Dementia risk reduction, why haven't the pharmacological risk reduction trials worked? An in-depth exploration of seven established risk factors.

Ruth Peters [a,b], John Breitner [c], Sarah James [d], Gregory A. Jicha [e], Pierre-Francois Meyer [f], Marcus Richards [d], A. David Smith [g], Hussein N Yassine [h], Erin Abner [e], Atticus H Hainsworth [i], Patrick G Kehoe[j], Nigel Beckett [k], Christopher Weber[l], Craig Anderson[m], Kaarin J Anstey [a,b], Hiroko H. Dodge[n]

a) Neuroscience Research Australia,
b) Department of Psychology University of New South Wales, Australia,
c) Douglas Hospital Research Center and McGill University, Quebec, Canada
d) MRC Unit for Lifelong Health and Ageing at UCL, University College London, UK
e) University of Kentucky, USA
f) Center for Studies on the Prevention of Alzheimer's Disease (PREVENT-AD), 6875 Boulevard LaSalle, Verdun, QC H4H 1R3, Canada
g) OPTIMA, Department of Pharmacology, University of Oxford, Oxford, UK
h) Departments of Medicine and Neurology, University of Southern California, CA, USA
i) Molecular and Clinical Sciences Research Institute, St Georges, University of London, UK; Department of Neurology, St George's Hospital, London UK.
j) Bristol Medical School University of Bristol, UK
k) Guys and St Thomas' NHS Foundation Trust, London UK
l) Alzheimer's Association
m) The George Institute for Global Health, Australia
n) Oregon Health & Sciences University, Portland, OR, USA

Emails
r.peters@neura.edu.au 0000-0003-0148-3617
john.breitner@mcgill.ca
sarah.n.james@ucl.ac.uk
gregory.jicha@uky.edu
pierre-francois.meyer@mail.mcgill.ca
m.richards@ucl.ac.uk
david.smith@pharm.ox.ac.uk
hyassine@usc.edu
erin.abner@uky.edu
ahainsworth@sgul.ac.uk AHH_0000_0001_7877_8013
Patrick.Kehoe@bristol.ac.uk 0000-0002-7542-1139
Nigel.Beckett@gstt.nhs.uk
cweber@alz.org
canderson@georgeinstitute.org.au
k.anstey@unsw.edu.au
dodgeh@ohsu.edu

Correspondence address: C Weber, Director, Global Science Initiatives, Alzheimer's Association, cweber@alz.org, *alz.org*

Ruth Peters is supported by the Australian National Health and Medical Research Centre (NHMRC), Dementia Centre for Research Collaboration and has received grants paid to her institution from the NHMRC and the University of New South Wales in the past 36 months. Leadership roles in the last 36 months include Chair of the Alzheimer's Association International Society to Advance Alzheimer's Research and Treatment (ISTAART) Clinical Trials and Methodology Professional Interest Area (unpaid).

John Breitner has received grants paid to his institution from the Canadian Institute for Health Research in the last 36 months. He has also participated on an data safety monitoring board or advisory board for which he has received honoraria in the last 36 months.

Sarah James reports no conflict of interest.

Gregory A. Jicha has received grants paid to his institution from NIH R01 AG061111, UH3 NS100606, R01 AG054130, R01AG061848, R01AG054029, R01AG063689, U19AG010483, R01 NS116990, R56 AG060608, U24 AG057437, R01 AG053798, P30 AG028383, U19AG068054, R01AG057187, R01NS116058, U19 AG024904 and research contracts with AbbVie, Alector, Biohaven, Esai, Lilly also paid to his institution in the last 36 months. He has also participated on a data safety monitoring board or advisory board for which he has received honoraria in the last 36 months and received honoraria for speaking in the last 36 months. Leadership roles in the last 36 months include ISTAART Clinical Trials and Methodology Professional Interest Area Chair (unpaid), and the NIH/NIA Clinical task Force and Clinical Core Steering Committee

Pierre-Francois Meyer is a full time employee of IQVIA Solutions Canada Inc.and reports no conflicts of interest

Marcus Richards has received grants from the UK Medical Research Council MC_UU_12019/1 and /3 and the UK Alzheimer's Society paid to his institution in the last 36 months. He is a member of several advisory groups and part of the steering committee for the Dementias Platform UK (DPUK) (unpaid).

David Smith is a member of the scientific advisory board for Elysium Health and a Consultant for Aprofol for which he has received payment. In the last 36 months he has been listed as an inventor on US Patent 10,966,947 B2

Hussein N Yassine has received grants paid to his institution R21AG056518, R01AG055770, R01AG054434, R01AG067063 from the National Institute on Aging in the last 36 months. He is a member of the steering committee of the National Institute on Aging Research and Education Core. Leadership roles in the last 36 months include ISTAART NMD Professional Interest Area Co-Chair (unpaid).

Erin Abner reports no conflict of interest. Leadership roles in the last 36 months include ISTAART Clinical Trials and Methodology Professional Interest Area Professional Interest Area Co-Chair (unpaid).

Atticus H Hainsworth has received grants paid to his institution from the Alzheimer's Society (UK) and Alzheimer's Drug Discovery Foundation (Project Ref 20140901) in the last 36 months. Dr. Hainsworth has also received honoraria from Eli Lilly and NIA. Leadership roles in the last 36 months include ISTAART Clinical Trials and Vascular Cognitive Disrders Professional Interest Area Chair (unpaid) and membership of the Vascular Experimental Medicine group within DPUK (unpaid).

Patrick G Kehoe has received grants paid to his institution from the Sigmund Gestetner Foundation Fellowship, the Alzheimer's Society, Alzheimer's Research UK, BRACE, the Bright Focus Foundation, the British Heart Foundation, the UK Medical Research Council, and the UK Natonal Institute of Health Research

(NIHR-EME) in the last 36 months. Leadership roles in the last 36 months include membership of the Alzheimer's Society UK Research Advisory Council. and as a Trustee to the Research into Care of the Elderly (RICE) Centre, Bath, UK (unpaid)

Nigel Beckett reports no conflict of interest. Leadership roles in the last 36 months include Committee member with responsibility for research of British Geriatric Society - Cardiovascular Specialist Interest Group (unpaid).

Craig Anderson has received grants from the NHMRC paid to his institution and honoraria from Takeda China in the last 36 months.

Kaarin J Anstey has received grants paid to her institution from the NHMRC, Australian Research Council, Australian Medical Reseach Futures Fund, Mindgardens Alliance, the NHMRC Dementia Centre for Research Collaboration, the Australian Government in the last 36 months. She has received honoraria from the American Association of Retired Persons, the University of British Columbia Member, Governance Committee of the Global Council on Brain Health. Leadership roles in the last 36 months include, Advisor, Staying Sharp platform for American Association of Retired persons, membership of the board of directors of the Dementia Australia Research Foundation.

Hiroko H. Dodge has received grants paid to her institution from the NIH R01AG051628 R01AG056102 R01AG069782 P30AG066518 R01AG072449 P30 AG008017 P30AG024978 U2CAG054397 R01AG056712 R01AG0380651 R21AG062679 P30 AG053760 U01NS100611 U2C AG057441 U01NS106670 R01AG054484 over the last 36 months. She has received honoraria from the Alzheimer's Clinical Trials Consortium (ACTC) and has also participated on data safety monitoring boards/advisory boards in the last 36 months. Leadership roles in the last 36 months include membership of the ISTAART Advisory Board.

This manuscript was facilitated by the Alzheimer's Association International Society to Advance Alzheimer's Research and Treatment (ISTAART), through the Clinical Trials and Methodology professional interest area (PIA). The views and opinions expressed by authors in this publication represent those of the authors and do not necessarily reflect those of the PIA membership, ISTAART or the Alzheimer's Association.

Abstract

Identifying the leading health and lifestyle factors for risk of incident dementia and Alzheimer's disease has yet to translate to risk reduction. To understand why, we examine the discrepancies between observational and clinical trial evidence for seven modifiable risk factors: type 2 diabetes, dyslipidaemia, hypertension, estrogens, inflammation, omega-3 fatty acids and hyperhomocysteinemia. Sample heterogeneity and paucity of intervention details (dose, timing, formulation) were common themes. Epidemiological evidence is more mature for some interventions (e.g. NSAIDs) than others. Trial data are promising for antihypertensives and B vitamin supplementation. Taken together these risk factors highlight a future need for more targeted sample selection in clinical trials, a better understanding of interventions and deeper analysis of existing data.

Introduction

The last 20 years have seen a substantial growth in research on risk factors for cognitive decline and dementia [1, 2]. In 2013 this led to an international petition to the G8 dementia summit asking governments to promote research into modifiable risk factors and the prevention of dementia [3]. In the evidence base, multiple longitudinal cohort and medical record studies have examined dementia risk factors and have been combined into systematic reviews and meta-analyses [1, 2], and the field is now starting to see reviews of reviews [4, 5]. However, recent attention has also focused on a critical examination of gaps in the current evidence base [6]. A key aspect of the latter is the contrast between the epidemiological evidence and the data from clinical trials where interventional trial results for dementia outcomes typically fail to reflect those of observational risk factor epidemiology. Despite consensus regarding the main risk factors for dementia, this contrast with trial results leaves the evidence in support of risk reduction still comparatively lacking, as demonstrated in evidence summaries used to inform the recent World Health Organisation (WHO) dementia risk reduction guidelines [7] .

Here, we discuss and explore possible explanations for the divergence in findings between the risk factor epidemiology and the risk reduction trials. We draw on expertise from the Alzheimer's Association ISTAART Professional Interest Area (PIA) on Clinical Trials and Methodology and leading international experts to appraise and synthesize the evidence, highlight areas of discrepancy, and propose needed next steps. We have selected seven exemplar core risk factors associated with altered dementia risk. For each of these, a plausible mechanism exists for the association between the risk factor and cognition. Even so, trial evidence for risk reduction remains incomplete. To reduce potential for bias in the trial evidence, the selected risk factors are those that lend themselves to blinded pharmacological intervention. These include the following risk factors for which pharmaceutical agents are already in use: type 2 diabetes and antidiabetic medications; dyslipidemias and statins; blood pressure and antihypertensive agents; inflammation and non-steroidal anti-inflammatory drugs [NSAIDs]; and estrogen and hormone replacement

therapy [HRT]. Alongside this we also examine two nutritional risk factors and nutritional interventions, omega 3 fatty acids and their supplementation and hyperhomocysteinemia and B vitamins. The review and commentary is divided into seven separate sections each considering one of these risk factors, with each section drafted and shaped separately by experts in the related field. Each section summarises the rationale, potential biological mechanisms, the epidemiological evidence for the risk factor, the clinical trial evidence for risk reduction and provides recommendations for future observational and clinical trial work.

1. TYPE 2 DIABETES MELLITUS

1.1. Diabetes and dementia, an introduction

Type 2 diabetes mellitus (T2DM) is a common chronic disorder characterised by hyperglycaemia, insulin secretion deficiency and insulin resistance. T2DM has a global prevalence of ~9%, and this is expected to rise with a younger age of onset, particularly in low to middle income countries [8]. It is associated with increased mortality and comorbidity due to microvascular (i.e. retinopathy, neuropathy, nephropathy) and macrovascular (i.e. cardiovascular and cerebrovascular disease) complications[9]. The causes of T2DM are multifactorial and include a complex interplay of genetics [10] and lifestyle factors, including obesity, a sedentary lifestyle and energy-dense but nutrient-poor diets [11]

1.2. Potential Mechanisms

The pathophysiological mechanisms underlying the link between T2DM and dementia are unclear [12]. Some plausible mechanisms include: 1) vascular pathways from comorbidities and complications of T2DM (e.g. hypertension and cerebrovascular disease [13]; 2) cerebral insulin resistance pathways contributing to neurodegeneration and disruption of cerebral proteins [12-14] (this discovery even led to suggestions that Alzheimer's Disease (AD) be considered as 'Type III diabetes''[14]; 3) pathways through which hyperglycemia may

accelerate amyloid plaque aggregation and tau neurofibrilliary tangle formation via accelerated formation of advanced glycation end-products[15].

1.3. Epidemiological evidence that T2DM is a risk factor for dementia

Longitudinal epidemiological studies have consistently demonstrated associations between T2DM and its associated features of hyperglycaemia and insulin resistance, with risk of cognitive impairment and dementia [16-20]. For example a meta-analysis of 28 prospective observational studies demonstrated that, compared to those without T2DM, persons with T2DM had a 73% increase in risk of all-cause dementia, 56% increased risk of AD and 127% increase of vascular dementia [19]. Caution must be applied, however, since the confounding that is a major challenge to inferring causality from epidemiological evidence is particularly pertinent in a complex disorder like T2DM that has many contributing factors, comorbidities and complications. For example, most studies investigating the link between T2DM and dementia do not adjust for common cause factors such as premorbid IQ, education and socioeconomic position, which are the biggest predictors of cognitive function and impairment later in life, and strong predictors of T2DM [21, 22]. Information on the mediating effects of complications and comorbidities (e.g., hypertension) are also often lacking. Additionally, these studies have relied on clinical rather than neuropathological diagnoses of AD and so are limited by misclassification of the outcome [23]. When T2DM has been examined as a risk factor for Alzheimer's pathology, no association is observed; T2DM is associated with cerebrovascular pathology, however [24] [25].

A further consideration is to what extent participants in epidemiological studies may have untreated, or undiagnosed, T2D, especially given the socially patterned and health care-dependent nature of diagnoses and treatment.

It would be useful for studies to incorporate more objective measures of the underlying disease underlying T2D, such as HbA1c level and insulin resistance which would help

elucidate more mechanistic processes. Whilst epidemiological studies have tried to link these T2D processes with dementia and cognition outcomes,[26] we need more evidence on large sample sizes assessing the association between T2D disease processes with the whole spectrum of dementia, including the impact on cognitive function and level and progression of neuropathology associated with dementia, prior to overt clinical expression [27-29]. This would help strengthen or weaken our evidence base for a causal association between the disease processes of T2D and Dementia.

Self-reported, or linkage with, medication records would also be beneficial and there have been efforts to use T2D medication data as a main exposure in epidemiological studies [30], but these have yielded inconsistent results. Careful consideration of timings of treatment, duration of treatment and compliance of treatment would help to elucidate some of these issues.

Mendelian randomisation studies use genetic predictors of T2DM as potential causal instruments to assess causality in settings where confounders are known to be unmeasured. To date, studies have reported null associations between the genetic risk of T2DM, glucose and insulin resistance and all-cause dementia and AD [31-34] perhaps indicating that there is not a causal relationship between T2DM and later-life dementia per se, but implicating other pathways related to T2DM [22, 24] . Other causal inference methods are increasingly becoming applicable for clinical medicine and observational studies [35], but as of yet have not been applied to investigate the association between T2DM and dementia.

Future studies should endeavour to measure confounding and mediating influences and may consider applying causal inference methods alongside more traditional methods to infer more accurate causal estimates of the impact of T2DM on cognitive impairment and dementia risk.

