

Inflammation and Cognitive Impairment in Beaver Dam, WI

By

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ABSTRACT

Background: As the number of older adults increases, dementia prevention will become an even greater public health concern. Increasing evidence suggests that chronic systemic inflammation may be involved in the development of dementia and other adverse outcomes, including atherosclerosis, CVD, diabetes, cancer, frailty, disability, and mortality. The role of inflammation in cognitive impairment is an important area of research, as inflammation levels are theoretically modifiable, for example through the use of anti-inflammatory medications, including non-steroidal anti-inflammatory drugs (NSAIDs) and statins. The goals of this dissertation were to determine whether elevated levels of inflammatory markers are associated with cognitive impairment, mild cognitive impairment (MCI), and dementia, and whether NSAID or statin use are associated with lower risk of cognitive impairment, MCI, and dementia.

Methods: In the population-based Epidemiology of Hearing Loss Study (EHLS), cognitive impairment was measured in 1998-2000, 2003-2005, and 2009-2010. MCI/dementia was measured in 2009-2010 only. Participants who were not cognitively impaired in 1998-2000 were considered at risk for cognitive impairment and MCI/dementia, and eligible to be included in these analyses. High-sensitivity C-reactive protein (CRP) and interleukin-6 (IL-6) levels were measured in 1988-1990, 1998-2000, and 2009-2010, and tumor necrosis factor-alpha (TNF- α) levels were measured in 1998-2000 only. Data on NSAID and statin medication use were prospectively collected at 5 time points between 1988-1990 and 2009-2010. Inflammatory marker and medication use data from baseline and prior to baseline were used to classify exposure history at baseline (1998-2000), and prospective analyses examined associations with subsequent cognitive impairment and MCI/dementia.

Results: Sustained high CRP level in both 1988-1990 and 1998-2000 was associated with decreased risk of cognitive impairment (hazard ratio (HR) = 0.53; 95% confidence interval (CI) = 0.28, 0.99), sustained high IL-6 was suggestively associated with increased risk (HR = 2.35; 95% CI = 0.90, 6.14), and baseline TNF- α was not associated with the cognitive outcomes. Participants using aspirin at baseline but not 5 years prior were more likely to develop cognitive impairment compared to never users (HR = 1.83; 95% CI = 1.15, 2.89), with weaker non-significant associations for longer term use. Non-aspirin NSAID use was not associated with the incidence of cognitive impairment or the odds of MCI/dementia. Statin use among women was associated with increased risk of cognitive impairment (current vs. never HR = 1.82; 95% CI = 1.07, 3.09). Among men, statin use was not associated with risk in the adjusted model (HR = 0.69; 95% CI = 0.32, 1.52). Those who were cognitively impaired 10 years after baseline were more likely to be former statin users. No significant associations were observed with the MCI/dementia outcome, although results were similar to those for cognitive impairment.

Conclusions: This research provided some additional evidence for a role of systemic inflammation in the development of cognitive impairment, as sustained high IL-6 levels were associated with increased risk. Inverse CRP associations might reflect underestimation due to hardy survivor bias or increased statin use over the study period. There was no support for a potential protective role of NSAIDs or statins, with null or positive associations observed. The positive associations could reflect indication bias. The results of this dissertation suggest that inflammation may be involved in cognitive impairment, but the relevant inflammatory mechanisms may be upstream from CRP and not affected by NSAID or statin use.

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ABBREVIATIONS

3MS	Modified Mini-Mental State examination
ACT	Adult Changes in Thought
AD	Alzheimer's disease
<i>APOE</i>	Apolipoprotein E gene
ARIC	Atherosclerosis Risk In Communities
AVLT	Auditory Verbal Learning Test
BBB	Blood-brain barrier
BDES	Beaver Dam Eye Study
BLSA	Baltimore Longitudinal Study of Aging
BMI	Body mass index
BOSS	Beaver Dam Offspring Study
CHAP	Chicago Health and Aging Project
CHS	Cardiovascular Health Study
CI	Confidence interval
CDR	Clinical Dementia Rating
CRP	C-reactive protein
CSHA	Canadian Study of Health and Aging
CV	Coefficient of variation
CVD	Cardiovascular disease
DSM	Diagnostic and Statistical Manual of Mental Disorders
DSST	Digit Symbol Substitution Test
EHLS	Epidemiology of Hearing Loss Study
ELISA	Enzyme-linked immunosorbent assay
EPESE	Established Populations for Epidemiologic Studies of the Elderly
EURODEM	European Community Concerted Action Epidemiology of Dementia
FHS	Framingham Heart Study
HAAS	Honolulu-Asia Aging Study
HDL-C	High-density lipoprotein cholesterol
HR	Hazard ratio
HRS	Health and Retirement Study
IMT	Intima-media thickness
IL-6	Interleukin-6

LLA	Lipid-lowering agent
MCI	Mild cognitive impairment
MI	Myocardial infarction
MMSE	Mini-Mental State Examination
MoVIES	Monongahela Valley Independent Elders Survey
MRC	Medical Research Council
MSSA	MacArthur Study of Successful Aging
NSAID	Non-steroidal anti-inflammatory drug
OR	Odds ratio
PAQUID	Personnes Agées Quid
RCT	Randomized controlled trial
ROS	Religious Orders Study
RR	Relative risk
SD	Standard deviation
SF-36 MCS	Short Form Health Survey mental component score
TC	Total cholesterol
TMT-A	Trail Making Test (part A)
TMT-B	Trail Making Test (part B)
TNF- α	Tumor necrosis factor-alpha
VaD	Vascular dementia
VFT	Verbal Fluency Test

CHAPTER 1

INTRODUCTION and LITERATURE REVIEW

1.A Cognitive impairment: dementia and mild cognitive impairment (MCI)

Dementia

Dementia is a syndrome characterized by loss of intellectual abilities severe enough to interfere with normal activities, and involves impairment in multiple cognitive domains such as memory, judgment, personality, and/or abstract thinking. It can be caused by a large variety of conditions, including Alzheimer's disease (AD), cerebrovascular disease, multiple sclerosis, Parkinson's disease, HIV infection, folic acid deficiency, brain trauma or tumors, and infection of the central nervous system.¹ Several diagnostic classification systems exist, including the *Diagnostic and Statistical Manual of Mental Disorders* (DSM), the *International Classification of Diseases* (ICD), and the Cambridge Examination for Mental Disorders for the Elderly (used mainly in the UK). Most recently, a set of criteria for "all-cause dementia" was published as part of the newly recommended criteria for "dementia due to Alzheimer's disease", from the National Institute on Aging -Alzheimer's Association (NIA-AA) workgroups on diagnostic guidelines for AD.² The NIA-AA workgroup outlined criteria for all-cause dementia including cognitive or behavioral symptoms that: 1) interfere with ability to function at usual activities; and 2) represent a decline from previous levels; and 3) are not due to delirium or a major psychiatric disorder; 4) cognitive impairment is detected through patient and informant interview and an objective cognitive assessment, either "bedside" mental status or neuropsychological testing; 5) cognitive or behavioral impairment involves at least of the following two domains: ability to acquire and remember new

information, reason and handle complex tasks, visuospatial abilities, language functions, changes in personality, behavior, or comportment.²

AD is by far the most commonly diagnosed type of dementia and a considerable portion of dementia research is focused on AD-type dementia. Alois Alzheimer first reported finding plaques and tangles in a non-elderly individual's brain.³ Oscar Fischer later reported plaques in brains of elderly demented individuals (Fischer 1907, in German, as cited in ⁴). For decades, the condition in the non-elderly was called AD, but in the elderly was considered normal aging, and was called senile dementia rather than AD. In a 1976 editorial, Katzman reasoned that both AD and senile dementia were names for the same process and should be considered a single disease, AD.⁵ The prevalence of senile dementia had increased substantially with increasing life expectancy over the twentieth century, and thus the prevalence of AD increased dramatically once it included senile dementia.

However, it appears that the field is currently moving towards a consensus that most dementia cannot be attributed to AD- type pathology alone, but rather co-dominant or mixed pathologies (e.g., AD and vascular lesions). Recent findings have questioned both the specificity of AD-type pathology to the clinical disease AD, and the commonness of pure AD pathology (i.e., only AD-type) in those with clinical AD. For example, several studies have reported poor agreement between clinical diagnosis and pathological diagnosis upon autopsy.⁶⁻⁸ In the Rush study, 50 of the 141 participants with autopsy data had clinical dementia (39 probable AD, 8 possible AD, 3 other dementia). Of these 50 with clinical dementia, the most common pathological diagnosis was AD with infarctions (38%), followed by AD with no other contributing pathology (30%). Among the 91 with no dementia, the most common pathologic findings were no chronic diagnostic abnormalities (29%), followed by AD with no other contributing pathology (24%), infarctions (18%), and AD with other contributing pathologies (18%). The authors'

major conclusion was that those with multiple types of pathology had much greater odds of dementia, compared with those with only one type of pathology (odds ratio (OR) = 2.8; 95% confidence interval (CI) = 1.2, 6.7).⁷ In the Medical Research Council (MRC) Cognitive Function and Ageing Study (UK), 100 of the 209 participants with autopsy data had dementia prior to death. Of those with dementia, 36% had pathological features meeting the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria for definite AD and 28% had features suggesting probable AD, but the remaining 36% had low plaque scores or no plaques. Conversely, of the 109 with no dementia, 33% had pathologic features indicative of definite or probable AD.⁸ In the Honolulu-Asia Aging Study (HAAS), possible or probable AD was considered the sole or dominant cause of 56% of dementia cases during life, but upon autopsy, AD type pathology was observed as the sole or dominant lesion type in only 19% of dementia cases. Mixed, or codominant, lesion types, usually microinfarcts along with AD type lesions, were observed in 40% of the decedents with dementia, although based on clinical evaluation during life, only 16% had been attributed to mixed etiologies.⁶ In fact, the newly recommended criteria for AD highlight major shifts in the collective understanding of the disease. Included is new recognition of the common coexistence of subclinical cerebrovascular disease and AD pathology, and the related need for conceptual and semantic distinction between the pathophysiological processes of AD and the clinical syndrome AD.⁹

In 2010, an estimated 35.6 million people worldwide had dementia and by 2050 the projected prevalence is 115.4 million.¹⁰ Estimates of the incidence of dementia vary widely across studies and increase steeply with age (Table 1-1, and for AD only, Table 1-2). There are significant differences in the study populations, length of follow-up, and the criteria used to define dementia.

Mild cognitive impairment (MCI)

Several similar terms and concepts have been used to describe the state between normal cognition and dementia, but MCI is the most established. In 1999, Petersen et al. published criteria for MCI which emphasized their conceptualization of MCI as memory-specific impairment preceding AD.¹¹ The original criteria were: memory complaint, abnormal memory for age, normal activities of daily living, normal general cognition, and absence of dementia.¹¹ Subsequently, the criteria for MCI were revised to reflect a growing consensus that MCI could involve impairment of domains other than memory.¹² Amnesic MCI (aMCI), a sub-type of MCI, became the new term for memory-specific MCI. More recently, the MCI criteria have been revised yet again. As per the NIA-AA workgroup, the recommended criteria for MCI are 1) a concern regarding a change in cognition; 2) impairment in one or more cognitive domains; 3) preserved independence in function abilities; 4) not demented.¹³ The criterion of objective impairment in one or more domains is particularly vague. Within the same population, varying the applied definition, such as stringency of impairment cutoff (e.g., -1.0, -1.5, or -2.0 standard deviations (SD) below the mean) or the source of the normative data (e.g., published or sample-derived) resulted in MCI prevalence estimates ranging from 4-70%.¹⁴

Etiologic heterogeneity may be even greater for MCI compared to dementia, as a larger variety of conditions (e.g., systemic, psychiatric) may cause the less severe symptoms of MCI.¹⁵ Even among large prospective cohort studies, large variation exists in the estimates of MCI incidence (Table 1-3), likely due to significant differences in the study populations, length of follow-up, and the criteria used to define MCI. Furthermore, relatively low rates of progression to dementia have been reported (e.g., 27% in 10 years,¹⁶ 11% over 3 years,¹⁷ 7% over 2 years¹⁸), while substantial portions of those with MCI may remain stable or revert to normal.¹⁶⁻¹⁸ The Nun Study observed that only age was associated with the odds of

transitioning from MCI to dementia, and suggested that dementia risk factors predispose individuals to MCI, but progression to dementia depends on time and competing causes of death.¹⁹

Summary

In summary, dementia is a major cause of death and disability. As the number of older people continues to grow, dementia is expected to continue to be an important public health concern. Despite conflicting estimates of the progression rate to dementia, MCI is thought to be a precursor to dementia and thus may represent a better stage for intervention. Research on MCI has increased, just as the focus for some other chronic diseases has shifted to recognize the importance of earlier stages of disease (e.g., subclinical atherosclerosis and cardiovascular disease). Identifying risk factors for both dementia and MCI will be important in order to better understand and prevent these conditions.

Table 1-1. Estimates of dementia incidence from previous studies													
	Setting	N at risk; age (y); (ave) f/u	Diagnostic criteria	Age-specific incidence (per 1000 person-years or annual %)									
				50-54	55-59	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-99
²⁰	Monongahela Valley, PA; MoVIES; voters	N=1298 Age: 65+ 9-11y f/u	DSM-III-R; CDR for severity (≥ 0.5 & ≥ 1.0 presented)				4.3 2.1	12.0 5.8	21.1 12.4	56.8 29.8	74.5 31.1	80.0 ^b 51.1 ^b	
²¹	4 communities; CHS; Medicare lists	N=3375 Age: 65+ 5.5y f/u	Non-standard (ADLs affected, impairment in ≥ 2 domains);					w:11.7 ^a B:19.3 ^a	w:32.0 B:32.6	w:57.5 B:88.4	w:95.9 ^b B:130.5 ^b		
²²	Framingham, MA; FHS; Census sample + volunteers (i)	N=2391 Age:30-59 (in 1950) 2-10y f/u ^c	3 domains impaired (<i>transformed from 5y KM</i>)				1.4	5.3	10.3	16.2	23.6		
²³	Seattle, WA; ACT; GHC members	N=2581; (2356 w f/u) Age: 65+; ~4.5y f/u	DSM-IV for dementia				4.7%	8.4%	12.0%	35.8%	54.1%	84.2% ^b	
²⁴	Bronx, NY; Bronx Aging Study; Volunteers	N=442 Age: 75-85y Max. 8y f/u	DSM-III for dementia (<i>transformed from per 100/y</i>)						13	35	60 ^b		
²⁵	Bronx, NY; Bronx Aging Study; Volunteers	N=442 Age: 75-85 Max. 21y f/u	DSM-III-R (<i>transformed from per 100/y</i>)						17.1	39.6	74.8	111.1	145.2 ^b
²⁶	Rochester, MN; Rochester Epi. Project	N = 14439 Age: 50-99 4 y f/u	DSM-IV; Medical records	0.356	0.402	1.292	1.652	5.211	13.29	25.63	41.45	54.94	69.87

	Setting	N at risk; age (y); (ave) f/u	Diagnostic criteria	Age-specific incidence (per 1000 person-years or annual %)									
				50-54	55-59	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-99
²⁷	~Laguna Woods, CA Leisure World Cohort Study	N=344; (330 w f/u); Age: 90+ 2.3y f/u	DSM-IV for dementia									127	212 (407 for 100+)
²⁸	Pooled from 8 European studies		See specific studies, mostly DSM-III-R				2.4	5.5	16.0	30.5	48.6	70.2 ^b	
²⁹	Denmark; Odense Study; Random sample of population	N=3086 (2452 w f/u) Age: 65-84 2.1y f/u	DSM-III-R; also CDR for severity				12.4	22.5	41.3	82.2			
				≤68	69-71	72-74	75-77	78-80	81-83	84-86	87-89	90-92	≥93
³⁰	Cache County, UT; Cache County Study; Pop-based	N=4614 (3308 w f/u) Age: 65+ (1995) ~3.2y f/u	DSM-III-R	2.21	3.57	9.51	17.8	38.1	52.5	73.89	101.74	121.74	110.01
				65-74			75-84			≥85			
³¹	Ibadan, Nigeria (NG) +Indianapolis, IN; IIDP	N = 2359 (NG) + 2147 (US); Age: 65+ 4.7y f/u (US) 5.1y f/u (NG)	DSM-III-R <u>and</u> ICD-10 (Note: corrected for mortality & sampling)	NG: 0.45% US: 1.74% (annual)			NG: 1.69% US: 4.29% (annual)			NG: 5.71% ^b US: 9.12% ^b (annual)			

DSM = Diagnostic and Statistical Manual of Mental Disorders; CDR = Clinical Dementia Rating (severity); IIDP = Indianapolis-Ibadan Dementia Project; ACT = Adult Changes in Thought; (i) = included institutionalized; (a) Incidence rate for this age group and younger (e.g., < 75); (b) Incidence rate for this age group and older (e.g., ≥ 85); (c) = 2 subsamples combined, different baselines (1976/1978 or 1982/1983); W = whites, B= black (not reported overall).

Table 1-2. Estimates of Alzheimer's disease incidence from previous studies													
	Setting	N at risk; age (y); Years of f/u	Criteria for AD	Age-specific AD incidence (per 1000 person-years or annual %)									
				50-54	55-59	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-99
²⁰	Monongahela Valley, PA; MoVIES; voters	N=1298 Age: 65+ 9-11y f/u	NINCDS-ADRDA probable & possible; CDR for severity (≥ 0.5 & ≥ 1.0 presented)				2.1 2.1	8.5 4.6	16.1 10.0	45.1 25.8	48.8 26.8	70.2 ^b 50.9 ^b	
²¹	4 communities; CHS; Medicare lists	N=3375 Age: 65+ 5.5y f/u	DSM-IV, NINCDS-ADRDA, CA ADDTTC, NINDS-AIREN					w:5.9 ^a B:10.8 ^a	w:16.7 B:11.6	w:28.0 B:46.3	w:48.9 ^b B:74.6 ^b		
³²	East Boston, MA; EPESE; census enumeration	N=2313 (1601 w f/u) Age: 65+ 4.3y f/u	NINCDS-ADRDA for probable AD;				0.6%	1.0%	2.0%	3.3%	8.4% ^b		
²²	Framingham, MA; FHS; Census sample + volunteers (i)	N=2391 Age:30-59 (in 1950) 2-10y f/u ^c	NINCDS-ADRDA for probable AD (<i>transformed from 5y KM</i>)				0.7	3.1	6.0	10.7	14.6		
²³	Seattle, WA; ACT; GHC members	N= 2581 (2356 w f/u) Age: 65+ ~4.5y f/u	NINCDS-ADRDA for probable & possible AD				2.8%	4.4%	7.8%	27.5%	41.9%	56.1% ^b	
²⁶	Rochester, MN; Rochester Epi. Project	N = 14439 Age: 50-99 4y f/u	DSM-IV; Medical records	0.213	0.161	0.369	1.032	3.151	9.894	19.44	32.49	46.56	56.77

	Setting	N at risk; age (y); Years of f/u	Criteria for AD	Age-specific AD incidence (per 1000 person-years or annual %)									
				50-54	55-59	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-99
³³	Baltimore, MD; BLSA; Volunteers	N=1236 Age: 55+ ~7.5y f/u	DSM-III-R for dementia, NINCDS-ADRDA for definite, probable, possible AD		0.0%	0.08%	0.13%	0.42%	0.89%	2.16%	6.48% ^b		
²⁸	Pooled from 8 European studies						1.2	3.3	9.1	21.8	35.3	53.5 ^b	
³⁴	Southwest France; PAQUID; Electoral rolls	N=2043 Age: 65-101 1-3y f/u	NINCDS-ADRDA for probable & possible AD				0.7	2.5	10.9	20.4	42.9	65.6 ^b	
²⁹	Denmark; Odense Study; Random sample of population	N=3086 (2452 w f/u) Age: 65-84 2.1y f/u	DSM-IIIR; CDR for severity, included “questionable”; NINCDS-ADRDA for probable AD				6.0	15.1	26.9	74.1			
	Setting	N at risk; age (y); Years of f/u	Criteria for AD	Age-specific AD incidence (per 1000 person-years or annual %)									
				≤68	69-71	72-74	75-77	78-80	81-83	84-86	87-89	90-92	≥93
³⁰	Cache County, UT; Cache County Study; Pop-based	N=4614 (3308 w f/u) Age: 65+ (1995) ~3.2y f/u	DSM-III-R for dementia; NINCDS-ADRDA for AD	2.21	1.97	6.03	9.24	18.84	36.01	57.91	73.28	96.11	73.58

	Setting	N at risk; age (y); Years of f/u	Criteria for AD	Age-specific AD incidence (per 1000 person-years or annual %)		
				65-74	75-84	≥85
³¹	Ibadan, Nigeria (NG) +Indianapolis, IN; IIDP	N = 2359 (NG) + 2147 (US); Age: 65+ 4.7y f/u (US) 5.1y f/u (NG)	NINCDS-ADRDA for probable & possible AD (corrected for mortality & sampling)	NG: 0.38% US: 1.38% (annual)	NG: 1.41% US: 3.29% (annual)	NG: 5.02% US: 7.07% (annual)
³⁵	Chicago, IL; census enumeration (i);	N=3838 Age: 65+ 4.1y f/u	NINCDS-ADRDA for probable AD (included MD cases); adjusted for sampling	1.45% (annual)	4.73% (annual)	9.11% (annual)
³⁶	N.Manhattan, NY; Medicare beneficiaries	N=1799 Age: 65+ ~2.9y f/u	NINCDS-ADRDA for probable & possible AD	1.3%	4.0%	7.9%

DSM = Diagnostic and Statistical Manual of Mental Disorders; NINCDS-ADRDA = National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association; NINCDS-AIREN = National Institute of Neurological and Communicative Disorders and Stroke-Association Internationale pour la Recherche et l'Enseignement en Neurosciences International Workshop; CDR = Clinical Dementia Rating (severity); IIDP = Indianapolis-Ibadan Dementia Project; ACT = Adult Changes in Thought; MD= mixed dementia; CA ADDTC = State of California Alzheimer's Disease Diagnostic and Treatment Centers; (i) = included institutionalized; (a) Incidence rate for this age group and younger (e.g., < 75); (b) Incidence rate for this age group and older (e.g., ≥ 85); (c) = 2 subsamples combined, different baselines (1976/1978 or 1982/1983); W = whites, B= black (not reported overall); Note: the NINCDS-ADRDA criteria for probable AD require dementia confirmed by neuropsychological testing with deficits in two or more areas of cognition.

Table 1-3. Estimates of mild cognitive impairment incidence from previous studies									
	Setting	N at risk; age (y); Years of f/u	Criteria	Age-specific incidence (per 1000 person-years)					
				60-64	65-69	70-74	75-79	80-84	85-89
³⁷	N. Manhattan, NY; Medicare recipients in 3 census tracts	N=1800 (w f/u); Age: 65+; 4.7 y f/u	MCI (Petersen 2001, 2004)		28.2	47.1	56.4	65.7 ^b	
³⁸	Sweden; Kungsholmen Project;	N=1070 Age: 75+; 9 y f/u (max)	aMCI (Petersen 1999, 2005), and OCIND;				5.3	9.6	16.3 ^b
³⁹	Conselice, Italy; CSBA; Whole pop. recruited (+ i, n=34)	N = 865 (685 w f/u); Age: 65+; 3.8 y f/u	MCI (Winblad 2004) ^c		49.5	69.3	93.8	94.9	89.7 ^b
⁴⁰	Italy (8 cities); ILSA; Stratified sample of electoral rolls (+ i)	N=1524 Age: 65-84; 3.5 y f/u (max)	MCI (Petersen 1999, 2001 w/o SMC criterion)		3.7	9.6	55.8	46.6	
⁴¹	France; PAQUID	N = 1265 Age: 65+ 5 y f/u (max)	MCI (Petersen 1999), also OCIND			12.4	7.7	9.5	12.4 ^b
				65-74		75-84		≥85	
⁴²	Indianapolis, IN; IIDP; census tracts	N=2041 Age: 65+ ND (2 and 5y f/u)	CIND/MCI;		34.3		64.4		96.2
⁴³	Germany; LEILA75+; population registry (+i, n=192)	N=732 (519 w f/u); Age: 75+; ~3.5y f/u	MCI (Winblad 2004) ^c		50.3		84.8		157.6
				Overall incidence rate per 1000 person-years (no age-specific provided)					
⁴⁴	Kuopio, Finland; Random sample of population reg. (+i)	N=747 (550 w f/u); Age: 60-76;3.3 y f/u	MCI (Petersen)	25.9					

LEILA75+ = Leipzig Longitudinal Study of the Aged; SMC = subjective memory complaint; CSBA = Conselice Study of Brain Ageing; (b) Incidence rate for this age group and older (e.g., ≥ 85); (c) = also gives rates by amnesic and non-amnesic; (i) = included institutionalized; OCIND = other cognitive impairment, no dementia (mutually exclusive with aMCI).

1.B Cognitive impairment: Risk factors

Age

Dementia incidence increases exponentially with age, at least until 90 years of age.^{45,46} Beyond 90 years of age, estimates are less certain given smaller sample sizes and losses to follow-up in the oldest age groups. Some studies report that the increases in incidence plateau at older ages,⁴⁵ while others report that rates continue to rise past the age of 100 years.²⁷ The authors of a meta-analysis of incidence studies in the U.S., including the Framingham Heart Study (FHS), the Bronx Aging study, and the East Boston Established Populations for Epidemiologic Studies of the Elderly (EPESE), and elsewhere (UK, Germany, Sweden, France, China, Japan) concluded that the incidence of both dementia and AD continues to increase until at least age 98 (their upper limit), but that the rate of increase slows at the oldest ages. They observed dementia incidence rates that tripled for each 5-year increase in age before age 63, doubled between 64 and 75, and by age 84 were only about 1.5 times greater per 5-year increase in age. The pattern was very similar for AD. They note that their observations may have been affected by survival effects.⁴⁵ Corrada et al. report incidence rates almost double every 5 years from 90 years of age to over 100 years, with no leveling off; annual incidence was 12.7% in those age 90-94 years, 21.2% for 95-99 year age group, and 40.7% in the 100+ year age group.²⁷

Age is well established as a strong risk factor for cognitive impairment. The nature of the association of age with dementia incidence could provide clues to the etiology of dementia. If dementia is an inevitable result of brain aging (i.e., age-dependent), rates will continue to increase into the oldest age groups, and if not for death, would eventually reach 100%. Conversely, one would not necessarily expect incidence to continue to increase at a constant rate if age is only a proxy for greater exposure to other

risk factors and co-morbidities (i.e., age-related). In the latter scenario, avoidance of risk factors would theoretically prevent disease.

Sex

Many, but not all, studies have found no evidence that dementia rates differ by sex, including the Rochester Epidemiology Project,⁴⁷ the FHS,²² the Baltimore Longitudinal Study of Aging (BLSA),³³ the East Boston EPESE,⁴⁸ the Chicago Health and Aging Project (CHAP),³⁵ the Cardiovascular Health Study (CHS),²¹ and the Adult Changes in Thought study (ACT).²³ The Duke EPESE found white women, compared to white men, had greater rates of dementia but the difference was not statistically significant (based on only 5 male and 25 female cases in whites).⁴⁹

Few studies have reported rate differences by sex, and more often for AD than dementia in general. Age- and education-adjusted incidence was 50% greater for men in the Monongahela Valley Independent Elders Survey (MoVIES) cohort for dementia with a Clinical Dementia Rating (CDR) = 0.5, or “possible/incipient dementia of any etiology”, although no significant difference by sex among the subgroup with CDR ≥ 1 or the subgroup with probable/possible AD and CDR = 0.5. The authors propose a possible increased outmigration of men with the greatest intellectual abilities due to the G.I. bill and lack of opportunity in the region, without similar outmigration in women.²⁰ Results from the Personnes Agées Quid (PAQUID) study indicated a sex by age interaction for AD incidence, such that men were at greater risk until age 80 years, after which women were at greater risk; no significant association between sex and dementia in general.⁵⁰ The European Community Concerted Action Epidemiology of Dementia (EURODEM) pooled analysis, which includes the PAQUID study (France), the Odense Study (Denmark), the Rotterdam study (Netherlands), and the MRC-ALPHA study (UK), reported that women

had a 50% increased risk of AD and a 20% increased risk for dementia, adjusted for age, age², study site, and education.⁵¹ In the ACT cohort, women had statistically significant lower rates of non-AD dementia.²³ Based on a meta-analysis of 7 studies (U.S. and other, excluded Rochester), Gao et al. reported greater incidence of AD in women (OR = 1.5, 95% CI: 1.2 - 2.1) but no sex effect for dementia incidence.⁴⁵ They suggest that the difference in sex effect between AD and dementia is due to the fact that men are at greater risk of vascular dementia. They include a few potential reasons for the increased incidence of AD in women: a potential sex interaction with Apolipoprotein E (*APOE*) genotype,⁵²⁻⁵⁴ lack of estrogen and other hormonal changes following menopause, which could increase risk, and differential survival effects.

Overall, the epidemiological evidence is inconclusive regarding a sex effect on dementia incidence. In general, the European studies reported increased risk among women, especially for AD; but the U.S. studies found no differences by sex. Although this geographic variation between European and U.S. studies has been noted in the literature,^{26,55} no satisfactory explanations exist, except a vague suggestion of differences in sex-specific exposures between these two regions. The studies that showed differences by sex showed those differences increasing at older ages. If women are at greater risk, the most often suggested reason is decreased estrogen following menopause. Another explanation for the observed sex difference could be survival effects, as women have longer life expectancy, perhaps they are more likely to survive until they develop dementia and/or it is documented.

Education

Many studies have reported lower rates of dementia among the more educated, including BLSA,³³ CHS,²¹ East Boston,⁵⁶ PAQUID,⁵⁰ Canadian Study of Health and Aging (CSHA),⁵⁷ Amsterdam Study of the

Elderly,⁵⁸ a Finnish study,⁵⁹ and Rotterdam.⁶⁰ In a pooled analysis of data from the EURODEM group, lower education levels were associated with dementia and AD incidence in women, but not men.⁶¹ In the MoVIES cohort, less education was significantly associated with greater incidence only in the broadest category ($CDR \geq 0.5$) and the subgroup $CDR = 0.5$ (see discussion in the section on sex above, re: outmigration from the region).²⁰ From the 90+ study, Corrada et al. report an interaction of sex and education such that very old women, but not men, with some graduate school education had a slightly decreased risk of dementia (vs. high school or less, relative risk (RR) =0.59, $p=0.09$). They believe this is most likely due to uncontrolled confounding.²⁷ Letenneur et al. observed a similar pattern for participants under 90 years of age in a EURODEM meta-analysis, with less education (< 12 years, compared to ≥ 12) associated with increased risk of AD and any dementia only among women, and null associations among men.⁶¹ These authors also suggest this finding could be due to confounding or chance.

A few studies have failed to find significant associations between education and dementia risk. Cobb et al. report that age and education were highly correlated in the FHS population; after adjusting for age, the association between education and incident dementia or probable AD was no longer significant. Yet there was a significant increased risk for non-AD dementia among those with less education.⁶²

Investigators from the Hisayama Study in Japan found no association between low education (6 years or less) and incident dementia, vascular dementia, or AD.⁶³ Among participants in the Religious Orders Study (ROS), cognitively stimulating activity was associated with reduced risk of incident AD (adjusted for education), but education itself was not associated with incident AD.⁶⁴ The authors state this could be due to the high levels of education in this cohort (88% with 16 years or more). Among participants in the Amsterdam Study of the Elderly, low intelligence (DART-IQ) predicted incident dementia better than

low education, and education was not significant in their final model,⁶⁵ although a later publication from this study, regarding analyses not including intelligence, did report an increased risk of AD among the less educated.⁵⁸

The education-dementia effect could be due to bias, perhaps from differential losses to follow-up by education or ascertainment bias due to better cognitive test performance in more educated participants. The former possibility was examined by sensitivity analyses in two studies, one Finnish⁶⁶ and one Dutch,⁵⁸ and both sets of authors concluded that differential losses to follow-up by education appear to influence the magnitude of the education-dementia association, but not be solely responsible for it. For example, in the Finnish Cardiovascular Risk Factors, Aging and Dementia (CAIDE) study, among participants whose dementia status was measured directly (screening, diagnostic evaluations), high education (≥ 9 years) compared to low education (≤ 5 years) was associated with reduced odds of dementia (OR = 0.16; 95% CI = 0.06-0.41).⁶⁶ Dementia status of non-participants at follow-up was ascertained from registries, and with the non-participants included in analyses, the association was attenuated but still significant (OR = 0.40; 95% CI = 0.22-0.72).⁶⁶ Ascertainment bias is also a possible explanation for the association, as a positive association between years of education and better cognitive test performance is fairly well established, probably at least in part because those with more education are more practiced and confident at test-taking and abstract problem-solving.⁶⁷ Yet it is not clear if this is a detection problem (resulting in under-diagnosis among better educated), or if the benefits of education are truly protecting against, or delaying, cognitive decline and dementia (see section on cognitive reserve, below).

Education could be indirectly associated with cognitive impairment through differences in behaviors, exposures, and health care. For many reasons, people with less education may generally have

unhealthier lifestyles and more cardiovascular risk factors, which have been shown to also increase dementia risk. However, the Finnish CAIDE study reported that the education-dementia association remained despite adjustment for midlife income, occupation, physical activity, smoking, blood pressure, obesity, cholesterol, and *APOE* ϵ 4 carrier status (OR = 0.15; 95% CI = 0.06-0.37, ≥ 9 years vs. ≤ 5 years of education). Additional adjustment for late-life myocardial infarction (MI), stroke, diabetes, and depressive symptoms resulted in attenuation of the effect (OR=0.34; 95% CI = 0.11-1.06).⁶⁶

Education may be directly linked to cognitive function, perhaps as postulated in cognitive reserve theory. Autopsy data has shown that education is associated with clinical dementia, but not brain pathology.^{68,69} In the ROS, the association of brain pathology to cognitive function was weaker for each additional year of education.⁶⁹ Such observations have led to the concepts of brain reserve⁷⁰ and cognitive reserve.⁷¹ Brain reserve is generally conceived as passive, with a fixed brain capacity reserve based on number of neurons and brain size, whereas cognitive reserve is conceived as a dynamic process to cope with brain damage through less susceptible, more efficient, or redundant existing brain networks (neural reserve) and/or novel compensatory mechanisms (neural compensation).⁷² Cognitive reserve is not fixed, but is likely affected by accumulated exposures, including factors such as brain reserve, education, occupation, cognitive-stimulating and productive activities, traveling, and socializing.⁷² Greater cognitive reserve is thought to decrease or delay the effects of brain pathology on cognitive functioning. Studies have shown that AD patients with higher levels of education have faster cognitive decline⁷³ and increased mortality.⁷⁴ These observations are in accordance with the cognitive reserve hypothesis, which predicts that at any given level of cognitive function, the underlying pathology is more advanced among those with more reserve.⁷²

In summary, the vast majority of evidence indicates there is an association between education and cognitive impairment. However, the reasons for this association are not well understood. Biases may account for a portion of the association. Education may be associated with dementia risk through other mechanisms, such as environmental exposures, health behaviors, access to health care, or health conditions such as MI, stroke, or diabetes. Finally, cognitive reserve theory asserts that education and other stimulation of the brain may result in brain networks that are less susceptible to deleterious pathology, and better able to compensate.

