy decision making.
However, for certain questions observational studies provide the only evidence.
Recommendations from observational studies are always stronger when supported by other evidence.

APPENDIX VI

Checklist draft from Nordic Council

Quality assessment tool for prospective cohort studies

Author: Year: Reference nr:

1. General questions and study design		Requires Yes for level A	B	C
a) Research question clearly formulated?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x	x	
b) Endpoint/outcome clearly defined?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x	x	
c) Was the study design suited to test the research hypothesis?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x	x	
2. Sampling ***(Ascertainment of cases and non-cases)***				
a) Source population/study base well defined? Recruitement done in an acceptable way?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x	x	
b) Response rate reported and acceptable?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x	x	
c) Criteria for inclusion/exclusion clearly formulated and acceptable?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x	x	
d) Participants and non-participants comparable with Nordic population?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x		
e) Time period of baseline examinations clearly identified?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x		
f) Endpoint clearly ascertained and assessed in a valid way?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x	x	
g) Follow-up period clearly identified?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x		
h) Time-exposure-variable clearly defined (i.e., period non-cases being exposed)?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x		
i) Loss to follow up < 20%?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x		
3. Dietary exposure				
a) Type of exposure (nutrients, food groups etc) reported in sufficient detail?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x	x	
b) Particulars of dietary assessment tool reported in sufficient detail?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x		
c) Food composition database reported?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x		
d) Concurrent validity (validation coefficients) of specific exposures reported?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x		
e) Associations between dietary exposures reported?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x		
f) Measurement errors in dietary reporting considered?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x	x	
g) Energy intake at a credible level?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x	x	

Quality assessment tool for prospective cohort studies

3. Dietary exposure....cont		Requires Yes for level A B C
h) Energy adjustment adequately done?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x
i) Repeat assessment of diet during follow up?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x
j) Use of dietary biomarkers adequate? Details of assessment and handling reported? Valid biomarker assay?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x if relevant
k) Time period between biomarker assessment and diagnosis acceptable?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x if relevant
5. Anthropometry		
a) Assessment details clearly reported and assessment adequately performed?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x
6. Physical activity		
a) Assessment details clearly reported and assessment adequately performed?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x
7. Confounding		
a) Were important confounders identified/ascertained and considered by authors?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x x
b) The distribution of confounders similar in cases and non-cases?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x
8. Statistical power		
a) Was the study power considered and sample size and power calculations reported?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x
b) In view of multiple tests, were by chance findings considered?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x
c) Sufficient size of study population and no. of outcomes/cases?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x
9. Statistical analysis		
a) Appropriately handled?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x
b) Relevant confounders adequately handled: Restriction, Stratified analysis, Multivariate modelling, Interaction tested?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x x
c) Ascertainment/detection bias considered (eg. cases detected due to screening)?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x
d) Cases detected early during the follow-up period removed?	Yes ☐ No ☐ Can´t tell ☐ NA ☐	x
10. Summary of the study quality	A ☐ B ☐ C ☐	

Quality assessment tool for prospective cohort studies

A Studies have the least bias and results are considered valid. These studies adhere mostly to the commonly held concepts of high quality including the following: a formal study design; clear description of the population, setting, interventions, and comparison groups; appropriate measurement of outcomes; appropriate statistical and analytic methods and reporting; no reporting errors; less than 20 percent dropout (over 50% response rate for cross-sectional studies) clear reporting of dropouts; and no obvious bias. Studies must provide valid estimation of nutrient exposure, from dietary assessments and/or biomarkers with reasonable ranges of measurement errors, and justifications for approaches to control for confounding in their design and analyses.

B Studies are susceptible to some bias, but not sufficient to invalidate the results. They do not meet all the criteria in category "A", they have some deficiencies but none likely to cause major bias. The study may be missing information, making it difficult to assess limitations and potential problems.

C Studies have significant bias that may invalidate the results. These studies have serious errors in design, analysis, or reporting; there are large amounts of missing information, or discrepancies in reporting.