1.4. Diabetes-related therapeutics dementia reduction trials

RCT results to date do not suggest that antidiabetic agents as used treat diabetes are associated with better cognitive outcomes [36] Efforts to summarise the effects of antidiabetic agents on cognitive impairment include a Cochrane review of 7 RCTs up to 2017 [36] that found no evidence to favour T2DM treatment to prevent cognitive impairment or dementia. Indeed, there have even been indications that antidiabetic agents seem to increase risk of cognitive impairment, potentially via hypoglycaemic episodes [36]. While there were initial indications of a potentially beneficial effect on the incidence of dementia with Pioglitazone [37], a thiazolidinedione insulin sensitizer thought to have a role in microglia regulation, two phase III trials in patients with Mild Cognitive Impairment (MCI) (ClinicalTrials.gov identifier: NCT01931566 and NCT02284906) were terminated early because of a lack of efficacy on primary outcomes, namely a change in composite cognitive score over 24 months, compared to placebo. Overall evidence from trials to date is deemed low quality due to risk of bias in the studies and imprecision of results, for example, lack of data on blinded assessment of outcomes, inconsistencies with the primary outcome measures, patient selection and exclusion criteria, low event rates and wide confidence intervals [36]. Furthermore, RCTs of antidiabetic medication as an intervention for dementia usually were in populations with MCI, mild dementia cases [38] or those genetically at risk for dementia [27, 29, 39-41], and mostly exclude participants with a diagnosis or treatment of T2D, and in some cases, exclude based on glucose level thresholds [42]. There are very limited studies which have included at least some participants with diabetes [43, 44], which in turn enables a different research question to be addressed of whether there are beneficial effects of AD disease progression in diabetic patients with AD. In these cases, the placebo group often continues their existing treatment for T2D, apart from the antidiabetic agent of interest in the trial. This is a significant challenge and more evidence is needed from larger studies enrolling patients with and without T2D, with a comprehensive history and a range of treatments to enable subgroup analyses.

We also recommend that epidemiological and RCT studies make it clearer in their documentation whether participants with T2D were excluded, and if so, how this is defined, given that this information is often not easily accessible.

1.5. Methodological differences between observational studies and trials, discussion and recommendations for future work

Epidemiological studies and RCTs have heterogeneity and methodological variations that make them difficult to compare. The two approaches often differ in diagnostic criteria and duration of T2DM; treatment, duration and dosage of antidiabetic agents; follow-up times; populations under investigation; and cognitive outcomes [19], with trials having been limited in their attempts to reproduce real-life exposures and outcome effects.

Recommendations detailing the potential for alleviating such limitations in future work in T2DM and cognition include:

i. Where randomisation in trials offer gains in precision of controlled exposure and removal of confounding, RCTs do not mimic real-life exposures. For example, many studies do not consider duration of T2DM, prior management and antidiabetic agent(s) of choice, or consider the underlying metabolic effect of treatment, such as the level of glycaemic control, hyperinsulinemia and insulin resistance, on cognitive impairment.

   Our recommendation is on measurement of exposure: Given the dynamic metabolic features of T2DM, complex risk factors, comorbidities and complications of T2DM, future RCTs and observational studies should take a thorough approach to life course phenotyping participants including measurement of underlying metabolic

features and comorbidities, duration of T2DM and medication history, which will enable suitable matching, monitoring and ability to better address these potential confounders and mediators in the study design.

ii. Randomisation may weaken the exposure signal since, however precisely isolated it is for the trial, it is likely to occur with complex comorbidities in real life.

Our recommendation for treatment:, given that dementia mostly results from complex progressive disorders, it may be reasonable to conduct trials with drugs that have actions at multiple targets [45] and for multi-modal trials for dementia [46]

iii. Existing RCTs in this area lack reliable measures to detect clinically relevant cognitive change and have frequently been of short duration when considering the assessment of cognitive change. Most studies have used the MMSE, which is not sensitive to early or subtle changes in cognition over short time periods and which may be less sensitive to vascular cognitive impairment [47].

Our recommendation on measure of outcome, future trials should aim to capture long enough follow up to measure clinically relevant change and to facilitate this using a battery of tests designed to cover a range of domains of cognitive function, capture individual-level changes in cognition [48] and differentiate premorbid abilities (i.e. using discrepancies between crystallised and fluid functioning, whereby the former is relatively spared in preclinical AD [49]

iv. Epidemiological studies and clinical trials have differing drivers for sample selection and attrition.

Our recommendation for sample selection and follow-up: future studies examining the relationship between diabetes and cognition should carefully characterise participants to include appropriate at-risk populations. Studies should also aim to build in mechanisms for longer-term outcome collection, ideally through longitudinal prospective data collection that integrates phenotyping of features of T2DM (hyperglycaemia and insulin resistance) across the life course when the exposure

may exert maximal influence and follow-up, even in the face of shorter-term differential attrition.

2. CHOLESTEROL / STATINS

2.1. Cholesterol, statins and dementia, an introduction

Multiple epidemiologic studies have shown an association between reduced dementia risk and statin use reporting odds ratios of 0.6-0.9. [50-57] Experimental data using both in vitro and in vivo animal models of AD suggest pleiomorphic effects of the statins in relation to the pathogenesis of degenerative disease [58]. Such effects include direct actions on cholesterol-lowering, influences on related cardiovascular risks including type 2 diabetes and hypertension, alterations in inflammatory pathways, modulation of intracellular trafficking and neurotransmitter release, as well as indirect effects on β-amyloid and tau -related alterations that are associated with neurodegeneration. [58]

2.2. The “Statin Paradox” Introduction and mechanisms

Statins exert their primary effect by competitively inhibiting HMG-CoA reductase, the first and key rate-limiting enzyme of the cholesterol biosynthetic pathway [58]. Statins mimic the natural substrate molecule, HMG-CoA, and compete for binding to the HMGCR enzyme. This directly leads to effects on overall circulating cholesterol levels. The indication for statin use includes reduction in hypercholesterolemia, which has been linked to increased risk of cardiovascular and cerebrovascular events. Such consequences can be directly responsible for the development of cognitive impairment and dementia; or, more frequently, can be associated with cerebrovascular disease that interacts additively and possibly even

synergistically with other neurodegenerative pathways [52]. Much research has also suggested that genetic alterations affecting cholesterol trafficking and modulating pathways are directly related to increased risk of AD, suggesting the potential for other risk reduction pathways [50].

2.3. Cholesterol and statins, the epidemiological evidence

The epidemiologic associations between statin use and reduced risk of dementia have been reviewed in several recent publications including an update of the Cochrane database [50, 53-57, 59, 60]. These data clearly demonstrate an association between statin use and a lowered risk for all-cause dementia and AD specifically, but interestingly provide conflicting results for the reduction of dementia caused by cerebrovascular disease. The influence of ageing adds complexity here since much work in the field is focused on the relationship of midlife rather than late life hypercholesterolemia in modulating dementia risk .[51]. Accordingly, some of the variability seen in epidemiologic studies may be related to timing and exposure characteristics for the statin therapy identified as possibly modulating risk for future decline in cognition and in the development of dementia. Yet other work has suggested that the various statin drugs are not uniform in their effects on degenerative disease processes but instead have specific characteristics that may differ. Consequently, when statins are clustered as a uniform exposure in epidemiologic association studies, such exposure may reduce the opportunity for clarity and may lead to inconsistent results [56, 61]. Major factors include type of statin, dosage, length of exposure, and timing in the life-course when exposure occurred. Yet, the data are conclusive enough to warrant clinical trials of statin therapy to reduce the risk and or delay the progression of cognitive decline and degenerative dementia.

2.4. Cholesterol and statins, the clinical trial evidence for statins and their influence on dementia risk

Several studies have therefore investigated the hypothesis that statin therapy may be beneficial for the treatment of dementia. However, despite the promising epidemiological and observational data, results have been disappointing [56, 61], as the trial data appear to contradict the epidemiologic data. Attempts at an explanation for this discrepancy have focused back on the multiple sources of low precision inherent in the epidemiologic studies, including again the type of statin, dosage, length of exposure, and timing of exposure in the life-course [56, 61]—figure 1. In addition, many trial design considerations may explain the discrepancy. These include: inclusion and exclusion criteria that restrict participants in ways that are inconsistent with observational studies, e.g., different population characteristics, selection of statin, and dose, duration of exposure, and timing in the life-course that are again discordant with observational results [22, 53, 61]. We consider each of these considerations in the sections below.

Inclusion/exclusion criteria: One critical difference between the many null-finding statin clinical treatment trials and observational studies is that persons enrolled in clinical trials were not recruited based on dysregulated lipid status [53, 61]. Indeed, some trials excluded from enrolment participants whose lipid status revealed dysregulation [53, 61]. The contrast with clinical use (and resultant observational studies) is obvious. Secondary analyses of the data from several clinical trials have implicated genetic background, especially *APOE ε4* status as a primary modulator of statin effects that may be related to risk of cognitive decline in dementia [50]. Further trials should take such considerations into account when designing maximally appropriate inclusion/exclusion criteria.

Selection of statin: Clinical trials of statins for cognitive outcomes have focused largely on atorvastatin and pravastatin. While other, smaller trials included other statins, meta-analytic studies of the potential beneficial effect of statin therapy have typically considered statins as a single group. Yet clinical experience suggests that the statins are quite diverse in their

effects on HDL, as well as LDL modulation. Common practice dictates that if a patient fails one statin, another agent should be tried. Such flexibility in selection of agents has not yet been incorporated into clinical trial methodology. Thus, many who are intolerant of the assigned statin in a trial might have benefited from an alternate drug.

Statin dose: the dosage of statins in clinical trials for the prevention of cognitive decline and dementia have typically been in the mid-range based on studies of systemic cholesterol modification, without the inclusion of adaptive trial design to enable maximum dosage for unique participants. This issue relates partially to the usual inclusion and exclusion criteria for such trials, which, unlike clinical use, do not consider the type or severity of dyslipidemia when selecting statin agent or dosage [53, 61]. At least with respect to dose, consideration of an adaptive design protocol might allow flexibility in optimizing dose, based on systemic pharmacodynamic profiles, for prevention of cognitive decline. To date, a CNS-specific pharmacodynamic profile that might guide optimal statin dosing for dementia prevention has not been established.

Duration of exposure: The majority of clinical trials testing statin use for the prevention of dementia or cognitive decline have had relatively short durations, typically about two years [53, 61]. By contrast, the observational data on cognitive consequences of statin use for modulation of cardiovascular risks suggests a much longer duration of exposure may be necessary for the desired effect on cognition [54, 56]. Prolonged trials of statin therapy should therefore be considered when designing new trials of statins for the prevention of cognitive deficits.

Timing of exposure across the life-course: As noted above, a critical issue with the discrepancy between observational and clinical trial data regarding potential benefits of statin therapy in preventing cognitive decline may be the timing of exposure across the life-course. Observational studies often include exposure at any point in the life-course,

especially in midlife or early old age [54, 56]. By contrast, most statin trials to date have enrolled persons at older age and several with some level of existing cognitive impairment, when, arguably, a great deal of neural damage is already evident [53, 61, 62]. While it would be prohibitively costly to conduct a clinical trial that tests later life cognitive consequences of midlife exposures, there may be ways to achieve the same aims, using new technologies to detect early changes of neurocognitive disorders or ancilliary cognitive studies of midlife trials, looking at the late life conversion to dementia, such studies may ultimately provide the answers as to whether statin therapy can intervene in the development of late life cognitive decline and dementia.

2.5. Statins and cognition, conclusions and recommendations for future work

While the number of prospective, randomized, placebo-controlled clinical trials that have failed to provide evidence for benefit of statin therapy in reducing the incidence of cognitive decline in dementia argue strongly against further investigations in this area, the data supporting the use of such therapy from observational studies is overwhelmingly supportive of further investigations.[50, 51, 53-61, 63, 64] The field is now poised to look back and reconsider essential clinical trial flaws in the design and conduct of such research in an attempt to improve on the critical confounds of: inclusion and exclusion criteria for the participants, selection of statin, statin dose, duration of exposure, and timing in the life-course when the exposure should maximally exert its influence.[56, 61] Understanding the discrepancies between observational and clinical trial data regarding the use of statins for the prevention of cognitive decline in dementia is critical to uncovering whether the observational data represents pure epi-phenomena, unrelated to the underlying disease course.

Recommendations for future clinical trials of statin therapy include

i. Selection of an appropriate population including those with cholesterol/lipid dysregulation
ii. adaptive design in the selection and dose of statin therapy
iii. enhanced duration of exposure with consideration of timing within the degenerative cascade when therapy may prove most beneficial. Creative approaches such as ancilliary cognitive studies of midlife trials, looking at the late life conversion to dementia are warranted.

3. BLOOD PRESSURE AND ANTIHYPERTENSIVES

3.1. Blood pressure and antihypertensives introduction

Epidemiological evidence has consistently shown a relationship between higher blood pressure [BP] and an increased risk of developing cognitive decline and dementia [65]. Several plausible mechanisms support the potential for raised BP driving impairment in brain structure and function [66]. Blood pressure reduction is possible via several established classes of antihypertensive medication that are widely available and present in treatment pathways for cardiovascular risk reduction [67]. However, relatively few trials of antihypertensive drugs have measured cognitive outcomes or incident dementia, and those that have, have been largely inconclusive.

3.2. Potential mechanisms linking raised blood pressure to impaired cognition

Mechanisms by which raised blood pressure may lead to impaired cognitive function and dementia have been summarised elsewhere [66, 68] . They include damage to the vascular structure (e.g. increased risk of clinical and sub-clinical stroke, promotion of atherosclerosis, vascular remodelling and stiffening reducing effective perfusion, small vessel disease

leading to white matter lesions and microvascular rarefaction leading to loss of microvessels), and to function (e.g. disruption of endothelial cell function leading to impaired microvascular flow, disruption of the neurovascular coupling attenuating the ability for cerebral blood flow to respond to neural activity, impaired autoregulation and loss of blood brain barrier integrity). [66, 68, 69] There is also evidence to suggest that high blood pressure and vascular risk may be associated with deposition of beta amyloid [66, 70-72]

3.3. Epidemiology of blood pressure and cognition

Alongside the plausible mechanisms there are a large number of epidemiological studies linking raised blood pressure to incident cognitive decline or dementia [73, 74]. This is particularly the case for raised blood pressure in midlife, implying a role for ageing similar to the evidence for raised cholesterol. A 2005 review highlights 11 of 13 studies reporting a relationship between higher blood pressure and incident cognitive decline or dementia in populations aged in their 40s-50s and followed for around 20 years [65]. In contrast, for populations in their 60s and 70s whilst high blood pressure remains a risk factor the evidence is more mixed. The same 2005 review found only 6 of 21 studies reporting higher pressures in later life associated with increased risk and a further 3 reporting a U-shaped relationship, with both low and high pressures associated with increased risk [65]. More recent work supports the need for a life course perspective highlighting characteristics particularly relevant to blood pressure [75, 76]. For example, chronicity, the change in diastolic and systolic pressure with ageing and the steeper rise and subsequent fall in pressure observed 2-5 years prior to dementia diagnosis and the potential for differential mortality in higher and lower BP populations. It is in the context of this epidemiology that we must examine evidence from the trials.

3.4. Antihypertensives, randomised controlled trials and dementia

Several randomised controlled and blinded trials of antihypertensives have assessed cognition or dementia outcomes. However, their results have been largely inconclusive [77, 78]. In general, cognition and incident dementia have been secondary endpoints, or assessed in ancillary studies, in trials designed primarily to examine the cardiovascular benefits of antihypertensive use in later life populations. This point has driven three main issues when considering evidence for the potential of antihypertensives to reduce risk of cognitive decline and dementia: (i) the length of follow up, (ii) the selection of an appropriately aged population and (iii) the assessment of cognitive function and cognitive decline.