Race and ethnicity

It is unclear whether dementia incidence rates vary by race and ethnicity, as there have been few incidence studies that included minority groups. Tang et al. found greater incidence of AD among African Americans and Caribbean Hispanics, compared to whites, among a cohort of Medicare beneficiaries in northern Manhattan.³⁶ The Duke EPESE study found no difference in the prevalence or 3-year incidence of dementia between black and white elderly in the Piedmont area of North Carolina.⁴⁹ The CHS found borderline significant racial differences (age- and education-adjusted), although they say this could be due to ascertainment methods, and conclude that incidence rates did not differ by race.²¹ Evans et al. found non-significant but suggestive increased odds of dementia among black participants (OR for black race = 1.84, 95% CI = 0.73- 4.66 with race* *APOE* ϵ 4 interaction, 1.28, 95%CI= 0.54-2.98 without interaction) and concluded that this could suggest black race in itself increases risk, although the main finding was a substantial difference in *APOE* ϵ 4 effect by race.³⁵

Period effects

In 1993, researchers at the Mayo Clinic reported that the incidence of dementia in Rochester, MN had generally increased between 1960 and 1984, although the incidence was inexplicably higher during the period from 1960-1964, compared with 1965-1974. They also reported, over time, a greater proportion of cases were attributed to mixed AD plus other etiology, while AD only cases remained stable, and there were fewer cases due to cerebral infarct or unknown etiology. Their data was based on medical records rather than systematic evaluations of all participants, and the observed increase in incidence may be due to ascertainment bias, as awareness of dementia and particularly AD was increasing during the 1970s and 1980s, along with nursing home admissions, which required a physician examination.⁷⁵ In a later publication, corrected incidence estimates were presented for the later portion of the same period (1975-1984) and investigators concluded that no major time trend exists for the period 1975-1984. They corrected the census figures used as denominators by subtracting prevalent cases. However, due to a lack of prevalence data for the period before 1975, they were unable to make similar corrections to the incidence estimates from the earlier period, in which the 1993-reported increase in incidence was apparent. Yet they argue that the stability from 1975-1984 is further evidence that the apparent increase prior to 1975 is due to ascertainment bias.⁴⁷

A 2011 article examined time trends in data from three studies (Rochester, MN, 1975-1994; Chicago Health and Aging Project, 1997-2008; Indianapolis-Ibadan Dementia Project, 1992 vs. 2001) and a national survey (Health and Retirement Study, HRS, 1993 vs. 2002) and found no significant trends in the Minnesota, Illinois, and Indiana data, but a substantial decrease in the prevalence of cognitive impairment consistent with dementia (CI-D) between the two waves of HRS (12.2% in 1993 to 8.7% in 2002, crude rates). Of note, CI-D in HRS was a composite outcome based on neuropsychological test

scores, rather than a criterion-based diagnostic outcome of dementia or AD.⁷⁶ Additional analyses suggested that 40% of the reduction in cognitive impairment prevalence was due to greater wealth and education among the 2002 sample, compared to the 1993 sample. The authors of this 2011 article discussed the opposing vascular disease trends that may contribute to increased or decreased dementia incidence. Improvements in some cardiovascular disease (CVD) risk factors and treatments, along with wealthier and better educated elderly could contribute to lower risk for cognitive impairments, but other factors may increase risk, such as more people with chronic diseases such as CVD surviving to older ages, and increased prevalence of obesity, diabetes, and hypertension. They conclude that the decreased prevalence in the HRS data, along with favorable trends in CVD, is reason for cautious optimism.⁷⁶

Physical activity

Many studies support an association between increased physical activity and decreased risk of cognitive impairment, cognitive decline, dementia, AD.^{63,77-89} A recent meta-analysis of 14 prospective cohort studies reported that those in the highest physical activity category, compared to the lowest, had a pooled RR for overall dementia of 0.72 (95% CI: 0.60-0.86) and a RR for AD of 0.55 (95% CI: 0.36 – 0.84).⁹⁰ There was significant heterogeneity in the association, across studies. Note that only five studies were considered high quality, and some studies had crude measures of physical activity (e.g., “any” vs. “none”).⁹⁰ Another recent meta-analysis looked at the association between physical activity and cognitive decline across 15 prospective cohort studies.⁹¹ Those reporting high levels of activity had significantly lower risk of cognitive decline (hazard ratio (HR) 0.62, 95% CI: 0.54 – 0.70) compared to those who were sedentary. No dose response was observed, as those reporting low to moderate activity

had similarly reduced risk (HR 0.65, 95% CI 0.57 – 0.75). No significant heterogeneity was detected across studies. Cognitive decline was most often defined by a three point decline on the Mini-Mental State Examination (MMSE) or a follow-up score of <24.⁹¹

Walking is a particularly common way for older adults to exercise, and even this low intensity activity has been shown to be associated with cognitive impairment. In the Nurses' Health Study, women's average physical activity over the previous 8-15 years, even walking as little as 1.5 hours/week, was associated with better baseline scores on several cognitive tests, as well as less decline in scores over a mean cognitive follow-up of 1.8 years.⁷⁷ In the Study of Osteoporotic Fractures, women in the highest quartile of blocks walked per week were less likely to develop cognitive decline (≥ 3 point decline on MMSE) over 6-8 years of follow-up.⁸⁸ Men (71-93 years of age) in the HAAS who walked the least (less than ¼ mile/day) at baseline were almost twice as likely to develop dementia over a 7 year follow-up as those who walked the most (more than 2 miles/day), with similar results for AD.⁷⁸

Not all studies found associations between physical activity and cognition.^{64,92-97} Some authors suggest that the association of physical activity and cognition is ambiguous because it is difficult to disentangle the effects of exercise from the effects of socialization, cognitive stimulation, and productive activities. For example, dancing was considered a social activity in some studies but a physical one in others; gardening and housework were included with physical activity in certain studies but as productive activity in others. Other than Yamada et al, the studies reporting null associations between physical activity and cognitive function all reported associations of cognitive activities with cognitive impairment,^{64,92-96} and at least one study found an association between physical activity and cognitive decline which was attenuated and no longer significant when adjusting for cognitive activities.⁹³

Randomized intervention trials exist, although most were short in duration with effects measured post-intervention, but not re-measured to check for sustained effects. Many significant effects have been reported, but were not consistent in terms of the affected cognitive domain(s). A Cochrane review of 11 randomized controlled trials (RCTs) concluded that aerobic activity that improves fitness can benefit cognitive function in healthy older adults, with effects observed in the domains of cognitive speed, auditory and visual attention, and motor function.⁹⁸ However, the authors note that the majority of comparisons yielded no significant results, with only two out of eleven subcategories of cognitive function showing a significant effect for exercise versus any intervention, and only two out of eleven showing a significant effect of exercise versus no intervention. Colcombe and Kramer did a meta-analysis of intervention studies through 2001 (16 studies, pooled $n=101$). They characterized their results as supporting “robust but selective benefits for cognition”, with the largest effect found for executive control. The youngest age group (55-65 years of age) had the smallest effect size (0.298; effect = mean post-intervention performance-mean pre-intervention performance/ pooled SD; i.e., Hedges g) for improved performance, while the 66-70 year olds (effect size = .693) had a similar benefit as the 71-80 year olds (effect size = 0.549).⁹⁹ Etnier et al performed a meta-analysis with the specific objective of testing the cardiovascular fitness hypothesis, which states that improved aerobic fitness is necessary for physical activity to affect cognition. Overall, across 37 studies (many not random assignment), they found a small effect size of physical activity on improved cognitive performance (0.34). However, they concluded that fitness did not moderate the physical activity – cognition association, and explained only 8% of the variation in cognition.¹⁰⁰

In summary, there appears to be an association between physical activity and performance on cognitive tests, but it is still unclear whether it is causal or due to confounding. Reverse causation could also

explain the association, wherein existing subtle decreases in cognitive function may contribute to reduced physical activity. However, in analyses of data from the Nurses' Health Study, the association remained when restricting exposure to 9 years prior to cognitive testing.⁷⁷ Confounding, such as by cardiovascular risk factors, or other health habits such as nutrition, may also explain the association, as not all studies adjusted for these. If it is a causal association, the mechanisms are unclear. Etnier et al concluded that the cardiovascular fitness hypothesis was not supported by the empirical literature.¹⁰⁰ It could be that physical activity affects cognition through mechanisms other than fitness, such as by hand-eye coordination, improved reaction time, cognitive stimulation, social interactions, improved mental health, or increased appetites. In addition, it may be that fitness is a precursor to another mechanism that impacts cognition, such as increased oxygen to the brain (i.e., cerebral circulation hypothesis),¹⁰¹ or increased availability of neurotransmitters.¹⁰² Physical activity is also associated with inflammation,¹⁰³⁻¹⁰⁵ which is hypothesized to have a role in cognitive function.

Alcohol consumption

Alcohol consumption is generally thought to have a J- or U-shaped association with cognitive impairment, such that alcohol nondrinkers and, in particular, heavy drinkers are more likely to be impaired, compared to moderate drinkers. Several large epidemiological studies of the alcohol-cognition association have reported such a U-shaped pattern, including a nested case-control study in the CHS,¹⁰⁶ the Rotterdam study,¹⁰⁷ and the HAAS.¹⁰⁸

Table 1-4. Studies of alcohol consumption and cognitive impairment				
Citation	Study	Findings	Examined “Heavy” drinking?	Notes
¹⁰⁶	CHS	U-shaped	(≥ 14/week; OR= 1.2)	
¹⁰⁷	Rotterdam	U-shaped	(≥ 4/day)	Nondrinking (ref) and ≥ 4/day (highest cat) had HR = 1; 1-3/day had lowest HR
¹⁰⁹	MoVIES	Not null, not u-shaped	No – too few reported	None, Minimal (1/month or less), and Moderate (more than 1/month)
⁵⁷	CSHA	Inverse		Reduced risk for at least weekly consumption (vs. none)
¹⁰⁸	HAAS	U-shaped association	(≥ 61oz/mon, or 4 drinks/day)	
¹¹⁰	ZARADEMP	Null		
¹¹¹	East Boston EPESE	Mainly null	No	“Very light” drinking (vs. none) was associated with better digit span test

Other studies reported that some amount of alcohol consumption was associated with better cognition, but did not report a U-shaped association for various reasons. The MoVIES study estimated trajectories of drinking frequency (not quantity) over time and defined three groups: no drinking, minimal ($\leq$ once/month), and moderate drinking ($\geq$ once/month). Compared to no alcohol consumption, both minimal and moderate drinking trajectories were associated with lesser decline on the Trail Making test (TMT) and MMSE. There were too few heavy drinkers to analyze.¹⁰⁹ In a CSHA case-control analysis of various risk factors for AD, investigators reported that at least weekly consumption of any type of alcohol was associated with an OR = 0.68 (95% CI = 0.47-1.00), with weekly consumption of wine associated with an OR = 0.49 (95% CI = 0.28- 0.88).⁵⁷ The odds ratios for consumption of beer and spirits were not statistically significant. Alcohol consumption was not the sole focus of the report, and each type of alcohol consumption was simply analyzed and reported as a dichotomous variable (at least weekly consumption).⁵⁷ In the PAQUID study in France, wine consumption was categorized as follows: “nondrinker” if no wine or no more than 1 glass/week, “mild” if 2 glasses/week to 2 glasses/day, “moderate” if 3-4 glasses/day, and “heavy” if $\geq$ 5 glasses/day (“heavy” excluded from analyses due to

small N). Compared to nondrinkers, moderate drinkers (and to a lesser, non-significant extent, mild drinkers) had reduced risk of incident dementia (RR = 0.56, 95% CI = 0.36-0.92) or AD (RR = 0.53, 95% CI = 0.30 – 0.95) over the 8-year follow-up period, adjusted for age, sex, and education only.¹¹²

Conversely, other studies have reported mainly null associations between alcohol consumption and cognition.^{110,111} The ZARADEMP project in Spain compared abstainers, former drinkers, and consumers of <12 g/day, 12-24 g/day, and, among men only, 24-40 g/day and > 40 g/day, and reported no statistically significant differences in the odds of severe cognitive decline (≥ 1 point/year decline on MMSE).¹¹⁰ In the East Boston EPESE sample, results were mainly null; compared to abstainers, low to moderate consumption was not associated with most outcomes, but very light consumption (<0.5 ounces of alcohol/day) was associated with better change scores on the digit-span test, compared to nondrinkers.¹¹³

Few studies separated the non-drinking category into former drinkers and lifetime abstainers, although both MoVIES and CHS have reported such results.^{106,109} The CHS separated long-term abstainers from quitters (since baseline) and former drinkers (quit prior to baseline) throughout their analyses and found that across all the models examined, the quitters and former drinkers had higher odds ($\sim +20\%$ - 60%) of dementia compared to long-term abstainers.¹⁰⁶ The MoVIES found that much of the difference in cognitive performance between drinkers and nondrinkers could be attributed to the inclusion of former drinkers in the nondrinker category. In analyses where former drinkers were excluded from the reference group of nondrinkers, many of the associations that had indicated a protective effect of moderate consumption became non-significant, although some strong protective associations remained (e.g., with MMSE and TMT performance).¹⁰⁹ Finally, in an analyses of HRS data for men only, problem drinkers (score ≥ 2 on CAGE scale) were more likely to develop a memory impairment during a 6-year

period, compared to non-problem drinkers (OR = 1.71, 95% CI = 1.14 – 2.56). But compared to consumption of 1-2 drinks/day, neither heavy consumption (≥ 5 drinks/day) nor non-problem abstinence was significantly associated with the risk of memory impairment, although the study may have been underpowered to detect an association for heavy consumption.¹¹⁴

If, in fact, some amount of alcohol consumption is protective against cognitive impairment, the healthy amount remains to be determined, as the number of drinks shown to be protective against cognitive decline varied by study. In the CHS, compared to no alcohol consumption, 1-6 drinks/week appeared protective (OR = 0.46, 95% CI = 0.27-0.77) but more than 14 drinks/week was associated with a statistically non-significant increased risk of dementia (OR = 1.22, 95% CI = 0.60 – 2.49).¹⁰⁶ In the slightly younger Rotterdam study (ages 55 and older), nondrinkers were the reference group, and 1-3 drinks/day was associated with the lowest risk of dementia (HR = 0.58, 95% CI = 0.38-0.90). The greatest consumption category was ≥ 4 drinks/day (HR = 1.00, 95% CI = 0.39-2.59).¹⁰⁷ In the HAAS, compared to no consumption, consuming as many as 4 drinks/day was associated with reduced risk of poor CASI score, with the lowest risk observed among those consuming up to 1 drink/day. Consuming more than 4 drinks/day was associated with the lowest CASI scores and an elevated risk ratio (RR=1.29, 95% CI = 0.83-2.01).¹⁰⁸ In the French PAQUID cohort, the lowest risk of dementia or AD was observed among those consuming 3-4 glasses of wine/day (highest consumption category in the analysis).¹¹²

In summary, heavy drinking or problem drinking has been associated with increased risk of cognitive impairment, and moderate drinking, especially wine drinking, has been associated with decreased risk. It is not clear if such associations are causal, or may be biased due to factors such as mortality or selection effects. Heterogeneity of nondrinker categories may have implications for results and interpretations. Conflicting evidence exists on what amount of alcohol might be considered protective. Further support

of these conclusions is found in a recent systematic review (through June 2007) and meta-analyses of 15 studies, which concluded that those who drink alcohol in late adulthood appear to have reduced risk of cognitive impairment, but that this observation could be due to selection or mortality effects.¹¹⁵

It is unclear what mechanisms might underlie a potentially causal association of moderate alcohol consumption and reduced risk of cognitive impairment. Such consumption is known to increase high-density lipoprotein cholesterol (HDL-C), which protects against CVD.^{116,117} Alcohol has been shown to have various other effects that improve vascular health, and may have favorable effects on inflammation status.^{118,119} For example, in the Northern Manhattan Study, a history of moderate alcohol consumption was associated with lower IL-6 levels, compared to nondrinkers or heavier drinkers.¹²⁰ Also see the section on factors associated with inflammation levels.

Smoking

In the past, controversy existed regarding the association of cigarette smoking and dementia.¹²¹ Some earlier case-control studies reported that smoking protected against dementia, in particular AD.¹²²⁻¹²⁴ More recently, meta-analyses and systematic reviews of longitudinal data have generally led to the opposite conclusion, that smoking is a risk factor for dementia.¹²⁵⁻¹²⁷ Some have stated that the tobacco industry is invested in showing that smoking is not a risk factor for AD, and in fact, that nicotine could potentially be therapeutic. These authors also suggested that tobacco industry affiliations have further contributed to the confusion regarding smoking and AD risk, citing as evidence their pooled analyses results showing an association between affiliation and conclusions more favorable to the industry.¹²⁵ However, the evidence linking smoking and dementia is not as strong as one might expect, most likely

because smoking is strongly associated with premature mortality.¹²⁸ Despite this, close inspection of better quality studies suggests smoking is a risk factor for cognitive impairment.

Table 1-5. Studies of smoking status and cognitive impairment			
Study	Citation	Result	Notes:
HAAS	¹²⁹	Some +, some null	"very heavy smoking" not associated
Rotterdam	¹³⁰	+	Only in non-carriers of <i>APOE</i> ε4 allele
Rotterdam	¹³¹	+ for current only	Only in non-carriers of <i>APOE</i> ε4 allele
East Boston	¹¹¹	Null	
CSHA	⁵⁷	Null	
ARIC	¹³²	Null	
Kaiser	¹³³	+	

Investigators from the HAAS analyzed the potential association in several ways.¹²⁹ Overall, neither current nor former smokers were at increased risk of AD or any dementia after 25 years, but current smokers had an increased OR for VaD (OR = 1.73, 95% CI = 1.03 -2.92). However, among participants without an *APOE* ε4 allele, mid-life current smokers were more likely to develop AD (OR = 1.77, 95% CI = 1.05 – 2.96); there was no association among those with an *APOE* ε4 allele. In analyses of pack-years among smokers, a dose-response effect was observed: compared to light smokers, medium and, to a greater extent, heavy smokers, were more likely to develop AD or any dementia (but not VaD). Interestingly, the odds of developing any outcome did not differ between very heavy smokers and light smokers; the authors indicate this could be due to a hardy survivor effect, as 45% of very heavy smokers died before the first cognitive exam (compared to 25% of light smokers). Finally, the authors examined the association between mid-life smoking and 5 different neuropathologic markers at autopsy (n=218). Medium and heavy smokers had significantly higher counts of neocortical neuritic plaques (but no difference in the other 4 markers they examined). In summary, hardy survivor effects may have reduced the ability to find an association between mid-life smoking status and dementia 25 years later, but the

authors documented that pack-years smoked was associated with both incident disease (dose-response pattern) and increased neuropathology on autopsy.¹²⁹

The EURODEM collaborative group analyzed pooled data on 9,209 participants in the Odense Study (Denmark), the PAQUID Study (France), the MRC- ALPHA Study (UK), and the Rotterdam Study (the Netherlands) and investigated the impact of smoking on yearly decline on the MMSE.¹³⁴ Current smokers, and to a lesser extent former smokers, had greater annual decline on the MMSE, compared to never smokers. Analyses were stratified by gender, family history of dementia, and study site. The effect varied by study site (but not significantly by gender or family history), with significant effects in Rotterdam and MRC ALPHA, borderline significant in Odense, and no effect in PAQUID. There was also a trend toward greater decline for more pack-years smoked, which was significant among former smokers only.¹³⁴ Of note, in two separate publications from the Rotterdam Study (2.1 year and 7.1 year follow-ups), stratified analyses revealed that the increased risk of dementia and AD observed for smokers was restricted to those not carrying an *APOE* ϵ 4 allele (i.e., no association among ϵ 4 carriers),^{130,131} similar to the HAAS findings.¹²⁹ The *APOE* ϵ 4 allele has been associated with increased risk for CVD, as has smoking, indicating that mortality was likely higher among smokers with the *APOE* ϵ 4 allele, likely resulting in a particularly selected, robust group of smokers who carry the *APOE* ϵ 4 allele.

From 1978-1985, a portion of 50-60 year old members of Kaiser Permanente Medical Care Program of Northern California participated in a voluntary Multiphasic Health Checkup where data was collected on smoking habits (n= 33, 108). In 1994, electronic medical record data on dementia diagnoses became available; 21,123 of the volunteer MHC participants were still alive and Kaiser members in 1994, and thus included in the analyses. A dose-response association was observed such that, compared to never smokers, current very heavy smokers (> 2 packs/day) had about twice the risk of dementia, AD, and

VaD; smokers of 0.5 – 1 or 1-2 packs/day were at intermediate risk. Former smokers and light smokers (< 0.5 pack/day) were not at increased risk.¹³³

Among approximately middle-aged participants in the Whitehall II study, current smokers were significantly more likely to have a concurrent deficit in memory and a decline in reasoning after 5 years, compared to never-smokers.¹³⁵ Among current smokers, there was no dose-response relationship between pack-years and prevalent cognitive deficit or prospective decline. Interestingly, former smokers were less likely to have prevalent deficits on several cognitive tests compared to never smokers, although this pattern was not observed for prospective decline. Quitting smoking was associated with improvements in dietary habits, such that former smokers are more similar to never smokers than to current smokers. The authors observed that smokers were significantly more likely to die or otherwise be lost to follow-up, and indicated this may have resulted in underestimation of the association.¹³⁵

In contrast, largely null associations were reported from the CSHA⁵⁷ and East Boston EPESE.¹¹¹ In the CSHA, over a 5-year follow-up period, smoking was not associated with an increased risk of dementia (OR = 0.82, 95% CI = 0.57, 1.17), although smoking was not the focus of the article and was modeled only as yes/no, with no explanation regarding treatment of former smokers.⁵⁷ Such a simplified approach may mask an underlying association. For example, in the HAAS, current smokers were not at increased risk for AD or dementia, but greater exposure among smokers, based on pack-years, was associated with greater risk.¹²⁹ In East Boston, neither current nor former smoking status was significantly associated with change on three tests of orientation, digit-span, and immediate memory. However, in a linear regression model adjusted for age, sex, education, income, and alcohol use, greater pack-years was significantly associated with improvement on the digit span (β = 0.001, 95% CI = 0.0003

to 0.002; normal score change per pack-year), but current and former smoking status (yes/no) were also in the model and had non-significant but negative beta coefficients. The net result, as per an example from the authors, is that a pack-a-day smoker for 30 years would be predicted to have digit-span normal change score of 0.017 for current smokers (improvement), but -0.032 for former smokers (decline). The authors suggest this is a result of unidentified confounding.¹¹¹

Wang et al. contrasted results from cross-sectional and longitudinal analyses using data from the Kungsholmen Project in Sweden.¹³⁶ The authors showed that smoking was negatively associated with dementia prevalence (OR = 0.6, 95% CI = 0.4-1.0; adjusted for age, sex, education) but not dementia incidence over a 3-year period (HR = 1.4, 95% CI = 0.8-2.7). Results were similar for the AD outcome and models that separated former smokers. The authors also observed that 5-year mortality was significantly elevated for smokers with dementia (HR = 3.5, 95% CI = 1.4-8.8) but not for smokers without dementia (HR = 0.8, 95% CI = 0.5-1.2). They concluded that their less-biased prospective analysis failed to confirm cross-sectional results, suggesting that differential mortality or other sources of bias could be responsible for previous cross-sectional reports of a protective effect of smoking on dementia.¹³⁶

In summary, several longitudinal studies reported null associations with cognitive decline or impairment when simply comparing current smokers to never smokers.^{57,111,129} Some studies have reported that smoking status is associated with cognition only among those not carrying an *APOE* ε4 allele.¹²⁹⁻¹³¹ Pack-years were shown to have a dose-response relationship with poorer cognitive outcome in some studies,^{129,133,134} but improved digit-span performance in another study, perhaps due to confounding.¹¹¹ Several of these studies attempted to gauge the possible effect of biases, for example, by looking at differential mortality or losses to follow-up, or contrasting cross-sectional and longitudinal analyses, and

concluded that the smoking-cognition association is likely to be underestimated due to mortality and selection effects.^{129,135,136}

Cholesterol

Epidemiological studies have reported increased risk of cognitive impairment in late-life among those with elevated cholesterol in mid-life¹³⁷⁻¹⁴⁰ or among those with greater decreases in cholesterol from mid- to late-life.^{141,142} Those studies using cholesterol levels measured in late-life more often found inverse^{40,143-146} or null associations.^{63,147-149} In a 2008 meta-analysis and review that included 18 studies, Anstey et al. conclude there is an association between greater total cholesterol (TC) at mid-life and subsequent increased risk of AD and any dementia, but no association with late-life TC. There was no evidence for an association between TC (mid- or late-life) and VaD.¹⁵⁰ In a cross-sectional analysis using data from the 1998-2000 follow-up of the Epidemiology of Hearing Loss Study (EHLS), among those 65-97 years of age, Carlsson et al. observed a 2-fold increased adjusted odds of cognitive impairment for those in the highest quartile of non-HDL-C, compared to the lowest quartile (no association of cognitive impairment with TC or HDL-C in adjusted models).¹⁵¹

A potential causal association between cholesterol and cognitive impairment is supported by several plausible biological mechanisms. The *APOE* gene codes for Apolipoprotein E (ApoE), a cholesterol transport protein, and the $\epsilon 4$ allele of the gene is the only well-established genetic risk factor for AD. In cell culture and animal studies, it is widely accepted that high cholesterol levels are associated with amyloid- β deposition, which is one of the major pathologies associated with AD.¹⁵² Notkola et al. reported that the association they observed between *APOE* $\epsilon 4$ carrier status and AD prevalence was attenuated when TC was added to the model, and suggested that some of the increased risk associated

with *APOE* $\epsilon 4$ may function through elevated cholesterol.¹³⁹ High cholesterol also promotes intima thickening and endothelial dysfunction,¹⁵³ which could adversely impact the brain. If cholesterol is causally related to cognition, lipid-lowering agents (LLA) should theoretically be effective in preventing or delaying cognitive declines and dementia. Furthermore, statins, a specific class of LLA, have additional (cholesterol-independent) effects, including improved endothelial function, anti-thrombotic effects, and anti-inflammatory effects,¹⁵⁴ and are further discussed in the Background section on anti-inflammatory medications.

In summary, it appears that high cholesterol at mid-life is associated with increased risk of dementia later in life, while declining and low late-life cholesterol are associated with greater odds of dementia, perhaps due to subclinical or prodromal dementia. Similar trajectories have been proposed for body mass index (BMI) and blood pressure in relation to dementia, with positive associations reported among studies of risk factors more than 10 years before dementia onset, but inverse associations observed with the same risk factors measured more proximally.

Diabetes mellitus

Epidemiological evidence indicates that people with diabetes are more likely to develop dementia, as outlined in recent reviews.^{155,156} Several large, prospective studies have reported significant positive associations.¹⁵⁷⁻¹⁶⁴ Most estimated that diabetes increases the risk of dementia between 50-100%, with a recent meta-analysis reporting a pooled effect size of 1.54 (95% CI: 1.33 – 1.79) for AD.¹⁵⁶ The association has been reported for various outcomes, including MCI,¹⁵⁹ any dementia,¹⁶⁰⁻¹⁶⁵ AD,^{157,158,160,161,164,165} and VaD.^{161,162,166} Of note, the Kungsholmen project and CSHA reported a null association for the outcome AD,^{162,166} and the CSHA reported a null association for mixed dementia.¹⁶⁶

Table 1-6. Studies of diabetes and cognitive impairment				
Study	Ref.	Result	Diabetes type; ascertainment	Notes:
Rotterdam	¹⁶⁰	+	2; glucose, meds (WHO)	Type 2 almost doubled risk of AD and dementia; Patients on insulin had RR = 4.3
Rochester	¹⁶⁵	+	Adult-onset diabetes; glucose, meds	Adult-onset diabetes; retrospective, registry
N.Manhattan	¹⁵⁹	+	2; self-report	NOTE: outcome was MCI, not dementia
FHS	¹⁵⁷	+	Unspecified; glucose, meds	Only in non-carriers of <i>APOE</i> ϵ 4. In whole sample, was n.s. (RR = 1.15).
Kungsholmen	¹⁶²	+ / null (AD)	Unspecified; glucose, meds, self-report	Diabetes associated with significant increased HR for any dementia, stronger for VaD, n.s. for AD; also interacted with HYP
ROS	¹⁵⁸	Mostly “+”	Unspecified, meds, self-report	Outcomes: AD (+), baseline cog test performance (-), and cog decline (null except speed)
CHS	¹⁶⁷	“+”	Unspecified; glucose, self-report	Outcome was cognitive decline; reported stronger effect in presence of <i>APOE</i> ϵ 4
CHS	¹⁶⁴	+ / null (VaD)	2; glucose, meds	AD(+), VaD (null), mixed AD (+), all dementia (+); diabetes and <i>APOE</i> ϵ 4 were each RFs, together led to highest risk (HR for AD = 4.6).
HAAS	¹⁶¹	+	2; glucose, meds, self-report	Outcomes: dem, AD, VaD (all +); stronger in ϵ 4 carriers, but null in non-carriers of ϵ 4 (but significant in the whole sample)
IIHD*	¹⁶³	+	Unspecified; glucose, meds, self-report	midlife diabetes, long follow-up (> 30 years)
CSHA	¹⁶⁶	+ / null	Unspecified; glucose, meds, self-report	+ for VaD and VaCIND, but null for AD, and null for mixed dementia
Kaiser CA	¹⁶⁸	+, dose-response	2; diabetes registry based on medical records	NOTE: exposure was hypoglycemic hospitalizations NOT diabetes itself; also, only compared among patients with Type 2 diabetes
* IIHD = Israeli Ischemic Heart Disease (Jewish male civil servants, 1963-2001)				

Studies have also observed that participants with diabetes had worse cognitive test performance at baseline,¹⁵⁸ and greater decrements in performance on follow-up.¹⁶⁷ Many studies did not specify Type 1 or Type 2 diabetes mellitus (Table 1-6), and most that did referred to Type 2 but did not provide explicit details on how Type 2 cases were differentiated, or how any Type 1 cases were included in analyses. The

publication from the Rotterdam Study stated there were no insulin-dependent participants among those using insulin therapy.¹⁶⁰

Several possible pathways have been proposed by which diabetes could increase the risk of dementia, although these mechanisms have not yet been extensively researched. Insulin degrading enzyme (IDE) is involved in the regulation of both insulin and amyloid- β protein and thus may link diabetes and dementia. Hyperinsulinemia, as a competitive substrate for IDE, may reduce clearance and increase accumulation of amyloid- β , or potentially, IDE hypofunction could contribute to both diabetes and dementia, as the IDE region of chromosome 10q has been linked to both disorders.¹⁶⁹ Advanced glycation end products (AGEs) are thought to form as part of normal aging, but may be increased with hyperglycemia in diabetes, and have also been associated with some of the pathological changes seen in neurodegenerative disorders, including AD (e.g., senile plaques and neurofibrillary tangles).¹⁷⁰ In the Health ABC study, investigators found that greater peripheral AGE levels (measured pentosidine in urine) were associated with steeper 9-year cognitive decline (Digit Symbol Substitution Test (DSST) and the Modified Mini-Mental State examination (3MS)) in participants with and without diabetes.¹⁷¹ It is also well-known that diabetes is associated with macro- and micro-vascular complications, CVD, and cerebrovascular disease. Thus, an association between diabetes and vascular dementia, or mixed dementia, is not surprising; given recent findings that indicate “pure AD” may be relatively uncommon, it may be that a vascular mechanism could also explain the diabetes – AD association reported by various studies. Furthermore, diabetes is a complex disorder that has been closely linked with other possible risk factors for dementia, including hypertension, obesity, physical activity, and atherosclerosis. It is unclear whether diabetes itself may be causally related to dementia, or if the observed associations

could be due to shared risk factors. Although some studies of the diabetes-dementia association adjusted for the effect of some of these factors, more extensive research is needed in this area.

Apolipoprotein E

Apolipoprotein E (*APOE* is the gene, ApoE is the protein) is a plasma protein that is produced in many organs, although the liver is the major source and contains the greatest concentration of ApoE mRNA. The brain contains the second largest quantity of ApoE mRNA.¹⁷² ApoE is involved in cholesterol transport; it is a ligand for low density lipoprotein receptors.¹⁷³ Production of ApoE is increased following tissue injury, such as nerve injury, indicating a role in repair response.¹⁷³ Namba et al. found ApoE bound to extracellular plaques and intracellular neurofibrillary tangles in the brains of AD patients, and in the amyloid of kuru plaques in the brains of Creutzfeldt-Jakob disease (CJD) patients.¹⁷⁴ Strittmatter et al. subsequently reported ApoE in cerebrospinal fluid (CSF) binds with high affinity to amyloid- β peptide, that ApoE is contained in plaques and tangles, and that the $\epsilon 4$ allele of the *APOE* gene is overrepresented in late-onset familial AD.¹⁷⁵ Saunders et al. observed an increased frequency of the $\epsilon 4$ allele in both familial and sporadic late-onset AD.¹⁷⁶ Corder et al. reported a dose effect for AD risk, with HRs of 2.84 and 8.07 for the presence of one or two $\epsilon 4$ alleles, respectively, relative to those with no $\epsilon 4$ allele.¹⁷⁷ The association between the *APOE* $\epsilon 4$ allele and cognitive impairment has since been replicated in many large studies including FHS, Atherosclerosis Risk in Communities (ARIC), CHS, and Rotterdam, with some debate about the strength of association and the portion of disease attributable to *APOE* genotype. The $\epsilon 4$ allele has also been associated with cognitive decline, increased CVD, and decreased longevity.¹⁷⁸

A community-based study in East Boston, MA among a mostly white population reported that participants with $\epsilon 3/\epsilon 4$ or $\epsilon 4/\epsilon 4$ genotypes were twice as likely to develop AD.⁵⁶ In the Rotterdam Study, compared to $\epsilon 3/\epsilon 3$, those with $\epsilon 3/\epsilon 4$ and $\epsilon 2/\epsilon 4$ genotypes had about twice the risk of dementia and those with $\epsilon 4/\epsilon 4$ had an 8-fold greater risk, while those with $\epsilon 2/\epsilon 3$ had about 40% reduced risk; results were similar for dementia subtypes AD and vascular dementia.¹⁷⁹ In the HAAS, carriers of the $\epsilon 4$ allele were at increased risk for any dementia (OR=1.50; 95%CI =1.03-2.20) and AD (OR=2.39; 95%CI =1.07-5.31), but not VaD (OR=1.27; 95%CI =0.33-4.83).¹⁸⁰ In the FHS, compared to those with $\epsilon 3/\epsilon 3$, increased AD risk was observed for $\epsilon 4$ homozygotes (RR=30.06; 95% CI=10.7-84.4) and for $\epsilon 3/\epsilon 4$ (RR=3.7; 95% CI=1.9-7.5), and decreased AD risk was suggested for $\epsilon 2$ carriers (RR=0.25; 95%CI=0.04-1.89).¹⁸¹

APOE genotype has also been associated with cognitive decline. In the Nurses' Health Study, those with at least one $\epsilon 4$ allele performed worse on several cognitive tests at baseline and had greater decline over 2 years.¹⁸² In the CHS, those with at least one $\epsilon 4$ allele, compared to those without any, had greater decrements in cognitive test performance over a 7-year period (for the 3MS, -2.94 vs. -0.42; for the DSST, -2.0 vs. -0.29). Additionally, they reported effect modification by *APOE*, such that the rate of cognitive decline associated with measures of atherosclerosis, peripheral vascular disease, and diabetes mellitus was greater among $\epsilon 4$ carriers.¹⁶⁷ In the middle-aged ARIC cohort, *APOE* $\epsilon 4$ was associated with greater declines in cognitive test scores (DSST and Verbal Fluency Test (VFT)) among Caucasians and possible effect modification was noted, such that the effect was stronger among those with hypercholesterolemia or diabetes; for African Americans, only change in the DSST was associated with *APOE* genotype.¹⁸³

The risk associated with the *APOE* $\epsilon 4$ allele may vary by age and race. In the Cache County Study, Breitner et al. reported that among participants with no $\epsilon 4$ alleles, the age-specific prevalence of AD was

greatest at age 95 years, and then declines at older ages.¹⁸⁴ For the $\epsilon 4$ heterozygotes, the maximum prevalence was observed at age 87 years, while the maximum prevalence for homozygotes was estimated to be at 73 years of age. Models included an age-squared term (negative coefficient) and interaction terms for age and the presence of one or two $\epsilon 4$ alleles, such that the *APOE* genotype – AD association is weaker at older ages. They also reported that female sex was associated with increased odds of AD among $\epsilon 4$ carriers only (no sex-AD association in $\epsilon 4$ non-carriers).¹⁸⁴ In a Chicago study, the $\epsilon 4$ allele was associated with an increased risk for AD (OR=2.73; 95%CI=1.40-5.32) in white participants, but not associated with AD risk among black participants (OR=1.02; 95%CI=0.39-2.68).³⁵ A 1997 meta-analysis reported that the increased risk associated with the $\epsilon 4$ allele declines at older ages among Caucasians (after age 60 years for $\epsilon 3/\epsilon 4$ and $\epsilon 4/\epsilon 4$, after age 70 years for $\epsilon 2/\epsilon 4$), is greater among Caucasian women than men, and is weaker in African American and Hispanic samples compared to Caucasian and Japanese.⁵⁴ It should be noted that the age- and sex-specific odds ratios for Caucasians were pooled estimates from clinic- and autopsy-based studies, but did not include the population-based studies, which were too heterogeneous to be combined. The authors did calculate pooled odds ratios for the overall association between *APOE* genotype and AD for Caucasians in the clinic and autopsy based studies (combined) and separately in the population-based studies. The estimates were similar, but weaker among the population-based studies; compared to $\epsilon 3/\epsilon 3$, $\epsilon 2/\epsilon 2$ had an OR (95% CI) of 0.9 (0.3 -2.8), $\epsilon 2/\epsilon 3$ was 0.6 (0.5-0.9), $\epsilon 2/\epsilon 4$ was 1.2 (0.8-2.0), $\epsilon 3/\epsilon 4$ was 2.7 (2.2 – 3.2), $\epsilon 4/\epsilon 4$ was 12.5 (8.8-17.7). They also conclude that the $\epsilon 2/\epsilon 3$ genotype is protective to a similar degree in all ethnic groups, although statistically significant only in the larger Caucasian groups.⁵⁴

Controversy exists regarding the predictive utility and population-attributable risk of *APOE* genotype. In 1993, Corder et al. reported that 91% of their study participants with $\epsilon 4/\epsilon 4$ genotype had AD, and that

homozygosity was “nearly sufficient” to cause AD by age 80.¹⁷⁷ However, that study was not population-based; it evaluated 42 families, with no specific details provided regarding sample selection.¹⁷⁷

Subsequently, Henderson et al. published data from a moderately large population-based study in Canberra, Australia that suggested about one-third of the $\epsilon 4$ heterozygotes and half of the $\epsilon 4$ homozygotes who survive to age 90 years will have dementia.¹⁸⁵ In 1995, Seshadri et al. estimated the lifetime risk of AD by *APOE* $\epsilon 4$ allele count using age-specific AD incidence data on 65-89 year olds from large studies in Framingham, MA (FHS) and Rochester, MN, U.S. census life-table data, and an a priori lifetime AD risk estimate of 15% (also from FHS data).¹⁸⁶ The lifetime risk of AD was estimated to be 29% for those with at least one $\epsilon 4$ allele, and only 9% for those with no $\epsilon 4$ allele. The authors emphasize that an individual with at least one $\epsilon 4$ allele thus has greater than two out of three chances of not developing AD, and that *APOE* genotyping is rarely clinically useful for patients concerned about AD risk.¹⁸⁶ Mayeux et al. used data from 26 AD centers to compare the sensitivity and specificity of *APOE* genotyping and clinical diagnosis of AD, using autopsy-based diagnosis as the gold standard. They reported that the *APOE* $\epsilon 4$ allele sensitivity was 65% and specificity was 68% and concluded that this was insufficient for use as a diagnostic test for AD. When used sequentially following clinical diagnosis, *APOE* genotype increased the specificity of clinical diagnosis from 55% to 84% and decreased the sensitivity from 93% to 61%.¹⁸⁷

Two review articles (with overlapping authorship; from 2004 and 2002) argue that genetic factors, most importantly *APOE*, account for the vast majority of AD risk in the U.S.^{188,189} Raber et al. estimate that as much as 95% of AD is attributable to *APOE* genotype (in part because the disease is so rare among $\epsilon 2$ homozygotes), and state that this statistic has been underestimated in studies of populations over age 65 years because they do not include those who develop dementia at younger ages.¹⁸⁸ Cache County

investigators estimated that the population risk attributable to the $\epsilon 4$ allele was 70%, yet they also note that Cache County is unusual; the AD risk is greater than that reported by most studies and the frequency of the $\epsilon 4$ allele (0.187) is 25% greater than found in most white populations.¹⁸⁴ In the Rotterdam Study, only an estimated 25% of dementia cases were attributed to the $\epsilon 4$ allele, and the low prevalence of the $\epsilon 2/\epsilon 3$ genotype resulted in only a minor protective effect on the population level from the $\epsilon 2$ allele (2.8%).¹⁷⁹ A community-based study in East Boston, MA reported that the fraction of AD attributable to the $\epsilon 4$ allele was 13.7%, in part due to the low prevalence (10%) of the $\epsilon 4$ allele in this population, which was predominantly white, largely Italian-American.⁵⁶

The relationship between *APOE* genotype and cognitive impairment is consistently reported across most studies, but the mechanisms underlying the association are not well understood. The main mechanism is thought to be through an effect on amyloid- β metabolism. Evidence indicates that the various apoE isoforms differentially affect amyloid- β aggregation, and/or clearance,¹⁹⁰ accumulation of which has been hypothesized by some to be the initiating factor in development of AD, as per the amyloid cascade hypothesis.¹⁹¹ Synaptic plasticity and neuroinflammation are also possible mechanisms.¹⁹⁰ Brain glucose metabolism is affected by $\epsilon 4$ carrier status, and is also a direct measure of synaptic activity.¹⁹² ApoE is also involved in lipid metabolism in the brain, which is related to membrane synthesis, release of neurotransmitters, and propagation of action potentials.¹⁹² The *APOE* $\epsilon 4$ allele is also associated with increased CVD risk,¹⁹³ which in turn may be associated with increased dementia risk. In addition, a growing number of studies have found the *APOE* $\epsilon 4$ allele associated with a more pro-inflammatory state,¹⁹⁴ which may increase the risk of cognitive impairment.