(i) The primary focus on cardiovascular outcomes has typically resulted in relatively short follow up for cognition, and some trials have even been stopped early following observed cardiovascular benefit. The early stopping and lack of long follow-up (most are less than the recommended minimum of 5 years [78, 79] has very likely exacerbated a lack of statistical power to detect cognitive and dementia outcomes, as these develop more gradually over time. For example, mean follow-up in antihypertensive trials that have measured dementia [double-blind randomised phase rather than longer term open label follow-up] ranges from 2.0 to 4.3 years [77]. (ii) A common focus of anti-hypertensive trials for elderly individuals may also mean that the intervention ignores the most relevant, younger (midlife, or earlier adult life) target population for cognition and antihypertensive use. The trial populations have, by design, been drawn from people in early late life or older. Most of the trials recruited populations entirely from later life (≥60 years) and even the trials open to including people in their 50s arrived at mean baseline ages in the mid-60s. Trials that report on cognitive outcomes show similar issues [78]. (iii) Most of the trials have also used a relatively insensitive cognitive screening instrument as the primary cognitive assessment tool. This limits their ability to detect more subtle cognitive change [78].

Trials in this area have also been constrained by the development of the cardiovascular evidence base. That is, as the cardiovascular evidence base has grown the drug prescribing

guidelines and thresholds for treatment have changed. Guideline changes to recommend treatment in a new population drives consequent ethical requirements to treat thus shaping the populations that can be selected for each subsequent trial, or having limiting effects on recruitment due to accommodating aspects around prior exposures [80]. This has driven each new trial to recruit to different baseline blood pressures, ages or cardiovascular risk profiles furthering the heterogeneity across the evidence base.

Despite these limitations, there is a growing evidence base for antihypertensive treatment as having a role in dementia risk reduction ,[81, 82]. Meta-analyses, particularly those that focus on double-blind trials generally find point estimates (Odds Ratio, Relative Risk, Hazard Ratio) around 0.9 in favour of antihypertensive treatment reducing risk of dementia [83, 84] and showing a potential for dose-response [77, 83, 85]. For example, trials that achieved greater than 10mmHg reduction in blood pressure between their two randomised arms had a combined 12% (95% Confidence Intervals 22%-2%) risk reduction for incident dementia compared to a non-significant result (RR0.98 (95% CI 0.88-1.09) in those who did not achieve this difference [77]. Questions remain as to the ideal range of blood pressure for brain health which may be specific to different levels of chronological, or more likely biological age and by prior BP exposure. Furthermore, recent and potentially paradoxical results from the Systolic Blood Pressure Intervention Trial (SPRINT-MIND) [85, 86] have highlighted the possibility of increased cognitive risk from lowering blood pressure too far [86]and served to once again highlight the complexities and knowledge gaps in this area.

3.5. Blood pressure and antihypertensives, summary and recommendations

In summary, although overall the direction of the epidemiology and clinical trial evidence is broadly congruent, and more congruent than some of the other risk factors, this is still insufficient to tell us whether reducing blood pressure for dementia risk reduction is effective.

Recommendations for future work on antihypertensives, blood pressure and cognition include:

i. new sophisticated analysis of the existing epidemiology and clinical trial data. Eg using causal inference methods and more appropriately taking account of competing risks alongside using more sophisticated modelling to examine the role of different achieved BP levels and attrition.
ii. new data collection is needed to evaluate relevant populations. In particular we need a clear understanding of the relationship between BP and cognition over the life-course, and at ages 20, 30 or 40 years prior to dementia onset for example by collecting longer term prospective or even retrospective data on both blood pressure, cognition and antihypertensives
iii. related to point (ii) above we also need a better understanding of the role of trajectories of change in blood pressure and any consequent change in ideal blood pressure ranges (alongside change in other dementia influencing factors)
iv. we need to start using sufficiently sensitive cognitive outcome measures.

4. ESTROGEN AND HORMONE THERAPY (HT)

4.1. Hormones, HRT, introduction and potential mechanisms

Estrogen and supplementation using oral HT have been proposed as a treatment for observed changes in memory and dementia risk of women experiencing menopause. There are several plausible biological mechanisms for cognitive benefits from estrogen supplementation after menopause [87, 88]. Estrogen receptors are widespread in the brain, and regulate synaptogenesis [89], particularly in the hippocampus [90]. For example, rats show reduced density of dendritic spines after oophorectomy [91] . Estrogen also interacts with or modulates neurotransmitters important for cognition such as dopamine and serotonin [89, 92]. Animal studies have also provided evidence for a ‘sensitive period’ during which the therapeutic benefit of estrogen supplementation may occur, and suggest that estrogen

mediated cognitive benefits may be lost if treatment is commenced before, or after a specific age [93] .

4.2. Epidemiology, HT and cognition

Systematic reviews of the epidemiological data have consistently shown that HT is associated with reduced risk of late-life dementia [4]. Most cohort studies that report on HT in relation to dementia outcomes make comparisons between women who have 'ever' used HT with those who have 'never' used HRT. [94]. Data are lacking on estrogen creams and use of HT for short periods e.g. less than six months [94].

Positive early observational findings [95-97] ranged from a 39%-50% effect size for reduction in AD risk associated with HT use. Comparable evidence was demonstrated in one review which showed that the strongest evidence for HT in AD risk reduction came from 2 cohort studies, and 10 case-control studies, which showed a pooled 34% decrease in AD risk [95% CI, 18%-47%] [89, 98]. An additional review found the pooled risk ratio of cohort studies using HT in AD prevention to be 39% reduction [95% CI, 24% - 54%][1]. More recent observational evidence has also suggested a benefit of HT on cognition in postmenopausal women, with longer duration associated with greater benefit in the population based Cache-County cohort study [97, 99]. The 12-year follow-up of the Cache-County study found a significant 'sensitive period' effect, with timing of HT commencement being significantly related to cognition [3MS scores] such that those commencing within 5 years of menopause performed better than those commencing HT six or more years following menopause, with greater benefit conferred to older women [99].

Early observational data were subject to significant confounding, with depression typically not controlled, and the women who were prescribed HT being more educated, in better overall health prior to HT commencement, and leading healthier lifestyles than women not given HT [100, 101]. LeBlanc et al. also note potential bias by contraindication in

observational studies whereby women who already have dementia are less likely to receive HT due to issues relating to compliance and interactive effects between the HRTs and existing medications [98]. Error may also be introduced in reporting, with many studies using proxy reports which could lead to bias due to the proxy being unaware of any previous HT use. A limitation of the meta-analyses of the observational data are the lack of consistency in the information on age of exposure [4]. When measures are taken several years apart in panel surveys the exact timing of HT in relation to menopause may not be clearly specified.

4.3. Clinical trial evidence, HT and cognition

A systematic review of the clinical trial evidence for the effect of HT on cognitive outcomes did not find benefit [102]. The Women's Health Initiative Memory Study [WHIMS] was a double-blind, placebo-controlled clinical trial examining 8300 women aged 65 and above over a 2-year period to observe effects of HRTs and dementia progression. The trial failed to find a beneficial effect for HT in reducing dementia risk, instead finding an increase in all types dementia [103, 104]. One explanation for the discrepancy between WHIMS and early observational findings is differences in timing of treatment onset. Whereas observational studies followed women who had commenced HT during menopause, in WHIMS, participants were randomly allocated long into the post-menopausal phase [94]. The 'sensitive period hypothesis' suggests both the observational and WHIMS findings may be accurate, with differences in effects being accounted for by the timing of treatment onset, rather than methodological concerns [94].

Examining variation in the timing of treatment initiation, one review of RCTs found little support for the effects of HT on cognition in older women [65 years and above], although cited potential benefits to younger women [<65 years] for HT across certain cognitive domains. The author found this was especially true of women who had symptomatic menopause and were more recently menopausal [105]. Despite this, the author noted that

while larger RCT data for older women with late-life HT exist, there is a dearth of larger RCTs that examine HT in younger women. One review of 22 double-blinded RCTs found that only 30% of women were 50-59 years old during baseline, the age at which women are mostly likely considered for HT to alleviate symptoms[102]and most likely relevant to the sensitive period hypothesis .

LeBlanc and colleagues [98, 106] reviewed RCTs on HT and cognition and found significant heterogeneity in the cognitive tests employed across HT RCTs. Across 9 RCTs, more than 40 different tests were utilised, and within consistently used tests only 7/40 were used across more than one study and with varied administration. Regarding treatment, RCTs were inconsistent in the duration of administration, specific dosage, and formulation used [only 2 studies used same formulation and dose] [98]. The authors concluded that there is currently insufficient data regarding the attenuating effects of varied formulations and dosages on cognition. These studies also tended to be of poorer quality [only one out of ten rated as 'good']. Other authors suggest effect sizes of RCT findings are often limited by a large age range [107] and inclusion of participants with early- and late-onset AD at baseline [100, 107]. Despite the above it is also important to note that given evidence for longer duration of HT use being associated with increased risk of cardiovascular disease, breast cancer, and stroke, HT is not currently recommended for treatment in prevention of cognitive decline, or dementia [102, 108]

4.4. Hormones and HT, summary and recommendations

In summary, despite biological plausibility for estrogen being neuroprotective, and some positive findings from observational studies, the potential of HT to reduce risk of cognitive decline and dementia is not found in RCTs to date. There are several important gaps in this literature.

Recommendations for future studies in HT and cognition include:

i. The effects of long-term HT use in perimenopausal women, and postmenopausal women aged 50 and below on cognition should be evaluated.
ii. The potential role for HT type should be considered in relation to risk of dementia, with other women's health variables such as hysterectomy and oophorectomy also included for consideration.
iii. Data are needed on the association between HT and VaD or other non-AD dementias in the observational literature [4]
iv. There is a greater need for evidence for more globally diverse data for HT in order to understand effects not only across the life-course, but across socio-demographic, racial and cultural backgrounds [4]

5. INFLAMMATION AND NSAIDs

5.1. Inflammation and NSAIDs, introduction

In 1988 Joseph Rogers and Patrick McGeer reported the presence of HLA-DR and other T-immune cell markers around neuritic plaques in AD brains [109, 110]. Sensing that such immune activity was probably contributory (not adaptive) to AD pathology, McGeer studied the relationship between rheumatoid arthritis (RA; almost always treated with anti-inflammatory drugs) and AD [111]. AD appeared to be rare in RA patients, and vice versa. Among four explanations for this finding, McGeer considered the possibility that "AD (does) indeed develop less often in the RA population, but this is unrelated to anti-inflammatory drugs." [111] Alternatively stated, he noted the possibility of confounding by indication.

5.2. NSAIDS, Further observational data

Two years later, a co-twin control study investigated a broad agnostic array of antecedent exposures in 50 AD-discordant twin pairs. This search revealed only that a history of arthritic conditions or anti-inflammatory treatments was inversely associated with occurrence of AD [112]. The study's authors then investigated NSAID use vs. AD in a sample of siblings from families with a multiplex history of AD dementia [113], finding an inverse association between a report of sustained NSAID use and onset of AD. These analyses considered a historical report of "arthritis" (not otherwise specified), which appeared not to modify onset except in those treated with NSAIDs. In the ensuing years, numerous epidemiological studies – some including attempts to control for confounding by indication and inclusion of a control exposure (acetaminophen / paracetamol) – suggested a benefit of sustained NSAID use. This trend reached its zenith with publication in the New England Journal of Medicine of findings from the Rotterdam Study [114]. The Rotterdam cohort was relatively youthful for an investigation of dementia (median age at entry mid- to late-60s). Relying on a prescription registry, it suggested a time-dependent inverse association between AD and NSAIDs, culminating in an 80% reduction in incidence for persons with $\geq$ 5 years of continuous NSAID use.

5.3. Contrast with randomized controlled trials of NSAIDS

The following years witnessed a series of carefully conducted randomized controlled trials (RCTs) that failed to affirm the observational findings. The Alzheimer's Disease Cooperative Study (ADCS) reported clinical trials of prednisone (a powerful immunosuppressant) and, a few years later, two NSAIDs (naproxen and rofecoxib). Both failed to show benefit in AD patients [115, 116]. A trial of the anti-malarial drug hydroxychloroquine (which also has substantial immunosuppressant activity) showed no benefit [117]. An RCT of rofecoxib (a selective COX-2 inhibiting NSAID) failed to suggest that drug's ability to postpone "conversion" of MCI to AD dementia. [118] Here, the hazard ratio (HR) for conversion to AD

with assignment to rofecoxib was a worrisome 1.46 (95% CI: 1.09 - 1.94). Shortly thereafter, the ADAPT research group reported similarly adverse findings in 25 incident cases, relating risk of incident AD dementia to treatment of asymptomatic elderly (age ≥ 70 years) with the COX-2 inhibitor celecoxib (HR 4.11, 95% CI: 1.30 - 13.0) or naproxen sodium (HR 3.57, 95% CI: 1.09 – 11.7) vs. placebo. [119]. Because ADAPT was stopped early, its incident AD cases became evident after no more than 3 years of treatment, suggesting that these persons had advanced pre-symptomatic disease when treatments were initiated. The latter conjecture was supported to some degree in the three-year ADAPT Follow-up Study, which showed dissipation of the adverse associations, [120] and by a detailed analysis of the original ADAPT data suggesting that naproxen treatment accelerated cognitive decline among the one-third of participants showing the greatest rate of decline. [121].

These findings seemed to suggest that the ideal population for NSAID treatment would be at-risk "young-elderly" persons without inflammatory disease. Participants should then be further removed from their possible age at onset of AD dementia. But the difficulty for such trials lay in measurement of the progression of pre-symptomatic AD. Only with such measurement could one expect to see that NSAID treatments would retard this progression. Attempting to address this problem, Canadian investigators assembled a younger (median age 63 yrs) asymptomatic cohort for PResymptomatic EValuation of Experimental or Novel Treatments for AD (PREVENT-AD cohort).[122] Their risk of AD was likely increased by a requirement that each had a parental or multiple-sibling history of AD dementia. They were evaluated annually using the 45-minute Repeatable Battery for Assessment of Neuropsychological Status, [123] and a broad array of other evaluative procedures, as detailed in reference [122] and a companion paper that describes the development of a composite indicator of pre-symptomatic AD progression, the "Alzheimer Progression Score" (APS). [121]

Some 200 members of the PREVENT-AD cohort were enrolled in INTREPAD, a two-year placebo-controlled (RCT) of naproxen sodium 220 mg BID [124]. The INTREPAD primary outcome was the APS – after validation efforts in the remaining ~175 PREVENT-AD participants had shown its excellent longitudinal stability and portability to the trial sample. Slightly more than half of INTREPAD participants also donated annual CSF samples for immune marker studies [125]. The trial results indicated: 1) a significant increase in participants' APS over the two-year trial interval; but 2) no suggestion of any mitigation in this change among naproxen-assigned individuals. No single component of the APS showed any suggestion of benefit from naproxen.

5.4. Later observational studies affirm the trial results and suggest adverse consequences of NSAID use among very elderly persons.

Perhaps resolving the discord between trial and observational study results, more recent observational data appear mostly to side with the available trial results. Since 2000, numerous investigations have shown null or worse association between NSAID exposure and AD incidence. A consistent feature of these later studies was their reliance on populations considerably older than the Rotterdam cohort. Thus, the elderly (age at entry 65 – 106 years) population-based MoViES cohort study found no association of NSAID use with occurrence of AD (data described in [126]) Similar results were observed in the Religious Orders Study – Memory and Aging Project (mean age at entry = 75 years with mean follow-up 12 yearrs). [127] Perhaps most surprising, results from the population-based Adult Changes in Thought observational study suggested a strong apparent *increase* in AD incidence among "heavy" users of NSAIDs (data from computerized prescription registry; Hazard Ratio 1.66 with 95% CI 1.24 – 2.24). [128] These persons had consumed $\geq$ 500 defined daily doses of NSAIDs over two or more years but were again quite elderly, with a median age at entry of 75 yrs and follow-up typically of a decade or more. Given the well-known epidemiologic relation of age to AD incidence (e.g., >20% cumulative incidence by

age 80), and recent awareness that AD pathological changes begin a decade or more prior to symptoms, cohorts in their late 70's and beyond would likely include >30% of participants with demonstrable evidence of (pre-symptomatic) AD pathology [129, 130]. In sum, the single most consistent finding of the observational data on NSAIDs appears to be a lack of benefit (and even a potential for harm) when persons in later old age are exposed to NSAIDs.

5.5. Should we attempt further RCTs for AD prevention using NSAIDs? Summary and recommendations.

The disappointing results from INTREPAD suggest that participants in any new trial should be even younger, probably under 60 years of age, and perhaps without prominent AD risk factors. The size and duration required for such a trial would likely render it prohibitively costly and difficult to execute. If this sort of trial were, nonetheless, contemplated, its sponsors should probably consider several other experimental findings:

- Should the trial choose a different NSAID intervention? Only a select group of NSAIDs have a capacity to inhibit gamma secretase activity, which is an important step in the cleavage of the amyloid precursor protein to Aβ fragments, ostensibly essential (if perhaps not causal) for early AD pathogenesis. [131]. Some authors have therefore lamented the fact that none of the completed NSAID RCTs tested ibuprofen or other "gamma secretase-modulating" (GSM) agents. But observational data, at least, suggest that GSM activity may not be important. A meta-analysis of six key cohort studies whose 17,000 participants had contributed 77,000 person-years of observation showed the familiar result of reduced dementia incidence among chronic NSAID users [126]. But the data failed to show any difference in

apparent “protection” offered by GSM NSAIDs compared with others, or in the apparent effects of their most common exemplars ibuprofen and naproxen.

- Will the chosen intervention cross the blood-brain barrier in sufficient concentration to modify the brain “inflammatory” (innate immune) changes that accompany AD pathogenesis? Findings among INTREPAD participants showed that treatment with low-dose naproxen (the conventional NSAID most commonly used in AD trials) produces appreciable levels in the CSF [125]. These levels represent only about 1% of concentrations found in plasma of treated subjects, but this result is not necessarily surprising given that about 99% of naproxen in plasma appears to be protein-bound (and therefore of doubtful effect.
- Will the chosen agent have appreciable effects on important immune and inflammatory markers in CSF (therefore, probably in brain)? Another finding from the study of INTREPAD CSF was that assignment to naproxen resulted in little or no consistent change in levels of important immune markers indicating “inflammatory” brain changes. Accordingly, there may be significant concern that none of the NSAID treatment or prevention trials used “anti-inflammatory” agents that would be likely to affect the changes described by Rogers, McGeer, et al.

5.6. Concluding thoughts on the disparity between NSAID trial and observational results.

The earliest published work on this topic considered the possibility of confounding by indication. None of the described observational studies was able in multivariate analyses to exclude the possibility that an apparent benefit with NSAIDs was attributable to confounding by an inflammatory diathesis. In particular, the above-cited meta-analysis of six cohorts [126] considered the possible influence of an “arthritis” (mostly osteoarthritis) variable. As in several other studies, this variable appeared to strengthen the inverse NSAID – AD association (arthritis sufferers are probably obligatory NSAID users). Notably, however, the “arthritis” variable itself was associated with diminished AD incidence, even after “adjusting”

for reported NSAID use. If reproducible, this finding suggests little reason to expect trial results to affirm a benefit of NSAIDs in persons without evidence of inflammatory disease (an exclusion criterion in all the cited trials). We have therefore come to have strong doubts about the possible benefit of NSAIDs for AD prevention. Instead, we recently conjectured, (as first discussed in McGeer's pioneering work) the aforementioned ". . . results may suggest re-consideration of . . . a pro-inflammatory diathesis (itself) as a possible explanation for the reduced AD incidence among (relatively young) NSAID users in observational studies," [124] i.e., confounding by indication.

Recommendations:

i. Future work on pharmaceutical interventions for dementia risk reduction must remain vigilant to potential sources of bias, not least those of reverse causality and confounding by indication.
ii. Any contemplated new trial of anti-inflammatory interventions for AD prevention should avoid enrolling very old participants or others with evidence of advancing pre-symptomatic AD pathology.

6. OMEGA-3 FATTY ACIDS AND SUPPLEMENTATION

6.1. Omega 3 and supplementation, introduction

Mediterranean [132], Mediterranean-Intervention for Neurodegenerative Delay (MIND)[133, 134] and prudent [135, 136] dietary patterns have been associated with slower cognitive decline and lower risk for developing AD. These associations may be attributable to the higher intake of plant-based foods and seafood dense in unsaturated fatty acids, vitamins and minerals, flavonoids and polyphenolic compounds and there is some evidence associating increased seafood consumption, omega-3 intake, or omega-3 blood levels with lower risk of dementia, or of cognitive decline [137]. Isolated components from these diets,

including the omega 3 polyunsaturated fatty acids (n-3 PUFA) [138, 139] and the homocysteine lowering B vitamins [140-142] (section 8 below) have been formally tested in slowing cognitive decline or AD progression but the results of randomised clinical trials have been inconsistent. This section, and the following section 8 provide updates and insights into n-3 PUFA and B vitamins respectively in the pursuit of developing more effective nutritional based interventions for prevention of age-related cognitive impairment and dementia.

Omega-3 polyunsaturated fatty acids (n-3 PUFA) have a variety of bioactive properties that regulate physiological functions and there are various potential mechanisms for the role of n-3 PUFAs in cognition. The two major n-3 PUFAs are docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]. DHA is quantitatively the most abundant n-3 PUFA in human brains, whereas EPA is present in very limited amounts [143]. The small concentration of EPA in the brain does not necessarily translate into a weak biological activity. Given that EPA and DHA can inter-convert in vivo[144], it is possible that both or either fatty acid may have similar neuroprotective effects. Although EPA is reported to have greater anti-inflammatory effects [145] and has been associated with greater white matter integrity [146], since the majority of preclinical studies to guide pharmacokinetics [PK] and pharmacodynamics [PD] were conducted using DHA[147], we focus on DHA in this review. Importantly neither EPA and DHA cannot be synthesized *de novo* but can be obtained from diet/supplementation.

In contrast to the pre-clinical studies in AD mouse models that bring some support a role for long-term and high dose omega-3 fatty acid intake in improving measures of cognition, clinical trials testing the effect of omega-3 supplementation on cognition have largely been disappointing. We examine the pharmacological properties of omega-3s in the brain in relation to study designs to understand this discrepancy.

6.2. Omega-3 and cognition, epidemiology

A possible role for PUFA n-3 consumption was also shown in a meta-analysis of 21 longitudinal studies (181,580 participants) with 4438 dementia cases reporting that a 1-serving/week increment of dietary fish was associated with lower risks of dementia (RR: 0.95; 95%CI: 0.90, 0.99; P = 0.042, I(2) = 63.4%) and AD (RR: 0.93; 95%CI: 0.90, 0.95; P = 0.003, I(2) = 74.8%). More specifically, the increment of dietary DHA intake was associated with lower risks of dementia (RR: 0.86; 95% CI: 0.76, 0.96; P < 0.001, I(2) = 92.7%) and AD (RR: 0.63; 95% CI: 0.51, 0.76; P < 0.001, I(2) = 94.5%) [137]. The KORA (KOoperativen Gesundheitsforschung in der Region Augsburg)-Age study have also reported a cross-sectional association between low omega 3 index (< 5.7 %) and cognitive impairment in an elderly population of 720 participants with cognitive status ranging from cognitively normal to suspected dementia [148].

6.3. Omega-3 cognition and clinical trials

Overall, the effects of omega-3 supplementation on cognition have been disappointing in several randomized clinical trials [149]. One possible explanation is the confounding effect observed in observational studies, where lower omega-3 levels could represent biomarkers of poor dietary networks[150] that affect several factors (other nutrient levels, lifestyles or risk factors) and therefore intake or levels of omega-3 per se may not be causally related to dementia. However, there is good biological evidence that omega-3 intake has neuroprotective effects in AD animal models [151]. It is plausible omega-3 supplementation started after the onset of significant neurodegeneration is too late, where the disease process may not be reversed by omega-3 supplements. There are many challenges for conducting prevention trials include indentifying an omega-3 dose that gets to the brain, the population that may benefit from supplementation, the duration of supplementation and sensitive cognitive outcomes.

6.3.1. Omega-3 fatty acids, dose and delivery,

Animal studies provide useful information on DHA brain pharmacodynamics with AD biomarkers as readouts [amyloid, tau, synaptic functions and makers of neurodegeneration]. In a systematic review, Hooijmans et al [151] reported cognitive and AD biomarker benefit using doses of DHA supplementation [0.6-0.24 g/kg/day]. Accounting for different body surface areas of mice and adult men with a correction factor of 0.08, [152] the equivalent human DHA doses to replicate these preclinical studies would range from 0.048 and 0.19 g/kg of DHA per day. This would be equivalent to providing 3.36 to 13.3 grams of DHA per day for a 70 kg individual [Table 2]. These large doses of triglyceride-DHA formulas are unrealistic for human consumption and implicate the need to develop alternative DHA formulations that can escape catabolism. The effects of DHA supplementation on behavioural and biochemical measures were demonstrated in rodent models carrying amyloid mutations[153-155] or *APOE ε4* allele knock-in models [156, 157] using higher doses and long term DHA supplementation to diet.

In humans, DHA is primarily consumed from oily fish while other sources include liver and eggs. DHA supplements are commonly provided in the form of an algal derived triacylglycerol [TG] form or in pure DHA ethyl esters [Table 1]. From a pharmacological perspective absorption of DHA is similar between TG and ethyl esters of DHA formulations [158]. Though DHA supplements penetrate into the brain, there are very few DHA dosing studies guiding the information on DHA penetration to the brain. In the omega-AD trial, 1720 mg of DHA [in ethyl esters] per day over 6 months was associated with only 11% increase in CSF DHA levels, as opposed to a two-fold [200%] increase in plasma DHA levels [159]. In the ADCS-sponsored DHA trial, 2 grams DHA daily [Algal TG derived], a 38% increase in CSF DHA levels was observed as opposed to 207% increase in plasma DHA levels [160]. In the DHA Brain Delivery Pilot trial that recruited cognitively normal older adults, 2 grams DHA daily [Algal TG derived], led to a 28% increase in CSF DHA levels[161]. Therefore, DHA doses of less than 2 grams per day may lead to relatively small [< 20%] increases in CSF or

brain DHA levels. This may provide an explanation whereby clinical trials using 1 gram or lower doses of omega-3 were negative for cognitive outcomes [162].

Furthermore, since the majority of ingested DHA is transported esterified to lipids, the half-life of DHA depends on the turnover of its carrier molecule. the half-life of DHA is approximately 3 weeks in plasma phospholipids and 4 months in red blood cell membranes [163]. In contrast, the half-life of DHA in tissue compartments is much slower. In the brain, Umhau et al demonstrated using ^{11}C DHA PET scans that DHA half-life is approximately two and a half years [164]. Even within the brain, different compartments may have different DHA turnover rates, with synaptic DHA turnover occurring at faster rate [165, 166] than other brain tissues. Similar to the brain, the half-life of polyunsaturated fatty acids in adipose tissues is around 3 years[167]. The slower turnover of DHA in the brain implies that a modest reduction in DHA intake or increase in DHA consumption may take several years to remodel brain DHA within neuronal membranes. Unless there is severe DHA depletion or deficiency secondary to strict dietary restriction or a metabolic defect, short-term DHA supplementation will less likely affect brain DHA levels.

Delivery of DHA to the brain may be enhanced using phospholipid DHA esters instead of TG DHA esters. Phospholipid DHA formulations have a longer plasma half-life [168], and associate with HDL metabolism. In addition, the incorporation of DHA into sn-1 position of dietary phospholipids can enhance its brain bioavailability [169] by limiting a phospholipase A_2 mediated loss of DHA during its peripheral circulation. Another strategy to enhance brain DHA delivery focuses on enhancing brain APOE lipidation. APOE lipidation is dependent on ABCA-1 activity[170]. DHA when added to the medium of glial cells in culture is incorporated into membrane phospholipids, and then secreted as the fatty acid moiety of phospholipids mostly to APOE-containing lipoproteins [171]. APOE containing DHA exhibits a strong effect on neurite outgrowth of hippocampal neurons by increasing the number of branches [171]. Therefore, enhancing brain APOE lipidation represents a mechanism to mobilize DHA from

glial stores into APOE lipoproteins and therefore facilitate its brain transport in tissues with greater APOE receptor expression such as the hippocampus.

6.3.2. Omega-3 fatty acid intake and the response to supplementation

An association has been shown between serum DHA and brain amyloid accumulation in persons at risk of dementia [149]. However, this association was largely driven by persons at the lowest quartile of serum DHA levels, ie, those who do not consume much seafood. The Multidomain Alzheimer Preventive Trial (MAPT), which was designed to assess the effects of DHA (800 mg) and EPA (to a maximum of 225mg), multidomain intervention in cognitive function in frail subjects with memory complaints aged over seventy. In the main analysis of MAPT, no significant effects of the interventions were found on cognition after adjustment for multiple testing. Exploratory sub-group analysis showed that participants on n-3 PUFA supplementation with a low omega-3 index (DHA + EPA ≤ 4.83 %, representing the lowest quartile of omega 3 index distribution) at baseline showed a trend towards less cognitive decline over 36 months in comparison to subjects on placebo with low baseline omega-3 index[172]. PREVENTE4 (NCT03613844) is testing whether high dose (2 grams per day) of TG-derived DHA supplementation over two years would benefit non-demented older individuals with low baseline omega-3 intake and who are at increased risk of dementia based on APOE genotype and cardiovascular risk factors

6.4. Omega-3 fatty acids, summary and recommendations

In summary, epidemiology studies might support a protective effect of increasing PUFA consumption when supplementation starts early and lasts for a considerable amount of time to allow n-3 to remodel within brain cells. Moreover, high dose and long-term DHA supplementation ameliorates AD pathology in rodent models. Short-term and low dose

omega-3 supplements are unlikely to produce meaningful effects sizes on cognitive outcomes with ongoing clinical trials, as these often include individuals with already-sufficient omega-3 blood levels or significant evidence of neurodegeneration, in which case reversing the pathology may not be possible. Furthermore, there are the complexities and confounding associated with dietary patterns and change in dietary patterns overtime in different populations.

i. Recommendations: Omega-3 clinical trials should begin with a focus on appropriate exposure level and sample selection, with clinical outcomes associated with lower PUFA intake and levels and responsive to supplementation and careful measure of confounding. Selection of participants at increased risk of dementia for example cognitively normal APOE4 carriers, may increase the likelihood of success

ii. Either greater doses of current TG-DHA formulations or better brain penetrant formulations may need to be tested over longer time frames and in those without significant evidence of neurodegeneration.