Anthropometry and obesity

Studies of the association between adiposity and dementia have reported conflicting findings (Table 1-7), yet together suggest that midlife obesity may be associated with increased risk, while late-life weight loss or underweight BMI may be symptoms of incipient dementia or other disease processes that are themselves associated with increased risk of dementia. Several studies have reported elevated midlife BMI or waist circumference were associated with increased risk of dementia¹⁹⁵⁻²⁰² or lower cognitive function.²⁰³ Some of these positive associations were sex-specific; in the Cache County study and BLSA, the association was present only in females,^{199,201}; in FHS, only in males²⁰³; and the HAAS has no female participants.²⁰² In the Swedish Twin study, the positive association was observed in generalized estimating equations models controlled for clustering of twin pairs, but much attenuated and no longer significant in the co-twin matched case-control analyses using conditional logistic models among the 137 dementia-discordant twin pairs. The authors suggest genetic and/or environmental factors from early life contribute to the association of midlife adiposity with dementia, although they acknowledge twins may also experience similar exposures later in life.¹⁹⁸

Although no cohorts have reported completely null results for midlife adiposity and later dementia risk, a few isolated reports of null analyses exist.^{199,201,203,206} One publication from the HAAS observed that neither midlife weight nor weight change between mid- and late-life was associated with dementia, but they note that midlife obesity was uncommon in the HAAS study population, and only men were studied. They did observe that increased weight loss over the last 5 years of follow-up was associated with increased risk of dementia.²⁰⁶ Also of note, an earlier publication from the HAAS, focused on the association of metabolic syndrome with dementia, reported an association between BMI measured 25 years earlier and prevalent dementia (RR= 1.21 per SD increase in BMI, adjusted for age and education,

95% CI = 1.05-1.40).²⁰² The other studies only failed to find this association in men,^{199,201} or women,²⁰³

but observed a positive association in the other sex.

Table 1-7. Studies of anthropometry or obesity and cognitive impairment			
Study	Citation	Result	Notes:
HAAS	²⁰²	+	Midlife BMI (per SD). No females in study. Focus was Metabolic Syn., BMI association adjusted for age and education only.
Swedish Twin Registry	¹⁹⁸	+	Midlife BMI. Attenuated and non-significant in co-twin control analyses, suggesting underlying genetic or early life factors.
CAIDE	¹⁹⁷	+	Midlife BMI
Kaiser CA	¹⁹⁵	+	Midlife BMI
Kaiser CA	¹⁹⁶	+	Focus on midlife central adiposity (sagittal abdominal diameter; SAD).
FHS	²⁰³	+ in males only	Null in females. Outcome was cognitive function, excluded demented. Only examined obesity (BMI), not waist.
Cache County	¹⁹⁹	+ in females only	Midlife obesity increased risk of AD in females only (null in males).
CHS	²⁰⁰	Mixed	Midlife obesity associated with increased risk, late-life obesity with reduced risk.
Rochester	²⁰⁴	Mixed	No association of weight 21-30y prior and dementia. Starting 11-20y prior, lower weight increased risk in women only.
N.Manhattan	²⁰⁵	Mixed	Association varied by measure (waist, BMI) and was modified by age
BLSA	²⁰¹	Mixed	Association varied by sex. Examined waist, BMI, and weight change.
HAAS	²⁰⁶	Null/-	Baseline (mid- to late-life) weight not associated, but late-life decreasing weight associated with increased risk.
Kungsholmen	²⁰⁷	-	Late-life overweight or obesity reduced risk. Large decreases in BMI ($\geq 10\%$) associated with increased risk. Symptom, not risk factor.
ROS	²⁰⁸	-	Lower BMI at baseline and decreasing BMI associated with increased risk. Symptom, not risk factor.
PAQUID	²⁰⁹	Null/-	Underweight associated with dementia risk over 8y, but null when exclude cases in first 3y. Suggest symptom, rather than risk factor.

Studies have also reported a negative association between late-life adiposity and dementia risk.^{200,204,206-}

²⁰⁹ Yet these studies do not necessarily contradict those studies reporting a positive association of midlife adiposity and dementia risk. Weight loss has been reported to precede dementia onset,²⁰⁵⁻²⁰⁸ and may precede the diagnosis of dementia by several decades.²⁰⁴ In the CHS, midlife obesity was associated with increased risk, but late-life obesity with reduced risk.²⁰⁰ In the study of Medicare recipients in northern Manhattan, there was a U-shaped association of BMI with dementia in those less than 76 years of age, but a negative association among those 76 years and older.²⁰⁵ From the PAQUID study in France, those with BMI < 21 were at increased risk of dementia compared to those with BMI between 23 and 26, but the association was attenuated and not significant after excluding those with dementia during the first 3 years of follow-up.²⁰⁹ The authors concluded that a low BMI was not an independent risk factor for dementia, but rather an early symptom.²⁰⁹

In summary, greater adiposity in midlife appears to be a risk factor for dementia, but decreasing adiposity or BMI later in life is associated with increased odds of dementia, as an early symptom of dementia or other conditions related to weight loss and dementia, or possibly a risk factor. A limitation of most of these studies was the use of BMI or weight, which are not ideal measures of adiposity, especially in the elderly. Fewer studies used measures of central adiposity, such as waist circumference, but one showed that associations varied depending on whether waist or BMI was used as the exposure measure.²⁰⁵ The studies discussed above generally controlled for many potential confounders, but given the complex associations between adiposity and many other cognition-associated factors such as blood pressure, physical activity, cholesterol, diabetes, depression, it is unclear whether adiposity has an independent, direct, or modifiable effect on dementia risk. Potential direct mechanisms exist. The fat-brain axis²¹⁰ and the hypothalamic-pituitary-adipose tissue axis²¹¹ may provide the basis for

mechanistic links between adiposity and cognition. Elmquist and Flier summarize fat-brain axis findings indicating that the hormone leptin, produced by adipocytes, may be an important regulator of synaptic plasticity and axon guidance in the hypothalamus.²¹⁰ Schaffler et al. hypothesized that there may exist a feedback loop between the hypothalamus/pituitary gland and adipose tissue, such that the function of adipocytes may be under direct control of the brain.²¹¹ Inflammatory processes are also involved in these complex signaling mechanisms, and obesity has been linked to increased release of interleukin-6 and TNF- α .²¹²

Summary of factors associated with cognitive impairment

Older age is strongly associated with increased risk for cognitive impairment. More education is associated with decreased risk, according to the majority of evidence, but the association could be due in part to confounding or bias. There is relatively little evidence for associations of sex or race with cognitive impairment, but some studies have suggested that women (in Europe) and African Americans have increased incidence rates. Moderate drinkers appear to have reduced risk, compared to non-drinkers, but previous heavy drinking among a subset of current non-drinkers could be partially responsible, as could confounding. Cigarette smoking is thought to be associated with increased risk of cognitive impairment, with most concluding that stronger evidence is lacking due to mortality and selection effects.

Much evidence indicates that physical activity is associated with lower incidence of dementia, but it is not clear if this is due to improved fitness or other related factors, such as cognitive stimulation or socialization. Body size and cholesterol each had time-dependent associations, with mid-life measures positively associated with dementia risk and late-life measures negatively associated with risk. There is

strong evidence linking diabetes to increased risk of cognitive impairment, although it is not clear whether diabetes is an independent risk factor for CI, shares the same risk factors as CI, or is in the pathway between other risk factors and CI.

Currently, *APOE* is the only well-established genetic risk factor for late-onset AD, although recent research has identified several other promising candidates not reviewed here.²¹³ Interestingly, newly identified loci are involved in pathways such as immune system function, cholesterol metabolism and synaptic cell membrane processes, rather than amyloid metabolism, which has been the dominant causal hypothesis for AD.²¹⁴ In the studies reviewed above, the *APOE* $\epsilon 4$ allele was consistently reported to be associated with increased risk for CI, although some studies demonstrated that the effect varied by age, or possibly by race. The effects of other risk factors, including age, sex, race, cigarette smoking, and diabetes, may be modified by the presence of one or more $\epsilon 4$ alleles.

In the literature reviewed, there were recurrent concerns that risk factor-dementia associations were underestimated or otherwise biased due to differential losses to follow-up and mortality effects, resulting in a hardy survivor effect. Such issues may explain certain conflicting findings or lack of stronger evidence (e.g., smoking). Timing of exposure, and exposure measurement, is also important in understanding the risk factors for dementia and may explain some controversy in the literature. It is thought that dementia has a long pre-clinical stage, and thus there is greater potential for underlying disease processes to affect exposure status.

1.C Inflammation

Markers of inflammation

Inflammation has been shown to be involved in the aging process and age-associated diseases. C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α) are the markers most consistently shown to be associated with these processes.²¹⁵ The markers affect one another. In response to infection, trauma, or stress, inflammatory cytokines are released, beginning with TNF- α , and followed shortly by IL-6.²¹⁶ IL-6 inhibits TNF- α expression,²¹⁷ stimulates acute-phase reactant production (e.g., CRP) in the liver,²¹⁸ and stimulates the hypothalamic-pituitary-adrenal axis.²¹⁹

TNF- α is a cytokine known for its pleiotropic effects and has been extensively studied for its role in human diseases. It is also known as cachetin, as chronic exposure may lead to cachexia, or wasting syndrome.^{220,221} Macrophages are thought to be the major source of TNF- α , although, similar to IL-6, it is also synthesized by other cell types including monocytes, astrocytes, microglial cells, neutrophils, lymphocytes, endothelial cells, and fibroblasts.^{220,222} The actions of TNF- α are both direct and indirect, as TNF- α triggers a variety of secondary mediators including cortisol, glucagon, growth hormone, IL-6, epinephrine, and norepinephrine. TNF- α affects fever, anorexia, and corticotropin-releasing factor secretion through direct effects on the hypothalamus, metabolic and endocrine processes through myocytes and adipocytes, and the synthesis of acute phase proteins and other cytokines by hepatocytes.^{220,221} In addition to its role in stimulating production of other cytokines and acute phase proteins, inflammation effects of TNF- α include leukocyte activation, promotion of inflammatory cell migration into the intercellular matrix, enhanced adherence of monocytes and neutrophils to endothelium, and fibroblast proliferation.²²¹ TNF- α has been implicated in a variety of diseases, including

cancer, multiple sclerosis, diabetes, and rheumatoid arthritis. Acute exposure to high levels may result in septic shock.²²¹ It is thought to be a reasonably reliable biomarker for use in epidemiological studies, with one study reporting intra-class coefficient (ICC) comparable to other biomarkers (e.g., 0.73 over 2 years),²²³ although another reported relatively low ICC when comparing levels based on single measurement with mean level from multiple measurements over 1 year (0.48).²²⁴

IL-6 is an inflammatory cytokine with a wide variety of actions, including not only immune, hepatic, and hematologic effects, but also endocrine and metabolic.²²⁵ It is produced by cells involved in the immune system (e.g., macrophages, monocytes, microglia, astrocytes, lymphocytes, mast cells),^{218,225} as well as many other types of cells and organs, including smooth muscle cells,²²⁶ intestinal epithelial cells,²²⁷ and osteoblasts.²²⁸ In young healthy individuals, IL-6 is tightly regulated and expressed at very low levels, except when there is infection, trauma, or other stress. In those conditions, IL-6 is rapidly produced and plays a role in the chain of inflammatory events, including leukocytosis, thrombocytosis, lymphocyte activation, acute-phase protein synthesis, and metabolic effects.²²⁹ IL-6 plays both proinflammatory (e.g., promoting CRP production) and anti-inflammatory roles (e.g., inhibiting TNF- α).²²⁵ Because IL-6 gene expression is down-regulated by estrogen and testosterone (among other factors), individuals' baseline IL-6 levels are often elevated following menopause or andropause, which is hypothesized to contribute to frailty and risk for chronic diseases including lymphoproliferative disorders (e.g., chronic lymphocytic leukemia), osteoporosis, or AD.²²⁹ Others have reported that IL-6 level from a single blood sample is useful as a marker of inflammation, based on good intra-class coefficient (0.73) in the comparison of single measure levels with mean level across several measurements over a year long period.²²⁴

CRP is an acute phase protein produced as part of the nonspecific response to most types of infection, inflammation, tissue damage, and malignant neoplasia.²³⁰ Synthesis of acute phase proteins, mainly by hepatocytes, is up-regulated in response to cytokine release, principally IL-6, from the site of pathology. CRP has a plasma half-life estimated to be 19 hours, with clearance reportedly not affected by disease state.²³¹ Thus, synthesis rate is thought to be the only determinant of CRP concentration, making CRP a good marker of the intensity of the pathological processes that stimulate its production.²³¹ Except for spikes due to acute infections, trauma, and inflammation, individuals have relatively stable CRP concentrations, with measures taken a year or more apart estimated to have correlation coefficients (e.g., 0.66, 0.5) similar to cholesterol, and no substantial seasonal variation.^{230,232} It is a very stable in plasma or serum and assays are well standardized and readily available, further increasing its value as a biomarker.²³⁰ Low-grade up-regulation predicts coronary events, perhaps in accordance to the severity of atherosclerotic lesions and their concomitant inflammation, although it could reflect inflammation elsewhere in the body, or even a certain metabolic state rather than inflammation or tissue damage.²³⁰ Statins have been shown to reduce CRP levels, independently of their effect on cholesterol.²³³ Values above 10 mg/L usually indicate a significant acute phase response rather than a stable usual level,²³⁴ and thus to avoid misclassification of usual level of CRP, sensitivity analyses excluding such values may be warranted.

Inflammation and chronic disease

Across various studies of older adults, relatively slight elevations in levels of inflammatory markers, including CRP, IL-6, and TNF- α , have been independently associated with CVD.^{234,235} It has been well established that inflammation is integrally involved in atherosclerosis,^{236,237} which is likely one

mechanism by which inflammation affects CVD and other chronic diseases. It is important to note that the literature indicates that within the “normal” range of these markers (i.e., not elevated to the extent indicating acute inflammation), those with relatively greater levels are at increased risk of CVD. Yet it is not clear whether inflammatory markers are markers of underlying early disease, markers of disease risk, or potentially modifiable risk factors. As markers of disease, their predictive usefulness beyond traditional risk factors is debated.²³⁸

A joint report from the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) concluded that hsCRP was the best marker of inflammation currently available, given that relatively less research or assay development had been done with other markers.²³⁴ The group also recommended cut-points for hsCRP: low risk (< 1.0 mg/L), average risk (1.0 to 3.0 mg/L), and high risk (> 3.0 mg/L), which are approximately equivalent to tertiles in adult populations. Those in the high risk category, compared to the lowest risk group, have approximately twice the odds of having had a major coronary event, based on a meta-analysis of prospective population-based studies.²³⁴

Although the association with CVD has been researched more extensively, evidence also supports the associations of inflammation with many chronic conditions, particularly diabetes²³⁹⁻²⁴¹ and cancer,²⁴² but also osteoporosis,²⁴³ rheumatoid arthritis,²⁴⁴ and anemia.²⁴⁵ In addition, inflammatory processes are associated with frailty, sarcopenia, disability, and mortality.^{229,246,247} Overall, CRP has been more extensively studied, especially in relation to CVD, where strong, positive associations have been observed in many large studies. In general, where both CRP and IL-6 have been investigated, it seems somewhat stronger associations are found with IL-6. Relatively fewer studies examined TNF- α .

Factors associated with inflammation

In cross-sectional analyses of data from the National Social Life, Health, and Aging Project, McDade et al. observed that socioeconomic factors (less education, fewer assets, non-Hispanic Black race/ethnicity) were associated with increased CRP levels among 57-85 year olds, but that these associations were substantially attenuated in a full model adjusting for medication use, health and lifestyle factors.²⁴⁸ In the full model, greater waist circumference, current and former smoker status, and use of sex hormones or immunosuppressives, and an interaction term for age x non-Hispanic Black race/ethnicity (such that black race was significantly associated with greater CRP only among the youngest age group) were significantly associated with higher CRP, while more education, household cleanliness and statin use were associated with lower CRP.²⁴⁸

The inflammatory markers IL-6 and CRP have been associated with obesity, especially central obesity, in the InCHIANTI study,²⁴⁹ abdominal visceral fat area (adjusted for total body fat) in the Health ABC study,²⁵⁰ visceral adipose tissue (even when controlling for BMI and waist circumference) and subcutaneous adipose tissue (without adjustment for BMI and waist circumference) in the Framingham Offspring Study.²⁵¹ Neither InCHIANTI²⁴⁹ nor Framingham Offspring Study²⁵¹ detected a statistically significant association between obesity or adiposity and TNF- α , which could be due to lack of association or measurement problems (low circulating TNF- α and short half-life). The Health ABC study reported TNF- α was inconsistently associated with abdominal visceral fat area, with a stronger association observed among women and no significant association in black men.²⁵⁰

Muscles produce IL-6 during contraction and increase the concentration of plasma IL-6.²⁵² Thus, strenuous physical activity causes an acute increase in IL-6,^{252,253} CRP,²⁵⁴ and possibly TNF- α ²⁵⁵ but self-

reported regular physical activity is associated with lower CRP levels in cross-sectional analyses of several large studies including NHANES-III,¹⁰⁵ CHS,¹⁰⁴ and the MacArthur Study of Successful Aging (MSSA),¹⁰³ adjusted for BMI and/or waist-hip ratio. In the MSSA, high level of self-reported recreational activity was also cross-sectionally associated with lower IL-6 levels.¹⁰³

Similarly, the effects of smoking on inflammatory marker levels are complex. A recent review summarizes that smoking both stimulates and suppresses pro- and anti-inflammatory processes through various pathways, but chronic smoking is ultimately associated with increased levels of acute phase reactants and pro-inflammatory cytokines, particularly IL-6 and TNF- α , and may be a risk factor for certain autoimmune-related disorders such as rheumatoid arthritis.²⁵⁶

In a recent review of large epidemiological aging studies with inflammatory markers, Singh and Newman concluded that there are few factors known to be associated with low-grade systemic inflammation,²¹⁵ although relatively good evidence exists for associations with greater adiposity (epidemiological evidence summarized above) and decreased sex hormones. The evidence for an association with sex hormones is not summarized here, as the current study does not include hormone levels, and the vast majority of females have experienced menopause. Singh and Newman noted that smoking, physical activity, depression, stress, and periodontitis have also been associated with inflammation in population-based studies.

In summary, greater deposits of adipose tissue appear to be strongly linked with increased inflammation. The relationships of inflammation with tobacco smoking and physical activity are complex, but in general, over time, smoking is associated with higher inflammation and regular physical activity is associated with lower inflammation. Socioeconomic status and/or education may be

associated with inflammatory marker levels, possibly via other factors such as adiposity, smoking, or physical activity.

1.D Inflammation and cognitive impairment

Evidence for potential mechanisms linking inflammation to cognition

In the past, the brain was viewed as relatively “immune privileged,” however it is now known that immune responses occur within the central nervous system (CNS). In fact, inflammation in the CNS is common in degenerative and trauma-related brain diseases such as AD, stroke, and Parkinson disease, which were traditionally considered noninflammatory brain diseases, in contrast to neuroinflammatory diseases such as meningitis or multiple sclerosis.²⁵⁷ Inflammatory molecules such as cytokines, complement factors, and chemokines have been observed in the CSF and plaques of AD patients.²⁵⁸ The amyloid- β cascade hypothesis of AD states that amyloid- β protein deposition is the initiating factor for AD pathology, and directly causes the other characteristics of AD, including neurofibrillary tangles, cell loss, vascular damage, and dementia.¹⁹¹ In accordance with that theory, AD is viewed as a primary neurodegenerative disease, with amyloid- β accumulation and neuron damage, which stimulates local microglia to produce an inflammatory response.^{257,259} Amyloid- β may or may not be the initiating factor, but it is likely that inflammation is a secondary phenomenon in AD and dementia, as inflammation is usually a response to some more primary stimuli. However, that does not mean that inflammation is not an important, or even necessary, cause of dementia. For example, participants without clinical dementia who qualified for a pathological diagnosis of AD on autopsy had only modestly elevated levels of inflammatory markers, greater than those participants with neither clinical dementia nor AD pathology, but much less than the AD patients.²⁶⁰ AD has been compared to other diseases with early and late onset types, including diabetes and arteriosclerosis. The early onset varieties are thought to be related to altered production of amyloid- β , insulin, and cholesterol, respectively, while the late onset

varieties are more associated with altered clearance of amyloid- β and lipoproteins, and insulin resistance, and have been linked to age-related low-level chronic inflammation.²⁶¹

It may also be that inflammation is associated with dementia through vascular mechanisms.

Inflammation is associated with cardiovascular²³⁷ and cerebrovascular disease,^{262,263} and both these diseases could contribute to dementia through major acute hypoxia, low-level chronic hypoperfusion, or increased inflammation. It is also possible that dementia and CVD are independent from each other, but share some underlying disease processes. There is a significant overlap in risk factors for dementia and vascular disease, including *APOE* genotype, high cholesterol, diabetes, smoking, physical activity, and obesity.²⁵⁹

Accumulating evidence indicates that cytokines play a central and complex role in numerous healthy state cognitive processes on the molecular level, through pathways such as synaptic plasticity, neurogenesis, and neuromodulation.²⁶⁴ Cytokines mediate cellular mechanisms involved in cognition such as cholinergic and dopaminergic pathways, and can facilitate neurodegeneration or regeneration.²⁶⁵ Although much remains unknown, some evidence indicates that peripheral cytokines may penetrate the blood-brain barrier (BBB), with direct mechanisms of entry through circumventricular regions less protected by the BBB,²⁶⁵ or indirectly via stimulation of the vagal nerve.^{266,267}

Previous studies

Although results are mixed and longitudinal evidence is relatively sparse, some investigators have reported that elevated inflammatory markers, including CRP,²⁶⁸⁻²⁷² IL-6,^{269,271,273,274} or TNF- α ,^{269,275,276} are associated with various measures of cognitive impairment. Others have reported null associations for

some or all analyses.^{272,273,275-279} This review focused mainly on longitudinal results for incident dementia and cognitive decline, except where noted. For the MCI outcome, one quasi-longitudinal analysis (cognition only ascertained at follow-up) and one cross-sectional analysis are reviewed, as no analyses were found for inflammatory markers with incident MCI (Table 1-8).

The HAAS was established in 1991 with 3734 participants of the original Honolulu Heart Program (n=8006), all Japanese American men born 1900-1919 and living on the island Oahu in 1965-1968. High-sensitivity CRP was measured from stored blood samples obtained in 1968-1970, when the men were 49- 70 years of age. Using a nested case-control design, investigators showed that men in the top 3 quartiles of hsCRP (vs. the lowest quartile) were at significantly increased risk (ORs ~3) of developing any dementia, AD, AD with CVD, or VaD by 1991-1993 or 1994-1996. Associations were similar when models included potential confounders measured at midlife (concurrently with hsCRP; age, education, *APOE* ε4, BMI, smoking, blood pressure, cholesterol, and years of follow-up to dementia assessment), and potential mediation by certain medical conditions reported up to the time of dementia assessment (stroke, CHD, LVH, atrial fibrillation, diabetes mellitus, ABI as a marker of peripheral subclinical atherosclerosis).²⁶⁸ Another report from the HAAS focused on the risk of cognitive decline (CASI score) and incorporated additional follow-up data from 1997-1999 and 1999-2000.²⁷⁰ There were no differences in baseline CASI score by hsCRP quartile, however those in the fourth quartile (compared to the first) showed a moderately significant ($p < 0.05$) increased rate of cognitive decline, adjusted for age, BMI, education, smoking, systolic blood pressure, TC, and *APOE* genotype. Interestingly, the difference was no longer statistically significant after excluding those with dementia.²⁷⁰ Limitations of these HAAS studies include the all-male, Japanese American sample, only one hsCRP measurement from 1968-1970, and prevalent dementia first ascertained in 1991-1993.

The original Cardiovascular Health Study (CHS) cohort included 5201 men and women aged 65 years or older, enrolled from 1989-1990 and an additional cohort of 687 African Americans were enrolled in 1992-1993. In 2005-2006, 1677 survivors participated in CHS All Stars study. Paired samples from 1996-1997 and 2005-2006 were assayed for high-sensitivity CRP and IL-6. Cognitive impairment was defined as a 3MS score < 80 out of 100. The associations between 9-year change in inflammatory markers and

Table 1-8. Previous studies of inflammation and cognitive impairment					
Citation	Study	Outcome	Predictor	F/u (max)	Summary of findings
Jenny et al. 2012 ²⁸⁰	CHS	Cognitive impairment	IL-6, CRP (2x; 9 years apart)	9 years	Change in IL-6 and CRP positively associated with odds of cognitive impairment.
Sundelof et al. 2009 ²⁸¹	UL-SAM (men)	AD, dementia	IL-6, CRP (2x; at ages 70 and 77y)	14 years	IL-6 at age 70 associated with non-AD dementia. Others null.
Engelhart et al. 2004 ²⁷¹	Rotterdam	Dementia (& AD, VaD)	IL-6, CRP	9 years	Positive (CRP less so)
Schram et al. 2007 ²⁷³	Rotterdam and Leiden 85+	Cognitive function & decline	CRP and IL-6	7 years/ 5 years	Mostly null, a few positive associations
Tan et al. 2007 ²⁷⁵	FHS	AD	CRP, IL-6 (serum), TNF- α , IL-1, IL-1RA	10 years	IL-1 and TNF-alpha increased risk, no association for IL-6 or CRP. TNF- α , IL-1, IL-1RA measured from PBMC.
Jefferson et al. 2011 ²⁷⁶	FOS	Cognitive function & decline	11 markers, including CRP, IL-6, TNF- α .	6.3 $\pm$ 1 year	Largely null, a few modest associations (TNF with TMT-B minus TMT-A). TNF- α only on a subset.
Schmidt et al. 2002 ²⁶⁸	HAAS (men)	Dementia (& AD, VaD)	CRP	25 years	Positive association
Laurin et al. 2009 ²⁷⁰	HAAS (men)	Cognitive decline	CRP	31 years	Modest association, disappears when exclude those with CI
Yaffe et al. 2003 ²⁶⁹	Health ABC	Cognitive decline (dichotomized)	IL-6, CRP	2 years	Only borderline (OR ~1.2) associations after full adjustment
Yaffe et al. 2004 ²⁸²	Health ABC	Cognitive decline ("cog impairment")	IL-6 and CRP (& combined)	5 years	Only those with both MetSyn and high inflammation were at increased risk of cog decline
Weaver et al. 2002 ²⁷⁴	MSSA	Cognitive decline (dichotomized)	IL-6	7 years	Positive (similar but non-significant association with continuous cog decline)
Alley et al. 2008 ²⁷⁹	MSSA	Cognitive decline	IL-6, CRP	7 years	Mostly null.
Dik et al. 2005 ²⁷⁷	LASA	Cognitive decline (dichotomized)	IL-6, CRP	3 years	Among those with cognitive impairment (MMSE 21-26), both IL6 and CRP were associated with MMSE decline (OR~3).
Weuve et al. 2006 ²⁷⁸	WHS	Cognitive fxn (continuous, 1 time)	CRP	4-8 years	All null.
Roberts et al. 2009 ²⁷²	Rochester	MCI, na-MCI, a-MCI	CRP, IL-6, TNF- α	Cross-sectional	Only CRP (top quartile) was associated (OR for MCI = 1.4).
Dlugaj et al. 2012 ²⁸³	Heinz Nixdorf Recall (HNR)	MCI, na-MCI, a-MCI	CRP	5 years	Positive strong associations. Cognition not measured at baseline.

concurrent onset of new cognitive impairment were examined using logistic regression. They found that a doubling in IL-6 over 9 years was associated with a 45% increased risk of cognitive impairment, with a 29% increase in risk observed for doubling in CRP. An important limitation was that the change in inflammatory markers was concurrent with onset of impairment, implying that the development of cognitive impairment might be causing the increased inflammation, rather than vice versa.²⁸⁰

The Uppsala Longitudinal Study of Adult Men (ULSAM) began in 1970, and recruited all 50-year old men (born 1920-24) living in Uppsala, Sweden. IL-6 and high-sensitivity CRP were measured in 1991-1995 (approximately age 70 years; n=1093) and again in 1997-2001 (approximately age 77 years; n = 777), with 704 participants measured at both times. Those with dementia at baseline were excluded. AD and dementia were determined by cognitive screening and medical records review. They examined whether levels at age 70 predicted incident dementia, and separately, whether levels at age 77 predicted incident dementia. Higher IL-6 levels at age 70 were associated with increased risk of non-AD dementia (HR = 1.28 per SD increase; 95% CI = 1.01-1.62). No other associations were significant. There was no association between longitudinal change in IL-6 or CRP over 7 years and AD or dementia.²⁸¹

In a case-cohort study within the Rotterdam Study, higher levels of IL-6, α 1-antichymotrypsin, and to some extent CRP were associated with greater risk of dementia with rate ratios of 1.28, 1.49, and 1.12 per SD in marker, respectively.²⁷¹ Adjustment for age, sex, education, current smoking, BMI, diabetes mellitus presence, and anti-inflammatory medication use did not change the results. Inclusion of PAD, carotid plaque, or intima-media thickness (IMT) in the model did not alter the results, indicating the effect may not be via atherosclerosis. Further sensitivity analyses excluding (a) those taking anti-inflammatory medications, (b) users of statins, or (c) those with possible acute infections (CRP > 15 mg/L), yielded similar results. Soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular

cell adhesion molecule-1 (sVCAM-1) were not associated with dementia risk, providing further evidence against the theory of mediation by atherosclerosis. Of note, the authors also examined the rate ratios for dementia by quintile of inflammatory marker to confirm the appropriateness of using a linear term to model the association. When comparing the fifth to the first quintile of each marker, the relative risks were non-significant for all markers except α 1-antichymotrypsin, and there was generally no pattern (e.g., no dose-response) by quintile, with wide and overlapping confidence intervals. Markers were measured from baseline blood samples (1990-93; all participants aged ≥ 55 y) and dementia follow-up continued through 1999.²⁷¹

Additional cognitive follow-up data (2001-2004) is available from the Rotterdam study in combined analyses with the Leiden 85-plus Study, providing maximum follow-up periods of 7 years and 5 years, respectively.²⁷³ In the Rotterdam data, higher levels of CRP and IL-6 were cross-sectionally associated with worse executive function and global cognition (but not MMSE or memory). Similar, but non-significant, associations with CRP were seen in the Leiden data. In the overall longitudinal analyses, neither CRP nor IL-6 level was significantly associated with annual cognitive decline in the Rotterdam Study; in the Leiden 85-plus Study, higher IL-6 level at baseline was associated with steeper decline in memory. When stratified by *APOE* ϵ 4 carrier status, Rotterdam data indicated higher IL-6 level was associated with greater decline in global cognition only among *APOE* ϵ 4 carriers. Leiden data showed a significant interaction with *APOE* ϵ 4 carrier status, such that the associations of higher CRP and IL-6 level with steeper decline in global cognition and memory were stronger in *APOE* ϵ 4 carriers. The authors also stratified by baseline CVD and atherosclerosis, and report “no consistent” influence of those factors on the associations. In the Rotterdam sample, effect modification by age was investigated, and again the authors use the language “no consistent” effect to describe the impact of age on the association.

Adjustment for depression symptoms, anti-inflammatory drug use, BMI, CVD, and diabetes mellitus did not substantially alter the results. Overall, the authors characterize their findings as modest, possibly due to chance (at least in the larger Rotterdam sample), and an indicator of the unsuitability of these markers for prediction. Yet they do not exclude the possibility of a true, possibly causal, association. They point out the discordance between these results and earlier results from the Rotterdam study, which used the more severe outcome of dementia,²⁷¹ as evidence of the importance of timing, and suggest that inflammation may only predict the late stages of cognitive decline. Also in contrast with previous cross-sectional findings from the Leiden study,²⁸⁴ Schram et al. failed to observe an interaction with CVD or atherosclerosis.²⁷³

In the FHS, investigators tested whether several inflammatory markers were associated with the risk of AD.²⁷⁵ For several markers (IL-1, IL-1RA, TNF- α), they used measures of spontaneous production by peripheral blood mononuclear cell (PBMC); for CRP and IL-6, they measured levels in serum. They found that those in the top tertile (vs. the bottom tertile) of PBMC IL-1 and TNF- α were at increased risk of AD (HR = 2.61, p=0.06 and HR = 2.59, p=0.03, respectively), adjusted for age, sex, *APOE* ϵ 4 allele status, stroke history, education, homocysteine, current smoking, BMI, and β -hydroxy- β -methylglutaryl-coenzyme A reductase inhibitor use. Although unclear why they adjusted for NSAID use in separate secondary analyses (not shown), they state “NSAID use did not effect a statistically significant change in the results.” They also looked at a composite variable, classifying participants as having favorable levels of PBMC cytokines (low IL-1, TNF- α) or unfavorable levels (> T1 for IL-1, > T2 for TNF- α , or < T2 for IL-1RA). Comparing the two groups, those with unfavorable levels (28% of females, 30% of males in the sample) were at greater risk of AD (HR = 1.61, p=0.009), and accounted for 42% of all AD cases. There were no associations with CRP or IL-6. However, limitations included: the use of low-sensitivity CRP,

potential survival effects, as IL-6 and CRP were significantly associated with increased risk of mortality during follow-up (HR, T3 vs. T1: for CRP = 1.57, $p=0.003$; for IL-6 = 1.76, $p=0.001$), while the PBMC cytokines were not, and finally, the length of follow-up is unclear, although biomarkers were measured in samples from the 22nd exam cycle (1990-1994), when AD follow-up began, with an additional “remote” CRP measure (1980-1982) on a subsample.²⁷⁵

The Framingham Offspring Study reported largely null associations between markers of inflammation and measures of neuropsychological functioning, based on longitudinal data for 1352 participants (1213 with TNF- α).²⁷⁶ Eleven markers (including CRP, IL-6, ICAM-1), plus TNF- α on a subset, were measured at exam 7 (1998-2001) on 3539 participants, although only 2208 enrolled in the ancillary neuro-study (1999-2004), with follow-up exams during 2005-2007. The vast majority of associations were null, a few were modest and borderline significant, and the most significant longitudinal association was TNF- α with standardized scores from TMT part B minus part A ($\beta = 0.04$ estimated annual change per SD in marker value, 95% CI = 0.01-0.07, $p < 0.004$). Given the very large number of associations tested (cross-sectional and longitudinal), the authors did some adjustment for multiple testing, but state that they did not use a strict Bonferroni correction, which would have nullified all results. Importantly, those with clinical dementia or stroke, and institutionalized participants, were excluded from these analyses (see Laurin et al. 2009, above, where HAAS findings became null when dementia cases excluded). It appears that inflammation was measured at one time point only.²⁷⁶

Investigators from the Longitudinal Aging Study Amsterdam (LASA) estimated 3-year reliable cognitive change, incorporating test reliability and regression to the mean, and then dichotomized by using a statistical cutoff ($p < 0.10$) to define clinically relevant decline.²⁷⁷ They reported that those in the highest tertile of $\alpha 1$ -antichymotrypsin were at increased risk for 3-year decline in MMSE (adjusted for age, sex,

education). No associations were found for IL-6, CRP, albumin, or the other four cognitive measures.

Level of IL-6 was not tertiled, but dichotomized around the detectable limit, as 89.7% of the sample had levels below the detectable limit for the IL-6 ELISA (5 pg/mL), and it is possible this assay was not sensitive enough. For CRP, the detection limit was 0.8 ng/mL. In a sensitivity analysis of cognitively impaired subjects (MMSE scores 21-26), high CRP and IL-6 were associated with MMSE decline (OR = 2.67, 95% CI = 1.21, 5.91 and OR=3.18, 95% CI = 1.33, 7.57, respectively).²⁷⁷

The Health, Aging, and Body Composition (ABC) Study reported that among 2538 well-functioning African American and white older adults, those in the highest tertile of IL-6 (>2.4 pg/mL) or CRP levels (>2.5mg/L), and to a lesser extent TNF- α (>3.7 pg/mL), were more likely to develop cognitive decline (≥ 5 point decrease on 3MS, dementia diagnosis, or cholinesterase inhibitor use).²⁶⁹ Adjusted for sex, education, age, race, smoking, alcohol use, BMI, self-reported health, co-morbidities, NSAID use, and baseline 3MS, the odds ratios were somewhat attenuated (ORs ~ 1.23) and were borderline significant. No significant interactions with race or NSAID use were detected. The 8% of participants who had all three markers in the highest tertile were more likely to have cognitive decline (adjusted OR=1.53, 95% CI =1.03, 2.28), compared to the 40% of the sample with no inflammatory markers in the top tertiles. Inflammatory markers were measured at baseline only, and follow-up was 2 years.²⁶⁹ In another publication by the Health ABC study, researchers found participants with metabolic syndrome were more likely to develop cognitive impairment (≥ 5 point decrease on 3MS at 3 or 5-year follow-up; adjusted RR = 1.20, 95% CI =1.02, 1.41).²⁸² Participants were classified as having “high inflammation” (25% of the sample) if they had levels above the median value for both CRP (≥ 2 mg/L) and IL-6 (≥ 2.0 pg/mL). A significant interaction was detected such that the presence of the metabolic syndrome was associated with increased risk among those with high inflammation (adjusted RR = 1.66, 95% CI = 1.19,

2.32) but not among those with low inflammation (adjusted RR = 1.08, 95% CI = 0.89, 1.30). Finally, those with high inflammation but no metabolic syndrome (n=317) were not at increased risk of cognitive impairment (adjusted RR = 0.81, 95% CI = 0.60, 1.08) compared to those with neither high inflammation nor metabolic syndrome. In a sensitivity analyses excluding participants with clinically significant hypertension, hyperlipidemia, or diabetes (n=797 of 2632), the association was attenuated and no longer statistically significant (adjusted RR = 1.35, 95% CI = 0.83, 2.19).²⁸²

The MSSA followed 70-79 years olds (1988) in the top third of the EPESE sample, in terms of physical and cognitive functioning (N=779, ~19% non-white).²⁷⁴ Investigators summed several subtest scores to obtain a total cognitive function score, as well as change scores. Cognitive decline was defined as having a change score in the bottom tertile of the sample distribution of change scores. Those in the top tertile of IL-6 were more likely to have cognitive decline at the 2.5 year follow-up (OR=2.03, 95% CI = 1.30, 3.19) and the 7-year follow-up (OR=1.90, 95% CI = 1.14, 3.18), adjusted for age, income, education, marital status, ethnicity, smoking status, alcohol use, physical activity, BMI, HBA1c, blood pressure, diabetes mellitus, cancer, MI, stroke, hip fracture, and other bone fracture. Similar, but non-significant trends were observed using a continuous measure of change as the outcome; the authors speculate this could be due to measurement error, or perhaps higher IL-6 level is only associated with substantial decrements in cognitive function, rather than linearly. Only a single baseline measure of IL-6 was used to classify exposure.²⁷⁴

Additional analyses of the MSSA data were reported in 2008,²⁷⁹ to determine whether inflammation was related to baseline cognitive performance or rate of change over 7 years, and they yielded mainly null results. Some subtest scores were associated with continuous IL-6 and CRP levels, but these effects disappeared in models adjusted for time, age, mortality and practice indicators, the other inflammatory

marker (IL-6 or CRP), sex, education, race, income, diabetes, MI, stroke, hip fracture, blood pressure, HBA1c, HDL cholesterol, waist circumference, NSAID use, alcohol use, physical activity, and smoking history. There were no significant associations between continuous markers and the rate of decline. In order to compare results to other studies, the authors also tested whether those in the highest tertile of CRP or the highest tertile of IL-6 were at greater risk for cognitive decline. Those in the highest tertile of IL-6 were at significantly greater risk of SPMSQ decline of 2 or more points (OR=1.67, 95% CI = 1.04, 2.67) in a fully adjusted model. The authors conclude that, if there is a relationship between inflammation and cognitive decline, it is only detectable when studying the extremes (highest inflammation levels and most substantial cognitive decline) and may not extend across the spectrum, or at least may be comparatively modest in populations that are not cognitively impaired. Limitations include the selection of a well-functioning sample and biomarker measurement at a single time point. Of note, these analyses differed from previous published articles by using growth curve analysis and adjusting for some factors not often controlled for, including mortality indicator, practice indicator, and inclusion of both IL-6 and CRP in analytic models.²⁷⁹

As part of the Women's Health Study, from 1992-1996, 4231 women aged 60-90 years provided blood samples for hsCRP measurement, and from 1998-2000, a battery of 5 cognitive tests was administered.²⁷⁸ There was no association between hsCRP quintile and any of the 5 cognitive test scores. Of note, the authors did not dichotomize the cognition outcome. The authors also reported that hsCRP levels were 57% greater, on average, among oral estrogen users, compared to non-users. Limitations include CRP measurement at one time point only, and cognitive assessment at one time only, done over the phone (although they stated it validated well against in-person assessments).²⁷⁸

A cross-sectional study in Olmsted County, MN residents, aged 70-89 years, reported an association of CRP (top vs. bottom quartile) with any-MCI (OR = 1.42, 95% CI 1.00, 2.01) and non-amnesic-MCI (na-MCI; OR = 2.05, 95% CI = 1.12, 3.78), but not amnesic-MCI (a-MCI), adjusted for age, sex, and education.²⁷² Additional adjustment for diabetes, hypertension, CHD, stroke, depression, and *APOE* genotype did not alter results. There were no associations with IL-6 or TNF- α , although lack of power may have been a problem, as these markers were only measured in 885 participants, while CRP was measured in 1883 participants. Limitations include lack of adjustment for important confounders (e.g., physical activity, obesity, smoking, alcohol, NSAID use), cross-sectional nature of the data, and modest participation rate (62%, or 2719 of 4398 eligible, agreed to participate; but only 2050 of those were in-person, and only 1890 with blood samples).²⁷² Subsequent cross-sectional analyses reported the next year investigated a possible interaction effect with metabolic syndrome. Metabolic syndrome was not associated with either type of MCI. Compared to those without metabolic syndrome and in the lowest tertile of CRP, those with metabolic syndrome and in the highest CRP tertile were more likely to have na-MCI (OR = 2.31, 95% CI = 1.07, 5.00) but not a-MCI, adjusted for age, sex, and education only.²⁸⁵

The Heinz Nixdorf Recall (HNR) study in the Ruhr area of Germany is a prospective population-based cohort study that began in 2000-2003 in order to investigate the use of coronary artery calcification as a predictor of MI and cardiac death (n=4814, ages 45-75 years).²⁸³ Participants were randomly selected from mandatory residence lists. Cognition was only measured at the 5-year follow-up, but hsCRP was measured from both baseline and follow-up examinations. A matched case-control analysis was used, with 148 MCI cases and 148 controls. Baseline CRP was strongly associated with the presence of MCI 5 years later (OR = 2.29, 95% CI = 1.01–5.15, 4th vs. 1st quartile). In cross-sectional analysis using the 5-year

follow-up marker data, CRP was even more strongly associated with prevalent MCI (OR = 3.60, 95% CI, 1.55–8.33).²⁸³

Table 1-9. Summary of evidence for inflammatory markers and cognitive impairment		
Marker	Outcome	Summary of evidence
CRP	Dementia (all)	1 positive (HAAS), 2 borderline (Rotterdam, ULSAM)
	Cognitive impairment	1 positive (CHS)
	AD	1 positive (HAAS), 1 borderline (Rotterdam), 2 null (FHS, ULSAM)
	VaD	2 positive (HAAS and Rotterdam)
	MCI	2 positive (HNR, quasi-longitudinal; Rochester, cross-sectional)
	Cognitive decline	1 positive (HAAS), 1 borderline (Health ABC), 6 null (Rotterdam, Leiden, FOS for 6/7 tests, MSSA, LASA, WHS for 5/6 tests)
IL-6	Dementia (all)	1 positive (Rotterdam), 1 borderline (ULSAM)
	Cognitive impairment	1 positive (CHS)
	AD	1 positive (Rotterdam), 2 null (FHS, ULSAM)
	VaD	1 borderline (Rotterdam), 1 positive (ULSAM; non-AD dementia)
	MCI	1 null (Rochester, cross-sectional)
	Cognitive decline	1 positive (MSSA), 2 borderline (Health ABC, Leiden), 4 null (Rotterdam, FOS, LASA, MSSA for 6/7 tests)
TNF- α	Dementia (all)	n/a
	AD	1 positive (FHS)
	VaD	n/a
	MCI	1 null (Rochester, cross-sectional)
	Cognitive decline	1 borderline (Health ABC), 1 null (FOS, for 5/7 tests)

In summary, the epidemiological evidence of an association between inflammatory markers and cognitive impairment is conflicting (Table 1-9). Very few studies of TNF- α were found, although this was the only marker associated with AD in the FHS, with a substantial adjusted OR of 2.59 (95% CI = 1.09–6.12) for the 3rd versus 1st tertile.²⁷⁵ For CRP, there was suggestive evidence for an association with dementia or AD, based on 2–3 studies with mixed findings, but more consistent evidence for an association with VaD (2/2 studies reporting positive associations), perhaps not surprising given research linking CRP to increased cardiovascular risk. For CRP and MCI, both the studies reviewed (one cross-

sectional)^{272,283} found a positive association with 4th vs. 1st quartile of CRP, but in the cross-sectional study, when CRP was alternatively modeled as tertiles, the association was not significant.²⁷² The weight of the evidence indicates there is not an association of CRP with cognitive decline, or at least not a strong association, as there were only a few significant associations among the many various cognitive decline measures tested (Table 1-9). For IL-6, only Rotterdam and FHS examined associations with AD, dementia, or VaD. The Rotterdam study reported significant but modest positive associations of IL-6 with all three outcomes,²⁷¹ while FHS results were null for AD.²⁷⁵ IL-6 was not associated with MCI in the cross-sectional analysis of data from the Rochester study, or with cognitive decline in the majority of studies reviewed, although a few significant associations were observed in MSSA, Leiden, and Health ABC (Table 1-9). Thus, for each marker there was at least one prospective association with cognition, particularly for the more severe outcomes dementia, AD, and VaD, but there were also multiple null associations, particularly for cognitive decline. More longitudinal studies are needed, given the lack of sufficient evidence.

Several factors may explain these contradictory results, including when inflammation and cognition were measured, how they were modeled, which covariates were adjusted for in the analyses, and whether interactions were explored. For example, some studies found interactions such that an association of inflammatory markers with a cognitive outcome was only evident, or more pronounced, among those with *APOE* $\epsilon 4$ ²⁷³ or metabolic syndrome.²⁸² The Rotterdam study investigated whether an inflammation – cognition association was mediated through atherosclerosis, by including variables for PAD, carotid plaque, or IMT, and based on results similar to their main models, concluded atherosclerosis was not mediating the association.²⁷¹

Importantly, it appears that the timing of exposure and outcome measurements may be key factors in understanding the association between inflammation and cognitive impairment. There is more evidence supporting an association of inflammation with AD, dementia, or VaD, compared to cognitive decline, leading some to suggest that inflammatory markers are only associated with later stages, or more severe cognitive decline.^{273,274,279} Another possible explanation is that inflammation is not necessarily a cause of cognitive decline, but rather that dementia may increase inflammatory marker levels, or some other factor is eventually causing both low-grade systemic inflammation and dementia. In fact, features characteristic of the AD brain, such as amyloid- β deposits and damaged neurons, are well-known to promote inflammatory processes.^{258,286} The relatively strong associations detected in the HAAS, where CRP was measured in midlife, indicate that an inflammation-cognition association is probably not exclusively due to existing dementia-related pathological features in the brain triggering inflammation.²⁶⁸ Almost all the risk factors for cognitive impairment (e.g., cholesterol, BMI) have varying strengths and directions of association, depending on whether they are measured earlier or later in life, probably because they both affect, and are affected by, disease processes in the brain. Such contrasting effects, by time of exposure measurement, have not yet been explicitly shown for the association of inflammation with cognition, as previous studies have measures of inflammation from only one time point.

The proposed dissertation aims will contribute to the evidence in several ways. First, most existing studies measured inflammatory markers at one time point only, with the exceptions of CHS, ULSAM, and FHS, which had an additional “remote” CRP measure available on 295 participants. For CHS, there was no temporal lag between the second measure of inflammation and outcome measurement. None of these studies with multiple inflammation measures examined sustained high levels versus sustained low

levels. A key advantage for the current proposal is that inflammatory markers have been measured from 2 (TNF- α) or 3 exam phases (IL-6 and CRP) with good quality control measures in place. Second, some of the existing studies were done among selected “healthy” participants, while others were among specific subgroups (e.g., only men, only women, only Japanese American men living on Oahu). The EHLS is a population-based study with good participation rates, and Beaver Dam, WI residents are likely to be similar to much of the U.S. population, except for the lack of racial diversity. Third, in addition to the inflammatory marker data from multiple time points and the expanded battery of cognitive tests from the most recent exam phase, the EHLS has good quality measures of other factors which are possible confounders of the inflammation-cognition association.

1.E Anti-inflammatory medication use and cognitive impairment

Non-steroidal anti-inflammatory drug (NSAID) use

An early observation of low AD prevalence among patients with rheumatoid arthritis inspired the hypothesis that anti-inflammatory drug use may be associated with decreased risk of AD.²⁸⁷ Some, but not all, prospective observational studies have reported lower risk of AD or dementia among those using anti-inflammatory drugs (Table 1-10, below), however randomized controlled trials (RCT) have found no benefit of anti-inflammatory drugs (NSAIDs) on the progression of disease among patients with AD. A meta-analysis concluded that much of the observed beneficial effects of NSAIDs is likely due to recall, prescription, and publication bias based on their pooled estimates of a 50% risk reduction from studies of prevalent dementia, 20% reduction for incident dementia studies, and no association overall among studies of cognitive decline.²⁸⁸ Thus, the evidence is contradictory, but one could argue that the overall pattern shows a lack of short-term benefits in preventing or slowing of progression of dementia, while suggesting that long-term use earlier in life may be associated with reduced risk.

The authors of the report from ACT²⁸⁹ suggested an explanation for contradictory results among various large observational studies. They state that NSAID use, and other potential protective factors, are thought to delay the onset of AD, but not necessarily prevent it. Such delays in onset would appear to reduce the incidence of disease at younger ages, but effectively increase the incidence at older ages. In support of this theory, they observe that studies of older cohorts, including the ACT (median age 83.5 y at onset), the CSHA, the ROS and the MoVIES, have failed to detect reduced risk among NSAID users, while studies among slightly younger cohorts, such as Rotterdam and Cache County, have reported such effects (Table 1-10).

Viewed together, the observational studies have identified a couple potential key factors in the association of NSAID use and cognitive impairment, including *APOE* genotype and duration of use or age of initiation. Some, but not all, of the studies that stratified by *APOE* $\epsilon 4$ carrier status found that NSAIDs were only associated with reduced risk of cognitive impairment among carriers of the $\epsilon 4$ allele. Most studies that examined duration or age of initiation found that longer duration and early age of initiation were more strongly associated with reduced risk of adverse cognitive outcomes. Several studies found duration of ≥ 2 years of use associated with more reduced risk than < 2 years of use, compared to non-users. It is often suggested that any protective effect of NSAIDs or other anti-inflammatory drugs is more likely to occur earlier in the disease process (or before it begins), but that there is little evidence to support any protective effect if initiated late in life, or in those with moderate or severe cognitive impairment. In fact, later in the disease, brain inflammation may be beneficial in combating the existing pathological features of AD and thus anti-inflammatory drugs may accelerate disease progression.

RCTs have failed to show beneficial effects of NSAIDs in slowing the progression of AD²⁹⁰ or preventing new disease.^{291,292} Null results from trials among participants who already have mild to moderate AD provide important information and limits regarding a potential association, but clearly do not preclude a possible protective effect of NSAIDs in delaying or preventing disease onset. The AD Anti-inflammatory Prevention Trial (ADAPT) was designed to determine whether celecoxib (a selective COX-2 inhibitor) or naproxen (a “dual-inhibitor” NSAID) could prevent AD or delay cognitive decline in volunteers ages 70 years and older who had family histories of AD but performed sufficiently well on a battery of cognitive tests. Regular NSAID users were excluded from participation, but low-dose aspirin users were not. The trial was ended early due an announcement from a different trial that celecoxib had been associated with significantly increased cardiovascular risk. The median duration of use for the treatments ranged

Table 1-10. Studies of NSAID use and cognitive impairment						
Study	Citation	Outcome	Exposure	Result	Method	Notes:
Rotterdam	In'T Veld et al. 2001 ²⁹³	AD, VaD	NSAIDs, oral salicylates	Inverse for NSAIDs – AD; positive for salicylates-VaD	Cox	Long-term use (but not short- or intermediate-term) of NSAIDs assoc. with reduced AD risk (RR = 0.20) but not assoc. with VaD risk. Oral salicylates (including aspirin) use was not assoc. with AD, but assoc. with increased risk for VaD (long term use RR = 4.9), perhaps due to indication bias. (Any use vs. no use was null (RR = .86))
BLSA	Waldstein et al. 2010 ²⁹⁴	Cognitive function	NSAIDs, aspirin	Inverse for NSAIDs; Positive for aspirin	Mixed effects	Less decline in performance on TMTB and Blessed IMC was observed in NSAID users. Aspirin use was associated with greater decline on Blessed IMC and Benton VRT. Use was yes/no at each exam (no frequency, quantity, duration info.).
BLSA	Stewart et al. 1997 ²⁹⁵	AD	NSAIDs, aspirin, acetaminophen	Inverse for NSAIDs, null for aspirin and acetaminophen	Cox	Exposure treated as binary user/nonuser over f/u period) and categorical (0, <2, or 2+ y use during f/u). Explored 0-5 y lag, found 2-5 y lag further emphasized duration effect.
ROS	Arvanitakis et al. 2008 ²⁹⁶	AD, AD pathology, cog decline	Nonaspirin NSAIDs, aspirin, ibuprofen	Null	Cox for AD; Mixed effects for change in cog.	Cumulative NSAID use was low, and potentially inconsistent, thus effects of regular, long-term use may not have been detected. Population was older at baseline (mean 75y) and earlier NSAID use is unknown.
CSHA	Wolfson et al. 2002 ²⁹⁷	AD	Nonaspirin NSAIDs	Null	Case-control with logistic regression	Used pharmacy database to avoid recall bias, but only examined prescriptions dispensed in the 3 years prior to onset of AD symptoms.
CHS	Szekely et al. 2008 ²⁹⁸	Dementia, AD, VaD	NSAIDs, (not aspirin, acetaminophen)	Inverse for dementia, AD (null for VaD);	Cox	Many thoughtful sensitivity analyses, none differed from main findings (except the <i>APOE</i> interaction). by <i>APOE</i> ε4, only significant in ε4 carriers

Study	Citation	Outcome	Exposure	Result	Method	Notes:
Cache County	Zandi et al. 2002 ²⁹⁹	AD	Nonaspirin NSAIDs, aspirin compounds, H2RA drugs, 3 “control” drug groups	Inverse for former or at least 2 year NSAID use (aspirin similar)	“discrete time survival analysis”	Any lifetime regular use of NSAIDs (for at least 1 month) was very marginally associated with reduced AD risk (null for other drug classes). When stratified, former but not current NSAID use was associated with reduced risk; and ≥ 2 years duration, but not $< 2y$ duration, was associated with reduced risk. In stratified analyses, aspirin effect showed similar pattern to NSAIDs.
Cache County	Hayden et al. 2007 ³⁰⁰	Cognitive function (3MS)	NSAIDs	Mixed (null among whole sample)	Mixed effects	NSAID effects modified by age of initiation and <i>APOE</i> $\epsilon 4$. In those initiating before age 65y, “protective” effect in $\epsilon 4$ carriers, null effect in non-carriers. In those initiating after age 65y, among $\epsilon 4$ carriers, NSAID use assoc. with higher baseline but no diff. over time; in non-carriers, NSAID use associated with greater decline. (When stratified by <i>APOE</i> only, NSAID use is null in non-carriers, but in carriers, associated with better baseline and less decline).
CHAP	Grodstein et al. 2008 ³⁰¹	Cognitive function	Nonaspirin NSAIDs, aspirin	Inverse for NSAID lifetime use; Null for aspirin	Mixed effects	Neither NSAID nor aspirin current use (in 2 weeks prior to baseline) was associated with decline. But longer lifetime duration use of NSAID was associated with less decline (significant trend).
ACT	Breitner et al. 2009 ²⁸⁹	Dementia, AD	NSAIDs	POSITIVE, heavy users, both outcomes	Cox	Suggest that their results, and those from other older cohorts, could reflect that NSAIDs simply delay the onset of disease.
U.S. VA	Vlad et al. 2008 ³⁰²	AD (diagnosis codes)	NSAIDs, all and 7 classes separately	Inverse, with longer duration more protective	Case-control, logistic regression	Slightly reduced risk in NSAID users ≤ 1 year, greater for longer use. By class, arylpropionic acids more pronounced, no effect for COX-2 inhibitors. By individual drugs, ibuprofen most protective.

from 546-561 days. Preliminary analyses showed an inconclusive trend toward increased risk of AD with either treatment.²⁹¹ A subsequent publication reported the changes in neuropsychological test performance in trial participants during 1-4 years following randomization, and concluded that neither drug improved cognitive function.²⁹² In summary, although the RCTs have failed to find protective effects, they were performed in a selected group of older adults for a relatively short period of time and thus do not necessarily contradict reports from observational studies indicating that prolonged use earlier in life may be protective, especially given indications that overt AD may be preceded by decades of latent disease processes.

There are several mechanisms by which NSAID use may protect against dementia, with anti-inflammatory actions perhaps the most obvious, but also including antiplatelet effects which reduce vascular disease and improve blood flow, and possible amyloid- β lowering effects among certain NSAIDs, such as ibuprofen. In fact, some of the observational studies have noted that most participants taking these drugs are using doses that are analgesic, but not thought to be anti-inflammatory,^{293,299} which may indicate that the main mechanism of action may not be anti-inflammation.

Statin medication use

Muangpaisan et al. published a systematic review in 2010 for which they identified 2 meta-analyses, 6 studies of dementia, and 2 of VaD, and concluded there was not convincing evidence that statin use prevents dementia or VaD and also suggested the protective effect noted in few observational studies could be a result of physicians prescribing statins to healthier patients.³⁰³ A recent Cochrane review concluded that there was good evidence that statins are not effective in preventing dementia, at least when given in late-life to individuals at risk of vascular disease. The review summarizes the inconsistent

findings from observational studies and two RCTs. Neither RCT found a significant cognitive effect, although both found significant effects of simvastatin or pravastatin on their primary outcomes, coronary and cerebral events and vascular disease. These RCTs were large but had weaknesses, including less than optimal cognitive assessments, losses to follow-up, and relatively short follow-up period, for a study of prevention of a disease thought to have origins in mid-life.¹⁵⁴

If cholesterol is causally related to cognition (reviewed in Background section on cholesterol), lipid-lowering agents (LLA) should theoretically be effective in preventing or delaying cognitive declines and dementia due to that mechanism. One particular class of LLAs, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, commonly called statins, are of particular interest in dementia research due in part to their additional (cholesterol-independent) effects, including improved endothelial function, anti-thrombotic effects, and anti-inflammatory effects (reviewed in ¹⁵⁴). Various statin medications exist, and are thought to differ in their potential for neuroprotection. A recent *in vitro* study of 9 different statins concluded that simvastatin had the best characteristics for neuroprotection, including higher efficiency in penetrating the BBB due to its small size and lipophilic nature, good safety profile, and ability to significantly lower cholesterol levels in neurons and glia cells, as well as hepatocytes.³⁰⁴

In a cross-sectional analysis using data from the 5-year follow-up of the EHLS (1998-2000) in an age-restricted subset 65-97 years of age, Carlsson et al. observed that use of any LLA and use of statin-LLA (but not use of non-statin LLA) were both associated with decreased odds of cognitive impairment in unadjusted analyses, but the association was much attenuated and non-significant with adjustment for age. As statin use was associated with factors that both positively and negatively affect cognition, indication bias is a major concern.¹⁵¹

The potential of statins to improve cognition is still unclear. Observational studies may fail to observe an association due to indication bias and RCTs indicate statins given in late-life do not protect cognition.

Although it is possible that statins begun in mid-life may protect against cognitive decline, such a long-term RCT would likely be infeasible.

1.F Potential interactions with *APOE* ϵ 4 carrier status

*Previous studies of inflammatory markers and cognitive outcomes by *APOE* ϵ 4 carrier status*

Two studies have reported significant effect modification by *APOE* ϵ 4 carrier status, although they reported opposite directions for the association among *APOE* ϵ 4 carriers. In the Leiden-85+ Study, higher CRP and IL-6 were associated with steeper decline in global cognitive function in carriers, with null associations among non-carriers.²⁷³ Conversely, a study of older Latinos reported an inverse association between CRP level and the incidence of the combined outcome cognitive impairment not dementia (CIND)/dementia among carriers, with a null association among non-carriers.³⁰⁵ Several other studies have reported a lack of significant effect modification by *APOE* ϵ 4 carrier status.^{270,272,275,277,281}

Additional studies have included *APOE* ϵ 4 carrier status as a covariate, but did not state whether interactions were tested, possibly due to either a lack of power to test the interaction, or a lack of significant interaction.

*Previous studies of NSAID use and cognitive outcomes by *APOE* ϵ 4 carrier status*

A couple previous studies have reported significant interactions for NSAID use and *APOE* ϵ 4 carrier status, with NSAID use associated with better outcomes in *APOE* ϵ 4 carriers, and null associations among non-carriers.^{298,300} Several other studies of NSAID use with cognitive outcomes reported that interaction terms with *APOE* ϵ 4 carrier status were not statistically significant.^{293,296,299} Interestingly, two of these studies used Cache County data, the one reporting a lack of significant interaction examined AD risk,²⁹⁹ and the one reporting a significant interaction examined cognitive function and decline.³⁰⁰ Yet

most large prospective epidemiological studies of the NSAID-cognition association did not state whether interactions with *APOE* ϵ 4 carrier status were tested.

Previous studies of statin use and cognitive outcomes by APOE ϵ 4 carrier status

Regarding statin use and cognitive outcomes, most studies that examined an interaction term for statin by *APOE* ϵ 4 carrier status reported no significant interaction.³⁰⁶⁻³¹² One of these studies, the Adult Changes in Thought (ACT) Study, had previously reported a suggestive trend toward reduced AD risk with statin use among only younger (65-79 years at entry) *APOE* ϵ 4 carriers (no risk reduction in older participants or younger non-carriers).³¹³ Although the subsequent ACT study reported that the interaction term was not significant ($p=0.65$), they found that the inverse association was stronger and significant among carriers (not significant among non-carriers).³⁰⁹

CHAPTER 2

SPECIFIC AIMS

As the number of older adults increases, dementia prevention will become an even greater public health concern. Many factors related to vascular health have also been associated with cognitive function and dementia. Inflammation may be one of the mechanisms through which such factors are associated with cognitive impairment, and thus we hypothesize that elevated levels of inflammatory markers will be associated with cognitive impairment, mild cognitive impairment (MCI), and dementia, and that anti-inflammatory medication use will be associated with lower risk of cognitive impairment, MCI, and dementia. The specific aims of this project are:

Aim 1: To determine the association of markers of systemic inflammation with the risk of cognitive impairment and the odds of MCI/dementia. We hypothesize that higher levels of inflammatory markers will be associated with increased risk of cognitive impairment and greater odds of MCI/dementia and that this relationship will be stronger for sustained high levels of inflammation.

Aim 2: To determine the association of anti-inflammatory drug use with the risk of cognitive impairment. We hypothesize that both NSAID use and statin use will be independently associated with decreased risk of cognitive impairment and lower odds of MCI/dementia and that these relationships will be stronger with longer duration of use.

Aim 3: To determine whether the associations in Aims 1 and 2 are modified by *APOE* genotype.

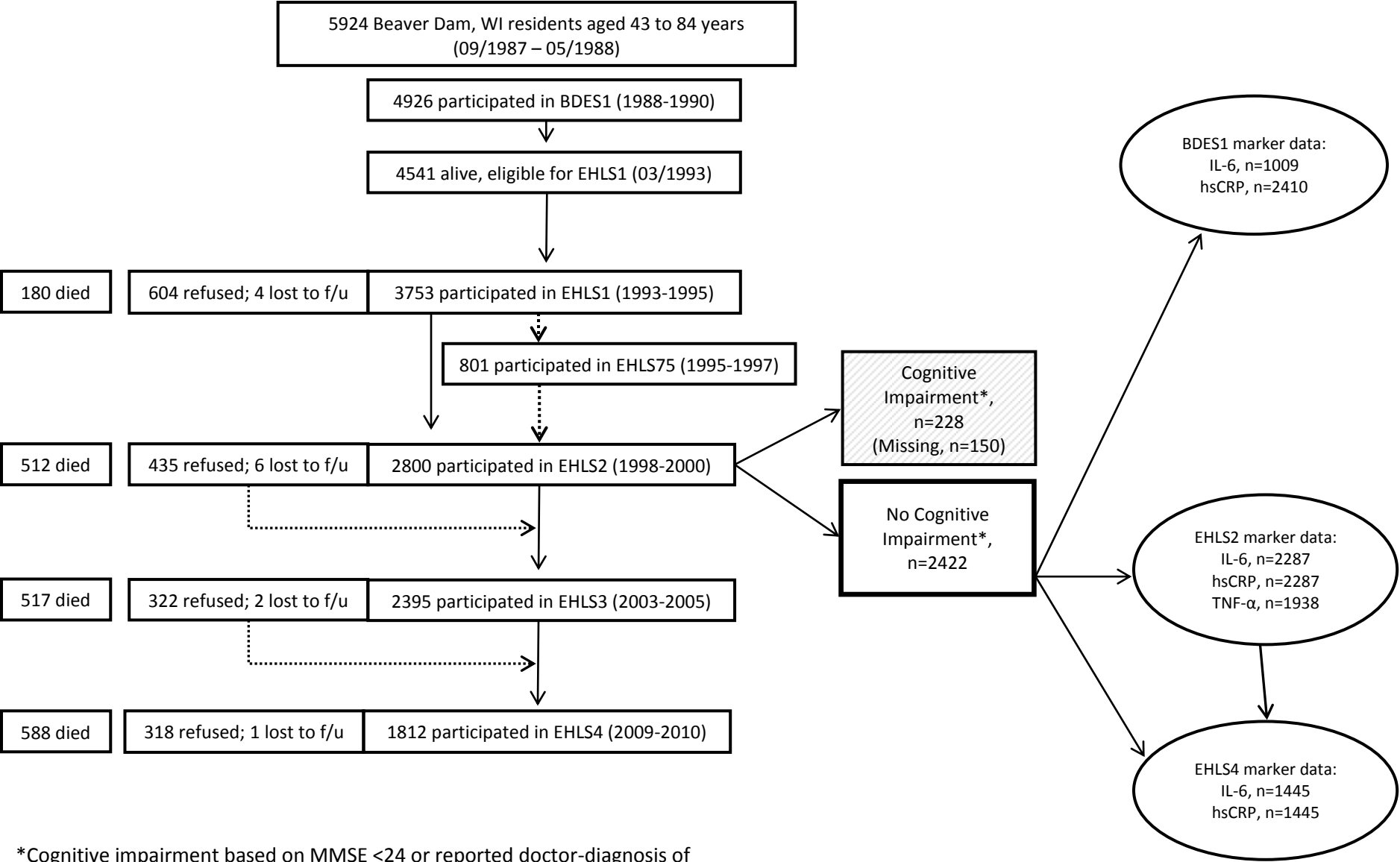
CHAPTER 3

METHODS

3.A Study population

The Epidemiology of Hearing Loss Study (EHLS) is a population-based longitudinal study of sensory loss and aging (1993-present). Participation over the course of the study is diagramed in Figure 4-1. In 1987-1988, a private census was conducted to identify 43 to 84 year old residents of the city or township of Beaver Dam, WI. There were 5924 eligible participants, and in 1988-1990, 4926 (83%) of these participated in the first examination phase of the Beaver Dam Eye Study (BDES).³¹⁴ The 4541 who participated in the baseline BDES and were alive on March 1, 1993 were eligible for the EHLS, and 3753 (82.6%) participated in the baseline EHLS examination phase from 1993-1995.³¹⁵ In 1998-2000, the EHLS five-year follow-up examinations took place and 2800 (82.2%) participated, of the 3407 EHLS participants alive as of March 1998.³¹⁶ At the 10-year follow-up examination phase in 2003-2005, 2395 (82.5% of survivors) participated.³¹⁷ In 2009-2010, of the 2243 eligible participants still alive before examinations began, 1812 participated (80.8%), 318 refused, 112 died without participating, and 1 was otherwise lost to follow-up. For the purposes of this project, the EHLS 5-year follow-up in 1998-2000 will be considered “baseline”, as that was the first point when cognition was measured for all participants.

Figure 3-1: Diagram of participation and available data, by exam phase, EHLS



*Cognitive impairment based on MMSE <24 or reported doctor-diagnosis of dementia or AD

3.B Data Collection

Examinations took place in study field offices at the Beaver Dam Community Hospital (BDCH) for most participants. For participants unable to visit the study offices, study staff visited individual homes, group homes, assisted living homes, and nursing homes to collect data. Informed consent was obtained for all participants.

Questionnaire data were obtained through interviews at each phase, and includes data on educational attainment, hearing, occupation, behavior and lifestyle factors, medication use, and medical history. For participants who were unable to provide their own interview data, proxy-respondent interviews were conducted. Of particular note for this project, reported diagnosis of AD was obtained by interview, and at the 10-year follow-up (2009-2010), two questionnaire items on memory problems were included (“Have you, your family, or your physician ever expressed concerns about your memory?” and “Do (your) memory loss symptoms interfere with your ability to do your own day-to-day activities?”).

Extensive examination data were collected, including data on hearing, blood pressure, and anthropometry. Olfaction, vascular, and cognition measures were first included in 1998-2000 (baseline for this project). One cognitive test, the MMSE, was administered in 1998-2000, 2003-2005, and 2009-2010. Additional cognitive tests were introduced in the 2009-2010 phase: Trail Making Test, parts A and B (TMT-A and TMT-B), Digit Symbol Substitution Test (DSST), Verbal Fluency Test (VFT), and the Auditory Verbal Learning Test (AVLT). Blood samples were obtained for both immediate testing and storage. Data on medication use were collected at each phase. Details on cognitive tests, inflammatory markers, medication use, and other variables are described below.

Cognitive tests

The MMSE was administered at the baseline, 5- and 10-year follow-up exams. At the 10-year follow-up exam, additional cognitive tests (TMTA, TMTB, DSST, VFT, and AVLT) were added to the exam.

The MMSE is a brief test of general cognitive function, and is often used as a screening device for cognitive impairment in clinical and research settings. Items assess orientation, registration, attention and calculation, short-term recall, and language.³¹⁸ The maximum score is 30 and cognitive impairment was defined as a score less than 24, a generally-accepted cutoff.³¹⁹ During the baseline (1998-2000) and 5-year follow-up examinations (2003-2005), when the cohort was relatively young and cognitively healthy, those participants who performed well on the first 5 parts of the test (≥ 15 out of the first 21 possible points) were not administered the remainder of the MMSE. Partial MMSE scores were adjusted to the full 0-30 point scale, based on the percent correct. Compared to the full MMSE, the partial MMSE had a sensitivity of 96% and a specificity of 93%, based on data collected in a 1995-1997 substudy of participants age 75 years and older.³²⁰ In 2009-2010, all participants were given the full MMSE.

The TMT requires participants to connect 25 circles in the correct order.³²¹ The TMT has two parts (A and B), part B is more difficult than part A. For part A, each circle has a number in it (i.e., 1, 2, 3, through 25) and participants were instructed to connect the circles in consecutive order as quickly as they could. In Part B, the circles are filled with numbers and letters, and participants were asked to alternate between numbers and letters as they connected the circles (i.e., 1, A, 2, B, 3, C and so on). Participants were given an abbreviated sample test to practice before each part. For each part, the score is the number of seconds it took to complete the task. If participants were unable to finish in 5 minutes, the examiner told them they could stop and the score was recorded as 301 seconds. If participants made a

mistake (e.g., 1, A, B), the examiner corrected the participant and asked them to return to the last correct circle (e.g., “You just connected A to B, but you are supposed to alternate between numbers and letters, 1 to A, A to 2, 2 to B, and so on. The last one you had correct was A, so go back to A, and try again from there.”).

For the DSST, participants were given a sheet of paper with 4 rows of paired boxes.³²¹ In each row, the top boxes have numerals (1 through 9), and the bottom boxes are empty. Participants were asked to fill in the empty boxes with the symbols that correspond to the numeral above, using a number-to-symbol key at the top of the testing sheet. Participants completed a small practice section before doing the actual test. During practice, the examiner corrected any mistakes made, and reiterated the instructions if necessary. Participants were told that they should fill in each box in order without skipping any boxes (i.e., not supposed to fill in the symbol for all the 1’s, then all the 2’s, etc.) and that they had 90 seconds to fill in as many boxes as possible. The maximum score is 93 symbols. The DSST score is the number of correct symbols completed by the time limit. Incorrect symbols were not counted in the score, but otherwise were not penalized.

The VFT asks participants to list as many words as possible that start with the letters “F”, “A”, and “S”.³²¹ Participants were given 1 minute for each letter. Examiners told participants that proper names (e.g., Boston, Bob, Buick) would not count and that the same word with different ending such as “-ing” would only count as one word (e.g., eat and eating). The score for each letter was based on the number of words the participant produced. The overall VFT score is the sum of the scores for the F, A, and S trials.

The EHLS utilized a modified version of the Rey AVLT.³²¹ The EHLS protocol for the AVLT instructed examiners to read aloud a list of 15 words (List A) at a steady pace, and then participants immediately

recalled as many words as possible, in any order. This process was repeated twice more, resulting in 3 trials with List A. Then the examiner read a different list (“distracter” List B) with 15 new words, and asked the participant to immediately recall as many as possible. Finally, the examiner asked the participant to recall as many words as possible from List A, without reading List A again. There was no explicit time limit for participants to recall the words, but examiners were trained to prompt participants (e.g., “Can you remember any more?”) if they became silent. For this project, the AVLT score is the number of words the participant was able to recall from List A during the final trial only (following the List B distracter trial). Modifications in EHLS, compared to the Rey AVLT, included fewer trials with List A prior to the distracter List B (3 trials versus 5), no delayed recall, and no recognition task.

Inflammatory markers

Inflammatory markers from the baseline (1998-2000) and 10-year follow-up (2009-2010) phases were measured together in 2011 by the Fairview Laboratory at the University of Minnesota from blood samples stored at -80°C. Serum hsCRP was measured using a latex particle-enhanced immunoturbidimetric assay kit (Roche Diagnostics, Indianapolis, IN 46250) on a Roche Modular P Chemistry analyzer (Roche Diagnostics). The inter-assay coefficient of variation (CV) for the laboratory was 4.5%. The reference range was 0-5 mg/L. Serum IL-6 was measured using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) QuantiKine High Sensitivity kit (R & D Systems, Minneapolis, MN). The intensity of color, reflecting the amount of IL-6, was measured on a SpectraMax spectrophotometer from Molecular Devices (Sunnyvale, California). For IL-6, the inter-assay CV was 11.7%. Serum TNF- α was measured with the human TNF- α QuantiGlo ELISA assay from R&D systems (Minneapolis, MN), with the amount of TNF- α measured from the intensity of emitted

light on a Packard luminometer (Packard Instrument Company, Meriden, CT, incorporated by Perkin Elmer, Boston, MA) or BioTek plate reader. The inter-assay CV was 13.0%.

Data on inflammatory markers from the baseline BDES in 1988-1990 were available. hsCRP was measured for all participants (n=4880) and IL-6 was measured for a random sample of participants (n=1763) as part of a substudy.³²² Serum hsCRP was measured using a latex particle-enhanced immunoturbidimetric assay kit from Kamiya Biomedical Co. (Seattle, Washington) on the Hitachi 911 Automatic Analyzer (Boehringer; Mannheim, Germany). The reference range was 0 to 0.5 mg/dL. The CV was 4.5%. Serum IL-6 from 1988-1990 was measured using the same methods for the IL-6 at the later time points, ELISA QuantiKine High Sensitivity kit (R & D Systems, Minneapolis, MN), with a SpectraMax spectrophotometer from Molecular Devices (Sunnyvale, California). The reported CV was 6.5-9.6%.³²²

Medication use

At each exam phase, participants were asked to bring to the examination all medications used in the past month, both prescriptions and over-the-counter, and examiners physically inventoried and recorded medication names directly from containers. Additional data on aspirin use were obtained as part of the questionnaire, participants were asked: “Do you take aspirin or aspirin products regularly, that is at least twice a week (for more than 3 months)?” Duration of use was not ascertained.