7. HOMOCYSTEINE AND B VITAMINS

Epidemiological studies have established that raised plasma total homocysteine (tHcy) - a marker of B vitamin status – and low-normal blood levels of the B vitamins folate, B6 and B12 are risk factors for dementia, including AD [173-177]. Plausible mechanisms for this association have been described [174, 175, 178, 179]; these include mediation by damage to the cerebral vasculature and the formation of phosphorylated tau, leading to brain atrophy. Several meta-analyses have estimated the population attributable risk (PAR) of dementia for raised tHcy. On the assumption that raised tHcy has a prevalence of some 30% in the elderly population, estimates of PAR range from 12% to 31% in four of the meta-analyses, with a fifth estimating that the PAR is 4.3% [176]. Thus, a substantial proportion of dementia may be caused by raised tHcy.

In view of the high PAR, it is important that raised tHcy can readily be lowered by the oral administration of three B vitamins (folate, B6 and B12). The doses of these vitamins that are required to lower tHcy are considerably larger than can readily be obtained from the diet. A limited number of trials have been carried out with these high doses in people with dementia, MCI or normal elderly but with conflicting results. Some of the reasons for these conflicting results have been discussed [174-176]

Here, we make recommendations specifying the conditions that should be fulfilled in any trial of homocysteine-lowering B vitamins in relation to cognition, based upon Table 2 in [176]

Appropriate sample selection is needed:

i. Elevated tHcy or suboptimal B vitamin status should be present in the participants so that benefit can occur. No benefit could be expected if the participants already have an adequate B vitamin status. Hence, it is crucial to measure tHcy or B vitamins at baseline. It is noteworthy that some trials have not done this, e.g. [180, 181]
ii. Study participants in the trial should be at risk of cognitive decline or already showing decline, but should not have a diagnosis of dementia. In patients with dementia it is likely, as is applicable for most interventions, that the degenerative process has proceeded too far for any clinically meaningful modification of the disease process to be possible. It was found, for example, in the ADCS trial [141] that patients with moderately severe dementia did not benefit from homocysteine-lowering treatment but those with mild dementia did show some benefit.

Appropriate outcomes must be measured:

iii. The outcome measured must be sufficiently sensitive to change over the duration of the trial. Screening tests like MMSE have often been used in trials but these are rarely sensitive enough to detect meaningful change over a short time. More specific

cognitive tests should be used and in addition, or alternatively, sensitive objective and physical measurements such as the rate of brain atrophy determined by MRI [182] can be used.

iv. The duration of the trial should be long enough to measure clinically-relevant change, such as cognitive decline, in the placebo group. This period should be at least 12 months and preferably 2 years, in particular if conversion to dementia is being assessed. It is noteworthy that many trials do not fulfil the criterion of cognitive decline in the placebo group: for example, in a New Zealand trial the placebo group had an MMSE score of 29.17±0.16 at baseline and 29.32±1.10 after two years; there was no effect of B vitamin treatment [183]. In the meta-analysis by Clarke [184], 76% out of 20,431 participants in the trials did not have baseline measures of cognition and so it was not possible to determine cognitive decline in the placebo group; this fact must cast doubt on the validity of the authors' conclusions.

The dose should be adequate.

v. The doses of the vitamins should be sufficient to lower tHcy in the majority of the participants, which means that food-based vitamins will not be adequate. Doses needed are typically: folate 0.4 to 0.8 mg, B6 10 - 20mg, B12 0.5 mg and these can be taken orally.

Analyses should take appropriate account of subgroups, confounding and interaction

vi. It is crucial that the analyses pre-specified in the trial protocol should include subgroup analysis in relation to baseline levels of tHcy and/or of the B vitamins. It may be that the beneficial effect will be the greater, the higher the baseline tHcy.

vii. The protocol should specify analyses adjusted, or stratified, according to other factors known to influence cognitive decline, such as age, APOE genotype and to factors like omega-3 fatty acids and antiplatelet drugs that appear to interact specifically with B vitamins (see below).

Relatively few published trials of B vitamins in relation to cognition have satisfied all the above criteria. These include the FACIT trial of folic acid over 3 years [140] ; the VITACOG trial of folic acid, B6 and B12 in MCI over 2 years, reviewed in Smith [175]; and two trials in MCI from China on folic acid for 2 years [185] and on folic acid and B12 for 6 months [186]. All of these trials reported a beneficial effect of the B vitamin treatment on cognitive or clinical function. Many trials that were deficient in one or more of the above criteria have been reported as negative, but in fact such conclusions cannot be drawn.

The VITACOG trial not only assessed cognitive and clinical measures but also measured total and regional brain atrophy. In the intention-to-treat analysis, the B vitamin treatment in 180 participants who had volunteered for MRI scans slowed whole brain atrophy by 30% overall, but in participants with tHcy in the top quartile (> 13 μmol/L) the B vitamin treatment slowed brain atrophy by 53%[182]. The slowing of brain atrophy by B vitamin treatment was not influenced by the *APOE ε4* allele status. Regional brain atrophy, in particular in the medial temporal lobe, was markedly slowed, by almost 90% [187].

Subsequent analysis showed that the beneficial effects of the B vitamins were restricted to participants who had a good omega-3 fatty acid status as well as elevated tHcy [188, 189]. Confirmation of this interaction has come from a trial showing that a combination of folic acid and DHA treatment was more effective in improving cognition in patients with MCI than either nutrient alone [190]. A theoretical basis for this interaction between two classes of nutrients has been proposed [175, 191, 192] Evidence that this interaction operates in the opposite direction as well, i.e. good B vitamin status (low tHcy) facilitates the cognitive improvement after administering omega-3 fatty acids, has been provided [193]

The VITACOG trial has drawn attention to several factors that can influence the response to treatment with B vitamins, such as the baseline level of tHcy, the possible influence of

omega-3 fatty acids and the use of aspirin by participants. For aspirin, it was found that those participants who regularly took aspirin, but not those taking other NSAIDS, showed no slowing of brain atrophy after B vitamin treatment [182] Similarly, aspirin use appeared to interfere with the beneficial cognitive effects of B vitamins in MCI (T. Kwok et al. unpublished). These factors, and possibly the use of other drugs such as lipid-lowering drugs, should always be taken into account when trials of B vitamins are designed.

It has been concluded that the VITACOG trial has already fufilled the criteria for disease modification in MCI [194], with the causal pathway shown in Figure 2. Trials of a combination of B vitamins and omega-3 fatty acids are now needed in people who have elevated tHcy, to see if this simple and safe treatment can slow, or prevent, the conversion from MCI to dementia.

Discussion

Although dementia risk reduction has never been more important, the evidence so far, at least for the risk factors we examined, is not yet sufficient to drive clear guidelines although some pointers have been identified. In particular, there are common areas of discrepancy between the observational and clinical trial evidence across the 7 risk factors.

Experts in the relevant field appraised each of the seven risk factors independently and yet when we pool all of these appraisals we find a series of commonalities. These are shown in figure 3 and can be summarised as those affecting population selection (age, subgroups, key characteristics, dementia type/pathology), those relating to the risk factor (level of baseline severity, relative importance of change in risk factor level) and those relevant to treatment (drug type/class, dosage, duration of treatment, need for combination treatment).

Limitations

We have chosen seven established risk factors that are all modifiable with pharmacological intervention although we acknowledge that risk factor interaction or clustering is possible and single interventions are not necessarily reflective of real life. There are also risk factors

where pharmacological intervention is not possible and/or where blinded clinical trials are not feasible and they two are likely to face some of the issues we have identified, examples might include air pollution, alcohol or social engagement. Related to this is the potential for commonalities amongst the mechanistic pathways. For example;a potential role for vascular and inflammatory etiologies are evident with vascular pathways most strongly but not exclusively linked to diabetes [13], cholesterol [52] blood pressure [66, 68, 69] and homocysteine [174, 175, 178, 179] and inflammatory pathways to estrogen [195] and omega-3 fatty acids [145], although this may not be the whole story with hyperglycemia [15] and blood pressure [66, 70-72] hypothesized to increase amyloid deposition and genetic alterations in cholesterol trafficking directly related to risk of AD [50]. Furthermore the work on NSAIDs reminds us to be 'vigilant to potential sources of bias, not least those of reverse causality and confounding by indication.' Further limitations come from the inherent differences between observational studies and clinical trials where the former is able to accrue long follow up but unlikely to modify the risk factor exposure or treatment. The latter by design has an intervention and is likely to be shorter. Finally, to take the first steps in moving the field forwards we have chosen to focus on the similarities between the different risk factor and treatment pairs rather than the differences. However, these are also a potential source of insight. For example, age of exposure seems more pertinent to some risk factors than others. Although a full evaluation of the differences is beyond this paper, we recommend they too are explored with a view to informing the next generation of research on dementia risk reduction.

Our use of expert appraisal could considered as both a limitation and a strength. We did not seek to carry out a systematic review as there are multiple systematic reviews already published for each of these seven risk factors. Instead, we have brought together expert perspectives in a consensus and critical commentary of the current evidence. In turn this has highlighted the different directions that the epidemiology and clinical trial evidence has taken across the different risk factors, for example, the availability of epidemiological evidence for some risk factors is heavily based around the risk factor exposure and outcome, (e.g. blood

pressure) whereas for others the evidence is greater for the association between the treatment and the outcome (e.g. hormone treatment). Altogether this underlines the importance of a critical lens when interpreting the existing evidence and a need for a more indepth understanding going forwards.

Overall we synthesize the challenges and opportunities (Table 3) faced across the risk factors and argue that the design of new observational studies and in particular new clinical trials should be both informed by the issues we raise and supported by careful analyses and understanding of the existing data, for example, using techniques such as causal inference[196].

We argue that to gain a greater understanding of the remaining areas of uncertainty and the issues associated with these is a requirement. Before planning future trials and when building a robust justification for future trials both targeted and methodologically sophisticated investigations are needed. Such evaluations might include re-examining past trials and observational data alongside a pragmatic approach remaining alert to the possibility that interventions may not modify the risk of dementia. In this context, overall, for NSAIDs the dementia risk reduction story seems close to complete. The current clinical trial evidence arguably holds the most promise for antihypertensive use and supplementation by B vitamins, but even for these and other interventions more work is needed to fully evaluate impact and reduce bias not least in greater understanding of the appropriate trial populations and interventions.

References

1. Xu, W., et al., *Meta-analysis of modifiable risk factors for Alzheimer's disease.* J Neurol Neurosurg Psychiatry, 2015. **86**(12): p. 1299-306.
2. Prince, M., Albanese, E., Guerchet, M., Prina, M., *World Alzheimer Report 2014 Dementia and Risk Reduction AN ANALYSIS OF PROTECTIVE AND MODIFIABLE FACTORS*. 2014: Alzheimer's Disease International.
3. Smith, A.D. and K. Yaffe, *Dementia (including Alzheimer's disease) can be prevented: statement supported by international experts.* J Alzheimers Dis, 2014. **38**(4): p. 699-703.

4. Anstey, K.J., et al., *A Systematic Review of Meta-Analyses that Evaluate Risk Factors for Dementia to Evaluate the Quantity, Quality, and Global Representativeness of Evidence.* J Alzheimers Dis, 2019. **70**(s1): p. S165-s186.
5. Peters, R., et al., *Common risk factors for major noncommunicable disease, a systematic overview of reviews and commentary: the implied potential for targeted risk reduction.* Therapeutic Advances in Chronic Disease, 2019. **10**: p. 2040622319880392.
6. Glymour, M.M. and R.A. Whitmer, *Using Cross-Cultural Studies to Improve Evidence on Dementia Prevention: Lessons from the Special Issue Sponsored by the International Research Network on Dementia Prevention (IRNDP).* J Alzheimers Dis, 2019. **70**(s1): p. S5-s10.
7. *Dementia_Guidelines_Evidence_Profiles*. Available from: https://www.who.int/mental_health/neurology/dementia/guidelines_risk_reduction/en/.
8. Bandosz, P., et al., *Potential impact of diabetes prevention on mortality and future burden of dementia and disability: a modelling study.* Diabetologia, 2020. **63**(1): p. 104-115.
9. da Rocha Fernandes, J., et al., *IDF Diabetes Atlas estimates of 2014 global health expenditures on diabetes.* Diabetes Res Clin Pract, 2016. **117**: p. 48-54.
10. Majithia, A.R. and J.C. Florez, *Clinical translation of genetic predictors for type 2 diabetes.* Curr Opin Endocrinol Diabetes Obes, 2009. **16**(2): p. 100-6.
11. Marín-Peñalver, J.J., et al., *Update on the treatment of type 2 diabetes mellitus.* World journal of diabetes, 2016. **7**(17): p. 354-395.
12. Shieh, J.C., P.T. Huang, and Y.F. Lin, *Alzheimer's Disease and Diabetes: Insulin Signaling as the Bridge Linking Two Pathologies.* Mol Neurobiol, 2020. **57**(4): p. 1966-1977.
13. Feinkohl, I., et al., *The impact of diabetes on cognitive decline: potential vascular, metabolic, and psychosocial risk factors.* Alzheimers Res Ther, 2015. **7**(1): p. 46.
14. Ahmed, S., Z. Mahmood, and S. Zahid, *Linking insulin with Alzheimer's disease: emergence as type III diabetes.* Neurol Sci, 2015. **36**(10): p. 1763-9.
15. Sasaki, N., et al., *Advanced glycation end products in Alzheimer's disease and other neurodegenerative diseases.* Am J Pathol, 1998. **153**(4): p. 1149-55.
16. Biessels, G.J., et al., *Risk of dementia in diabetes mellitus: a systematic review.* Lancet Neurol, 2006. **5**(1): p. 64-74.
17. Kloppenborg, R.P., et al., *Diabetes and other vascular risk factors for dementia: which factor matters most? A systematic review.* Eur J Pharmacol, 2008. **585**(1): p. 97-108.
18. Cheng, G., et al., *Diabetes as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies.* Intern Med J, 2012. **42**(5): p. 484-91.
19. Gudala, K., et al., *Diabetes mellitus and risk of dementia: A meta-analysis of prospective observational studies.* J Diabetes Investig, 2013. **4**(6): p. 640-50.
20. Crane, P.K., et al., *Glucose levels and risk of dementia.* N Engl J Med, 2013. **369**(6): p. 540-8.
21. Lu, K., et al., *Cognition at age 70: Life course predictors and associations with brain pathologies.* Neurology, 2019. **93**(23): p. e2144-e2156.
22. James, S.N., et al., *The effect of mid-life insulin resistance and type 2 diabetes on older-age cognitive state: the explanatory role of early-life advantage.* Diabetologia, 2019. **62**(10): p. 1891-1900.
23. Beach, T.G., et al., *Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005-2010.* J Neuropathol Exp Neurol, 2012. **71**(4): p. 266-73.
24. Abner, E.L., et al., *Diabetes is associated with cerebrovascular but not Alzheimer's disease neuropathology.* Alzheimers Dement, 2016. **12**(8): p. 882-9.
25. Pruzin, J.J., et al., *Review: Relationship of type 2 diabetes to human brain pathology.* Neuropathol Appl Neurobiol, 2018. **44**(4): p. 347-362.
26. Rawlings, A.M., et al., *The Association of Late-Life Diabetes Status and Hyperglycemia With Incident Mild Cognitive Impairment and Dementia: The ARIC Study.* Diabetes Care, 2019. **42**(7): p. 1248.

27. Koenig, A.M., et al., *Effects of the Insulin Sensitizer Metformin in Alzheimer Disease: Pilot Data From a Randomized Placebo-controlled Crossover Study.* Alzheimer Disease & Associated Disorders, 2017. **31**(2).
28. Craft, S., et al., *Intranasal Insulin Therapy for Alzheimer Disease and Amnestic Mild Cognitive Impairment: A Pilot Clinical Trial.* Archives of Neurology, 2012. **69**(1): p. 29-38.
29. Watson, G.S., et al., *Preserved cognition in patients with early Alzheimer disease and amnestic mild cognitive impairment during treatment with rosiglitazone: a preliminary study.* Am J Geriatr Psychiatry, 2005. **13**(11): p. 950-8.
30. Bendlin, B.B., *Antidiabetic therapies and Alzheimer disease.* Dialogues in clinical neuroscience, 2019. **21**(1): p. 83-91.
31. Hagenaars, S.P., et al., *Cognitive ability and physical health: a Mendelian randomization study.* Scientific Reports, 2017. **7**(1): p. 2651.
32. Hagenaars, S.P., et al., *Shared genetic aetiology between cognitive functions and physical and mental health in UK Biobank (N=112 151) and 24 GWAS consortia.* Molecular Psychiatry, 2016. **21**(11): p. 1624-1632.
33. Østergaard, S.D., et al., *Associations between Potentially Modifiable Risk Factors and Alzheimer Disease: A Mendelian Randomization Study.* PLoS Med, 2015. **12**(6): p. e1001841; discussion e1001841.
34. Larsson, S.C., et al., *Modifiable pathways in Alzheimer's disease: Mendelian randomisation analysis.* BMJ, 2017. **359**: p. j5375.
35. Robins, J.M., M.A. Hernán, and B. Brumback, *Marginal structural models and causal inference in epidemiology.* Epidemiology, 2000. **11**(5): p. 550-60.
36. Areosa Sastre, A., et al., *Effect of the treatment of Type 2 diabetes mellitus on the development of cognitive impairment and dementia.* Cochrane Database Syst Rev, 2017. **6**(6): p. Cd003804.
37. Chou, P.S., B.L. Ho, and Y.H. Yang, *Effects of pioglitazone on the incidence of dementia in patients with diabetes.* J Diabetes Complications, 2017. **31**(6): p. 1053-1057.
38. Budur, K., et al., *P4-073: A PHARMACOGENETICS-SUPPORTED CLINICAL TRIAL TO DELAY ONSET OF MILD COGNITIVE IMPAIRMENT DUE TO ALZHEIMER'S DISEASE USING LOW-DOSE PIOGLITAZONE: AN UPDATE ON THE TOMORROW STUDY.* Alzheimer's & Dementia, 2014. **10**(4S_Part_22): p. P809-P810.
39. Reger, M.A., et al., *Intranasal Insulin Administration Dose-Dependently Modulates Verbal Memory and Plasma Amyloid-β in Memory-Impaired Older Adults.* Journal of Alzheimer's Disease, 2008. **13**: p. 323-331.
40. Muñoz-Jiménez, M., et al., *Antidiabetic Drugs in Alzheimer's Disease and Mild Cognitive Impairment: A Systematic Review.* Dementia and Geriatric Cognitive Disorders, 2020. **49**(5): p. 423-434.
41. Luchsinger, J.A., et al., *Metformin in Amnestic Mild Cognitive Impairment: Results of a Pilot Randomized Placebo Controlled Clinical Trial.* Journal of Alzheimer's Disease, 2016. **51**: p. 501-514.
42. Risner, M.E., et al., *Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease.* The Pharmacogenomics Journal, 2006. **6**(4): p. 246-254.
43. Valcarce Carmen , D.I., Soeder Tom , Burstein Aaron and H.P. 1 vTv Therapeutics LLC, NC, USA ; 2 CATO Research Ltd., Durham, NC, USA, *Is RAGE the missing link between diabetes and dementia?*

Results from a subgroup analysis of the STE AD FAST trial

in *vTv Therapeutics Presents Positive Data on the Effect of Azeliragon in Patients with Alzheimer's and Diabetes at the 11th Clinical Trials on Alzheimer's Disease (CTAD) Conference*. 2018.

44. Sato, T., et al., *Efficacy of PPAR-γ agonist pioglitazone in mild Alzheimer disease.* Neurobiology of Aging, 2011. **32**(9): p. 1626-1633.

45. Huang, L.K., S.P. Chao, and C.J. Hu, *Clinical trials of new drugs for Alzheimer disease.* J Biomed Sci, 2020. **27**(1): p. 18.
46. Orrell, M. and C. Brayne, *Dementia prevention: call to action.* Lancet, 2015. **386**(10004): p. 1625.
47. Ritchie, C.W., G.M. Terrera, and T.J. Quinn, *Dementia trials and dementia tribulations: methodological and analytical challenges in dementia research.* Alzheimers Res Ther, 2015. **7**(1): p. 31.
48. Dodge, H.H., et al., *Use of High-Frequency In-Home Monitoring Data May Reduce Sample Sizes Needed in Clinical Trials.* PLoS One, 2015. **10**(9): p. e0138095.
49. McDonough, I.M., et al., *Discrepancies between fluid and crystallized ability in healthy adults: a behavioral marker of preclinical Alzheimer's disease.* Neurobiology of aging, 2016. **46**: p. 68-75.
50. Geifman, N., et al., *Evidence for benefit of statins to modify cognitive decline and risk in Alzheimer's disease.* Alzheimers Res Ther, 2017. **9**(1): p. 10.
51. Lin, F.C., et al., *Early Statin Use and the Progression of Alzheimer Disease: A Total Population-Based Case-Control Study.* Medicine (Baltimore), 2015. **94**(47): p. e2143.
52. McGuinness, B., et al., *Statins for the prevention of dementia.* Cochrane Database of Systematic Reviews, 2016(1).
53. Mejias-Trueba, M., M.A. Perez-Moreno, and M.A. Fernandez-Arche, *Systematic review of the efficacy of statins for the treatment of Alzheimer's disease.* Clin Med (Lond), 2018. **18**(1): p. 54-61.
54. Poly, T.N., et al., *Association between Use of Statin and Risk of Dementia: A Meta-Analysis of Observational Studies.* Neuroepidemiology, 2019: p. 1-13.
55. Sinyavskaya, L., et al., *Comparative effect of statins on the risk of incident Alzheimer disease.* Neurology, 2018. **90**(3): p. e179-e187.
56. Wong, W.B., et al., *Statins in the prevention of dementia and Alzheimer's disease: a meta-analysis of observational studies and an assessment of confounding.* Pharmacoepidemiol Drug Saf, 2013. **22**(4): p. 345-58.
57. Zhang, X., J. Wen, and Z. Zhang, *Statins use and risk of dementia: A dose-response meta analysis.* Medicine (Baltimore), 2018. **97**(30): p. e11304.
58. Mohammad, S., et al., *Pleiotropic Effects of Statins: Untapped Potential for Statin Pharmacotherapy.* Curr Vasc Pharmacol, 2019. **17**(3): p. 239-261.
59. Kelley, B.J. and S. Glasser, *Cognitive effects of statin medications.* CNS Drugs, 2014. **28**(5): p. 411-9.
60. McGuinness, B., et al., *Statins for the treatment of dementia.* Cochrane Database Syst Rev, 2014(7): p. CD007514.
61. Pandey, R.D., et al., *Role of statins in Alzheimer's disease: a retrospective meta-analysis for commonly investigated clinical parameters in RCTs.* Int J Neurosci, 2013. **123**(8): p. 521-5.
62. Richardson, K., et al., *Statins and Cognitive Function: A Systematic Review.* Annals of Internal Medicine, 2013. **159**(10): p. 688-697.
63. Freedman, D.M. and R.M. Pfeiffer, *Ascertainment Bias in Statin Use and Alzheimer Disease Incidence.* JAMA Neurol, 2017. **74**(7): p. 868.
64. Hendrie, H.C., et al., *Statin Use, Incident Dementia and Alzheimer Disease in Elderly African Americans.* Ethn Dis, 2015. **25**(3): p. 345-54.
65. Qiu, C., B. Winblad, and L. Fratiglioni, *The age-dependent relation of blood pressure to cognitive function and dementia.* Lancet Neurol, 2005. **4**(8): p. 487-99.
66. Iadecola, C., et al., *Impact of Hypertension on Cognitive Function: A Scientific Statement From the American Heart Association.* Hypertension, 2016. **68**(6): p. e67-e94.
67. Williams, B., et al., *2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension: The Task Force for the management of*

arterial hypertension of the European Society of Cardiology and the European Society of Hypertension. J Hypertens, 2018. **36**(10): p. 1953-2041.
68. Walker, K.A., M.C. Power, and R.F. Gottesman, *Defining the Relationship Between Hypertension, Cognitive Decline, and Dementia: a Review.* Curr Hypertens Rep, 2017. **19**(3): p. 24.
69. Iadecola, C. and R.F. Gottesman, *Neurovascular and Cognitive Dysfunction in Hypertension.* Circ Res, 2019. **124**(7): p. 1025-1044.
70. Gottesman, R.F., et al., *Association Between Midlife Vascular Risk Factors and Estimated Brain Amyloid Deposition.* JAMA, 2017. **317**(14): p. 1443-1450.
71. Petrovitch, H., et al., *Midlife blood pressure and neuritic plaques, neurofibrillary tangles, and brain weight at death: the HAAS. Honolulu-Asia aging Study.* Neurobiol Aging, 2000. **21**(1): p. 57-62.
72. Palmer, J.C., et al., *Zibotentan, an Endothelin A Receptor Antagonist, Prevents Amyloid-β-Induced Hypertension and Maintains Cerebral Perfusion.* Journal of Alzheimer's Disease, 2020. **73**: p. 1185-1199.
73. Skoog, I., et al., *15-year longitudinal study of blood pressure and dementia.* Lancet, 1996. **347**(9009): p. 1141-5.
74. Gottesman, R.F., et al., *Midlife Hypertension and 20-Year Cognitive Change: The Atherosclerosis Risk in Communities Neurocognitive Study.* JAMA Neurology, 2014. **71**(10): p. 1218-1227.
75. Walker, K.A., et al., *Association of Midlife to Late-Life Blood Pressure Patterns With Incident Dementia.* Jama, 2019. **322**(6): p. 535-545.
76. Peters, R., et al., *Trajectory of blood pressure, body mass index, cholesterol and incident dementia: systematic review.* Br J Psychiatry, 2020. **216**(1): p. 16-28.
77. Peters, R., et al., *Blood pressure and dementia: What the SPRINT-MIND trial adds and what we still need to know.* Neurology, 2019. **92**(21): p. 1017-1018.
78. Elias, M.F., R.V. Torres, and A. Davey, *Clinical Trials of Blood Pressure Lowering and Antihypertensive Medication: Is Cognitive Measurement State-of-the-Art?* American Journal of Hypertension, 2018. **31**(6): p. 631-642.
79. Skoog, I., *Antihypertensive treatment and dementia prevention.* The Lancet Neurology, 2008. **7**(8): p. 664-665.
80. Whelton, P.K., et al., *2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines.* Hypertension, 2018. **71**(6): p. 1269-1324.
81. Peters, R., et al., *Investigation of antihypertensive class, dementia, and cognitive decline.* Neurology, 2020. **94**(3): p. e267.
82. Ding, J., et al., *Antihypertensive medications and risk for incident dementia and Alzheimer's disease: a meta-analysis of individual participant data from prospective cohort studies.* Lancet Neurol, 2020. **19**(1): p. 61-70.
83. Hughes, D., et al., *Association of Blood Pressure Lowering With Incident Dementia or Cognitive Impairment: A Systematic Review and Meta-analysis.* JAMA, 2020. **323**(19): p. 1934-1944.
84. McGuinness, B., et al., *Blood pressure lowering in patients without prior cerebrovascular disease for prevention of cognitive impairment and dementia.* Cochrane Database of Systematic Reviews, 2009(4).
85. Williamson, J.D., et al., *Effect of Intensive vs Standard Blood Pressure Control on Probable Dementia: A Randomized Clinical Trial.* Jama, 2019. **321**(6): p. 553-561.

86. Pajewski, N., *Lessons Learned from Cognitive Outcomes in SPRINT: Neuropsychological Test Scores, Domain-Specific Cognitive Function, and Adjudicated Outcomes.* Journal of Prevention of Alzheimers Disease, 2019. **6**(S1).
87. Boss, L., et al., *Endogenous Sex Hormones and Cognitive Function in Older Adults: A Systematic Review.* Western Journal of Nursing Research, 2013. **36**(3): p. 388-426.
88. Gurvich, C., et al., *Sex Differences and the Influence of Sex Hormones on Cognition through Adulthood and the Aging Process.* Brain Sci, 2018. **8**(9).
89. Compton, J., T. van Amelsvoort, and D. Murphy, *HRT and its effect on normal ageing of the brain and dementia.* British journal of clinical pharmacology, 2001. **52**(6): p. 647-653.
90. Bean, L.A., L. Ianov, and T.C. Foster, *Estrogen receptors, the hippocampus, and memory.* The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry, 2014. **20**(5): p. 534-545.
91. Silva, I., et al., *Onset of estrogen replacement has a critical effect on synaptic density of CA1 hippocampus in ovariectomized adult rats.* Menopause, 2003. **10**(5): p. 406-11.
92. Chavez, C., et al., *The effect of estrogen on dopamine and serotonin receptor and transporter levels in the brain: an autoradiography study.* Brain Res, 2010. **1321**: p. 51-9.
93. Bean, L.A., et al., *Re-Opening the Critical Window for Estrogen Therapy.* The Journal of neuroscience : the official journal of the Society for Neuroscience, 2015. **35**(49): p. 16077-16093.
94. O'Brien, J., et al., *Postmenopausal hormone therapy is not associated with risk of all-cause dementia and Alzheimer's disease.* Epidemiol Rev, 2014. **36**(1): p. 83-103.
95. Kawas, C., et al., *A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: the Baltimore Longitudinal Study of Aging.* Neurology, 1997. **48**(6): p. 1517-21.
96. Tang, M., et al., *Superior and distinct antioxidant effects of selected estrogen metabolites on lipid peroxidation.* Metabolism, 1996. **45**(4): p. 411-4.
97. Zandi, P.P., et al., *Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study.* Jama, 2002. **288**(17): p. 2123-9.
98. LeBlanc, E.S., et al., *Hormone replacement therapy and cognition: systematic review and meta-analysis.* Jama, 2001. **285**(11): p. 1489-99.
99. Matyi, J., et al., *Lifetime estrogen exposure is associated with cognitive status in late life: The Cache County Study.* Innovation in Aging, 2018. **2**(Suppl 1): p. 884.
100. Hogervorst, E., et al., *The nature of the effect of female gonadal hormone replacement therapy on cognitive function in post-menopausal women: a meta-analysis.* Neuroscience, 2000. **101**(3): p. 485-512.
101. Hogervorst, E., et al., *Hormone replacement therapy to maintain cognitive function in women with dementia.* Cochrane Database Syst Rev, 2009. **2009**(1): p. Cd003799.
102. Marjoribanks, J., et al., *Long‐term hormone therapy for perimenopausal and postmenopausal women.* Cochrane Database of Systematic Reviews, 2017(1).
103. Shumaker, S.A., et al., *Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: A randomized controlled trial.* JAMA, 2003. **289**(20): p. 2651-2662.
104. Shumaker, S.A., et al., *Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial.* Jama, 2003. **289**(20): p. 2651-62.
105. Maki, P.M., *A systematic review of clinical trials of hormone therapy on cognitive function: effects of age at initiation and progestin use.* Ann N Y Acad Sci, 2005. **1052**: p. 182-97.
106. LeBlanc, E.S., et al., *Hormone replacement therapy and cognition: systematic review and meta-analysis.* JAMA, 2001. **285**(11): p. 1489-1499.
107. Mulnard, R.A., et al., *Estrogen replacement therapy for treatment of mild to moderate Alzheimer disease: A randomized controlled trial.* JAMA, 2000. **283**(8): p. 1007-1015.