Medications were coded based on active ingredients and variables were created to indicate whether a participant was taking a medication containing a particular type of drug, in particular, aspirin, non-aspirin NSAID, or statin. Any NSAID use was based on both aspirin and non-aspirin NSAID medications, with a participant considered an NSAID user if they used either aspirin or non-aspirin NSAID containing medications, and a non-user of NSAIDs if they used neither.

Blood pressure

At baseline and follow-up examinations, a random zero sphygmomanometer was used to measure blood pressure, using a protocol modified from that used in the Hypertension Detection and Follow-Up Program.³²³ In brief, participants' systolic and diastolic blood pressures were calculated as the average of two blood pressure readings, measured 30-second apart. Anti-hypertensive medications use was also ascertained by physical inventory of the participants' current medications.

Cholesterol

Both non-fasting serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) levels were measured. Cholesterol levels from 1998-2000 examinations were measured on a Vitros chemistry analyzer at the Beaver Dam Community Hospital Laboratory, Beaver Dam, WI, using an enzymatic-based test. In order to measure the HDL-C levels, low-density lipoprotein and very-low-density lipoprotein components were precipitated out of samples using dextran sulfate. Measurement of 2009-2010 TC and HDL-C levels were performed at the Collaborative Studies Clinical Laboratory, Fairview-University Medical Center, Minneapolis, MN on a Roche Modular P chemistry analyzer. TC was measured using a cholesterol oxidase method (Roche), and the laboratory CV was 1.6%. HDL-C was measured on the same instrument using a double enzyme (cholesterol esterase, cholesterol oxidase) reaction, after the other lipoprotein components were precipitated out with divalent cations (Mg^{2+}) and polyanions (dextran sulfate). The laboratory CV was 2.9% for HDL-C. The assay was regularly calibrated as per the CDC/NHLBI Lipid Standardization Program.³²⁴

Diabetes and glycated hemoglobin

Serum glycated hemoglobin from 1998-2000 was measured with the IMx glycated hemoglobin test, an ion-capture boronate affinity-binding assay (Abbott Laboratories). The reference laboratory was Consultant Physician Laboratory, Beaver Dam, WI. Hemoglobin A1c is the major subfraction of the normal glycated hemoglobin, and was calculated using a conversion factor as per the assay documentation. For the 2003-2005 and 2009-2010 follow-up phases, hemoglobin A1c was measured on the Tosoh HPLC G7 Glycohemoglobin Analyzer (Tosoh Medics, Inc., San Francisco, CA, 94080), at the Collaborative Studies Clinical laboratory, Fairview-University Medical Center, Minneapolis, MN. The laboratory CV range was 1.4-1.9%. The normal reference range for A1c was 4.3-6.0%. Methods were calibrated to the National Glycohemoglobin Standardization Program (NGSP) standard values. Participants were also asked whether a physician had ever told them they had diabetes and whether they were taking any medications for diabetes. Participants were categorized as having diabetes if they reported a doctor diagnosis, were using medications for diabetes (injections or pills), or had a measured (or estimated) hemoglobin A1C level $\geq 6.5\%$.

Anthropometry and obesity

Height, weight, and waist measurements were obtained by trained examiners. Weight was measured after participants removed their shoes and pocket contents. Height and weight were measured on the Health-O-Meter scale (BDES, 1998-2000). Waist circumference was measured with a measuring tape while participants were standing. BMI was calculated by dividing weight (in kilograms) by height (in meters) squared. BMI $\geq 30 \text{ kg/m}^2$ was considered obese.

Mental Health

General mental health was measured with the mental component score (MCS) of the Short Form Health Survey (SF-36), in which higher scores indicate better mental health.³²⁵

Physical activity

At each phase, physical activity was ascertained by the questions “At least once a week do you engage in a regular activity long enough to work up a sweat?” and “On average, how many times per week do you do this?” Responses were categorized as: 0 times/week, 1-2 times/week, or 3+ times/week.

Alcohol consumption

At each phase, participants were first asked if they have consumed any alcohol in the past year. If they responded yes, they were asked whether they consumed any light beer, beer, wine, or liquor, respectively. For each type of alcohol they have consumed in the past year, they were asked to estimate the number of drinks they have in an *average* week. The frequencies and quantities were transformed into estimated number of grams/week. In analyses, the categories will include: No consumption of alcohol in the past year, >0-14 grams/week, 15-74 grams/week, 75-140 grams/week, 141 or greater grams/week. They were also asked if they had ever consumed ≥ 4 drinks/day on a regular basis (3 months or longer), if so, they were considered a heavy drinker.

Smoking history

At each phase, the questionnaire asked whether the participant had ever smoked at least 100 cigarettes in their lifetime. If they responded “no”, they were categorized as a never smoker. If they responded “yes”, additional questions were asked including the number of years they smoked and whether they were still smoking. If they were no longer smoking, they were asked to estimate how many cigarettes they smoked per day *on average* during the time they smoked. If they were still smoking, they were asked how cigarettes per day they currently smoked on average. Participants’ smoking status will be included in analyses with the following categories: never (less than 100 in life), past (≥ 100 in lifetime, but not currently smoking), current (report current smoking).

Cardiovascular disease history

At each phase, participants were asked several questions about their cardiovascular history. These included questions about whether a doctor had ever told them they had angina, a myocardial infarction (MI), a transient ischemic attack (TIA) or a stroke.

Arthritis history

Participants were asked whether a doctor had ever told them they had arthritis.

3.C Cognitive outcome definitions

Cognitive impairment outcome

The MMSE was administered at baseline, 5- and 10-year follow-up exams, as described in the Data Collection section. Cognitive impairment was defined as MMSE score < 24 or reported AD or dementia diagnosis. This outcome is available from 1998-2000, 2003-2005, and 2009-2010.

MCI/Dementia outcome

At the 10-year follow-up exam, additional cognitive tests were added (TMT-A, TMT-B, DSST, VFT, AVLT), as described in the Data Collection section of this dissertation. These additional cognitive test data enabled the creation of a second cognitive outcome (MCI/dementia) based on the revised criteria for MCI¹³ and all-cause dementia², as per the workgroups assembled by the NIA and Alzheimer's Association. The recommended criteria for MCI are 1) a concern regarding a change in cognition; 2) impairment in one or more cognitive domains; 3) preserved independence in function abilities; 4) not demented¹³. In this study, the criteria used to define an MCI-like outcome (hereafter called MCI for brevity) were: subjective concern about memory, objective impairment in at least one domain, and not meeting the criteria for dementia (Figure 1-1). The recommended criteria for dementia require cognitive impairment affecting at least two domains and symptoms interfering with ability to function at work or in other activities². The latter criterion is the major distinction between MCI and dementia. For this project, the criteria used to define a dementia-like outcome (hereafter called dementia) were two impaired domains and a report that memory loss symptoms interfered with daily activities (Figure 1-1).

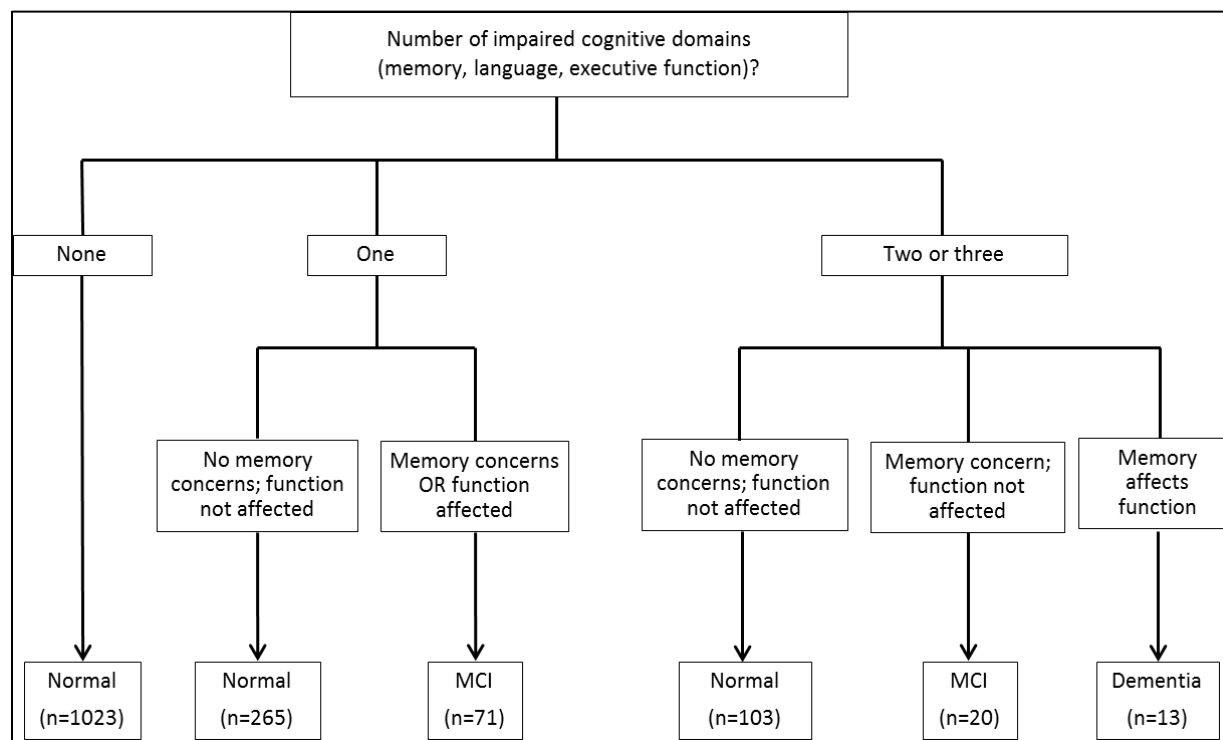


Figure 3-2. Decision tree for the classification of participants as normal, MCI, or dementia. Frequencies are among those with no missing domains, no AD, and no legal representative (n=1497); others were classified based on a modified algorithm if possible, or individual review.

The criteria do not specify how to operationalize the objective impairment criteria. For this dissertation, age-specific cutpoints for impaired performance on each test were derived from the distribution of scores in published normative data (1.5 standard deviations (SD) below the mean; or roughly equivalent, 3rd-5th percentile; see Table 3-1 for cutpoints). Three cognitive domains were measured by the cognitive tests: executive function (DSST, TMT-A, TMT-B), memory (AVLT), and language (VFT). Impairment in executive function was defined as impaired performance on any one of the DSST, TMT-A, or TMT-B. Impaired memory and language were defined as impaired performance on the AVLT and VFT, respectively.

Table 3-1. Age-specific cut-points used to define impaired performance on cognitive tests

	Ages (y)	Impaired performance	Source
AVLT (words)			
	< 66	≤ 4	Ivnik et al. 1992 ³²⁶
	66-71	≤ 3	MOANS
	≥ 72	≤ 2	3 rd -5 th percentile*
VFT (words)			
	< 80	≤ 8.65 (0-8y education) ≤ 16.85 (9-12y educ.) ≤ 23.85 (13-21y educ.)	Tombaugh et al. 1999 ³²⁷ Volunteers & CSHA 1.5 SD below the mean
	≥ 80	≤ 10.1 (0-8y educ.) ≤ 12.7 (9-12y educ.) ≤ 20.2 (13-21y educ.)	
TMT-A (sec)			
	< 66	≥ 56	Ivnik et al. 1996 ³²⁸
	66-68	≥ 58	MOANS
	69-71	≥ 63	3 rd -5 th percentile*
	72-77	≥ 81	
	78-80	≥ 83	
	≥ 81	≥ 84	
TMT-B (sec)			
	< 69	≥ 155	Ivnik et al. 1996 ³²⁸
	69-71	≥ 212	MOANS
	72-74	≥ 225	3 rd -5 th percentile*
	75-80	≥ 237	
	≥ 81	≥ 240	
DSST (symbols)			
	< 66	≤ 31	Ivnik et al. 1992 ³²⁹
	66-68	≤ 28	MOANS
	69-71	≤ 26	3 rd -5 th percentile*
	72-74	≤ 25	
	75-77	≤ 24	
	78-80	≤ 20	
	81-83	≤ 19	
	84-86	≤ 18	
	≥ 87	≤ 17	

*3rd-5th percentile = cut-point based on performance of the bottom 5%. For example, for TMT-A, the 3rd-5th percentile performance corresponded to 56-60 seconds. For TMT, higher scores indicate WORSE performance, the 5th percentile is the better end of this range (56 seconds), so scoring at or above is considered impaired performance; MOANS = Mayo's Older American Normative Studies; CSHA = Canadian Study of Health and Aging

To categorize each participant as normal, MCI, or dementia, the number of impaired domains was combined with subjective report of memory problems, as described in Figure 1-1. For participants who could not be categorized based on these criteria, additional sources of data were reviewed, including MMSE performance, reported AD, need for legal representative, whether a surrogate completed the questionnaire, examiner notes, and scheduling notes. If there was not enough evidence to categorize participants as normal, MCI, or dementia, they were assigned a missing value. Because non-proportional odds precluded the use of ordinal logistic regression with the 3-level outcome, and there were too few dementia cases to separately examine each impaired category versus normal in two binary logistic regression models, those categorized as MCI or dementia were combined into one MCI/dementia group, and compared to those with normal cognition.

3.D Statistical Methods

Table 3-2. Overview of specific aims	
1	To determine the association of markers of systemic inflammation with the risk of cognitive impairment.
2	To determine the association of anti-inflammatory medication use with the risk of cognitive impairment.
3	To determine whether the associations in Aims 1 and 2 are modified by <i>APOE</i> genotype.

All analyses were performed using SAS software. The first time cognitive status was measured in the entire cohort was 1998-2000, and thus this phase is referred to as the “baseline”. Accordingly, other time points are referred to in relation to this baseline exam. For example, “10-year follow-up” refers to the 2009-2010 exam phase.

Means, standard deviations (SD), and percentages were used to characterize the study population. Among those at risk of cognitive impairment at baseline, participant characteristics were compared between those included in analyses and those excluded due to missing exposure or outcome data.

Important potential covariates were identified based on the literature (Table 3-3, below, and Introduction and Literature Review chapter). The relationships of such factors with exposures and outcomes, respectively, were examined. Minimal models included age, sex, and education, and a quadratic term for age if needed. Additional factors were retained in the model for a given association if they were statistically significant ($p < 0.05$) or if they substantially changed the association of interest. A final, parsimonious model was constructed by including all potential confounders and sequentially removing non-significant factors. Some factors removed from the model were re-inserted after other

factors were removed, if there was reason to believe they may have substantially altered the association in the reduced model. Covariate values were obtained from baseline data (1998-2000).

Table 3-3. Summary of key potential covariates and definitions	
Key variables	Definition
Physical activity	Self-reported regular activity: 0 times/week, 1-2 times/week, or ≥ 3 times/week.
Smoker status	Never smokers are those who have not smoked more than 100 cigarettes in their lifetime. Past smokers are those who have smoked more than 100 cigarettes in their lifetime but are not currently smoking. Current smokers are those who report smoking at the time of data collection.
Alcohol consumption	Heavy alcohol consumption: 4 or more drinks per day (ever). Grams of ethanol/week: No consumption of alcohol in the past year, >0-14 grams/week, 15-74 grams/week, 75-140 grams/week, 141 or greater grams/week.
Anthropometry	BMI = weight (kg)/height (m^2)
Cholesterol	Serum non-HDL cholesterol, HDL cholesterol; hypercholesteremia if > 200 mg/dL.
Hypertension	Systolic blood pressure ≥ 140 mmHg, diastolic pressure ≥ 90 mmHg, a self-reported physician diagnosis of hypertension, or the current use of hypertension medications
Diabetes	Reported physician diagnosis, treatment for diabetes, or A1C ≥ 6.5 .
History of CVD	Reported physician diagnosis of myocardial infarction, stroke, or angina.
Stroke	Reported physician diagnosis of stroke.
Arthritis	Reported physician diagnosis of arthritis.
Self-rated health	Excellent, good, or fair/poor.
Mental health	Mental Component Score (MCS) on the SF-36 (higher scores are better mental health).

Quadratic age terms were tested in the models to determine whether there were nonlinear age effects, and retained if their inclusion altered the coefficients for the main exposure variables. In the Cox models, the proportional hazards assumption was tested by including an interaction term for time x the exposure variable. For example, Time x Tertile 2 = 0 and Time x Tertile 3 = 0 were tested together using the test statement in PHREG to obtain the joint p-value for the two terms together. The proportional hazards assumption was considered valid if the joint p-value for the interaction terms had a non-significant p-value ($p > 0.05$).

Aim 1: To determine the association of markers of systemic inflammation with the risk of cognitive impairment.

Marker levels were modeled as categories. This allowed for comparison of results to other studies that modeled inflammation this way, and allowed for further categorization of 10- or 20-year inflammatory patterns (e.g., in the highest category at multiple time points). Levels of hsCRP were categorized based on the CDC/AHA recommended cut-points (low risk < 1.0 mg/L; average risk 1.0 to 3.0 mg/L; and high risk > 3.0 mg/L). Levels of IL-6 and TNF- α were categorized based on tertiles in the at-risk cohort. More specifically, for the analyses with baseline levels only, the cutpoints were determined based on tertiles in the distribution of 1998-2000 marker levels among those at risk of cognitive impairment (for IL-6, tertile 1 $\leq$ 1.187, tertile 2 $\leq$ 2.26; for TNF- α tertile 1 $\leq$ 1.01 and tertile 2 $\leq$ 1.477). Separately, for the 10-year IL-6 exposure patterns (high-high, all other, low-low), the cutpoints were determined based on tertiles in the distribution of 1988-1990 IL-6 levels among those at risk of cognitive impairment, and applied to both the 1988-1990 levels and the 1998-2000 levels (tertile 1 $\leq$ 1.64, tertile 2 $\leq$ 2.64). The reference category was the lowest inflammation category.

Cox regression models were used to model the association between baseline inflammatory markers, or 10-year inflammatory patterns, and the 10-year cumulative incidence of cognitive impairment, among those not cognitively impaired at baseline and with exposure data. Use of the Cox model allowed subjects with incomplete follow-up to be included in these analyses. They were modeled with two time scales: time on study and age. With time on study as the time scale, discrete-time models were used to reflect the data collection process. Because Cox models are semi-parametric, using age as the time scale avoided making any assumptions about the relationship between age and risk.³³⁰ Logistic regression

models were used to estimate the association between inflammation exposure variables and MCI/dementia at the 10-year follow-up, among those without cognitive impairment at baseline.

Outliers in these data were not necessarily suspected of inaccuracy, however, from the perspective of relevance to the hypotheses of this project, where *chronic* inflammation was the exposure of interest, the extreme values were more likely to represent short-term, acute, elevations in inflammation. To gauge the effect of the extreme values on the estimated effects, sensitivity analyses excluded those with hsCRP values >10mg/L, which may indicate acute infection.²³⁴

Additional details of the primary statistical methods for Aim 1 are included in the first manuscript (Chapter 1), and additional details regarding supplementary analyses are included in the appendix with the results of the supplementary analyses.

Aim 2: To determine the association of anti-inflammatory medication use with the risk of cognitive impairment.

The primary analyses for NSAID use are described in detail in the second manuscript (Chapter 5).

Similarly, the primary analyses for statin use are described in detail in the third manuscript (Chapter 6).

Briefly, in preliminary analyses, relevant characteristics were compared between users and nonusers, with chi-square and t-tests used as appropriate to test for statistically significant differences (see manuscripts). These informed evaluation of indication bias as well as confounders. The main analyses examined exposure status at baseline (1998-2000) with subsequent cognitive impairment 5 or 10 years later, or MCI/dementia in 2009-2010. Additional analyses investigated exposure status in 2009-2010 (utilizing data from the previous 20 years) with cognitive impairment in 2009-2010, or MCI/dementia in 2009-2010. Although the latter set of analyses does not establish a temporal relationship and is more likely affected by reverse causality or bias, it provided additional information about the relationships that was useful for interpretation of the overall results in the context of previous studies. Further sensitivity analyses were performed in order to address potential indication bias and to interpret our results in the context of previous study results (see Appendix).

Medication use was operationalized as never, former, or current. In addition, for current users only, duration was approximated based on the first phase since which use had been consistently reported.

Cox regression models were used to examine the associations of baseline medication use with incident cognitive impairment 5 or 10 years later. The main approach reported in the manuscripts did not utilize time-dependent medication use variables, as there was concern that exposure status at the intermediate time point (2003-2005) might not only be a predictor but also a response, potentially

limiting the power to detect associations of baseline medication use with cognitive impairment 5 or 10 years later. Details of the statistical analyses and major results are in the manuscripts (Chapter 4 for NSAIDs; Chapter 5 for statins) and complete results are the in Appendix. Alternative Cox models did include medication use as a time-dependent variable. Another set of Cox models used age as the time scale (additional details of the statistical methods and the results are in the Appendix).

Baseline inflammatory marker levels were added to the medication use models to test for confounding or mediating effects, but had no substantial effect of the medication use associations with cognitive outcomes.

Aim 3: To determine whether the associations in Aims 1 and 2 are modified by APOE genotype.

APOE genotype was operationalized with a dichotomous variable $\epsilon 4$ carrier status, as there were few participants homozygous for the $\epsilon 4$ allele. Participant characteristics were compared between carriers, non-carriers, and those with missing genotype data. To the models examined in Aims 1 and 2, exposure x carrier status interaction terms were added to models to test for significant interactions.

CHAPTER 4

MANUSCRIPT 1: INFLAMMATORY MARKERS AND COGNITIVE IMPAIRMENT

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Key words: older adults, population-based, cognition, dementia, inflammation, epidemiology

Abbreviations:

OR	odds ratio
CI	confidence interval
EHLS	Epidemiology of Hearing Loss Study
BDES	Beaver Dam Eye Study
AD	Alzheimer's disease
CRP	C-reactive protein
IL-6	Interleukin-6
TNF- α	Tumor necrosis factor-alpha
CHS	Cardiovascular Health Study
FHS	Framingham Heart Study
MMSE	Mini-Mental State Examination
ELISA	enzyme-linked immunosorbent assay
CV	coefficient of variation
<i>APOE</i>	Apolipoprotein E gene
BMI	body mass index
SF-36	Short Form Health Survey

Abstract

Background: Evidence suggests inflammation is associated with cognitive impairment, but previous epidemiological studies have reported conflicting results.

Methods: The prospective association of inflammatory marker levels with 10-year incidence of cognitive impairment was examined among participants of the population-based Epidemiology of Hearing Loss Study (EHLS) who were not cognitively impaired in 1998-2000 (n=1947). High-sensitivity C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α) levels from 1998-2000 were measured; CRP and IL-6 levels were also available from 1988-1990 and 2009-2010, enabling further examination of cognitive impairment with consistent elevation or change in inflammatory marker levels during a 20-yr period.

Results: Compared to those with low IL-6 levels in both 1988-1990 and 1998-2000, those with high levels at both time points had marginally significant increased risk of cognitive impairment (hazard ratio (HR) = 2.35; 95% confidence interval (CI) = 0.90, 6.14), adjusted for multiple covariates. Change in IL-6 level between 1988-1990 and 2009-2010 was also associated with increased odds of cognitive impairment (odds ratio = 1.40 per two-fold increase, 95% CI = 1.04, 1.89). Conversely, those with high CRP at both time points had significantly lower risk of cognitive impairment (HR = 0.53; 95% CI = 0.28, 0.99). Baseline CRP levels in the medium range, compared to low, were borderline significantly associated with lower risk of cognitive impairment after adjustment for covariates (HR = 0.66; 95% CI = 0.43, 1.00). Single baseline measures of IL-6 and TNF- α were not associated with cognitive impairment. Results were generally similar for a second outcome based on diagnostic criteria for MCI and dementia.

Conclusion(s): This study was consistent with previous reports of positive associations between IL-6 levels and cognitive impairment. The inverse relationship with CRP conflicts with most but not all previous studies, and may reflect an effect of statin medications or survivor bias. Further studies of long-term inflammation patterns and cognitive impairment are needed to better understand this complex association.

Introduction

Dementia is a significant public health burden. A recent study estimated that 35.6 million people worldwide had dementia in 2010 and this number is expected to double every 20 years, to 115.4 million in 2050.¹ Many of the shared risk factors for cardiovascular disease (CVD) and other chronic conditions are also thought to be associated with dementia.²⁻⁴ There is evidence to suggest that inflammation may be such a shared risk factor.

Relatively slight elevations in levels of inflammatory markers, including C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α), have been independently associated with CVD,^{5,6} diabetes,⁷⁻⁹ cancer,¹⁰ frailty, sarcopenia, disability, and mortality.¹¹⁻¹³ It is well established that inflammation is integrally involved in atherosclerosis,^{14,15} which is one mechanism by which inflammation might also be involved in the development of cognitive impairment. Inflammatory molecules have been observed in the cerebrospinal fluid and amyloid plaques of AD patients.¹⁶ Prior longitudinal epidemiological studies have reported mixed results from analyses of inflammatory marker levels with cognitive impairment.¹⁷⁻²⁶

In the current study, IL-6 and CRP levels measured from three examination phases were used to characterize usual or chronic inflammation exposure status. The aim of the study was to determine whether inflammation, is associated with incident cognitive impairment in a large population-based cohort.

Methods

Study population

The Epidemiology of Hearing Loss Study (EHLS) is a population-based longitudinal study of sensory loss and aging in Beaver Dam, WI (1993-present). In 1987-1988, a private census was conducted

to identify 43 to 84 year old residents of the city or township of Beaver Dam, WI. There were 5924 eligible participants, and in 1988-1990, 4926 (83%) of these participated in the first examination phase of the Beaver Dam Eye Study (BDES).²⁷ Those who participated in the baseline BDES and were alive on March 1, 1993 (n=4541) were eligible for the EHLS, and 3753 (82.6%) participated in the first EHLS examination phase from 1993-1995.²⁸ The EHLS five-year follow-up examinations were conducted from 1998-2000 and, of the 3407 EHLS participants alive as of March 1998, 2800 (82.2%) participated.²⁹ From 2003-2005, 2395 (82.5% of survivors) participated in the 10-year follow-up examination phase.³⁰ In 2009-2010, of the 2243 eligible participants alive when examinations began, 1812 participated (80.8%), 318 refused, 112 died without participating, and 1 was lost to follow-up.

Cognition was first measured for all participants at the EHLS 5-year examination in 1998-2000, thus that phase will be considered the 'baseline' in these analyses. Accordingly, other time points are referred to in relation to this baseline exam (e.g., '10-year follow-up' refers to the 2009-2010 exam phase). Participants without cognitive impairment at baseline who had inflammatory marker data and follow-up for cognitive impairment were included in the analyses of inflammation and cognitive impairment (n=1947). This study was approved by the Health Sciences Institutional Review Board of the University of Wisconsin and informed consent was obtained from each participant.

Outcome

The Mini-Mental State Examination (MMSE) was administered at the baseline, and at the 5- and 10-year follow-up examinations.³¹ The MMSE is a brief test of general cognitive function, often used as a screening device for cognitive impairment.³² Cognitive impairment was defined as a MMSE score less than 24 (out of 30) or a self- or proxy-reported diagnosis of AD or dementia.

In 2009-2010, additional cognitive tests were administered: Trail Making Test, parts A and B, Digit Symbol Substitution Test, Verbal Fluency Test, and the Auditory Verbal Learning Test, as previously described for the EHLS cohort.³³ Two new questionnaire items regarding memory were also added: “Have you, your family, or your physician ever expressed concerns about your memory?” and “Do (your) memory loss symptoms interfere with your ability to do your own day-to-day activities?” Based on recommended criteria for mild cognitive impairment (MCI)³⁴ and all-cause dementia,³⁵ cognitive test performance and responses to these memory questions were used to categorize participants as having normal cognition, MCI, or dementia. Those categorized as MCI or dementia were combined into one MCI/dementia group, and compared to those with normal cognition.

Inflammatory markers

Inflammatory markers from the baseline (1998-2000) and 10-year follow-up (2009-2010) phases were measured together in 2011 by the Fairview Laboratory at the University of Minnesota from blood samples stored at -80°C. Serum CRP was measured using a high-sensitivity latex particle-enhanced immunoturbidimetric assay kit (Roche Diagnostics, Indianapolis, IN 46250) on a Roche Modular P Chemistry analyzer (Roche Diagnostics). The inter-assay coefficient of variation (CV) for the laboratory was 4.5%. The reference range was 0-5 mg/L. Serum IL-6 was measured using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) QuantiKine High Sensitivity kit (R & D Systems, Minneapolis, MN). The intensity of color, reflecting the amount of IL-6, was measured on a SpectraMax spectrophotometer from Molecular Devices (Sunnyvale, California). For IL-6, the inter-assay CV was 11.7%. Samples from 1998-2000 were assayed for serum TNF- α with the human TNF- α QuantiGlo ELISA assay from R&D systems (Minneapolis, MN), with the amount of TNF- α measured from

the intensity of emitted light on a Packard luminometer (Packard Instrument Company, Meriden, CT, incorporated by Perkin Elmer, Boston, MA) or BioTek plate reader. The inter-assay CV was 13.0%.

Data on inflammatory markers from 10 years prior to baseline (1988-1990) were available from the BDES. CRP was measured for all BDES participants and IL-6 was measured for a sample of participants as part of a substudy.³⁶ Serum CRP was measured using a high-sensitivity latex particle-enhanced immunoturbidimetric assay kit from Kamiya Biomedical Co. (Seattle, Washington) on the Hitachi 911 Automatic Analyzer (Boehringer; Mannheim, Germany). The reference range was 0 to 0.5 mg/dL. The CV was 4.5%. Serum IL-6 from 1988-1990 was measured using the same methods for the IL-6 at the later time points, ELISA Quantikine High Sensitivity kit (R & D Systems, Minneapolis, MN), with a SpectraMax spectrophotometer from Molecular Devices (Sunnyvale, California). The reported CV was 6.5-9.6%.³⁶

Covariates

Unless otherwise noted, covariate data was obtained from the baseline exam phase (1998-2000). Education was self-reported and categorized as: <12 years, 12 years, 13-15 years, or ≥ 16 years. General mental health was measured with the mental component score (MCS) of the Short Form Health Survey (SF-36), in which higher scores indicate better mental health.³⁷ Participants self-rated their overall health as excellent, good, fair, or poor. Participants who reported smoking at least 100 cigarettes in their lifetime were considered current smokers if they were still smoking at the time of their examination, or past smokers if they were not. Never smokers were those who had smoked fewer than 100 cigarettes in their lifetime. Ever heavy alcohol drinking was defined as 4 or more drinks per day, self-reported during any exam phase between 1988 and 2000. Participants self-reported the number of times per week that they engaged in regular activity long enough to work up a sweat. Height and weight

were measured and BMI was calculated by dividing weight (in kilograms) by height (in meters) squared. BMI ≥ 30 kg/m² was considered obese. Among participants of the 5-year follow-up phase (2003-2005), Apolipoprotein E (*APOE*) genotype was measured (Children's Hospital of Philadelphia, Center for Applied Genomics) and available for 73% (n=1773) of the participants at risk of cognitive impairment in 1998-2000. *APOE* $\epsilon 4$ carrier status was defined as the presence of one or more $\epsilon 4$ alleles. History of stroke and history of transient ischemic attack (TIA) were both based on self-reported physician diagnosis. Cardiovascular disease (CVD) was defined as a self-reported physician diagnosis of myocardial infarction, stroke, or angina. Arthritis was also defined as a self-reported physician diagnosis. Diabetes was defined as a self-reported physician diagnosis, treatment for diabetes, or hemoglobin A1C $\geq 6.5\%$. Systolic and diastolic blood pressure were measured based on the Hypertension Detection and Follow-Up Program protocol.³⁸ Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic pressure ≥ 90 mmHg, a self-reported physician diagnosis of hypertension, or the current use of hypertension medications. Participants were asked to bring to the examination all medications used in the past month, both prescriptions and over-the-counter, and examiners inventoried and recorded medication names directly from containers.

Statistical analyses

Statistical analyses were conducted using SAS software (version 9.2; SAS Institute, Inc., Cary, NC). Among the participants at risk of cognitive impairment, participant characteristics at baseline were compared between those included in any analyses (n=1947) and those not included in any analyses (n = 475) because they were missing exposure or outcome data (mostly losses to follow-up due to death). These comparisons used the Cochran-Mantel-Haenszel statistic for general association³⁹ and were age- and sex-adjusted, except for the age and sex comparisons, which were adjusted for sex only and age

only, respectively. The Kaplan-Meier method was used to estimate the 10-year cumulative incidence of cognitive impairment.

Marker levels were modeled primarily as categorical variables to accommodate the skewed data. Levels of IL-6 and TNF- α were categorized based on tertiles in the at-risk cohort. Levels of CRP were categorized based on the Centers for Disease Control/American Heart Association recommended cut-points (low risk < 1.0 mg/L; average risk 1.0 to 3.0 mg/L; and high risk > 3.0 mg/L).⁵ Ten-year exposure patterns were defined as high-high, all other, and low-low (reference), based on 1988-1990 and 1998-2000 marker categories (e.g., for each marker, those in the top tertile or highest category at both time points were categorized as high-high for that marker). Similarly, 20-year exposure was modeled as high-high-high, all other, and low-low-low (reference), based on 1988-1990, 1998-2000, and 2009-2010 marker categories.

Discrete-time Cox regression models were used to model the association between baseline inflammatory marker levels and the 10-year cumulative incidence of cognitive impairment, as well as the association between 10-year inflammation exposure (based on levels in 1988-1990 and 1998-2000) and the cumulative incidence of cognitive impairment during the following 10 years. An event was defined as the first occurrence of cognitive impairment, at either the 5- or 10-year follow-up examinations. Participants who died or failed to participate in subsequent follow-up phases were censored as of the time of the last follow-up phase in which they did participate. There were no significant violations of the proportional hazards assumption. Logistic regression models were used to examine the association between 20-year CRP exposure category and the odds of incident cognitive impairment at the last time point (2009-2010).

The second outcome, MCI/dementia, was only measured in 2009-2010, and all analyses with this outcome used logistic regression. Analyses estimated the associations between the odds of

MCI/dementia in 2009-2010 and each exposure variable: baseline inflammatory marker categories, 10-year CRP and IL-6 categories, and 20-year CRP categories.

Models were minimally adjusted for age, sex, and education. Other potential confounders were tested in the model, and retained in the full multivariate model if they were statistically significant, or if their inclusion substantially altered the inflammatory marker estimates. During the modeling process, within each outcome, similar sets of covariates were retained for each inflammation exposure variable, with discordant variables making little difference. Thus, one standard set of covariates was used in all fully-adjusted models with the cognitive impairment outcome, and similarly, a second standard set of covariates was used for the MCI/dementia models. Age-squared terms were tested in all models, but did not alter the results and were not included in the models presented. Interaction terms were used to test for effect modification by *APOE* ϵ 4 carrier status. Due to the fact that statin use may lower inflammation levels,⁴⁰⁻⁴³ sensitivity analyses excluded those using statins during a given exposure period (e.g., for models with 10-year inflammation categories, based on levels at baseline and 10 years prior, those reporting statin use at baseline, 5, or 10 years prior were excluded from the sensitivity analyses, etc.). Additional sensitivity analyses excluded those with CRP levels greater than 10mg/L, as such elevated levels may be indicative of acute inflammatory response, for example due to acute infection.⁵ Further sensitivity analyses excluded those with reported stroke or TIA history at any phase.

In order to compare results to a previous study,²⁰ secondary analyses modeled the change in CRP and IL-6 levels over the 20-year period as continuous variables using a log (base 2) transformation to accommodate the skewed data. Logistic regression models were used to examine the associations between 20-year change in inflammatory marker levels and the odds of incident cognitive impairment in 2009-2010. The same analyses were repeated with the second outcome, MCI/dementia in 2009-2010.

Because CRP trajectories have been shown to differ considerably among statin users, these 20-year change analyses were repeated among only those participants reporting statin non-use at all 5 phases.

Results

Compared to the analytic sample (n=1947), those at risk of cognitive impairment but not included in any analyses due to missing exposure or outcome data (n=475) were more likely to be older, to have diabetes, hypertension, or CVD, and to self-report worse health. Additionally, they were less likely to exercise weekly or to be obese, and had worse mental health as measured by the SF-36 MCS score (Table 4-1). Among the 1947 in the analytic sample, the 10-year cumulative incidence of cognitive impairment was 10.3%.

Adjusting for age, sex and education (Model 1, Table 4-2) participants with medium levels of CRP at baseline were less likely to develop cognitive impairment than those with low CRP. Adjusted for additional covariates, this association was attenuated and borderline significant (Model 2, Table 4-2). Participants with high CRP in both 1988-1990 and 1998-2000 were significantly less likely to develop cognitive impairment in the subsequent 10 years, compared to those with low CRP at both time points, but they were also less likely to have follow-up cognitive testing (Table 4-3, Model 2). Similar but non-significant results were found when comparing odds of cognitive impairment in 2009-2010 for those with high CRP at all three time points (including 2009-2010), compared to those with low CRP all three times (OR = 0.41; 95% CI = 0.14, 1.22, adjusted for Model 2 covariates). In secondary analyses, cognitive impairment was not associated with change in CRP among the whole sample. However, among those reporting no statin use at each of the 5 exam phases, change in CRP over the 20-year period had a significant positive association with cognitive impairment, adjusted for Model 2 covariates (OR = 1.32

per 2-fold increase, 95% CI = 1.06, 1.65; N=584). Other results did not substantially differ when statin users were excluded.

Baseline IL-6 and TNF- α levels were not associated with the 10-year risk of cognitive impairment (Table 4-2). Although not statistically significant, those with the highest IL-6 levels at both earlier time points (1988-1990 and 1998-2000) were estimated to have more than a 2-fold increased risk of cognitive impairment (Table 4-3). This association was statistically significant among those not using statin medications at baseline or prior phases (HR = 3.35; 95% CI = 1.09, 10.3; n=604 adjusted for standard set of Model 2 covariates). Compared to the 188 excluded from this sensitivity analyses due to statin use or missing data on statin use, these 613 non-users of statins were less likely to have hypertension, high cholesterol, or cardiovascular disease, and were more likely to be never-smokers and self-report their health as “excellent”. In secondary analyses, change in IL-6 over the 20-year period (1988-1990 to 2009-2010) was associated with increased odds of incident cognitive impairment in 2009-2010 (OR = 1.40 per 2-fold increase, 95% CI = 1.04, 1.89; n=560, adjusted for Model 2 covariates). Except where noted, these IL-6 results were generally similar upon exclusion of statin users.