108. Lethaby, A., et al., *Hormone replacement therapy for cognitive function in postmenopausal women.* Cochrane Database Syst Rev, 2008. **2008**(1): p. Cd003122.
109. Rogers, J., et al., *Expression of immune system-associated antigens by cells of the human central nervous system: relationship to the pathology of Alzheimer's disease.* Neurobiol Aging, 1988. **9**(4): p. 339-49.
110. McGeer, P.L., et al., *Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains.* Neurology, 1988. **38**(8): p. 1285-91.
111. McGeer, P.L., et al., *Anti-inflammatory drugs and Alzheimer disease.* Lancet, 1990. **335**(8696): p. 1037.
112. Breitner, J.C., et al., *Inverse association of anti-inflammatory treatments and Alzheimer's disease: initial results of a co-twin control study.* Neurology, 1994. **44**(2): p. 227-32.
113. Breitner, J.C., et al., *Delayed onset of Alzheimer's disease with nonsteroidal anti-inflammatory and histamine H2 blocking drugs.* Neurobiol Aging, 1995. **16**(4): p. 523-30.
114. in 't Veld, B.A., et al., *Nonsteroidal Antiinflammatory Drugs and the Risk of Alzheimer's Disease.* New England Journal of Medicine, 2001. **345**(21): p. 1515-1521.
115. Aisen, P.S., et al., *A randomized controlled trial of prednisone in Alzheimer's disease. Alzheimer's Disease Cooperative Study.* Neurology, 2000. **54**(3): p. 588-93.
116. Aisen, P.S., et al., *Effects of rofecoxib or naproxen vs placebo on Alzheimer disease progression: a randomized controlled trial.* Jama, 2003. **289**(21): p. 2819-26.
117. Van Gool, W.A., et al., *Effect of hydroxychloroquine on progression of dementia in early Alzheimer's disease: an 18-month randomised, double-blind, placebo-controlled study.* Lancet, 2001. **358**(9280): p. 455-60.
118. Thal, L.J., et al., *A randomized, double-blind, study of rofecoxib in patients with mild cognitive impairment.* Neuropsychopharmacology, 2005. **30**(6): p. 1204-15.
119. *Naproxen and celecoxib do not prevent AD in early results from a randomized controlled trial.* Neurology, 2007. **68**(21): p. 1800.
120. Breitner, J.C., et al., *Extended results of the Alzheimer's disease anti-inflammatory prevention trial.* Alzheimers Dement, 2011. **7**(4): p. 402-11.
121. Leoutsakos, J.M., et al., *'Alzheimer's Progression Score': Development of a Biomarker Summary Outcome for AD Prevention Trials.* J Prev Alzheimers Dis, 2016. **3**(4): p. 229-235.
122. Breitner, J.C.S., et al., *Rationale and Structure for a New Center for Studies on Prevention of Alzheimer's Disease (StoP-AD).* The journal of prevention of Alzheimer's disease, 2016. **3**(4): p. 236-242.
123. Randolph, C., et al., *The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS): preliminary clinical validity.* J Clin Exp Neuropsychol, 1998. **20**(3): p. 310-9.
124. Meyer, P.F., et al., *INTREPAD: A randomized trial of naproxen to slow progress of presymptomatic Alzheimer disease.* Neurology, 2019. **92**(18): p. e2070-e2080.
125. Meyer, P.-F., et al., *No apparent effect of naproxen on CSF markers of innate immune activation.* Annals of clinical and translational neurology, 2019. **6**(6): p. 1127-1133.
126. Szekely, C.A., et al., *No advantage of A beta 42-lowering NSAIDs for prevention of Alzheimer dementia in six pooled cohort studies.* Neurology, 2008. **70**(24): p. 2291-8.
127. Arvanitakis, Z., et al., *Relation of NSAIDs to incident AD, change in cognitive function, and AD pathology.* Neurology, 2008. **70**(23): p. 2219-25.
128. Breitner, J.C.S., et al., *Risk of dementia and AD with prior exposure to NSAIDs in an elderly community-based cohort.* Neurology, 2009. **72**(22): p. 1899-1905.
129. Jansen, W.J., et al., *Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis.* JAMA, 2015. **313**(19): p. 1924-1938.
130. Roberts, R.O., et al., *Prevalence and Outcomes of Amyloid Positivity Among Persons Without Dementia in a Longitudinal, Population-Based Setting.* JAMA Neurol, 2018. **75**(8): p. 970-979.

131. Weggen, S., et al., *Evidence that nonsteroidal anti-inflammatory drugs decrease amyloid beta 42 production by direct modulation of gamma-secretase activity.* J Biol Chem, 2003. **278**(34): p. 31831-7.
132. Scarmeas, N., et al., *Mediterranean diet and risk for Alzheimer's disease.* Ann Neurol, 2006. **59**(6): p. 912-21.
133. Morris, M.C., et al., *MIND diet slows cognitive decline with aging.* Alzheimers Dement, 2015. **11**(9): p. 1015-22.
134. Morris, M.C., et al., *MIND diet associated with reduced incidence of Alzheimer's disease.* Alzheimers Dement, 2015. **11**(9): p. 1007-14.
135. Shakersain, B., et al., *The Nordic Prudent Diet Reduces Risk of Cognitive Decline in the Swedish Older Adults: A Population-Based Cohort Study.* Nutrients, 2018. **10**(2).
136. Shakersain, B., et al., *Prudent diet may attenuate the adverse effects of Western diet on cognitive decline.* Alzheimers Dement, 2016. **12**(2): p. 100-109.
137. Zhang, Y., et al., *Intakes of fish and polyunsaturated fatty acids and mild-to-severe cognitive impairment risks: a dose-response meta-analysis of 21 cohort studies.* The American Journal of Clinical Nutrition, 2016. **103**(2): p. 330-340.
138. Freund-Levi, Y., et al., *ω-3 Fatty Acid Treatment in 174 Patients With Mild to Moderate Alzheimer Disease: OmegAD Study: A Randomized Double-blind Trial.* Archives of Neurology, 2006. **63**(10): p. 1402-1408.
139. Quinn, J.F., et al., *Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial.* JAMA, 2010. **304**(17): p. 1903-1911.
140. Durga, J., et al., *Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial.* Lancet, 2007. **369**(9557): p. 208-16.
141. Aisen, P.S., et al., *High-dose B vitamin supplementation and cognitive decline in Alzheimer disease: a randomized controlled trial.* Jama, 2008. **300**(15): p. 1774-83.
142. Smith, A.D., et al., *Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trial.* PloS one, 2010. **5**(9): p. e12244-e12244.
143. Chen, C.T., et al., *The low levels of eicosapentaenoic acid in rat brain phospholipids are maintained via multiple redundant mechanisms.* Journal of lipid research, 2013. **54**(9): p. 2410-2422.
144. Metherel, A.H., et al., *Retroconversion is a minor contributor to increases in eicosapentaenoic acid following docosahexaenoic acid feeding as determined by compound specific isotope analysis in rat liver.* Nutr Metab (Lond), 2017. **14**: p. 75.
145. Sierra, S., et al., *Dietary eicosapentaenoic acid and docosahexaenoic acid equally incorporate as decosahexaenoic acid but differ in inflammatory effects.* Nutrition, 2008. **24**(3): p. 245-54.
146. Witte, A.V., et al., *Long-chain omega-3 fatty acids improve brain function and structure in older adults.* Cerebral Cortex, 2014. **24**(11): p. 3059-3068.
147. Hooijmans, C.R., et al., *The effects of long-term omega-3 fatty acid supplementation on cognition and Alzheimer's pathology in animal models of Alzheimer's disease: a systematic review and meta-analysis.* Journal of Alzheimer's Disease, 2012. **28**(1): p. 191-209.
148. Lukaschek, K., et al., *Cognitive Impairment Is Associated with a Low Omega-3 Index in the Elderly: Results from the KORA-Age Study.* Dement Geriatr Cogn Disord, 2016. **42**(3-4): p. 236-245.
149. Yassine HN, F.Q., Azizkhanian I, Rawat V, Castor K, Fonteh AN, Harrington MG, Zheng L, Reed BR, DeCarli C, Jagust WJ, Chui HC, *Association of Serum Docosahexaenoic Acid With Cerebral Amyloidosis.* JAMA Neurology, 2016. **73**(10): p. 1208-1216.
150. Samieri, C., et al., *Using network science tools to identify novel diet patterns in prodromal dementia.* Neurology, 2020. **94**(19): p. e2014-e2025.

151. Hooijmans CR, P.-d.J.P., de Vries RB, Ritskes-Hoitinga M, *The effects of long-term omega-3 fatty acid supplementation on cognition and Alzheimer's pathology in animal models of Alzheimer's disease: a systematic review and meta-analysis.* Journal of Alzheimer's Disease, 2012. **28**(1): p. 191-209.
152. Reagan-Shaw, S., M. Nihal, and N. Ahmad, *Dose translation from animal to human studies revisited.* The FASEB Journal, 2007. **22**(3): p. 659-661.
153. Oksman, M., et al., *Impact of different saturated fatty acid, polyunsaturated fatty acid and cholesterol containing diets on beta-amyloid accumulation in APP/PS1 transgenic mice.* Neurobiol Dis, 2006. **23**(3): p. 563-72.
154. Hooijmans, C., et al., *DHA and cholesterol containing diets influence Alzheimer-like pathology, cognition and cerebral vasculature in APP SWE/PS1 dE9 mice.* Neurobiology of disease, 2009. **33**(3): p. 482-498.
155. Arsenault, D., et al., *DHA improves cognition and prevents dysfunction of entorhinal cortex neurons in 3xTg-AD mice.* PLoS One, 2011. **6**(2): p. e17397.
156. Kariv-Inbal, Z., et al., *The isoform-specific pathological effects of ApoE4 in vivo are prevented by a fish oil (DHA) diet and are modified by cholesterol.* Journal of Alzheimer's Disease, 2012. **28**(3): p. 667-683.
157. Chouinard-Watkins, R., et al., *Docosahexaenoic acid prevents cognitive deficits in human apolipoprotein E epsilon 4-targeted replacement mice.* Neurobiol Aging, 2017. **57**: p. 28-35.
158. Nordoy, A., et al., *Absorption of the n-3 eicosapentaenoic and docosahexaenoic acids as ethyl esters and triglycerides by humans.* Am J Clin Nutr, 1991. **53**(5): p. 1185-90.
159. Freund Levi, Y., et al., *Transfer of omega-3 fatty acids across the blood-brain barrier after dietary supplementation with a docosahexaenoic acid-rich omega-3 fatty acid preparation in patients with Alzheimer's disease: the OmegAD study.* J Intern Med, 2014. **275**(4): p. 428-36.
160. Yassine, H.N., et al., *The effect of APOE genotype on the delivery of DHA to cerebrospinal fluid in Alzheimer's disease.* Alzheimers Res Ther, 2016. **8**(1): p. 25.
161. Arellanes, I.C., et al., *Brain delivery of supplemental docosahexaenoic acid (DHA): A randomized placebo-controlled clinical trial.* EBioMedicine, 2020. **59**.
162. Yassine, H.N., et al., *Association of Docosahexaenoic Acid Supplementation With Alzheimer Disease Stage in Apolipoprotein E epsilon4 Carriers: A Review.* JAMA Neurol, 2017.
163. Arterburn, L.M., *Bioequivalence of docosahexaenoic acid from different algal oils in capsules and in a DHA-fortified food.* Lipids, 2007. **42**.
164. Umhau, J.C., et al., *Imaging incorporation of circulating docosahexaenoic acid into the human brain using positron emission tomography.* J Lipid Res, 2009. **50**(7): p. 1259-68.
165. DeMar, J.C., Jr., et al., *Half-lives of docosahexaenoic acid in rat brain phospholipids are prolonged by 15 weeks of nutritional deprivation of n-3 polyunsaturated fatty acids.* J Neurochem, 2004. **91**(5): p. 1125-37.
166. Rapoport, S.I., M.C. Chang, and A.A. Spector, *Delivery and turnover of plasma-derived essential PUFAs in mammalian brain.* J Lipid Res, 2001. **42**(5): p. 678-85.
167. Dayton, S., et al., *Composition of lipids in human serum and adipose tissue during prolonged feeding of a diet high in unsaturated fat.* J Lipid Res, 1966. **7**(1): p. 103-11.
168. Liu, L., et al., *Higher efficacy of dietary DHA provided as a phospholipid than as a triglyceride for brain DHA accretion in neonatal piglets.* Journal of Lipid Research, 2014.
169. Subbaiah, P.V., et al., *Enhanced incorporation of dietary DHA into lymph phospholipids by altering its molecular carrier.* Biochimica et biophysica acta, 2016. **1861**(8 Pt A): p. 723-729.
170. Yassine, H.N., et al., *ABCA1 - Mediated Cholesterol Efflux Capacity to Cerebrospinal Fluid Is Reduced in Patients With Mild Cognitive Impairment and Alzheimer's Disease.* Journal of the American Heart Association, 2016. **5**(2): p. e002886.
171. Nakato, M., et al., *Neurite outgrowth stimulation by n-3 and n-6 PUFAs of phospholipids in apoE-containing lipoproteins secreted from glial cells.* Journal of lipid research, 2015. **56**(10): p. 1880-1890.

172. Andrieu, S., et al., *Effect of long-term omega 3 polyunsaturated fatty acid supplementation with or without multidomain intervention on cognitive function in elderly adults with memory complaints (MAPT): a randomised, placebo-controlled trial.* The Lancet Neurology, 2017. **16**(5): p. 377-389.
173. Beydoun, M.A., et al., *Epidemiologic studies of modifiable factors associated with cognition and dementia: systematic review and meta-analysis.* BMC Public Health, 2014. **14**(1): p. 643.
174. McCaddon, A. and J.W. Miller, *Assessing the association between homocysteine and cognition: reflections on Bradford Hill, meta-analyses and causality.* Nutr Rev, 2015. **73**(10): p. 723-35.
175. Smith, A.D. and H. Refsum, *Homocysteine, B vitamins, and cognitive impairment.* Annu Rev Nutr, 2016. **36**: p. 211-39.
176. Smith, A.D., et al., *Homocysteine and dementia: An international consensus statement.* J Alzheimers Dis, 2018. **62**(2): p. 561-570.
177. Yu, J.-T., et al., *Evidence-based prevention of Alzheimer's disease: systematic review and meta-analysis of 243 observational prospective studies and 153 randomised controlled trials.* Journal of Neurology, Neurosurgery & Psychiatry, 2020. **91**(11): p. 1201.
178. Obeid, R. and W. Herrmann, *Mechanisms of homocysteine neurotoxicity in neurodegenerative diseases with special reference to dementia.* FEBS Lett, 2006. **580**(13): p. 2994-3005.
179. Zhuo, J.M., H. Wang, and D. Pratico, *Is hyperhomocysteinemia an Alzheimer's disease (AD) risk factor, an AD marker, or neither?* Trends Pharmacol Sci, 2011. **32**(3): p. 562-71.
180. Kang, J.H., et al., *A trial of B vitamins and cognitive function among women at high risk of cardiovascular disease.* Am J Clin Nutr, 2008. **88**(6): p. 1602-10.
181. Grodstein, F., et al., *Long-term multivitamin supplementation and cognitive function in men: a randomized trial.* Ann Intern Med, 2013. **159**(12): p. 806-14.
182. Smith, A.D., et al., *Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment. A randomized controlled trial.* PLoS ONE, 2010. **5**(9): p. e12244.
183. McMahon, J.A., et al., *A controlled trial of homocysteine lowering and cognitive performance.* N Engl J Med, 2006. **354**(26): p. 2764-72.
184. Clarke, R., et al., *Effects of homocysteine lowering with B vitamins on cognitive aging: meta-analysis of 11 trials with cognitive data on 22,000 individuals.* Am J Clin Nutr, 2014. **100**(2): p. 657-666.
185. Ma, F., et al., *Effects of folic acid supplementation on cognitive function and Abeta-related biomarkers in mild cognitive impairment: a randomized controlled trial.* Eur J Nutr, 2019. **58**(1): p. 345-356.
186. Ma, F., et al., *Effects of folic acid and vitamin B12 , alone and in combination on cognitive function and inflammatory factors in the elderly with Mild Cognitive Impairment: a single-blind experimental design.* Curr Alzheimer Res, 2019.
187. Douaud, G., et al., *Preventing Alzheimer's disease-related gray matter atrophy by B-vitamin treatment.* Proc Natl Acad Sci U S A, 2013. **110**(23): p. 9523-8.
188. Jernerén, F., et al., *Brain atrophy in cognitively impaired elderly: the importance of long-chain omega-3 fatty acids and B vitamin status in a randomized controlled trial.* Am J Clin Nutr, 2015. **102**(7): p. 215-21.
189. Oulhaj, A., et al., *Omega-3 fatty acid status enhances the prevention of cognitive decline by B vitamins in Mild Cognitive Impairment* J Alzheimer's Dis, 2016. **50**(2): p. 547-557.
190. Li, M., et al., *Effect of folic acid combined with docosahexaenoic acid intervention on mild cognitive impairment in elderly: a randomized double-blind, placebo-controlled trial.* Eur J Nutr, 2020.
191. Selley, M.L., *A metabolic link between S-adenosylhomocysteine and polyunsaturated fatty acid metabolism in Alzheimer's disease.* Neurobiol Aging, 2007. **28**(12): p. 1834-1839.

192. Smith, A.D., F. Jernerén, and H. Refsum, *ω-3 fatty acids and their interactions.* The American Journal of Clinical Nutrition, 2021. **113**: p. 775-778.
193. Jerneren, F., et al., *Homocysteine status modifies the treatment effect of omega-3 fatty acids on cognition in a randomized clinical trial in mild to moderate Alzheimer's disease: The OmegAD study.* J Alzheimers Dis, 2019. **69**(1): p. 189-197.
194. Smith, A.D. and H. Refsum, *Dementia prevention by disease-modification through nutrition.* J Prev Alz Dis, 2017. **4**(3): p. 138-9.
195. Au, A., et al., *Estrogens, inflammation and cognition.* Frontiers in Neuroendocrinology, 2016. **40**: p. 87-100.
196. Hernán, M.A. and J.M. Robins, *Authors' Response, Part I: Observational Studies Analyzed Like Randomized Experiments: Best of Both Worlds.* Epidemiology, 2008. **19**(6).

Figure legends

Figure 1 Confounds that have plagued clinical trials of statin therapy

Figure 2. Directed acyclic graph analysis of B vitamin treatment and consequential changes in brain structure and function in MCI. The mediating pathway shows the optimal Bayesian network that explains the findings from the VITACOG trial.

Abbreviations:
tHcy-total homocysteine,
CDR-Clinical dementia rating scale
MMSE-Mini-mental state exam

Figure 3 Showing the common areas of discrepancy identified by expert review for each of the seven risk factors

Table 1 Existing DHA formulations

DHA Ester	Formulation	properties
Triacylglycerol ester	DHA esterified to triacylglycerol backbone	Most abundant natural form of DHA
Ethyl Esters	DHA esterified to ethanol	Synthetic form that converts into TG or PL DHA after absorption
Phospholipid Esters	DHA esterified to phosphatidyl choline or phosphatidyl serine	Demonstrates greater brain uptake compared with the other forms

Table 2 Comparison of omega-3 study designs between human and animal trials

	Human trials using omega-3 supplementation	Animal studies using a DHA dietary intervention
Dose	0.003-0.03 g/kg/day	0.6-0.24 g/kg/day
Age at the onset of intervention	>65	3-4 months
Duration of intervention	4 weeks-5 years	12 weeks-8 months
Effects on Cognition	Null	Enhanced cognitive functions
Effects on Abeta/Tau	No change in CSF Abeta/tau[160]	Decrease tau and abeta
Effects on synaptic functions	Not directly studied	Enhanced expression of synaptic proteins

Table 3 The mismatch between the epidemiological and clinical trial evidence, challenges and opportunities

	Challenges	Opportunities
Target population in terms of age The epidemiological evidence is generally strongest for risk factor exposure in midlife, however the majority of the clinical trials have taken place in later life populations with short duration of followup. .	Unrealistic to develop clinical trials that modify risk and protective factors during mid-life and examine its effects on late life dementia. Therefore, the trial efficacy is often examined under a hypothesis (or assumption) that given the treatment/intervention could be provided at later age, it would still show efficacy.	Important to examine differential efficacy levels across different age groups to develop sensitive outcome measures for the reliable detection of changes. Additional opportunities could include more sophisticated use of epidemiological data to understand risk factor variation and interactions over time/life-course, causal analyses of observational data [196] or the selection of future clinical trial participants with fully characterized past histories.
Target population in terms of characteristics of the participants There is a lack of data on the potential for different levels of benefit in different sub-groups	The more subgroups we include, the smaller sample size for each subgroup, lowing the statistical power. Harmonized diagnosis of dementia sub-types are often lacking in epidemiological studies	Careful selection of trial populations. Additional epidemiological work (new studies or further precise reporting from existing data) may be required to understand the risk factor/outcome relationship across cohorts with

E.g. risk factor level/severity or co-occurrence, a genetic risk or variations in the balance of different contributory dementia pathologies.	Risk factor levels/severity and clustering may differ in clinical trial participants and epidemiological cohorts.	different risk scores, chronic conditions, lifestyle factors and baseline disease severity and pathologies.
Target intervention. Type and dose of intervention drug or combination of drugs	We have not yet identified the levels of each risk factor that are associated with the best outcomes for cognition nor whether this differs by prior exposure.	Additional epidemiological work to identify potential targets for change (goals/biomarker change etc) supplemented by a greater understanding of the physiological processes and their potential inter-connectivity alongside trials looking at different goals or treatment targets.

Figure 1

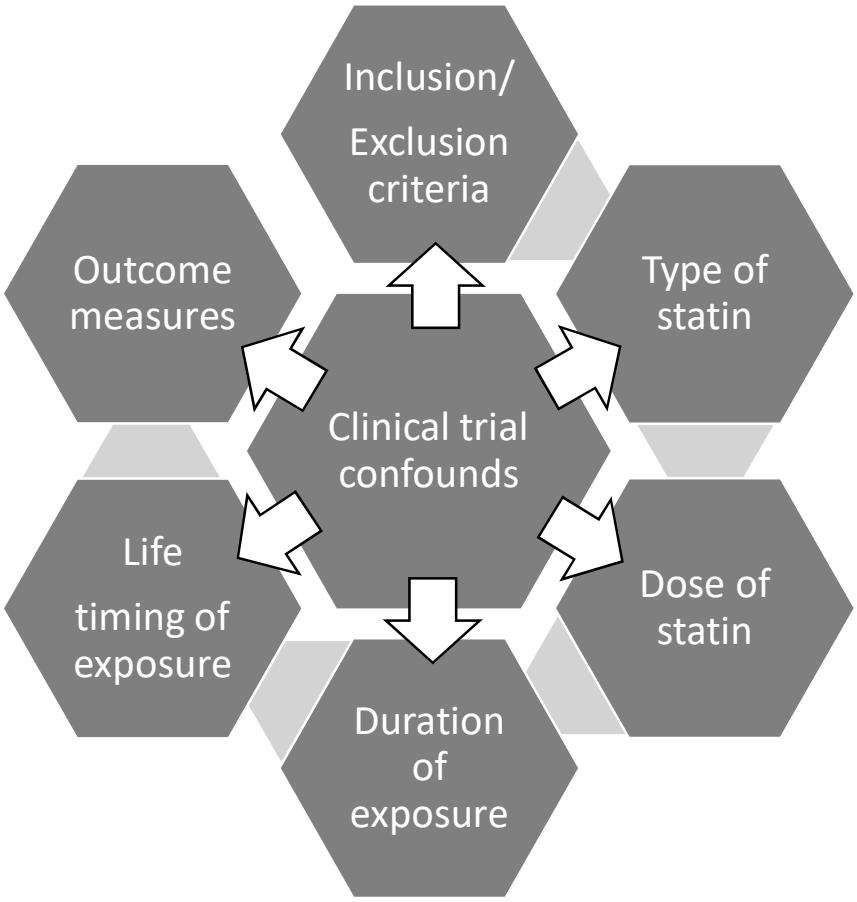
Inclusion/
Exclusion
criteria
Type of
statin
Dose of
statin
Duration
of
exposure
Life
timing of
exposure
Outcome
measures
Clinical trial
confounds

Figure 2.

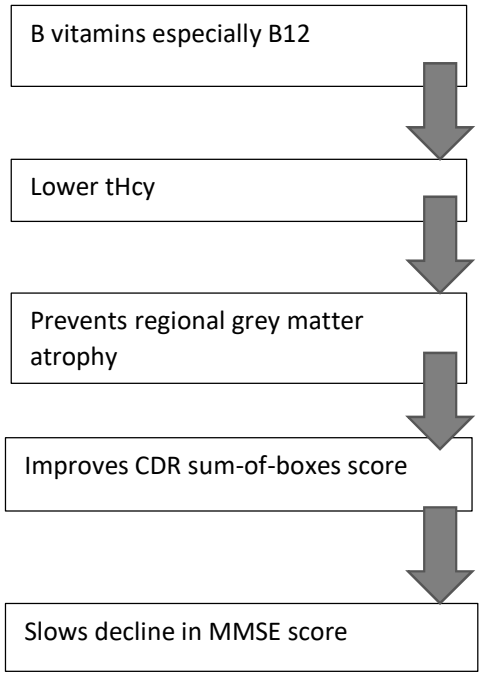
B vitamins especially B12
Lower tHcy
Prevents regional grey matter atrophy
Improves CDR sum-of-boxes score
Slows decline in MMSE score

Figure 3

Figure 3 Showing the common areas of discrepancy identified by expert review for each of the seven risk factors

Key:
Areas where discrepancies have been identified between the observational and clinical trial evidence base [X]
No discrepancy identified or no evidence available [ ]

	High blood pressure and anti-hypertensives	High cholesterol and statins	Diabetes and treatment of diabetes	Hormone regulation and hormone therapy	Omega 3 fatty acid and supple-mentation	Homo-cysteine and Vitamin B	Inflammation and NSAIDs
Target population (age). The epidemiological evidence is generally most robust for risk factor exposure at a particular time in the life-course, e.g., midlife. However, most clinical trials have taken place in populations at different ages, e.g., late-life.	X	X		X			X
Population subgroups to consider. Different subgroups may respond differently to risk reduction (e.g., there may be differences between those with and without a genetic risk profile). These may need to be selected for in trial populations.	X	X	X	X	X	X	X
Level of baseline risk factor /level of severity. Risk factor levels may differ in clinical trial and epidemiological samples. E.g., population samples will likely include people with a greater range of severity than a selective clinical trial population.	X	X	X	X	X	X	
Dementia type, balance of pathology/severity. Population samples are likely to show a range of dementia severity and pathology whereas interventions may need to be targeted to a specific at risk group.	X	X		X	X		X
Type of treatment/drug class/specific drug. Some drugs may have direct effects on cognition and therefore be more effective than others. Trials are usually selective in their choice of treatment whereas observational studies will have a range of treatment types.	X	X	X	X	X		X
Combined treatments. Combined treatments changing multiple risk factors may be required to achieve benefit. Trials are likely to have focused on individual treatments.					X	X	
Dose of intervention. Trials usually select a restricted range of doses which may miss the therapeutic level needed for cognition. Epidemiological studies are more likely to have a range of doses but often do not report details of doses.	X	X	X		X	X	
Expected goal level/size of the change in risk factor required. To select an at risk population and test the efficacy of risk reduction in a trial population we need more evidence to understand the risk factor levels that are associated with the best cognitive outcomes.	X	X	X		X		
Duration of intervention /length of clinical trials. Treatment is usually required long-term, whereas trials run for a few years at most.	X	X	X	X	X	X	X

Dementia risk reduction, why haven't the pharmacological risk reduction trials worked? An in-depth exploration of seven established risk factors.

1. Systematic review: The authors have reviewed and critically appraised the current evidence for pharmacological risk modification and dementia risk reduction for seven leading modifiable dementia risk factors (type 2 diabetes, dyslipidaemia, hypertension, estrogens, inflammation, omega-3 fatty acids and hyperhomocysteinemia).
2. Interpretation: Critical appraisal of the evidence base uncovered overlapping themes and knowledge gaps common to multiple risk factors. Sample heterogeneity and paucity of intervention details (dose, timing, formulation) were common.
3. Future directions: There remains a potential for dementia risk modification, particularly for antihypertensive use and vitamin B supplementation. Further work is needed to fully establish this, evaluating impact and reducing bias. Targeted and methodologically sophisticated investigations are now urgently needed to drive forward our understanding in this area and to inform recommended targets for concrete and effective risk reduction strategies.

..