For the other outcome, MCI/dementia, results were largely consistent with those for the cognitive impairment outcome, although the strength and statistical significance of associations differed slightly. When modeled using categories, CRP was inversely associated with MCI/dementia, with the associations statistically non-significant for baseline CRP (Table 4-4), borderline significant for 10-year CRP categories (Table 4-5), and significant for 20-year CRP category (OR = 0.35 for high-high-high vs. low-low-low; 95% CI = 0.15, 0.84; n=1340, adjusted for Model 2 covariates). When 20-year change in CRP was examined with MCI/dementia, results were the same as with the cognitive impairment outcome, the association was null in the whole sample, but stronger and significant among those not using statins (OR = 1.24 per 2-fold increase; 95% CI = 1.03, 1.49; n=585 adjusted for Model 2 covariates).

Mostly positive associations were observed between IL-6 and MCI/dementia, but none were statistically significant (Tables 4-4 and 4-5). Among those not using statins at baseline, 5, or 10 years prior, the association for sustained high IL-6 with MCI/dementia was substantially stronger but did not reach statistical significance (OR = 2.42, high-high vs. low-low; 95% CI = 0.66, 8.98; n=443, adjusted for Model 2 covariates). There was no association between 20-year change in IL-6 and MCI/dementia.

The main results were not considerably different when analyses excluded those participants with CRP levels > 10 mg/L (results not shown). In sensitivity analyses excluding those with stroke or TIA, most associations were generally similar, except that the positive association between sustained high IL-6 and cognitive impairment was substantially weaker (data not shown). Interaction terms for exposure categories by statin use were not significant. No significant interactions were observed between exposure categories and *APOE* ϵ 4 carrier status, although there was limited power to detect such interactions. Among those with genotype data, *APOE* ϵ 4 carrier status did not act as a confounder of the inflammation – cognitive impairment associations.

Discussion

Consistently high CRP levels were associated with lower risk of cognitive impairment over the subsequent 10 years. Conversely, among those not using statins at or before baseline, consistently elevated IL-6 levels were associated with a 3-fold increased risk of cognitive impairment. This IL-6 association was weaker and statistically non-significant among the whole sample. Change in IL-6 over 20 years was positively associated with increased odds of concurrent onset of cognitive impairment, and among statin non-users, a similar positive association was observed for 20-year change in CRP. Consistent results were observed with the MCI/dementia outcome, except 20-year IL-6 change was not associated with MCI/dementia.

The IL-6 results are generally in agreement with previous studies, although few studies have included IL-6 levels measured at multiple times, and they have not examined consistently higher levels compared to consistently lower levels. The CHS All Stars study reported that change in IL-6 level over a 9-year period was associated with a 35% increased risk of cognitive impairment, per 2-fold increase.²⁰ In secondary analyses to compare our results to these from the CHS, 20-year change in IL-6 was associated with a 40% increased odds of cognitive impairment per 2-fold increase in IL-6. A study in Uppsala, Sweden of men born from 1920-1924 had IL-6 measures from ages 70 and 77 years. Longitudinal change was not associated with risk of AD or dementia, but high IL-6 level at age 70 years was associated with increased risk of non-AD dementia (HR = 2.2 for above vs. at/below median, 95% CI = 1.23-3.95).²² Others have also observed null associations between IL-6 measured at a single time point and poor cognitive outcomes,^{23,24,44} as did the current study, although some studies found positive associations.^{18,19,21} Consistently elevated IL-6 levels were associated with subsequent cognitive impairment only among those not using statins at or before baseline. The association was weaker and statistically non-significant among the whole sample. This might suggest that any association of IL-6 with cognitive impairment could be attenuated by statin use, but other explanations also exist, including selection bias, as the non-users of statins appeared generally healthier. For example, low IL-6 levels could be a marker of poor immune function in less healthy participants.

The CRP results are more difficult to interpret. In the primary analyses, consistently high CRP levels were associated with lower risk of cognitive impairment, while secondary analyses of 20-year change in CRP revealed a positive association with greater odds of cognitive impairment among those not using statins. Few studies have reported results for CRP and cognitive impairment using more than one measure of CRP, and they have not compared those with multiple high CRP levels to those with multiple low CRP levels. Instead, they have modeled change in CRP or separately examined CRP level at

each time point. The CHS found that 9-year change in CRP was associated with an 18% increased odds of cognitive impairment per 2-fold increase.²⁰ A Swedish study with CRP measured at ages 70 and 77 years reported that neither CRP level was associated with dementia or AD, and there was no association with 7-year CRP change.²² The current study found that 20-year change in CRP was associated with a 37% increased odds of cognitive impairment per 2-fold increase, among non-users of statin medications. The association was substantially weaker and non-significant among the whole sample, most likely due to the large increase in statin use between baseline and the 10-year follow-up. In this population, from 1998-2000 to 2009-2010, CRP levels decreased on average among statin users, but not among non-users.⁴³ The Framingham Heart Study (FHS) concluded neither baseline CRP levels nor CRP levels from about 10 years prior to baseline were associated with incident AD over the subsequent 10 years.²³ However, in a model adjusted for only age and sex, high CRP at the earlier time point was associated with lower risk of AD (HR = 0.23, 95% CI = 0.06-0.91; n=295), consistent with the inverse association reported in the current study.²³ A more recent study from the FHS examined CRP levels from only one time point and observed an inverse association with all-cause dementia (HR = 0.78 per standard deviation, adjusted for age, BMI, and weight change; 95% CI = 0.65 – 0.93).⁴⁵ Further adjustment for *APOE* ϵ 4 allele, plasma docosahexaenoic acid, and education resulted in attenuation to statistical non-significance (HR = 0.83; 95% CI = 0.67-1.03), but also a notable decrease in sample size, from 805 to 634 participants.⁴⁵ In the MacArthur Study of Successful Aging, CRP levels were not significantly associated with cognitive decline, although it was notable that the estimated associations were almost all inverse for CRP, but positive for IL-6.²⁶ Other prospective studies that investigated CRP levels measured at a single time point have reported positive,^{17,18} null,^{24,25,46} or mixed^{19,44} associations with poor cognitive outcomes.

Several potential mechanisms might explain an association between inflammation and cognitive impairment. It may be that inflammation is associated with dementia through vascular mechanisms. There is a significant overlap in risk factors for dementia and vascular disease, including *APOE* genotype, high cholesterol, diabetes, smoking, physical activity, and obesity.³ Inflammation is associated with cardiovascular¹⁵ and cerebrovascular disease,^{47,48} and both these diseases could contribute to dementia through major acute hypoxia, low-level chronic hypoperfusion, or increased inflammation. In the current study, it appeared that a portion of the positive association between sustained higher IL-6 levels and cognitive impairment risk could be explained by stroke or TIA, as the effect was attenuated upon exclusion of those with such history. It is also possible that inflammation is associated with dementia independent of CVD. Accumulating evidence indicates that cytokines play a central and complex role in numerous healthy state cognitive processes on the molecular level, through pathways such as synaptic plasticity, neurogenesis, and neuromodulation.⁴⁹ Cytokines mediate cellular mechanisms involved in cognition such as cholinergic and dopaminergic pathways, and can facilitate neurodegeneration or regeneration.⁵⁰ Although much remains unknown, some evidence indicates that peripheral cytokines may penetrate the blood-brain barrier (BBB), with direct mechanisms of entry through circumventricular regions less protected by the BBB,⁵⁰ or indirectly via stimulation of the vagal nerve.^{51,52}

When inflammation exposure was modeled as consistently high inflammation levels and compared to consistently low levels, the CRP association with cognitive impairment was inverse and the IL-6 association was positive. Several possible explanations exist. First, it is likely that both the IL-6 and CRP associations were underestimated due to hardy survivor bias as there was more loss to follow-up among the high inflammation groups, but it is possible that the underlying CRP association was either null or weaker than the IL-6 association. Few large longitudinal cohort studies have examined both IL-6 and CRP in relation to cognition, but they have generally reported somewhat stronger associations with

IL-6, versus CRP.^{19,20,26} Building on other evidence indicating that CRP may not be a causal factor in coronary heart disease (CHD), a recent large Mendelian randomization meta-analysis concluded that CRP concentration was unlikely to be even a modest causal factor, despite significant positive associations between CRP levels and CHD.⁵³ Conversely, a smaller meta-analysis using Mendelian randomization suggested that IL-6 was a causal factor in coronary artery disease, although given the smaller sample and less available research on IL-6 in general, there is less certainty for this finding.⁵⁴ If CRP is only a marker and IL-6 a true causal risk factor for cardiovascular disease, the same may be true for cognitive impairment, and might partially explain the different results observed for CRP and IL-6 in this study. Second, statin use increased markedly among this cohort during the course of the study, and may have biased the CRP estimates toward the null, more so than the IL-6 associations. Evidence suggests that statin use decreases CRP levels.⁴⁰⁻⁴² A previous study in this cohort characterized the long-term variability in inflammatory markers and observed decreasing CRP levels among statin users only.⁴³ Conversely, IL-6 levels did not differ by statin use.⁴³ Third, some have suggested that high CRP with normal cognition at older ages may characterize a resilient subgroup, although few potential protective factors have been indicated.⁵⁵ However, a recent study among cognitively intact adults 75 years and older observed lower risk of dementia among relatives of those with high CRP, suggesting familial, possibly genetic, factors may contribute to resiliency.⁵⁶

Strengths and limitations of the current study should be considered when interpreting the results. Those included in these analyses were generally healthier than those who were not included due to missing data, as expected in a long-term study of older adults. Thus, these results may only apply to those who are relatively healthier and surviving to older ages. In addition, among those with exposure data, greater proportions were lost from the higher inflammation categories. It is possible that selection bias, such as a hardy survivor bias, may have affected our findings, although it is likely such bias would

lead to underestimation of associations. However, this is a large, prospective, population-based cohort with consistently high participation rates and home visits and nursing home visits were provided in order to minimize losses to follow-up. This population is predominately non-Hispanic white and so results may not be generalizable to minority groups. The cognitive impairment outcome was based on MMSE performance or reported AD diagnosis, rather than diagnostic evaluations. Yet results were largely similar for a second outcome available only in 2009-2010, MCI/dementia, which was based on additional cognitive tests and memory concerns. Due to budget cuts, TNF-alpha was only measured from baseline samples, eliminating the possibility of examining consistently elevated levels with cognitive impairment. However, CRP and IL-6 levels were obtained from 3 time points over a 20-year period, enabling a more specific categorization of long-term exposure.

In conclusion, this study supports existing evidence of an association between IL-6 and cognitive impairment and demonstrated the importance of multiple measures of IL-6 over time. The CRP results may be due to the effects of statin medications, hardy survivor bias, or a true inverse association. Additional prospective population-based studies with multiple inflammatory marker measures are needed to confirm or refute these findings.

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Table 4-1. Participant characteristics in 1998-2000, by inclusion in the current analyses, EHLS

Characteristic	Included (N=1947)	Not included (N=475)	Age- and sex- adjusted p-value [¥]
	N (%)	N (%)	
Male sex	796 (40.9)	194 (40.8)	0.19
Age, y			
48-59	511 (26.3)	56 (11.8)	<0.0001
60-69	734 (37.7)	108 (22.7)	
70-79	541 (27.8)	162 (34.1)	
80-92	161 (8.3)	149 (31.4)	
Education, y			
< 12	269 (13.8)	112 (23.6)	0.13
12	971 (49.9)	226 (47.7)	
13-15	339 (17.4)	76 (16.0)	
≥ 16	368 (18.9)	60 (12.7)	
Obese (BMI ≥ 30)	922 (48.2)	160 (38.2)	0.02
Smoking status			
Never	909 (47.2)	202 (46.4)	0.27
Past	816 (42.4)	195 (44.8)	
Current	201 (10.4)	38 (8.7)	
Ever heavy drinking	455 (23.6)	113 (25.4)	0.09
Exercise ≥ 1/week	851 (44.2)	132 (30.3)	0.003
Diabetes	220 (11.4)	81 (18.6)	0.001
Hypertension	1107 (57.0)	303 (68.1)	0.03
CVD†	251 (13.0)	114 (26.3)	0.001
History of stroke	32 (1.7)	21 (4.7)	0.08
Self-rated health			
Excellent	497 (26.0)	84 (18.6)	<0.0001
Good	1207 (63.1)	264 (58.4)	
Fair	192 (10.0)	96 (21.2)	
Poor	17 (0.9)	8 (1.8)	
NSAID use	889 (46.2)	167 (38.4)	0.07
Aspirin use	325 (16.9)	60 (13.9)	0.70
Statin use	398 (20.7)	70 (16.1)	0.11
APOE ε4-carrier*	245 (14.5)	---	---
	Mean (sd)	Mean (sd)	
SF-36 MCS score	55.6 (7.2)	54.3 (8.5)	0.001

*Note: Percentages are of the number with data for a given covariate and counts may not sum to the total N for the column, due to missing data. ¥ P-values are adjusted for age and sex, except the P-values for sex and age, which are adjusted for age only and sex only, respectively. *APOE genotype data was only available for 82 (17.3%) of those not included in the analyses; for 1691 (86.9%) of those included in the analyses.*

Table 4-3. Cox regression models for the association of prior 10-year pattern of inflammatory marker levels with incident cognitive impairment, EHLS, 1988-2010

[illegible]

Table 4-4. Logistic regression models for the association baseline inflammatory marker categories with MCI/Dementia in 2009-2010

	N at risk	% with follow-up	Cases	% with MCI/Dementia (of those w f/u)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
CRP					N=1490	N=1456
Low	474	68.4	42	13.0	Reference	Reference
Med	904	65.8	65	10.9	0.80 (0.52, 1.23)	0.87 (0.55, 1.38)
High	909	62.9	62	10.8	0.84 (0.54, 1.30)	0.78 (0.48, 1.28)
					P=0.57	P=0.62
IL-6					N=1490	N=1456
Tertile 1	762	77.6	52	8.8	Reference	Reference
Tertile 2	762	66.5	69	13.6	1.37 (0.92, 2.03)	1.42 (0.93, 2.16)
Tertile 3	763	51.5	48	12.2	1.34 (0.87, 2.06)	1.28 (0.80, 2.05)
					P=0.25	P=0.26
TNF-α					N=1489	N=1455
Tertile 1	653	78.6**	63	12.3	Reference	Reference
Tertile 2	643	78.5**	54	10.7	0.80 (0.54, 1.20)	0.76 (0.49, 1.16)
Tertile 3	655	72.1**	51	10.8	0.73 (0.48, 1.10)	0.70 (0.46, 1.08)
					P=0.29	P=0.23

Model 1 covariates: baseline age, sex, and education. Model 2 covariates: Model 1 + baseline smoking status, ever heavy drinking, BMI, hypertension, diabetes, myocardial infarction, SF-36 MCS, and arthritis. **TNF was only measured in those with some follow-up.

Table 4-5. Logistic regression models for the association of 10-year pattern of inflammatory marker levels with MCI/Dementia in 2009-2010

	N at risk	% with follow-up	Cases	% with MCI/Dementia (of those w f/u)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
CRP					N=1486	N=1453
Low-Low	299	72.6	29	13.4	Reference	Reference
All Other	1529	65.8	115	11.4	0.84 (0.53, 1.32)	0.84 (0.51, 1.39)
High-High	452	58.4	25	9.5	0.64 (0.35, 1.16)	0.51 (0.26, 1.01)
					P=0.33	P=0.12
IL-6					N=593	N=578
Low-Low	222	80.2	16	9.0	Reference	Reference
All Other	573	61.3	42	12.0	1.01 (0.52, 1.94)	0.90 (0.44, 1.84)
High-High	164	39.0	9	14.1	1.57 (0.62, 3.98)	1.36 (0.44, 4.27)
					P=0.55	P=0.70
Model 1 includes age at E2, sex, and education. Model 2 includes Model 1 + baseline smoking status, ever heavy drinking, BMI, hypertension, diabetes, myocardial infarction, SF-36 MCS, and arthritis.						

CHAPTER 5

MANUSCRIPT 2: NSAID USE AND COGNITIVE IMPAIRMENT

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Abstract

Background: Non-steroidal anti-inflammatory drugs (NSAIDs) may be associated with cognitive impairment risk. Previous studies have yielded conflicting results.

Objective: To determine the associations of aspirin use and non-aspirin NSAID use with the incidence of cognitive impairment in a population-based cohort with 20 years of prospectively-collected medication data and 10 years of follow-up for cognitive impairment.

Methods: In a population-based cohort, medication use data were prospectively collected from 1988-1990 through 2009-2010. Among 2422 participants free of cognitive impairment in 1998-2000, Cox regression models were used to estimate the associations between NSAID use history and duration in 1998-2000 and incident cognitive impairment 5 or 10 years later. Logistic regression analyses were used to estimate the associations with a second outcome, MCI/dementia, available in 2009-2010. Aspirin use and non-aspirin NSAID use were separately examined.

Results: Participants using aspirin at baseline but not 5 years prior were more likely to develop cognitive impairment over the subsequent 10 years (adjusted hazard ratio (HR) = 1.83; 95% confidence interval (CI) = 1.15, 2.89), with weaker non-significant associations for longer term use. Non-aspirin NSAID use was not associated with the incidence of cognitive impairment or the odds of MCI/dementia.

Conclusions: The results provided no evidence to support a potential protective effect of NSAIDs on cognition. Rather, aspirin use was associated with greater risk of cognitive impairment. Non-aspirin NSAID use was not associated with cognitive impairment.

Introduction

Cognitive impairment is a significant public health burden. In 2010, an estimated 35.6 million people worldwide had dementia and by 2050 the projected prevalence is 115.4 million.¹ A recent panel of experts reported a lack of sufficient high quality evidence to firmly link any modifiable risk factor or potential intervention with Alzheimer's disease (AD) incidence.² Inflammatory processes are involved in neurodegeneration and vascular disease, and could play an important role in the development of cognitive impairment.³ Non-steroidal anti-inflammatory medications (NSAIDs) may protect against dementia due to their anti-inflammatory actions, or other mechanisms, such as anti-amyloidogenic effects of some NSAIDs.⁴

An early observation of low AD prevalence among patients with rheumatoid arthritis supported the potential association of NSAID use with decreased risk of AD.⁵ Randomized controlled trials have found no benefit of non-aspirin NSAIDs on the progression of disease among patients with AD,⁶ and mixed results regarding the prevention of disease among those at risk.⁷⁻⁹ Randomized controlled trials of aspirin use for the prevention of cardiovascular disease have reported no overall cognitive benefits of aspirin use in women only¹⁰ or both sexes.¹¹ Large prospective epidemiological studies have investigated associations of aspirin or non-aspirin NSAID use with various cognitive impairment outcomes, including AD, vascular dementia (VaD), and all-cause dementia and reported mixed results.¹²⁻²¹ Some studies observed substantially different associations for aspirin versus non-aspirin NSAIDs, with reported inverse associations for non-aspirin NSAIDs and null or positive associations for aspirin use.^{12,17,18} Many previous studies had relatively short follow-up periods, and some incorporated only 1 or 2 year lag periods between exposure and outcome measurement, or used concurrent measures. Most previous studies did not separate former users, a group that might have substantially different cognitive impairment risk. These limitations imply greater susceptibility to bias, including prescription bias.

Using 20 years of prospectively collected data from a population-based cohort, the current study investigated the relationship between NSAID use and incident cognitive impairment. Aspirin use and non-aspirin NSAID use were separately examined. With exposure data from multiple time points over 20 years, including from 5 and 10 years prior to baseline, former users could be identified and separately examined. This study also provided up to 10 years of follow-up for the incidence of the main cognitive impairment outcome, allowing for a more biologically plausible lag time between exposure and outcome.

Methods

Study population

A private census was conducted in 1987-1988 to identify all 43 to 84 year old residents of Beaver Dam, WI. In 1988-1990, 4926 (83%) of the 5924 eligible participated in the first examination phase of the Beaver Dam Eye Study (BDES).²² These original BDES participants were eligible for the Epidemiology of Hearing Loss Study (EHLS), a population-based longitudinal study of sensory aging in Beaver Dam, WI. During 1993-1995, the first phase of the EHLS examined 3753 participants (82.6% of the 4541 BDES participants still alive on March 1, 1993).²³ Additional data collection phases in this EHLS cohort took place approximately every 5 years thereafter, in 1998-2000 (n= 2800),²⁴ 2003-2005 (n= 2395),²⁵ and 2009-2010 (n= 1812), with each phase achieving greater than 80% participation rates (not including deceased). BDES examinations took place concurrently with EHLS examinations, and provided some of the data used in this study, including medication data. In 1998-2000, cognition was measured in all participants, and this phase will be considered the 'baseline'. Participants who were free of cognitive impairment at baseline (n=2422) were eligible to be included in these analyses. This study was approved

by the Health Sciences Institutional Review Board of the University of Wisconsin and informed consent was obtained from each participant.

Outcomes

The Mini-Mental State Examination (MMSE) is a brief test of general cognitive function, often used as a screening device for cognitive impairment.²⁶ It was administered at the baseline (1998-2000), and at the 5- and 10-year follow-up examinations.²⁷ Cognitive impairment was defined as a self- or proxy-reported diagnosis of AD or dementia, or a MMSE score less than 24 (out of 30).

Ten years later, at the examination in 2009-2010, additional cognitive tests were added: Trail Making Test, parts A and B (TMT-A; TMT-B), Digit Symbol Substitution Test (DSST), Verbal Fluency Test (VFT), and the Auditory Verbal Learning Test (AVLT). Two questionnaire items on memory problems were also included: “Have you, your family, or your physician ever expressed concerns about your memory?” and “Do (your) memory loss symptoms interfere with your ability to do your own day-to-day activities?” Mild cognitive impairment (MCI) and dementia were defined from performance on these tests and responses to these memory questions, based on the revised criteria for MCI²⁸ and all-cause dementia.²⁹ Participants classified as MCI or dementia were combined into one MCI/dementia category and compared to participants who did not meet the criteria for MCI or dementia.

NSAID medication use

Participants were asked to bring to examinations all medications used in the past month, both prescriptions and over-the-counter, and examiners inventoried and recorded medication names directly from containers. Medications were coded based on active ingredients. Additional data on regular aspirin

use were obtained in the questionnaire using the item: “Do you take aspirin or aspirin products regularly, that is at least twice a week (for more than 3 months)?” Duration of use was not ascertained.

Data on aspirin-containing medications and non-aspirin NSAID-containing medications were available from 5 and 10 years prior to baseline (1988-1990 and 1993-1995), baseline (1998-2000), and 10 years after baseline (2009-2010), from the BDES or the EHLS data collection phases. Aspirin use was defined based on the medication inventory as well as the supplemental question about regular aspirin use. Non-aspirin NSAID use was defined based on the medication inventory at each examination.

Covariates

Covariate data were ascertained at the baseline examination phase (1998-2000). Smoking status was defined based on self-report. Never smokers were those who had smoked fewer than 100 cigarettes in their lifetime. Participants who reported smoking at least 100 cigarettes in their lifetime were considered current or past smokers, depending on whether they were still smoking at the time of their examination. Heavy alcohol consumption was defined as ever consuming 4 or more drinks per day, reported at or before the baseline examination phase. Participants’ self-reported education levels were categorized as: <12 years, 12 years, 13-15 years, or ≥ 16 years. The mental component score (MCS) of the Short Form Health Survey (SF-36) was used to measure general mental health (higher scores indicate better mental health).³⁰ Overall health was self-rated by participants as excellent, good, fair, or poor; poor and fair were combined into one fair/poor category due to relatively few responses of poor health. Participants reported the number of times per week that they engaged in regular activity long enough to work up a sweat; responses were categorized as 0 times/week, 1-2 times/week, and ≥ 3 times/week.

BMI was calculated by dividing measured weight (in kilograms) by measured height (in meters) squared. Self-reported physician diagnoses were used to define a history of stroke. Cardiovascular

disease (CVD) was defined as a self-reported physician diagnosis of angina, myocardial infarction, or stroke. Diabetes was defined as a self-reported physician diagnosis, hemoglobin A1C $\geq 6.5\%$, or diabetes treatment. Arthritis was also defined as a self-reported physician diagnosis. Blood pressure was measured based on the Hypertension Detection and Follow-Up Program protocol.³¹ Hypertension was defined as a self-reported physician diagnosis of hypertension, systolic blood pressure ≥ 140 mmHg, diastolic pressure ≥ 90 mmHg, or the current use of hypertension medications. For those who participated in 2003-2005, Apolipoprotein E (*APOE*) genotype was measured (Children's Hospital of Philadelphia, Center for Applied Genomics), and was available for 1773 (73%) of the 2422 participants at risk of cognitive impairment in 1998-2000. *APOE* $\epsilon 4$ carrier status was defined as the presence of one or more $\epsilon 4$ alleles.

Inflammatory markers from the baseline (1998-2000) examination phase were measured in 2011 (Fairview Laboratory at the University of Minnesota) from blood samples stored at -80°C . Serum CRP was measured using a high-sensitivity latex particle-enhanced immunoturbidimetric assay kit (Roche Diagnostics, Indianapolis, IN 46250) on a Roche Modular P Chemistry analyzer (Roche Diagnostics). The reference range was 0-5 mg/L. The inter-assay coefficient of variation (CV) for the laboratory was 4.5%. Serum IL-6 was measured using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) QuantiKine High Sensitivity kit (R & D Systems, Minneapolis, MN). The intensity of color, reflecting the amount of IL-6, was measured on a SpectraMax spectrophotometer from Molecular Devices (Sunnyvale, California). The inter-assay CV for IL-6 was 11.7%. Serum TNF- α was measured with the human TNF- α QuantiGlo ELISA assay (R&D systems, Minneapolis, MN), with the amount of TNF- α measured from the intensity of emitted light on a Packard luminometer (Packard Instrument Company, Meriden, CT, incorporated by Perkin Elmer, Boston, MA) or BioTek plate reader. The inter-assay CV was 13.0% for TNF- α . Marker levels were modeled as categorical

variables. Levels of IL-6 and TNF- α were categorized based on tertiles in the at-risk cohort. Levels of CRP were categorized based on the Centers for Disease Control/American Heart Association recommended cut-points (low risk < 1.0 mg/L; average risk 1.0 to 3.0 mg/L; and high risk > 3.0 mg/L).³²

Statistical analyses

Aspirin ever users (current or former) at baseline were compared to aspirin never users, and non-aspirin NSAID ever users were compared to non-aspirin NSAID never users. For categorical variables, age- and sex-adjusted p-values were based on Cochran-Mantel-Haenszel statistics for general association; for continuous variables, age- and sex-adjusted p-values were obtained with the LSMEANS statement in PROC GLM.

For the main analyses, baseline aspirin and non-aspirin NSAID exposure, respectively, were categorized as current, former, or never (reference), using data from the baseline and two prior examinations. Baseline non-users were considered never users if they were non-users 5 and 10 years prior to baseline, or considered former users if they were users either 5 or 10 years prior. Although duration of use was not ascertained directly, current users at baseline were further classified according to the first examination since which they have persistently reported use (e.g., “current, not 5y prior” includes participants using at baseline but not 5 years prior, regardless of use 10 years prior; other categories were “current and 5y prior” and “current, 5y and 10y prior”).

To further include exposure data from follow-up, exposure status in 2009-2010 was categorized using data from 2009-2010, baseline, 5 years prior, and 10 years prior (approximately a 20-year period). Never use was defined as non-use at all 4 time points, former use was defined as non-use in 2009-2010 but use at one of the 3 earlier points, and current use was defined as use in 2009-2010. Current users were further categorized based on the first examination phase since which they persistently reported

use, in order to examine potential duration effects (2009-2010, not 10y prior; 10y prior; 15y prior; and 20y prior). For non-aspirin NSAID use, there were relatively few participants who began reporting persistent use at the earlier phases, thus the 10, 15, and 20 year prior groups were collapsed into one category for the duration analyses.

Discrete-time Cox regression models with time on study as the time scale were used to estimate the longitudinal associations of baseline exposure status variables with the incidence of cognitive impairment over the subsequent 10 years. For the MCI/dementia outcome, logistic regression models were used to estimate the association of baseline exposure status variables with the odds of MCI/dementia 10 years later. For both outcomes, logistic regression models were used to estimate the cross-sectional associations of exposure history in 2009-2010 with concurrent onset of cognitive impairment and MCI/dementia, respectively. Never users were the reference group for all analyses. For the duration analyses, former users were excluded.

All models were minimally adjusted for age, age², sex, and education. Each additional potential covariate was tested in the minimally-adjusted never/former/current models. All covariates that were significant at $p \leq 0.25$, or substantially altered the estimated NSAID effects were then included together. Covariates that were not statistically significant ($p < 0.05$) were sequentially removed, beginning with the least significant. As warranted, removed covariates were re-tested in the full model of significant covariates. Based on that process, final models consisted of covariates that substantially altered the association of interest or were significantly associated with the cognitive outcome. In addition to being tested as a potential confounder, *APOE* $\epsilon 4$ carrier status was tested as a potential effect modifier; interaction terms with NSAID exposure variables were tested in each of the never/former/current models.

Results

Compared to aspirin never users in 1998-2000, ever users were older, had a greater proportion of men, had a different distribution of educational attainment, and reported more frequent exercise (Table 5-1). Aspirin ever users also had a greater prevalence of diabetes, myocardial infarction, angina, and statin use at baseline, adjusted for age and sex (Table 5-1). Compared to non-aspirin NSAID never users, non-aspirin NSAID ever users were less likely to be men, were more likely to be past smokers and have high CRP levels, and less likely to rate their health as excellent (Table 5-1). Non-aspirin NSAID ever users also had a greater prevalence of diabetes and arthritis (Table 5-1).

Neither former nor current aspirin use at baseline was significantly associated with incident cognitive impairment during the subsequent 10 years, although positive hazard ratios suggested both former and current use may be associated with increased risk, compared to never use (Table 5-2). In the duration analyses, current aspirin users in 1998-2000 but not 5 years prior were more likely to develop cognitive impairment over the subsequent 10 years; longer term use was not associated with cognitive impairment and the overall p-value was not significant (Table 5-3). Non-aspirin NSAID use status at baseline was not associated with cognitive impairment over the following 10 years (Table 5-2). Null associations were observed in the duration analysis for non-aspirin NSAID use at baseline (data not shown). No significant violation of the proportional hazards assumption was detected in any model.

In the logistic regression analyses of the same exposures, but using MCI/dementia as the outcome, results were generally similar. Former or current aspirin use at baseline was not significantly associated with greater odds of MCI/dementia 10 years later, with point estimates that were closer to null than those with the cognitive impairment outcome (data not shown), although there was still a suggestion of increased risk for those using aspirin at baseline but not 5 years prior (Table 5-4). For non-aspirin NSAID use at baseline, neither former nor current use was associated with the odds of

MCI/dementia (data not shown), but the duration analyses revealed a non-significant reduced odds among long-term users of non-aspirin NSAIDs (used at baseline, 5, and 10 years prior), compared to never users (OR = 0.44; 95% CI = 0.18, 1.06, adjusted for baseline age, age², sex, education, smoking, SF-36 Mental Component score, arthritis, and self-rated health).

When exposure status was updated with medication data from 2009-2010, no significant associations were observed with either outcome, although former aspirin users had moderately increased odds of both outcomes, compared to never users (Table 5-5 and 5-6). In the duration analyses, no significant associations were observed with either outcome, although the pattern of results for the duration of aspirin use in 2009-2010 with cognitive impairment was similar to that for duration of aspirin use in 1998-2000, in that the subgroup of current users who began reporting persistent use at baseline (1998-2000) had the greatest odds of cognitive impairment, compared to never users (OR = 1.72; 95% CI = 0.79, 3.76, adjusted for baseline age, age², sex, education, hypertension, stroke, and self-rated health). Inflammatory marker levels and *APOE* ε4 carrier status were not confounders, and were not retained in any model.

To investigate possible mechanisms or indication bias that might explain the increased risk of cognitive impairment among those using aspirin at baseline but not 5 years prior, post hoc sensitivity analyses examined the association among those without a history of stroke or, separately, any cardiovascular disease. Restricted to those without a history of stroke or transient ischemic attack (TIA) at baseline or during follow-up, the association was slightly attenuated and marginally significant (HR = 1.64; 95% CI = 0.99, 2.71; adjusted for Model 2 covariates, see Table 5-3). Restricted to those without a history of myocardial infarction, angina, or stroke at baseline, the association was somewhat stronger (HR = 2.00; 95% CI = 1.23, 3.24, adjusted for Model 2 covariates).

Discussion

In the current study, there was some evidence that aspirin use may be associated with increased risk of cognitive impairment. Those using aspirin at baseline but not 5 years prior were more likely to develop cognitive impairment over the subsequent 10 years, compared to never users of aspirin. No significant increased risk was observed for those with other patterns of aspirin use, although some point estimates suggested possible increased risk associated with current and former use. Conversely, non-aspirin NSAID use was not significantly associated with cognitive outcomes. In general, the current results do not support a potential protective effect of NSAID use on cognitive impairment.

Aspirin use was significantly associated with increased risk of vascular dementia (VaD) in the Rotterdam Study¹⁷ and the Cardiovascular Health Cognition study,¹⁸ but not significantly associated with AD risk in the Cardiovascular Health study and only marginally associated with AD in the Rotterdam Study, among those using for more than 1 month but less than 2 years (adjusted RR = 1.30; 95% CI = 0.97-1.74).¹⁷ The Kungsholmen Project also reported that aspirin use was associated with increased risk of dementia.²¹ Other large prospective epidemiological studies have reported null^{12,13,15} or inverse associations¹⁹ for aspirin use with cognitive impairment. Randomized controlled trials of aspirin use for the prevention of cardiovascular disease have reported no overall cognitive benefits of aspirin use.^{10,11} Thus, some previous research shows aspirin to be positively associated with VaD but perhaps less associated with AD, which could be due in part to how AD is defined with respect to vascular disease. Our current results were a mix of null and positive associations, perhaps reflecting that our cognitive outcomes likely capture a mix of AD and VaD.

Aspirin use could contribute to increased risk of cognitive impairment through increased risk of hemorrhagic stroke,³³ although in general any such increased risk would likely be offset by simultaneous decreased risk of ischemic stroke.³⁴ Furthermore, baseline stroke history was included as a covariate as

indicated, including in the model that showed significant increased risk for those using aspirin at baseline but not 5 years prior. In that model, stroke was significantly and positively associated with cognitive impairment, but its inclusion did not substantially alter the significant aspirin effect. A sensitivity analysis restricted to those without a history of stroke or TIA at baseline or during follow-up showed a slightly attenuated and marginally significant association. Aspirin use could also be associated with greater risk of microbleeds.³⁵ Microbleeds are thought to be a marker of, and possible contributor to, cerebral small vessel disease.³⁶ Some evidence indicates that microbleeds are associated with cognitive function, even after controlling for other markers of cerebral small vessel disease.³⁷ Conversely, indications for aspirin use may be associated with increased risk of cognitive impairment. For example, aspirin is often used for vascular disease prophylaxis, and cardiovascular disease may be a risk factor for cognitive impairment. In the current study, aspirin ever users were more likely to have a history of myocardial infarction and angina. Although these factors were tested as potential covariates and retained in models as needed, and results were similar in a sensitivity analysis restricted to participants with no history of angina, myocardial infarction or stroke at baseline, residual confounding by indication could explain the observed positive association of aspirin use and cognitive impairment.

The lack of significant association between longer term aspirin use and cognitive impairment is seemingly inconsistent with either a harmful effect of aspirin or indication bias, but upon further consideration could have several explanations. Long-term medication users are enriched with those who tolerate the medication relatively well, compared to those who became former users. Those most susceptible to potential harmful effects of aspirin are less likely to be long-term users, and may not have been included in the at-risk cohort due to prevalent cognitive impairment at baseline or loss to follow-up prior to baseline. There may be important biological mechanisms that are time-dependent. Relatively short-term effects of a medication on certain biological processes would be expected to manifest in

earlier events among susceptible individuals, while longer-term effects may be differently related to cognitive impairment risk. For example, some research on hormone therapy suggests that it might increase early risk of coronary heart disease events in a subset of susceptibles (e.g., due to prothrombotic mechanisms) while possibly decreasing later risk in others (e.g., due to benefits for atherosclerosis).^{38,39} Similar, time-dependent, effects have also been suggested for non-aspirin NSAID to explain unexpected results from a large randomized controlled trial (RCT),⁹ discussed further below.

There were no significant associations of non-aspirin NSAID use with cognitive impairment or MCI/dementia. In contrast to the aspirin results, most point estimates for non-aspirin NSAID use were close to null. This is consistent with the Religious Orders Study, which found baseline non-aspirin NSAID use was not associated with incident AD or AD pathology.¹⁵ In addition, large randomized controlled trials have mostly failed to show beneficial effects of NSAIDs in slowing the progression of AD⁶ or preventing new disease.^{7,8} The primary prevention trial was ended early due to safety concerns, but preliminary results indicated neither celecoxib nor naproxen were protective, and instead suggested increased AD risk,⁷ and worse cognitive function over time⁸ in both treatment groups, versus placebo. However, the most recent analyses found time-dependent effects, with increased AD rates in both treatment arms during the first 2.5 years after randomization, but subsequently no difference in AD risk (celecoxib) or decreased risk (naproxen).⁹ The authors suggest that NSAIDs have a protective effect in healthy brains and an adverse effect in the later stages of AD pathogenesis, but acknowledge that the observed protective effect of naproxen in the later period could be due to selective removal of the most vulnerable in the earlier period.⁹ A large observational study that combined self-reported NSAID use with extensive pharmacy data failed to find any evidence for an inverse association, and instead showed heavy NSAID use was associated with increased risk of dementia.¹⁶ Our study did not examine dosage effects, so it is possible that a harmful high-dose effect was missed.

In contrast, several prospective observational studies have reported inverse associations for non-aspirin NSAID use and cognitive outcomes. The Canadian Study of Health and Aging observed decreased risks, that were attenuated and, for all-cause dementia, no longer significant, upon exclusion of those with cognitive impairment not dementia at baseline.¹³ The Baltimore Longitudinal Study of Aging reported stronger AD risk reduction with ≥ 2 years of use, but it appears that they did not exclude prevalent cases at baseline.¹² A large case-control study using medical record and pharmacy data from the U.S. Veterans Affairs Health Care system reported significant 24% reduced odds of AD for > 4 years of use, with weaker significant associations for shorter term use (2-10%).¹⁴ The Rotterdam Study found reduced AD risk in those using NSAIDs for ≥ 24 months (only 3 exposed AD cases), with null associations for VaD, and for shorter term use.¹⁷ Those results were from time-dependent Cox models that used concurrent exposure and outcome data. In the Cache County Study, any regular lifetime use was not significantly associated with the risk of AD, but more than two years of use, or former use of any duration, were each associated with reduced risk.¹⁹ Importantly, recall bias was possible, as NSAID use prior to baseline was based on recall and follow-up was only 3 years. The Cardiovascular Health Cognition study reported reduced risk of AD only among carriers of the *APOE* $\epsilon 4$ allele.¹⁸ Like Rotterdam, they used time-dependent Cox models with concurrent exposure and outcome data, and state only that there was no evidence of greater risk reduction with lagging. These previous studies are limited by the use of concurrent exposure and outcome data, relatively short follow-up periods, or failure to exclude prevalent cases at baseline. The observed inverse associations for non-aspirin NSAIDs and cognitive impairment could reflect reverse causality, reporting or prescription bias. In fact, a meta-analysis concluded that much of the observed beneficial effect of NSAIDs is likely due to recall, prescription, and publication bias based on their pooled estimates of a 50% risk reduction from studies of prevalent

dementia, 20% reduction for incident dementia studies, and no association overall among studies of cognitive decline.⁴⁰

Our study had prospectively collected data prior to baseline, so baseline non-users could be divided into those who were former or never users during the previous 10 years, groups in which we often observed substantially different incidence rates. Inclusion of former users with referent non-users might explain some of the previously reported inverse associations with NSAID use, as few studies separated former users. In addition, dementia and AD are thought to develop over an extended period, which raises conceptual questions about whether inverse associations observed by studies with 1-2 year lag periods are likely to be causal. Those with cognitive impairment at baseline were excluded from the current study, and in the prospective analyses, outcomes were ascertained 5 or 10 years after exposure, reducing the likelihood that underlying early cognitive decline affected exposure ascertainment.

Mechanisms through which NSAID medications might affect cognitive impairment are complex and not completely understood. A major therapeutic action of NSAIDs is the inhibition of cyclooxygenase (COX), which makes prostaglandins and is central to inflammatory processes.⁴¹ Some evidence from animal studies suggests that NSAIDs reduce microglial activation, reduce glutamate-induced neurotoxicity, and possibly scavenge or inhibit production of free radicals.⁴ Certain NSAIDs may inhibit the formation or deposition of amyloid through COX-independent mechanisms.⁴ Importantly, the role of inflammation in the development of AD and dementia is not clear, for example, whether it is a cause or a response to brain pathology, or whether it may be beneficial or harmful in various contexts or time periods.⁴² Greater knowledge of the links between inflammation and cognitive impairment could improve understanding of conflicting NSAID results and inform future research.

The current study has both limitations and strengths. NSAID-containing medication use was divided into aspirin and non-aspirin NSAID use, but specific NSAID medications were not examined.

Although certain NSAIDs may be differently associated with cognitive impairment, previous studies have failed to find significant differences or consistent patterns for specific NSAIDs.^{14,18} Dosage was not examined. Past month medication use was ascertained approximately every 5 years, and used to approximate duration of use, but direct duration data were not available, and duration may have been misclassified. It is possible that an association with long-term use was undetected. However, with multiple time points over 20 years, it was possible to separate former users from the referent group of never users. Studies which examine current use versus non-use, without separating former and never users, should be interpreted carefully. The cognitive impairment outcome for which longitudinal data was available was based on a single cognitive test (MMSE) and reported physician-diagnosed AD or dementia, and may have misclassified some participants' cognitive status, potentially biasing results towards the null. However, results were generally similar or weaker for a second outcome (MCI/dementia) that was constructed to include milder cognitive impairment as well as dementia, using additional cognitive tests and memory questions administered only in 2009-2010. Residual confounding, particularly by indication, may have been present, although these analyses were adjusted for many possible confounders, including factors associated with NSAID use.

In conclusion, the current analyses suggested that aspirin users may have greater risk of cognitive impairment. Conversely, non-aspirin NSAID use was not associated with cognitive impairment. Investigations of these associations are challenging given that the relevant exposure period and necessary lag period for disease development are not well understood, but are likely to be lengthier than most studies. This study examined medication use over a 20-year period and found no evidence to support a protective effect of NSAID use on cognitive impairment, but rather observed a positive prospective association that could reflect a potential adverse effect of aspirin on cognition.

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Table 5-1. Baseline characteristics by baseline NSAID use, among those at risk of cognitive impairment (n=2422)

Characteristic	Aspirin			Non-aspirin NSAID		
	Never users (N=753)	Ever users (N=1583)	p-value	Never users (N=1212)	Ever users (N=1111)	p-value
	N (%)	N (%)		N (%)	N (%)	
Male sex	257 (34.1)	704 (44.5)	<0.01	571 (47.1)	387 (34.8)	<0.01
Education			0.05			0.84
<12y	105 (14.0)	255 (16.1)		189 (15.6)	167 (15.0)	
12y	404 (53.7)	751 (47.4)		592 (48.9)	555 (50.0)	
13-15	115 (15.3)	284 (17.9)		199 (16.4)	199 (17.9)	
≥16y	128 (17.0)	293 (18.5)		231 (19.1)	190 (17.1)	
Smoking status			0.21			<0.01
Never	374 (49.7)	726 (45.9)		588 (48.5)	505 (45.5)	
Past	290 (38.5)	710 (44.9)		486 (40.1)	509 (45.8)	
Current	89 (11.8)	147 (9.3)		138 (11.4)	97 (8.7)	
Ever heavy drinking	166 (22.1)	383 (24.2)	0.61	298 (24.6)	245 (22.1)	0.36
Exercise			0.03			0.96
0	461 (61.2)	905 (57.2)		697 (57.5)	658 (59.2)	
1-2 times/week	99 (13.2)	219 (13.8)		167 (13.8)	151 (13.6)	
3 times/week	193 (25.6)	459 (29.0)		348 (28.7)	302 (27.2)	
Hypertension	413 (55.0)	962 (60.9)	0.17	715 (59.0)	650 (58.7)	0.88
Diabetes	76 (10.1)	222 (14.0)	0.04	143 (11.8)	154 (13.9)	0.04
MI	22 (2.9)	184 (11.6)	<0.01	123 (10.2)	81 (7.3)	0.12
Angina	22 (2.9)	215 (13.6)	<0.01	133 (11.0)	102 (9.2)	0.59
Stroke	10 (1.3)	43 (2.7)	0.13	33 (2.7)	18 (1.6)	0.07
Statin use	102 (13.6)	363 (22.9)	<0.01	231 (19.1)	226 (20.3)	0.16
Arthritis	332 (44.2)	737 (46.6)	0.75	403 (33.3)	668 (60.2)	<0.01
Self-reported Health			0.22			<0.01
Excellent	143 (19.0)	305 (19.3)		268 (22.2)	180 (16.2)	
Good	512 (68.0)	1019 (64.5)		780 (64.5)	743 (66.9)	
Fair/Poor	98 (13.0)	256 (16.2)		162 (13.4)	187 (16.9)	
C-reactive protein			0.73			0.04
Low	140 (19.7)	327 (21.6)		263 (22.6)	200 (19.0)	
Medium	273 (38.3)	607 (40.1)		471 (40.5)	404 (38.4)	
High	299 (42.0)	581 (38.4)		429 (36.9)	449 (42.6)	
Interleukin-6			0.29			0.16
Tertile 1	232 (32.6)	520 (34.3)		409 (35.2)	338 (32.1)	
Tertile 2	246 (34.6)	496 (32.7)		389 (33.5)	354 (33.6)	
Tertile 3	234 (32.9)	499 (32.9)		365 (31.4)	361 (34.3)	
Tumor necrosis factor- α			0.62			0.54
Tertile 1	220 (35.4)	420 (32.5)		341 (34.4)	298 (32.5)	
Tertile 2	194 (31.2)	435 (33.7)		325 (32.8)	299 (32.6)	
Tertile 3	207 (33.3)	436 (33.8)		325 (32.8)	319 (34.8)	
	Mean (sd)	Mean (sd)		Mean (sd)	Mean (sd)	
Age	66.6 (9.0)	69.0 (9.4)	<0.01	68.5 (9.2)	68.0 (9.4)	0.05
BMI	30.2 (6.3)	29.8 (5.7)	0.36	29.2 (5.6)	30.9 (6.1)	<0.01
Non HDL cholesterol	164.0 (41.4)	161.4 (38.9)	0.51	160.7 (39.3)	163.7 (39.8)	0.25

Table 5-1 (continued). Baseline characteristics by baseline NSAID use, among those at risk of cognitive impairment (n=2422)

Characteristic	Aspirin			Non-aspirin NSAID		
	Never users (N=753)	Ever users (N=1583)	p-value	Never users (N=1212)	Ever users (N=1111)	p-value
	Mean (sd)	Mean (sd)		Mean (sd)	Mean (sd)	
HDL cholesterol	52.1 (17.6)	50.2 (16.5)	0.55	50.5 (16.9)	51.0 (16.9)	0.10
Cholesterol	216.3 (40.6)	211.2 (39.3)	0.20	211.2 (39.2)	214.3 (39.9)	0.82
SF-36 Mental Score	55.2 (7.8)	55.4 (7.4)	0.51	55.5 (7.2)	55.2 (7.8)	0.60

* Note: Age- and sex-adjusted p-values for categorical variables are based on Cochran-Mantel-Haenszel statistics for general association, for continuous variables, from LSMeans in PROC GLM. Percentages are of those with non-missing data, and thus may not equal the number with the characteristic divided by the column total.

Table 5-2. Cox proportional hazards models for the association of baseline NSAID use with 10-year incidence of cognitive impairment

	At risk (n)	Follow-up (%)	Cases (n)	Cumulative incidence (%)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
					N=1995	N=1982
Aspirin						
Never user	753	86.5	42	7.13	Reference	Reference
Former user	333	82.3	30	12.67	1.46 (0.88, 2.43)	1.46 (0.87, 2.44)
Current user	1250	85.7	109	11.65	1.36 (0.93, 1.99)	1.43 (0.96, 2.12)
					P=0.22	P=0.18
Non-aspirin NSAID					N=1987	N=1973
Never user	1212	84.6	97	10.77	Reference	Reference
Former user	337	83.1	27	10.84	1.09 (0.69, 1.72)	1.01 (0.62, 1.64)
Current user	774	88.2	56	9.19	0.97 (0.68, 1.38)	0.92 (0.63, 1.36)
					P=0.90	P=0.90

*Model 1 adjusted for baseline age, age², sex, and education. All Model 2s are further adjusted for baseline smoking status, ever heavy drinking, SF-36 Mental Component score, and self-rated health. Additional Model 2 covariates depended on exposure as follows. Aspirin: myocardial infarction and stroke; Non-aspirin NSAID: stroke and arthritis.

Table 5-3. Cox proportional hazards model for the association of duration of aspirin-containing medication use in 1998-2000 with cognitive impairment during the subsequent 10 years

	At risk (n)	Follow-up (%)	Cases (n)	Cumulative incidence (%)	Model 1	Model 2
					HR (95% CI)	HR (95% CI)
Duration of aspirin use					N=1699	N=1689
Never user	753	86.5	42	7.13	Reference	Reference
Current, not 5y prior	486	87.2	48	12.60	1.67 (1.07, 2.62)	1.83 (1.15, 2.89)
Current and 5y prior	440	85.2	33	10.36	1.18 (0.72, 1.92)	1.30 (0.79, 2.15)
Current, 5y and 10y prior	294	85.0	26	11.91	1.25 (0.73, 2.12)	1.16 (0.66, 2.04)
					P=0.15	P=0.07

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 also includes baseline smoking status, ever heavy drinking, myocardial infarction, stroke, SF-36 Mental Component score, self-rated health.

Table 5-4. Logistic regression model for the association of duration of aspirin-containing medication use in 1998-2000 with MCI/dementia in 2009-2010, among those at risk of cognitive impairment in 1998-2000.

	At risk (n)	Follow-up (%)	Cases (n)	MCI/Dementia (%)	Model 1	Model 2
					OR (95% CI)	OR (95% CI)
Duration of use					N=1308	N=1301
Never user	753	68.7	48	9.28	Reference	Reference
Current, not 5y prior	486	70.0	46	13.53	1.26 (0.80, 1.99)	1.38 (0.86, 2.21)
Current and 5y prior	440	64.3	32	11.31	1.04 (0.63, 1.71)	1.08 (0.65, 1.82)
Current, 5y and 10y prior	294	57.5	23	13.61	1.13 (0.64, 1.99)	1.04 (0.57, 1.88)
					P=0.77	P=0.57

*Model 1 adjusted for baseline age, age²(ns), sex, and education. Model 2 is further adjusted for baseline smoking status, SF-36 Mental Component score, arthritis, and self-rated health.

Table 5-5. Associations between NSAID use history in 2009-2010 and cognitive impairment in 2009-2010, among those at risk of cognitive impairment at baseline.

	N	Incident cases (n)	Cognitive impairment (%)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Aspirin				N=1511	N=1490
Never users	271	13	4.80	Reference	Reference
Former users	314	30	9.55	1.76 (0.87, 3.60)	1.64 (0.79, 3.40)
Current users	927	61	6.58	1.11 (0.58, 2.12)	1.05 (0.54, 2.05)
				P=0.13	P=0.20
Non-aspirin NSAID				N=1496	N=1479
Never users	675	46	6.81	Reference	Reference
Former users	505	34	6.73	1.06 (0.65, 1.72)	0.89 (0.52, 1.50)
Current users	317	20	6.31	1.31 (0.73, 2.34)	1.09 (0.59, 2.03)
				P=0.66	P=0.80

Notes: Model 1 adjusted for baseline age, age², sex, and education. Model 2s are further adjusted for baseline hypertension and self-rated health, stroke (aspirin only), and arthritis (non-aspirin NSAID only). N is those with exposure data and cognitive follow-up. There were relatively few that had exposure data and no cognitive f/u, so N at risk and % f/u not included in the table.

Table 5-6. Associations between NSAID use history in 2009-2010 and MCI/dementia in 2009-2010, among those at risk of cognitive impairment at baseline.

	N	Incident cases (n)	MCI/Dementia (%)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Aspirin				N=1516	N=1489
Never users	272	24	8.82	Reference	Reference
Former users	314	46	14.65	1.49 (0.87, 2.57)	1.54 (0.89, 2.69)
Current users	931	97	10.42	1.00 (0.61, 1.62)	1.02 (0.62, 1.68)
				P=0.11	P=0.11
Non-aspirin NSAID				N=1501	N=1479
Never users	680	67	9.85	Reference	Reference
Former users	504	66	13.10	1.43 (0.98, 2.09)	1.10 (0.73, 1.66)
Current users	318	30	9.43	1.18 (0.73, 1.88)	0.84 (0.51, 1.40)
				P=0.18	P=0.58

Notes: Model 1 adjusted for baseline age, age², sex, and education. Model 2 is further adjusted for baseline SF-36 Mental Component score, arthritis, self-rated health (non-aspirin NSAID only), and ever heavy drinking (non-aspirin NSAID only). N is those with exposure data and cognitive follow-up. There were relatively few that had exposure data and no cognitive f/u, so N at risk and % f/u not included in the table.

CHAPTER 6

MANUSCRIPT 3: STATIN MEDICATION USE AND COGNITIVE IMPAIRMENT

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Abbreviations

AD	Alzheimer's disease
<i>APOE</i>	Apolipoprotein E gene
BDES	Beaver Dam Eye Study
BMI	Body mass index
CI	Confidence interval
CRP	C-reactive protein
EHLS	Epidemiology of Hearing Loss Study
HDL-C	High-density lipoprotein cholesterol
HR	Hazard ratio
IL-6	Interleukin-6
MCI	Mild cognitive impairment
MI	Myocardial infarction
MMSE	Mini-Mental State Examination
NSAID	Non-steroidal anti-inflammatory drug
OR	Odds ratio
RCT	Randomized controlled trial
SD	Standard deviation
SF-36 MCS	Short Form Health Survey mental component score
TC	Total cholesterol
TNF- α	Tumor necrosis factor- α
VaD	Vascular dementia

Abstract

Background: Statin use has been proposed to prevent cognitive impairment, but previous studies have yielded conflicting results.

Objective: To determine the association of statin use with the incidence of cognitive impairment in a population-based cohort with 20 years of prospectively collected medication data and 10 years of follow-up for cognitive impairment.

Methods: In a population-based cohort, medication use data were prospectively collected from 1988-1990 through 2009-2010. Among 2422 participants free of cognitive impairment in 1998-2000, Cox regression models were used to estimate the associations between statin use history and duration in 1998-2000 and incident cognitive impairment 5 or 10 years later. Logistic regression analyses were used to estimate the associations with a second outcome, MCI/dementia, available in 2009-2010. Additional logistic regression analyses further considered statin use during the entire 20-year period in estimating the association of statin use with each outcome in 2009-2010.

Results: Among women, there was a positive association (current vs. never HR = 1.82; 95% CI = 1.07, 3.09) between current statin use at baseline and incident cognitive impairment. Among men, an inverse association was attenuated and non-significant in the more adjusted model (HR = 0.69; 95% CI = 0.32, 1.52). Those who were cognitively impaired 10 years after baseline were more likely to be former statin users. No significant associations were observed with the MCI/dementia outcome.

Conclusions: Statin use does not appear to protect against cognitive impairment overall, and may be associated with increased risk among women. Increased odds of cognitive impairment among former statin users at follow-up could indicate that those with cognitive impairment are more likely to stop using statins, which might explain some previously reported inverse associations.

Introduction

An estimated 35.6 million people worldwide had dementia in 2010, and the prevalence is projected to increase to 115.4 million by 2050.¹ There is a lack of sufficient high-quality evidence supporting potential interventions to decrease the incidence of Alzheimer's disease (AD), according to an expert panel focusing on lifestyle behaviors, chronic disease risk factors, nutritional supplements and dietary factors.² However, given that many traditional vascular risk factors have been associated with AD risk, and accumulating evidence indicates late-life cognitive impairment is due to a mix of AD pathology and microvascular damage in the brain, control of vascular risk factors could help prevent cognitive impairment.³ Some prospective studies suggest that statin medications might protect against cognitive impairment,⁴⁻⁷ but others report conflicting results.⁸⁻¹⁴

Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase, thereby reducing low density lipoprotein (LDL) cholesterol and triglycerides and increasing high density lipoprotein (HDL) cholesterol. Randomized controlled trials (RCTs) indicate that statins reduce major vascular events and mortality among those at high risk of cardiovascular disease,^{12,13} and one trial observed such effects regardless of initial cholesterol level,¹² suggesting additional, cholesterol-independent mechanisms. Recent studies indicate that pleiotropic effects of statins might include decreased inflammation and oxidative stress, increased atherosclerotic plaque stability, improved endothelial function, and anti-thrombotic effects.¹⁵ Any one of these actions, or a combination, could plausibly explain a potential preventive effect of statins on cognitive impairment.¹⁶ For example, inflammatory processes are involved in neurodegeneration and vascular disease, and could play an important role in the development of cognitive impairment.¹⁷

A previous cross-sectional analysis in this cohort used data from 1998-2000 and found that statin use among 65-97 year old participants was associated with decreased odds of cognitive impairment in unadjusted analyses, but the association was much attenuated and non-significant with adjustment for age.¹⁸ Indication bias was a concern, as statin use was associated with factors that both positively and negatively affect cognition. Using additional longitudinal data collected approximately 5 and 10 years after the 1998-2000 baseline cognitive examinations, we investigated the prospective association of statin use with incident cognitive impairment. Extensive adjustment for confounding factors and assessment of indication bias were possible with the rich data on a variety of health-related factors, including inflammatory marker levels. This population-based cohort also offered a less selected sample than some previous studies, and prospectively collected medication data, including from 5 and 10 years prior to the cognitive baseline, enabled separation of never and former statin users and evaluation of duration effects.

Methods

Study population

During 1987-1988, a private census was conducted in Beaver Dam, WI to identify all 43 to 84 year old residents. The Beaver Dam Eye Study (BDES) began in 1988-1990, with 4926 (83%) of the 5924 eligible participating.¹⁹ Those participants were eligible for the Epidemiology of Hearing Loss Study (EHLS), and 3753 participated in the first phase of the EHLS from 1993-1995 (82.6% of the 4541 BDES participants still alive on March 1, 1993).²⁰ Subsequent EHLS follow-up examinations took place approximately every 5 years, in 1998-2000 (n= 2800),²¹ 2003-2005 (n= 2395),²² and 2009-2010 (n= 1812), with each phase achieving greater than 80% participation rates (not including deceased). BDES examinations took place

concurrently with EHLS examinations, and provided some of the data used in this study, including medication data. For the current study, the 1998-2000 examination phase was considered the baseline, as that was the first time when cognition was measured in all participants. The study was approved by the Health Sciences Institutional Review Board of the University of Wisconsin and informed consent was obtained from each participant.

Outcomes

The Mini-Mental State Examination (MMSE) was administered to participants in 1998-2000, 2003-2005, and 2009-2010.²³ The MMSE is a brief test of general cognitive function and is often used as a screening device for cognitive impairment.²⁴ Participants with an MMSE score less than 24 (out of 30), or with a self- or proxy-reported diagnosis of AD or dementia, were classified as cognitively impaired.

Additional cognitive tests were added at the 10-year follow-up examination in 2009-2010: Trail Making Test, parts A and B, Digit Symbol Substitution Test, Verbal Fluency Test, and the Auditory Verbal Learning Test.²⁵ Two new questionnaire items on memory were also included: “Have you, your family, or your physician ever expressed concerns about your memory?” and “Do (your) memory loss symptoms interfere with your ability to do your own day-to-day activities?” Based on the new recommended criteria for mild cognitive impairment (MCI)²⁶ and all-cause dementia,²⁷ performance on these tests and responses to these memory questions were used to categorize participants as having normal cognition, MCI, or dementia. MCI and dementia were collapsed into one MCI/dementia category and normal cognition was the reference category.

Statin medication use

At each examination phase, participants were asked to bring to the examination all medications used in the past month (prescriptions and over-the-counter). Some of the medication use data were collected by the BDES. Examiners recorded medication names directly from containers to a medication list. Medications were coded based on active ingredients. Duration of use was not directly ascertained.

Covariates

Covariate data were obtained from the baseline examination phase (1998-2000). Participants self-reported smoking status. Those who had smoked fewer than 100 cigarettes in their lifetime were categorized as never smokers. Those who had smoked at least 100 cigarettes in their lifetime were categorized as current smokers if they were still smokers at the time of the examination, or as former smokers if not. Ever heavy alcohol drinking was based on self-report and defined as 4 or more drinks per day, at or before the baseline examination phase. Years of education were self-reported and categorized as: <12 years, 12 years, 13-15 years, or ≥ 16 years. General mental health was measured by the mental component score of the Short Form Health Survey (SF-36 MCS); higher scores indicate better mental health.²⁸ Overall health was self-rated by participants as excellent, good, fair, or poor. Poor and fair were collapsed into one category (fair/poor) due to few responses of poor health. Participants were asked how many times per week they engaged in regular activity long enough to work up a sweat; responses were categorized as 0 times/week, 1-2 times/week, and ≥ 3 times/week.

Height and weight were measured and body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. History of stroke was based on self-reported physician diagnosis. Participants also self-reported on physician diagnoses of angina, myocardial infarction (MI), and arthritis. Diabetes was defined as hemoglobin A1C $\geq 6.5\%$, self-reported physician diagnosis, use of medications

for diabetes. Blood pressure was measured using the Hypertension Detection and Follow-Up Program protocol.²⁹ Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic pressure ≥ 90 mmHg, a self-reported physician diagnosis of hypertension, or the current use of hypertension medications. Both non-fasting serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) levels were measured on a Vitros chemistry analyzer at the Beaver Dam Community Hospital Laboratory, Beaver Dam, WI, using an enzymatic-based test. In order to measure the HDL-C levels, low-density lipoprotein and very-low-density lipoprotein components were precipitated out of samples using dextran sulfate. Non-HDL cholesterol (Non-HDL-C) levels were calculated by subtracting HDL-C from TC. Hypercholesterolemia was defined as a measured baseline cholesterol level ≥ 200 mg/dL. Apolipoprotein E (*APOE*) genotype was measured (Children's Hospital of Philadelphia, Center for Applied Genomics) for participants seen in 2003-2005, and was available for 1773 of the 2422 at risk for cognitive impairment. *APOE* $\epsilon 4$ carriers are those with one or more $\epsilon 4$ allele(s).

Inflammatory markers from the baseline (1998-2000) examination phase were measured (Fairview Laboratory at the University of Minnesota) from blood samples stored at -80°C . Serum interleukin-6 (IL-6) was measured using the quantitative sandwich enzyme technique of the enzyme-linked immunosorbent assay (ELISA) QuantiKine High Sensitivity kit (R & D Systems, Minneapolis, MN). A SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California) was used to measure the intensity of color, reflecting the amount of IL-6. The IL-6 inter-assay CV was 11.7%. Serum C-reactive protein (CRP) was measured using a high-sensitivity latex particle-enhanced immunoturbidimetric assay kit from Roche Diagnostics (Indianapolis, IN 46250) on a Roche Modular P Chemistry analyzer (Roche Diagnostics). The reference range was 0-5 mg/L. The inter-assay coefficient of variation (CV) for the laboratory was 4.5%. Serum tumor necrosis factor- α (TNF- α) was measured with the human TNF- α

QuantiGlo ELISA assay (R&D systems, Minneapolis, MN), with the amount of TNF- α measured from the intensity of emitted light on a luminometer (Packard Instrument Company, Meriden, CT, incorporated by Perkin Elmer, Boston, MA) or BioTek plate reader. The inter-assay CV for TNF- α was 13.0%.

Categorical variables were used to model marker levels. IL-6 and TNF- α categories were defined from tertiles in the at-risk cohort. CRP categories were defined from the Centers for Disease

Control/American Heart Association recommended cut-points (low risk < 1.0 mg/L; average risk 1.0 to 3.0 mg/L; and high risk > 3.0 mg/L).³⁰

Statistical analyses

Baseline statin use was categorized as never, former, or current, using medication data from the baseline examination and the examinations 5 and 10 years prior. Baseline non-users were considered former users if they had been statin users either 5 or 10 years prior to baseline, or considered never users if they were non-users both 5 and 10 years prior to baseline. Although duration of use was not directly measured, current users were further categorized into two subgroups: new users (reported statin use at baseline but not 5 years prior) and continuing users (reported use at baseline and 5 years prior).

To further examine statin use during the follow-up period, statin medication data from all 5 examination phases (1988-1990, 1993-1995, baseline, 2003-2005, 2009-2010) were used to categorize statin use history in 2009-2010. Never use was defined as non-use at all 5 time points, former use was defined as non-use in 2009-2010 with use at one or more of the earlier points, and current use was defined as use in 2009-2010. In order to examine potential duration effects, current users were further categorized based on the first examination phase since which they persistently reported use. Short-term users were

those who began using in 2009-2010 or 5 years prior, and long-term users were those who began use 10, 15, or 20 years prior and have reported use at each phase since.

Characteristics of current statin users were compared to never users at baseline, separately for men and women. For categorical variables, age-adjusted p-values were based on Cochran-Mantel-Haenszel statistics for general association; for continuous variables, age-adjusted p-values were obtained with the LSMEANS statement in PROC GLM.

Prospective associations of baseline exposure status variables with the incidence of cognitive impairment over the subsequent 10 years were examined using discrete-time Cox regression models, among those not cognitively impaired at baseline. Logistic regression models were used to estimate the association of baseline exposure status variables with the odds of MCI/dementia 10 years later. For both outcomes, logistic regression models were used to estimate the cross-sectional associations of exposure history in 2009-2010 with cognitive impairment and MCI/dementia in 2009-2010, respectively, among those not cognitively impaired at baseline. Never users were the reference group for all analyses. For the duration analyses, former users were excluded.

Models were minimally adjusted for age, age², sex, and education. Additional potential covariates were each tested in the minimally-adjusted model. All covariates that were significant at $p \leq 0.25$, or substantially altered the estimated statin beta coefficient were then included together in the model, along with age, age², sex, and education. Beginning with the least significant variable, covariates that were not statistically significant ($p < 0.05$) were sequentially removed. As needed, removed covariates were re-inserted in the full model of significant covariates. Based on that process, final models consisted of covariates that were significantly associated with the cognitive outcome or that substantially altered

the association of interest. Interaction terms for hypercholesterolemia x statin use, *APOE* ε4 carrier x statin use, and sex x statin use were tested in the models.

Results

Factors associated with current statin use, versus never use, at baseline were examined separately by sex (Table 6-1). In both sexes, current statin users were more likely to be past smokers, use non-steroidal anti-inflammatory drugs (NSAIDs), have hypertension, angina, and MI history, but less likely to have high cholesterol; they also had a greater mean BMI, and lower mean total cholesterol and non-HDL cholesterol levels, compared to never users at baseline. Among women only, current users were significantly more likely to have diabetes, were less likely to report their health as excellent, and had lower mean HDL cholesterol levels. Among men only, current users were more likely to report frequent exercise.

Statin use history at baseline (1998-2000) was not prospectively associated with subsequent cognitive impairment over the following 10 years in the whole sample (Table 6-2). However, a statistically significant interaction with sex was observed ($p=0.0004$). Sex-stratified results showed that current use in women was associated with increased risk of impairment in the fully-adjusted model, while current use in men was associated with decreased risk of impairment in the minimally-adjusted model, with attenuated and non-significant reduced risk in the more adjusted model (Table 6-2). There were too few former users ($n=16$) to examine past use, and these 16 were excluded in order to compare current users to never users. Duration of statin use was not associated with cognitive impairment, although the vast majority of current users had reported statin use for the first time at baseline, limiting the ability to examine longer term use.

To further aid in interpretation of the observed sex difference in the prospective association, additional analyses estimated the cross-sectional association between baseline statin use and prevalent cognitive impairment. Among 2497 men and women, the odds ratio for current statin use, versus never use, was 0.82 (95 % CI = 0.52, 1.27), adjusted for age, age², sex, and education. Among 1439 women, the OR was 0.45 (95% CI = 0.19, 1.08), while among 1058 men, the OR was 1.07 (95% CI = 0.63, 1.83).

When statin exposure was classified based on statin history in 2009-2010, former statin use was cross-sectionally associated with increased odds of incident cognitive impairment in 2009-2010, compared to never use (Table 6-3). Current use was not associated with cognitive impairment. Duration of use by 2009-2010 was not associated with cognitive impairment (Table 6-3). To further describe the temporal relationship between statin cessation and onset of cognitive impairment, cognitive impairment at 2003-2005 was examined among the 18 former statin users who were impaired in 2009-2010. Most (n=13) became cognitively impaired during the same interval that they stopped using statins.

Additional analyses with the second cognitive outcome, MCI/dementia, revealed no significant associations, and results that were generally closer to null than those with the cognitive impairment outcome (Tables 6-4 and 6-5). There were few persistent statin users at baseline with MCI/dementia follow-up, limiting our ability to examine duration effects (Table 6-4).

Further adjustment for baseline total cholesterol levels or inflammatory marker categories did not appreciably alter any estimates. Interaction terms for hypercholesterolemia and statin use were non-significant in all models. Interaction terms for *APOE* ϵ 4 carrier and statin use were non-significant in all models. Other than the single significant sex interaction discussed above, no other significant sex interactions were detected.

Discussion

In this large population-based cohort study with 10 years of cognitive follow-up, current statin use at baseline was prospectively associated with increased cognitive impairment risk among women only. Among men, an inverse association was attenuated and no longer significant upon adjustment for additional covariates. In 2009-2010, 10 years after baseline, former users were more likely to be cognitive impaired. Upon further examination, most of these cases occurred during the same interval as statin cessation, so it is unclear which came first, and causality in either direction cannot be ruled out. It is possible that declining cognitive status contributed to the statin cessation, or that statin cessation could have contributed to accelerated cognitive decline, or that other factors, such as comorbid disease or aging, could be influencing both statin cessation and cognitive impairment.

To our knowledge, previous studies have not reported significant sex differences in the association of statin use with cognitive impairment, and we detected no significant sex interactions in the association of baseline statin use with MCI/dementia, or the associations of statin use history in 2009-2010 with either outcome. Yet it is possible that statins could have different effects on cognition by sex, perhaps due to sex differences in pharmacodynamics or metabolic pathways. However, it is also likely that prescribing patterns differ by sex, and we noted some differences by sex in the characteristics associated with statin use at baseline. Among females, statin use was significantly associated with greater diabetes prevalence, lower likelihood of reporting excellent health, and lower mean HDL cholesterol levels. Conversely, among males but not females, current statin users were more likely to report frequent exercise compared to never users, possibly indicating that statin use in men may be accompanied by other good health habits. Thus, the increased risk associated with statin use in women could be due to greater indication bias among women. The inverse association observed among men

could be due to increased hardy survivor bias in men, as men may experience cardiovascular disease at an earlier age than women, and the most vulnerable male statin users may have been less likely to be included in the at-risk cohort due to death or loss to follow-up prior to baseline, or cognitive impairment at baseline. The cross-sectional association of statin use and prevalent cognitive impairment at baseline was further investigated within sex strata, to investigate the possibility that male statin users were already cognitively impaired at baseline and so excluded from the at-risk cohort, leading to an apparent inverse prospective association. However, among men, the cross-sectional association with prevalent impairment was null. Surprisingly, among women, there was a non-significant inverse cross-sectional association. Thus, the sex difference observed in the cross-sectional association was almost opposite of the pattern seen in the prospective association. Among women, a possible interpretation for the inverse cross-sectional association and positive prospective association could be that statin use delayed the onset of cognitive impairment but did not prevent it altogether. Finally, it is possible that the sex interaction is due to chance. Although the Cardiovascular Health Study (CHS) and the Cache County Study each reported no significant interactions by sex,^{8,9} most studies did not report investigating sex interactions.^{4-7,10,11,31-33} In the large Three City Study from France, no significant sex interaction was detected for statin use, but the estimated hazard ratios (HR) for dementia substantially differed by sex: for men the HR was 0.81 (95% CI = 0.53–1.23); for women the HR was 1.20 (95% CI = 0.88–1.64), and a significant sex interaction was detected for fibrate use and a single measure of cognitive decline, with an adverse effect noted only in women.¹⁴ The significant sex interaction detected in the current results should be interpreted with caution pending confirmation from other studies.

In general, the overall results for men and women together are consistent with those from the CHS, which reported a null prospective association of current statin use with dementia (HR = 0.90; 95% CI =

0.61-1.35), but a significant positive association of former statin use (HR = 1.88; 95% CI = 1.05-3.36), compared to never use of statins.⁸ The Cache County study,⁹ the Adult Changes in Thought Study,¹⁰ the Religious Orders Study,¹¹ and the Three-City Study¹⁴ reported that statin use was not prospectively associated with dementia or AD risk, and did not examine former statin use. Results from two large RCTs also indicated that statins do not protect against cognitive impairment¹² or cognitive decline,¹³ despite significant effects of simvastatin or pravastatin, respectively, on their primary outcomes, coronary and cerebral events and vascular disease. Limitations of the trials included less than optimal cognitive assessments, losses to follow-up, and relatively short follow-up periods. A recent Cochrane review considered these two RCTs and observational studies and concluded that there was good evidence that statins are not effective in preventing dementia, at least when given in late-life to individuals at risk of vascular disease.¹⁶ Muangpaisan et al. published a systematic review in 2010 for which they identified 2 meta-analyses, 6 studies of dementia, and 2 of vascular dementia (VaD), and concluded there was not convincing evidence that statin use prevents dementia or VaD and also suggested the protective effect noted in few observational studies could be a result of physicians prescribing statins to healthier patients.³⁴ Yet several prospective observational studies have reported reduced risk of AD or dementia among statin users,⁴⁻⁷ only one of which was included in the systematic review by Muangpaisan et al.^{5,34}

Statin use may not affect cognitive impairment, as the bulk of the evidence for a protective effect is derived from cross-sectional and case-control studies,^{31-33,35,36} which are more susceptible to various forms of bias. Supporting this possibility, three⁸⁻¹⁰ of the five cohort studies that did not observe inverse associations in their prospective analyses demonstrated the ability to do so when they included exposure data from the same time point as the outcome data. Together, those findings imply that people with cognitive impairment may be less likely to use statins, but do not support a role for statins

in preventing cognitive impairment. In the current study, analyses that considered statin use throughout the follow-up period and concurrent with outcomes further supported this interpretation. We separated former and never users, and observed that former users were much more likely to be cognitively impaired than never users. Although not statistically significant, the point estimate for current use was less than 1, in contrast to our prospective analysis, where current use was associated with point estimates greater than 1 (also non-significant). While it is possible that discontinuation of statin therapy might contribute to cognitive impairment, it seems more likely that cessation is a marker for worsening health. Dementia, in particular, may substantially alter the risk-benefit ratio of treatment, given decreased life expectancy and increased difficulty making decisions, adhering to treatment, and reporting potential adverse effects.³⁷ Despite concerns regarding the risks and benefits of statin therapy at older ages, evidence appears to support the use of statins to manage cardiovascular risk in elderly patients at high risk who are otherwise reasonably healthy,³⁸ suggesting that statin use, or particularly initiation of therapy, may be a marker of relatively good health.

Alternatively, several limitations could explain why statin use was not prospectively associated with cognitive impairment risk in our study. Most importantly, statin use was not randomly assigned, and uncontrolled confounding could have overwhelmed a potential protective effect. In our study population, statin use at baseline was associated with some good health habits, such as more frequent exercise and lower odds of current smoking, but also with increased prevalence of comorbid conditions, including diabetes, MI, angina, and hypertension. Vascular risk factors, including smoking, hypertension, diabetes, and high cholesterol at midlife, have been implicated as potential risk factors for dementia.^{39,40} However, we examined all these factors, and others, as potential confounders and adjusted analyses revealed no significant inverse association. We also detected no statistically significant difference in the

association by hypercholesterolemia. Past month medication use was ascertained approximately every 5 years, and used to approximate duration of use, but direct duration data were not available, and duration may have been misclassified. Furthermore, statin use was relatively uncommon in the early years of our study (e.g., 0.5% in 1988-1990, 19.8% in 1998-2000, and 47.4% in 2009-2010, among those at risk of cognitive impairment in 1998-2000) and most of those who developed cognitive impairment were likely older when they initiated statin therapy. It is possible that any protective effect of statin medications may require initiation earlier in life with longer duration of use, but the younger participants, who might have initiated statin use relatively earlier in life, were generally too young to have developed cognitive impairment by 2009-2010. Continued follow-up of this cohort is needed to explore this issue. The cognitive impairment outcome for which longitudinal data was available was based on reported physician-diagnosed AD or dementia, and a single cognitive test (MMSE), and potential misclassification of cognitive status could have biased results towards the null. However, analyses with a second outcome (MCI/dementia) that was based on additional cognitive tests and memory questions, administered only in 2009-2010, revealed no significant associations. The expanded cognitive battery will be re-administered at the next phase of the study, providing an additional 5 years of follow-up for the MCI/dementia outcome.

In this study, statin use was not prospectively associated with the risk of cognitive impairment overall, but among women only, it was associated with increased risk. Additional results suggested that cognitive impairment may be associated with discontinuation of statin therapy. This study adds to the literature indicating that prescription or cessation bias might explain previously reported inverse associations, and highlights the need to separate former users and incorporate sufficient lag time between exposure and outcome measurement. Potential sex differences in the association of statins

with cognitive impairment need to be confirmed in other cohorts. The current results do not support a potential protective effect of statins on cognitive impairment.

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Table 6-1. Baseline characteristics by statin use and sex

	Women			Men		
Characteristic	Never (N=1100)	Current (N=245)	p*	Never (N=735)	Current (N=223)	p*
	N (%)	N (%)		N (%)	N (%)	
Education			0.48			0.82
<12y	167 (15.2)	30 (12.2)		121 (16.5)	35 (15.7)	
12y	566 (51.5)	137 (55.9)		337 (45.9)	97 (43.5)	
13-15	205 (18.7)	49 (20.0)		105 (14.3)	36 (16.1)	
≥16y	161 (14.7)	29 (11.8)		172 (23.4)	55 (24.7)	
Smoking status			<0.01			<0.01
Never	646 (58.7)	133 (54.3)		251 (34.2)	52 (23.3)	
Past	333 (30.3)	100 (40.8)		397 (54.0)	157 (70.4)	
Current	121 (11.0)	12 (4.9)		87 (11.8)	14 (6.3)	
Ever heavy drinking	103 (9.4)	25 (10.2)	0.68	320 (43.5)	93 (41.7)	0.59
Exercise			0.42			0.01
0	708 (64.4)	147 (60.0)		396 (53.9)	95 (42.6)	
1-2 times/week	135 (12.3)	31 (12.7)		113 (15.4)	38 (17.0)	
3 times/week	257 (23.4)	67 (27.4)		226 (30.8)	90 (40.4)	
Cholesterol ≥ 200 mg/dL	802 (73.6)	147 (60.5)	<0.01	417 (57.2)	63 (28.4)	<0.01
Hypertension	637 (58.0)	179 (73.1)	<0.01	390 (53.2)	144 (64.6)	<0.01
Diabetes	103 (9.4)	49 (20.0)	<0.01	99 (13.5)	41 (18.4)	0.07
MI	52 (4.7)	32 (13.1)	<0.01	58 (7.9)	60 (26.9)	<0.01
Angina	61 (5.6)	38 (15.6)	<0.01	62 (8.5)	74 (33.3)	<0.01
Stroke	25 (2.3)	7 (2.9)	0.47	16 (2.2)	2 (0.9)	0.26
NSAID use	759 (69.1)	195 (79.9)	<0.01	504 (68.6)	194 (87.0)	<0.01
Arthritis	536 (48.8)	134 (54.9)	0.17	289 (39.4)	92 (41.3)	0.57
Self-reported Health			<0.01			0.42
Excellent	222 (20.2)	28 (11.5)		146 (19.9)	48 (21.5)	
Good	713 (64.9)	169 (69.3)		491 (66.9)	141 (63.2)	
Fair/Poor	164 (14.9)	47 (19.3)		97 (13.2)	34 (15.3)	
C-reactive protein			0.08			0.99
Low	200 (19.2)	36 (15.6)		171 (24.2)	54 (24.9)	
Medium	378 (36.3)	103 (44.6)		301 (42.5)	91 (41.9)	
High	464 (44.5)	92 (39.8)		236 (33.3)	72 (33.2)	
Interleukin-6			0.74			0.68
Tertile 1	341 (32.7)	74 (32.0)		244 (34.5)	82 (37.8)	
Tertile 2	356 (34.2)	75 (32.5)		237 (33.5)	67 (30.9)	
Tertile 3	345 (33.1)	82 (35.5)		227 (32.1)	68 (31.3)	
Tumor necrosis factor-α			0.20			0.57
Tertile 1	313 (34.7)	60 (29.0)		191 (32.3)	67 (35.1)	
Tertile 2	288 (32.0)	65 (31.4)		212 (35.8)	61 (31.9)	
Tertile 3	300 (33.3)	82 (39.6)		189 (31.9)	63 (33.0)	
	Mean (sd)	Mean (sd)		Mean (sd)	Mean (sd)	
Age	68.9 (9.8)	68.8 (8.5)	0.94	67.3 (9.1)	67.1 (7.8)	0.77
BMI	29.3 (6.3)	31.2 (6.3)	<0.01	30.3 (5.0)	31.1 (5.9)	0.04
Non HDL cholesterol	166.8 (42.7)	157.2 (34.7)	<0.01	161.8 (35.9)	142.9 (30.5)	<0.01
HDL cholesterol	56.7 (17.3)	52.6 (15.3)	<0.01	43.6 (14.4)	42.7 (10.8)	0.38

Table 6-1 (continued). Baseline characteristics by statin use and sex

Characteristic	Women			Men		
	Never (N=1100)	Current (N=245)	p*	Never (N=735)	Current (N=223)	p*
	N (%)	N (%)		N (%)	N (%)	
Cholesterol	223.5 (40.4)	209.6 (33.8)	<0.01	205.3 (35.9)	185.4 (30.8)	<0.01
SF-36 Mental CS	54.8 (8.3)	55.8 (7.5)	0.07	55.6 (6.4)	56.6 (6.2)	0.05

* Note: Age- adjusted p-values for categorical characteristics are based on Cochran-Mantel-Haenszel statistics for general association, p-values for continuous variables are based on LSMeans in PROC GLM. Percentages are of those with non-missing data, and thus may not equal the number with the characteristic divided by the column total.

Table 6-2. Cox proportional hazards models for the association of baseline statin use with the incidence of cognitive impairment

	At risk (n)	Follow-up (%)	Cases (n)	Cumulative incidence (%)	Model 1	Model 2
					HR (95% CI)	HR (95% CI)
Statin status in 1998-2000						
All participants					N=1971	N=1902
Never users	1835	84.7	141	10.2	Reference	Reference
Current users	468	89.1	39	11.0	0.98 (0.67, 1.43)	1.25 (0.82, 1.90)
					P=.90	P=0.30
Women only					N=1164	N=1133
Never users	1100	85.9	76	9.1	Reference	Reference
Current users	245	89.8	30	15.4	1.59 (0.99, 2.53)	1.82 (1.07, 3.09)
					P=0.05	P=0.03
Men only					N=807	N=769
Never users	735	83.0	65	11.8	Reference	Reference
Current users	223	88.3	9	5.7	0.41 (0.20, 0.84)	0.69 (0.32, 1.52)
					P=0.01	P=0.36
Duration of use in 1998-2000						
All participants					N=1959	N=1890
Never users	1835	84.7	141	10.2	Reference	Reference
New users	377	89.4	30	10.4	0.91 (0.60, 1.39)	1.11 (0.70, 1.75)
Continuing users	77	88.3	6	10.4	0.98 (0.41, 2.31)	1.51 (0.62, 3.67)
					P=0.91	P=0.63

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 includes Model 1+ baseline smoking status, ever heavy drinking, HDL cholesterol, non-HDL cholesterol, stroke, SF-36 Mental Component Score, self-rated health, MI, and angina. There were only 16 "former" users in 1998-2000, excluded from analyses due to small sample. **The sex x statin status interaction term was significant at p = 0.004.** No sex interaction was tested in the duration model, given small sample size for the persistent users.

Table 6-3. Association of statin use history in 2009-2010 with cognitive impairment in 2009-2010

	N	Incident cases (n)	Cognitive impairment (%)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Statin status in 2009-2010				N=1462	N=1449
Never users	643	43	6.7	Reference	Reference
Former users	99	18	18.2	2.43 (1.27, 4.66)	2.46 (1.27, 4.77)
Current users	721	40	5.6	0.92 (0.57, 1.48)	0.76 (0.46, 1.26)
				P=0.01	P=0.003
Duration of use in 2009-2010				N=1322	N=1320
Never users	643	43	6.7	Reference	Reference
Short-term users	449	19	4.2	0.73 (0.41, 1.30)	0.62 (0.34, 1.14)
Long-term users	231	17	7.4	1.11 (0.60, 2.05)	0.89 (0.47, 1.69)
				P=0.44	P=0.31

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 is adjusted for Model 1 + stroke, hypertension, and self-rated health.

Table 6-4. Association of statin use history at baseline with MCI/Dementia in 2009-2010

	At risk (n)	Follow-up (%)	Cases (n)	MCI/Dementia (%)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Statin status in 1998-2000					N=1509	N=1500
Never users	1835	64.7	135	11.4	Reference	Reference
Current users	468	68.8	39	12.1	0.95 (0.64, 1.41) P=0.80	0.93 (0.60, 1.42) P=0.73
Duration of use in 1998-2000					N=1501	N=1492
Never users	1835	64.7	135	11.4	Reference	Reference
New users	377	68.7	34	13.1	1.05 (0.69, 1.60)	1.04 (0.66, 1.64)
Continuing user	77	71.4	3	5.5	0.39 (0.12, 1.30) P=0.29	0.27 (0.06, 1.17) P=0.20

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 is adjusted for Model 1 factors + baseline smoking, MI, self-rated health, SF-36 Mental Component Score, and arthritis.

Table 6-5. Association of statin use history in 2009-2010 with MCI/Dementia in 2009-2010

	N	Cases (n)	MCI/Dementia (%)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Statin status in 2009-2010				N=1465	N=1447
Never users	645	69	10.7	Reference	Reference
Former users	99	17	17.2	1.36 (0.74, 2.49)	1.33 (0.71, 2.49)
Current users	722	72	10.0	0.98 (0.68, 1.42) P=0.57	0.91 (0.62, 1.33) P=0.49
Duration of use in 2009-2010				N=1325	N=1320
Never users	645	69	10.7	Reference	Reference
Short-term users	450	46	10.2	1.07 (0.71, 1.62)	1.03 (0.67, 1.57)
Long-term users	231	22	9.5	0.84 (0.50, 1.42) P=0.69	0.85 (0.49, 1.48) P=0.80

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 is adjusted for Model 1 factors + baseline smoking, diabetes, SF-36 Mental Component Score, and arthritis.

CHAPTER 7

CONCLUSIONS

This research provided some additional supporting evidence for a role of systemic inflammation in the development of cognitive impairment, as sustained high and increasing IL-6 levels were associated with increased risk. If inflammation contributes to cognitive impairment, medications with anti-inflammatory actions would be expected to protect against cognitive impairment. However, there was no support for a potential protective role of NSAIDs or statins, with some results indicating that aspirin users and female statin users are more likely to develop cognitive impairment. Importantly, those observed positive associations could reflect a relationship between the underlying reasons for using the medications and cognitive impairment, rather than an actual effect of the medication.

Nash et al. previously reported that statin users in the EHLS cohort, and to some extent NSAID users, have decreasing CRP levels but no difference in IL-6 levels,³³¹ and another study also reported statin users had lower CRP levels, but no difference in other markers (IL-6, IL-1 β , or TNF- α).³³² Evidence suggests that statin use lowers IL-6-induced CRP production directly in hepatocytes through inhibition of STAT3 phosphorylation, but does not affect the IL-6 receptor complex.³³³ Thus, the lack of statin effect on IL-6 might explain the lack of a protective statin effect on cognitive impairment, as our results indicated that elevated IL-6 was associated with increased risk. Taken together, it appears that inflammation may be involved in cognitive impairment, but the relevant inflammatory mechanisms are likely upstream from CRP and not affected by NSAID or statin use. Clearly, further investigation of these complex relationships is needed at both the basic science and population levels.

Table 7-1. Summary of findings		
Aim 1: Inflammatory Markers		
	IL-6	CRP
Baseline High vs. Low (1998-2000)	Null.	Null with adjustment.
Sustained High High-High vs. Low-Low (1988-1990 to 1998-2000)	All: HR = 2.35 (0.90, 6.14) Statin non-users: 3.35 (1.09, 10.3)	All: HR = 0.53 (0.28, 0.99) Statin non-users: 0.50 (0.24, 1.03)
Increasing Continuous change (1988-1990 to 2009-2010)	All: OR = 1.40 (1.04, 1.89) per 2-fold Statin non-users: 1.55 (0.93, 2.60)	All: OR = 1.06 (0.92, 1.23) Statin non-users: 1.32 (1.06, 1.65)
Aim 2a: NSAID use		
	Aspirin use	Non-aspirin NSAID use
History at baseline (1998-2000 and prior)	Former: HR = 1.46 (0.87, 2.44) Current: HR = 1.43 (0.96, 2.12) Duration: only Current, not 5y prior was significant; HR = 1.83 (1.15, 2.89)	Former: HR = 1.01 (0.62, 1.64) Current: HR = 0.92 (0.63, 1.36) Duration: All null.
History in 2009-2010 (1988-1990 to 2009-2010)	Former: OR = 1.64 (0.79, 3.40) Current: OR = 1.05 (0.54, 2.05) Duration: Varied, none significant	Former: OR = 0.89 (0.52, 1.50) Current: OR = 1.09 (0.59, 2.03) Duration: All null.
Aim 2b: Statin use		
	Statin use	
History at baseline (1998-2000 and prior)	Former: too few to examine (n=16), excluded Current (women): HR = 1.82 (1.07, 3.09) Current (men): HR = 0.69 (0.32, 1.52) Duration (all): New users: HR = 1.11 (0.70, 1.75) Continuing users: HR = 1.51 (0.62, 3.67) Notes: significant sex interaction for current vs. never (p=0.004); for duration, too few continuing users (n=6) to stratify (interaction not tested).	
History in 2009-2010 (1988-1990 to 2009-2010)	Former (all): OR = 2.46 (1.27, 4.77) Current (all): OR = 0.76 (0.46, 1.26) Duration (all): Short-term users: OR = 0.62 (0.34, 1.14) Long-term users: OR = 0.89 (0.47, 1.69) Notes: no significant sex interaction	
* Outcome is cognitive impairment. Results for the MCI/dementia outcome were generally consistent, but not significant. Reference group for medication exposures was never users.		

Aim 1: Inflammatory markers and cognitive impairment

The first aim of this dissertation research was to determine the associations between 3 different markers of systemic inflammation and cognitive impairment. The hypothesis was that higher levels of the inflammatory markers, particularly sustained high levels, would be associated with increased risk of cognitive impairment and increased odds of MCI/dementia. The results showed that sustained high CRP and IL-6 had substantially different prospective associations with incident cognitive impairment and TNF- α , available from one time point only, was not associated with the cognitive outcomes.

The IL-6 results generally supported the hypothesized positive association between inflammation and cognitive impairment. Baseline IL-6 was not associated with cognitive impairment risk, but sustained high IL-6, at baseline and 10 years prior, was associated with more than a 2-fold increased risk. Although the association was not significant in the whole sample, it was significant and stronger among those not using statins. Change in IL-6 over 20 years was also positively associated with increased odds of cognitive impairment, indicating that increasing inflammation could also represent an adverse inflammatory profile for cognition. No significant associations were observed for IL-6 and the MCI/dementia outcome, although consistent positive associations were observed.

Conversely, the primary categorical CRP results did not support the hypothesis. Sustained high CRP levels were associated with lower risk of cognitive impairment over the subsequent 10 years, with similar results upon exclusion of those using statins at or before baseline. However, secondary analyses with a continuous variable for change in CRP over 20 years did partially support the hypothesis for Aim 1. Among those not using statins at any phase, 20-year change in CRP was positively associated with risk

of cognitive impairment; the association was null in the whole at-risk sample. Results were similar for the MCI/dementia outcome.

Inflammation and cognitive impairment: Potential for bias

It is possible that both the IL-6 and CRP associations were underestimated due to hardy survivor bias. There was more loss to follow-up among the higher inflammation categories, and those lost to follow-up were generally less healthy and probably more likely to have developed cognitive impairment. A systematic review of attrition in longitudinal studies among older adults concluded that the two main independent factors predicting attrition were greater age and cognitive impairment.³³⁴ Therefore, associations between risk factors and cognitive impairment may be underestimated for risk factors that are also strongly associated with mortality or loss to follow-up. For example, a previous study suggested that estimated effects of smoking on cognitive decline could be underestimated by as much as 56% to 86%, due to attrition.³³⁵ Like smoking, higher inflammation has also been associated with increased mortality,^{336,337} including in the EHLS cohort (unpublished, Nash dissertation). Thus, the estimated associations between inflammatory marker levels and cognitive impairment are likely to have been underestimated at least to some extent, and perhaps substantially. This is likely to explain the inverse associations observed for CRP. For example, if the underlying association would have otherwise been null, underestimated incidence in the high inflammation category could have resulted in a biased inverse association. Similarly, the IL-6 associations might be underestimates of stronger positive associations.

Yet death is the major reason for loss to follow-up in this cohort, and one could argue that the observed association therefore reflects reality among those who survive long enough to develop cognitive impairment. As discussed in the Literature Review section, previous reports have shown that some risk

factors, such as BMI and cholesterol, when measured in older adults have opposite direction of association with cognitive impairment as when measured in midlife. Those who survive to old age are likely different from those who die at younger ages, particularly those with disease risk factors, who may have some counterbalancing protective factors. Some have suggested that high CRP with normal cognition at older ages may characterize a resilient subgroup,³³⁸ and a recent study among cognitively intact adults 75 years and older observed lower risk of dementia among relatives of those with high CRP, suggesting familial, possibly genetic, factors may contribute to resiliency.³³⁹

Inflammation and cognitive impairment: Different results for CRP and IL-6

Although both are markers of systemic inflammation, CRP and IL-6 had substantially different prospective associations with incident cognitive impairment. Sustained high CRP level was associated with reduced risk, while sustained high IL-6 level was associated with increased risk. There are two likely explanations for this difference.

First, it may be that IL-6 is a better marker for the inflammatory processes that are important in cognitive impairment. Some evidence supports this possibility. Although CRP has been shown to predict CVD, some evidence suggests it is not causally related. A recent large Mendelian randomization meta-analysis concluded that CRP concentration was unlikely to be even a modest causal factor in coronary heart disease (CHD), despite significant positive associations between CRP levels and CHD.³⁴⁰ Conversely, a smaller meta-analysis using Mendelian randomization suggested that IL-6 was a causal factor in coronary artery disease, although given the smaller sample and less available research on IL-6 in general, there is less certainty for this finding.³⁴¹ If CRP is only a marker but IL-6 is a risk factor for cardiovascular disease, it might partially explain the different results observed for CRP and IL-6 in this study. The

underlying relationships that make CRP a good marker for CVD risk might not generalize to cognitive impairment risk. Although inflammatory processes are complex and involve feedback loops, CRP is generally characterized as downstream from IL-6, as CRP is mainly produced in response to cytokines, particularly IL-6.²³⁰ To the extent that CRP is downstream from IL-6, it may be susceptible to factors that could affect CRP levels without affecting inflammation more broadly. For example, as discussed in detail at the beginning of this Conclusions section, statin use appears to reduce CRP levels without impacting IL-6 levels.^{331,333} Other factors may feasibly affect CRP levels without affecting other portions of the inflammatory system, perhaps indicating CRP might not be the best inflammatory marker, at least for some outcomes that are not primarily cardiovascular. Furthermore, there are few large longitudinal cohort studies that have examined both IL-6 and CRP in relation to cognition, but they have generally reported somewhat stronger associations with IL-6, versus CRP.^{271,279,280} There are many mechanisms through which IL-6 could influence the development of cognitive impairment, as IL-6 is remarkable for its pleiotropic effects, including involvement in metabolic and endocrine processes,²²⁵ and was recently suggested to be a pinnacle of the pathological signaling cascade in brain inflammatory processes, along with IL-1, IL-17, and TNF- α .³⁴²

Differential statin effects on CRP and IL-6 could also contribute to the different associations between each marker and cognitive impairment. Statin use increased markedly among this cohort during the course of the study, and may have affected the CRP estimates more than the IL-6 associations, as evidence suggests that statin use decreases CRP levels.^{233,332,343} As mentioned above, a previous study in this cohort characterized the long-term variability in inflammatory markers and observed decreasing CRP levels among statin users only.³³¹ Conversely, IL-6 levels did not differ by statin use.³³¹ To try to address this possibility, sensitivity analyses examined each association after exclusion of participants

using statins during the exposure period. The inverse association for sustained high CRP was unchanged, but the association between 20-year change in CRP level and cognitive impairment was significant and positive among non-users of statins (was null in the whole sample). The remaining inverse association of sustained high CRP with cognitive impairment among non-users of statins does not necessarily imply that the inverse association is not related to the statin-CRP relationship, as a large portion of the sample (statin users) was excluded, leaving a selected and potentially biased subpopulation. Thus, although the substantially different effect of statins on CRP, compared to IL-6, represents an appealing potential explanation for the disparate CRP and IL-6 results, it is largely speculative without a satisfactory method to evaluate it in this observational study.

Inflammation and cognitive impairment: Sustained high CRP vs. increasing CRP

The original aim of this research was to determine whether chronic low-grade inflammation was associated with increased risk of cognitive impairment, so the main analyses compared those who remained in the highest category of inflammation to those who remained in the lowest category. However, a recent report of positive associations for increasing inflammation levels with cognitive impairment²⁸⁰ motivated a set of secondary analyses to determine whether those results could be replicated in the EHLS cohort. These two approaches yielded results that are intuitively conflicting, but may not actually be conflicting, given that they technically address two different questions and were susceptible to different biases. Sustained high CRP level, compared to sustained low level, was associated with decreased risk of cognitive impairment, but increasing CRP level was associated with increased odds of impairment (per 2-fold increase; among statin non-users only). As discussed above, the most likely explanation for the inverse association is an underestimation of a null relationship. It is

plausible that sustained high CRP levels are not associated with cognitive impairment risk 5 or 10 years later, but that CRP levels increase with the development of cognitive impairment.

Importantly, the secondary approach was cross-sectional, with CRP levels increasing concurrently with development of cognitive impairment, and provides less evidence for a causal association. Although there are biologically plausible explanations for why increasing CRP, but not sustained high levels, might be associated with cognitive impairment development, they seem less likely than non-causal explanations. For example, increasing CRP level, more so than sustained high levels, might reflect dysregulation of the immune system, which could contribute to cognitive impairment. However, such an explanation would lead to questions about what led to the dysregulation, and whether those factors might be directly causing cognitive impairment, with increasing CRP levels only correlated. In fact, two good explanations exist, other than a causal effect of increasing inflammation on cognitive impairment. First, although the role of inflammation in the development of cognitive impairment is still unclear, evidence indicates that neurodegeneration stimulates an inflammatory response. The positive cross-sectional association between increasing CRP levels and cognitive impairment among non-users of statins could reflect a systemic inflammatory response to neurodegeneration. Second, increasing age has been associated with increasing CRP levels (among non-users of statins), cognitive impairment, and many other chronic conditions. The cross-sectional association between increasing CRP level and cognitive impairment could reflect that some underlying mechanisms, related to either disease or biological aging, are responsible for both increasing CRP and cognitive impairment.

The comparison of those with sustained high levels to those with sustained low levels provides a contrast between the two extremes of the inflammatory burden spectrum. If a relationship exists between inflammation and cognitive impairment, it was expected to be most apparent in this approach,

and it was for IL-6. Unfortunately, there was also greater loss to follow-up in the sustained high inflammation groups, partially reinforcing the expectation that these participants represented the severe end of the inflammatory spectrum. In the context of likely underestimation due to hardy survivor bias, the estimated association between sustained high CRP and cognitive impairment risk probably reflects a null relationship. Given that there was no clear increased risk among those with sustained high CRP compared to those with sustained low, and there was no temporal lag in the positive cross-sectional association between increasing CRP levels and cognitive impairment odds, it seems most likely that the latter association is due to reverse causation or a non-causal correlation. Additional investigation of the factors associated with sustained high inflammation levels versus increasing levels is certainly needed, as few studies have measured levels at multiple time points. One might hypothesize that sustained high levels are more likely to be partially determined by genetics, so future genetic epidemiologic research is warranted, and discussed in the Future Studies section.

In summary, for both CRP and IL-6, stronger associations with cognitive impairment were observed for sustained high levels measured at two points 10 years apart, compared to categorizing exposure based on a single measurement. Greater losses to follow-up among the higher inflammation categories may have resulted in underestimated associations, relative to what might have been observed with complete follow-up. The CRP results may have been more affected by the substantial increase in statin use over the study period. The observed inverse association for sustained high CRP did not support the hypothesis, but might be explained by several factors, most likely underestimation of an underlying null association. The IL-6 results supported the hypothesis that increased inflammation is associated with increased risk of cognitive impairment. IL-6 may be a better marker of the inflammatory processes involved in cognitive impairment.

Aim 2: Anti-inflammatory medication use and cognitive impairment

The second aim of this dissertation was to determine the associations of anti-inflammatory medication use, specifically NSAID use and statin use, respectively, with the risk of cognitive impairment. The hypothesis was that NSAID use and statin use would each be associated with reduced risk of cognitive impairment. There was no evidence to support this hypothesis. Instead, we observed null associations for non-aspirin NSAID use, a positive association for aspirin use, and a positive association for statin use among women. Importantly, because medication use was not randomly assigned, the associations we observed might have been affected by indication bias, and such bias could have obscured a protective effect. However, given that randomized controlled trials have failed to find convincing protective effects, and observational study results are conflicting, with methodological limitations potentially explaining the previously reported inverse associations, there is a lack of current evidence to support the use of NSAIDs or statins to prevent cognitive impairment.

NSAIDs and cognitive impairment: Different results for aspirin and non-aspirin NSAIDs

Aspirin use was associated with increased risk of cognitive impairment, while non-aspirin NSAID use was not associated. There are a couple potential explanations for the difference. Although all NSAIDs inhibit cyclooxygenase (COX) by blocking access to its active site, aspirin does so in an irreversible manner, which prevents the synthesis of thromboxanes by platelets and results in an anti-clotting effect that makes aspirin useful for cardiovascular disease prevention.³⁴⁴ Thus, aspirin and non-aspirin NSAID have some differences in both biological effects and indications for use, either of which could explain the different associations observed with cognitive impairment.

Aspirin use could contribute to increased risk of cognitive impairment through increased risk of hemorrhagic stroke,³⁴⁵ although in general any such increased risk would likely be offset by simultaneous decreased risk of ischemic stroke.³⁴⁶ Furthermore, the significant positive association between new aspirin use at baseline and cognitive impairment was adjusted for baseline stroke history, because stroke was significantly and positively associated with cognitive impairment, but its inclusion did not substantially alter the significant aspirin effect. Conversely, a sensitivity analysis restricted to those without a history of stroke or TIA at baseline or during follow-up showed a slightly attenuated and marginally significant association, lending some support to a stroke mechanism partially explaining the association. Aspirin use could also be associated with greater risk of microbleeds.³⁴⁷ Microbleeds are thought to be a marker of, and possible contributor to, cerebral small vessel disease.³⁴⁸ Some evidence indicates that microbleeds are associated with cognitive function, even after controlling for other markers of cerebral small vessel disease.³⁴⁹

The other likely explanation for the different results between aspirin and non-aspirin NSAIDs is that indications for aspirin use may be associated with increased risk of cognitive impairment more so than the indications for non-aspirin NSAID use. For example, aspirin is often used for vascular disease prophylaxis, and cardiovascular disease may be a risk factor for cognitive impairment. In the current study, aspirin use was associated with increased prevalence of myocardial infarction and angina history, but non-aspirin NSAID use was not associated with these conditions. Although these factors were tested as potential covariates and retained in models as needed, and results were similar in a sensitivity analysis restricted to participants with no history of angina, myocardial infarction or stroke at baseline, residual confounding by indication could explain the observed positive association of aspirin use and cognitive impairment. Null effects of aspirin on cognitive performance in randomized controlled

trials,^{350,351} which avoid indication bias, support the possibility that our observed positive association is due to indication bias.

Non-aspirin NSAIDs and cognitive impairment: Methodological explanations for reported inverse associations from previous studies

In contrast to the null results observed for non-aspirin NSAID use and cognitive outcomes in the current study, several prospective observational studies have reported inverse associations.^{293,295,298,299,302,352}

These previous studies were limited by relatively short follow-up periods, the use of concurrent exposure and outcome data, or failure to exclude prevalent cases at baseline. Thus, the observed inverse associations for non-aspirin NSAIDs and cognitive impairment could reflect reverse causality, reporting or prescription bias. In fact, a meta-analysis concluded that much of the observed beneficial effect of NSAIDs is likely due to recall, prescription, and publication bias based on their pooled estimates of a 50% risk reduction from studies of prevalent dementia, 20% reduction for incident dementia studies, and no association overall among studies of cognitive decline.²⁸⁸ In addition, despite more recent *post hoc* analyses suggesting naproxen eventually reduces AD risk after an initial increased risk,³⁵³ the main results from a large randomized controlled trial indicated neither celecoxib nor naproxen were protective, and instead suggested increased AD risk,²⁹¹ and worse cognitive function over time²⁹² in both treatment groups, versus placebo.

Statin use and cognitive impairment: Different results by sex

In this dissertation, the prospective association between statin use at baseline and incident cognitive impairment was significantly modified by sex, with a positive association observed among women and a

non-significant association among men. It is possible that statins could have different effects on cognition by sex, perhaps due to sex differences in pharmacodynamics or metabolic pathways. However, this finding could have several explanations, and should be interpreted with caution pending replication in another cohort, as we did not observe significant sex interactions in the other statin models we examined, and two previous statin studies reported no significant interactions by sex,^{310,312} and most studies did not report investigating sex interactions.^{307-309,313,354-358} Another study, the large Three City Study from France, reported no significant sex interaction for statin use, although the estimated hazard ratios (HR) for dementia substantially differed by sex: for men the HR was 0.81 (95% CI = 0.53–1.23); for women the HR was 1.20 (95% CI = 0.88–1.64), and a significant sex interaction was detected for fibrate use and a single measure of cognitive decline, with an adverse effect noted only in women.³⁰⁶

Previous studies have observed that women have lower rates of lipid-lowering therapy use than men, among those with indication for use.³⁵⁹⁻³⁶¹ In the EHLS cohort at risk of cognitive impairment, a gender bias was also noted. Among those with cholesterol ≥ 200 mg/dL who were not using statin medications in 1993-1995, male sex was significantly associated with increased odds of statin use 5 years later (OR = 1.73; 95% CI = 1.34, 2.22, adjusted for age, education, and total cholesterol; n = 1838). Similarly, among those with non-users of statins with hypercholesterolemia in 1998-2000, men were twice as likely as women to be on statins by the next phase in 2003-2005 (adjusted OR = 2.03; 95% CI = 1.51, 2.74; n = 1053). Thus, it appears that statin prescribing patterns differ by sex, and we noted some differences by sex in the characteristics associated with statin use at baseline. Among females only, current statin use at baseline was significantly associated with greater diabetes prevalence, lower likelihood of reporting excellent health, and lower mean HDL cholesterol levels, compared to never use at baseline. Conversely, among males only, current statin users were more likely to report frequent exercise compared to never

users, possibly indicating that statin use in men may be accompanied by other good health habits. Thus, the increased risk associated with statin use in women could be due to greater indication bias among women. The inverse association observed among men could be due to increased hardy survivor bias in men, as men have a shorter life expectancy than women, and the most vulnerable male statin users may have been less likely to be included in the at-risk cohort due to death or loss to follow-up prior to baseline, or cognitive impairment at baseline. Supporting this possibility, additional adjustment for confounders resulted in attenuation and loss of statistical significance for the inverse association among men.

The cross-sectional association of statin use and prevalent cognitive impairment at baseline was further investigated within sex strata to investigate the possibility that male statin users were already cognitively impaired at baseline and so excluded from the at-risk cohort, leading to an apparent inverse prospective association. However, among men, the cross-sectional association with prevalent impairment was null. Surprisingly, among women, there was a non-significant inverse cross-sectional association. A possible interpretation for the inverse cross-sectional association and positive prospective association among women could be that statin use delayed the onset of cognitive impairment but did not prevent it altogether. However, for the reasons above, greater indication bias among women is the most likely explanation for the positive association of statin use with cognitive impairment.

Statins and cognitive impairment: Methodological explanations for reported inverse associations from previous studies

Statin use may not affect cognitive impairment, as several prospective epidemiological studies,^{306,307,310,312,313} and two randomized clinical trials^{362,363} have reported that statin use was not

prospectively associated with cognitive outcomes. The majority of the conflicting evidence, supporting a protective effect, is derived from cross-sectional and case-control studies,^{356-358,364,365} which are more susceptible to various forms of bias. Reinforcing that the cross-sectional results could be due to methodological approach, three^{310,312,313} of the five cohort studies that did not observe inverse associations in their prospective analyses demonstrated the ability to do so when they included exposure data from the same time point as the outcome data. Together, those findings imply that people with cognitive impairment may be less likely to use statins, but do not support a role for statins in preventing cognitive impairment. In the current study, analyses that considered statin use throughout the follow-up period and concurrent with outcomes further supported this interpretation. We separated former and never users, and observed that former users were much more likely to be cognitively impaired than never users. In addition, although not statistically significant, the point estimate for current use versus never use was less than 1, in contrast to our prospective analysis, where current use was associated with point estimates greater than 1 (also non-significant). While it is possible that discontinuation of statin therapy might contribute to cognitive impairment, it seems more likely that cessation is a marker for worsening health. Dementia, in particular, may substantially alter the risk-benefit ratio of treatment, given decreased life expectancy and increased difficulty making decisions, adhering to treatment, and reporting potential adverse effects.³⁶⁶ Despite concerns regarding the risks and benefits of statin therapy at older ages, evidence appears to support the use of statins to manage cardiovascular risk in elderly patients at high risk who are otherwise reasonably health,³⁶⁷ suggesting that statin use, or particularly initiation of therapy, may be a marker of relatively good health.

Aim 3: Effect modification in Aims 1 and 2 by *APOE* ϵ 4 carrier status

The third aim of this dissertation was to determine whether the associations in Aims 1 and 2 were modified by *APOE* genotype. Interaction terms were not statistically significant in any association. Furthermore, *APOE* ϵ 4 carrier status was not a confounder of any association. The ability to detect significant interactions may have been limited by a lack of statistical power. However, it may be that *APOE* ϵ 4 carrier status does not modify these associations, as the previous evidence supporting potential effect modification is relatively weak. Two studies found significant interactions with inflammatory markers, and both reported null associations among non-carriers, but they reported opposite effects among carriers.^{273,305} Two other studies reported significant interactions for NSAID use and *APOE* ϵ 4 carrier status, with NSAID use associated with lower AD risk²⁹⁸ or less cognitive decline³⁰⁰ only among *APOE* ϵ 4 carriers. But the latter of these was from the Cache County cohort,³⁰⁰ which separately reported no significant interaction with the outcome AD.²⁹⁹ For statins, the evidence was particularly weak, with only one study that reported a borderline significant interaction with *APOE* ϵ 4 carrier status among younger participants only,³¹³ and a later study from the same cohort reporting a non-significant interaction.³⁰⁹ The interactions reported were at least consistent in showing no effect of inflammatory markers, NSAID use, or statin use, among non-carriers, suggesting that *APOE* ϵ 4 carriers could represent a subgroup in which inflammation and anti-inflammatory medication use are more closely related to cognitive decline or impairment. However, our results for Aim 3 are consistent with the majority of studies that have examined potential *APOE* ϵ 4 interactions with inflammatory markers,^{270,272,275,277,281} NSAID use,^{293,296,299} or statin use³⁰⁶⁻³¹² and reported no significant interactions. The balance of the evidence indicates that *APOE* ϵ 4 carrier status does not substantially modify these associations.

Summary

The results of this dissertation provide limited support for a role of inflammation in cognitive impairment, and demonstrate the complexity of inflammatory processes, medication effects, and potential biases in longitudinal studies. Many possible explanations for conflicting or unexpected findings were considered and, although the others cannot be firmly excluded, some were determined to be most likely. Sustained high IL-6 was marginally associated with increased risk of cognitive impairment, which most likely reflects a role for inflammation in the development of cognitive impairment. Despite a role for inflammation in general, CRP specifically is probably not causally related to the development of cognitive impairment. The inverse association observed for sustained high level probably reflects hardy survivor effects on a null relationship, and the positive cross-sectional association for increasing CRP level most likely reflects non-causal correlation due to common mechanisms, such as aging, leading to increased inflammation and cognitive impairment. There was no evidence that statin or NSAID medication use protects against cognitive impairment, and the positive associations observed most likely reflect relationships between the reasons for use and cognitive impairment. Although they may affect some portions of the inflammatory system, the lack of effect on IL-6 level indicates their mechanisms of action are not sufficient to protect against cognitive impairment. This research also highlighted the importance of measuring multiple inflammatory markers at multiple time points and collecting longitudinal medication use data to better capture the complex and dynamic nature of inflammatory processes.

Strengths and Limitations

This dissertation used prospectively collected data from a population-based cohort. As with all longitudinal studies, some participants were lost to follow-up and such losses may have led to different results than would have been obtained with complete follow-up. A greater proportion of participants were lost from the higher inflammation exposure categories, and because they tended to be older and less healthy in general, they may also have been more likely to develop cognitive impairment. Thus, it is possible that associations were underestimated due to hardy survivor bias. In particular, as discussed above, one of the most likely explanations for the unexpected inverse association for sustained high CRP and cognitive impairment is that hardy survivor bias drove a null association to be inverse. However, extensive efforts were made to obtain follow-up data, including home visits, nursing home visits, and surrogate interviews, and excellent participation rates were achieved at each phase of the study. Most losses to follow-up were due to death and one could argue that losses to follow-up for this particular reason led to results that should not be considered underestimates, but rather reflect the reality that those who have died cannot later develop cognitive impairment. Conversely, another perspective holds that people are living longer, and because current research will inform future research and hopefully future preventive efforts, there is some merit in considering how inflammation-cognition associations may have differed if complete follow-up had been obtained for all participants.

Potential measurement error is another limitation. The main cognitive outcome, cognitive impairment, was based on MMSE score and reported diagnoses of dementia or AD. It is possible that some participants with subtle or focal cognitive deficits may have been classified as not impaired by our definition. To address this limitation, additional cognitive tests were administered at the most recent

phase which measure a variety of cognitive domains and may capture more subtle problems. Some previous studies have used diagnostic evaluations to define dementia or AD outcomes, which were not available in this study. However, the additional cognitive tests and memory questions available at the most recent phase were used to create a second cognitive outcome to represent MCI-like and dementia-like impairment, based on the new diagnostic criteria for MCI and dementia. It is well understood that this outcome is not equivalent to clinically-diagnosed MCI or dementia, although the outcome was referred to as MCI/dementia for simplicity. Additional follow-up in this cohort, with the expanded cognitive battery and memory questions, will enable prospective analyses with the MCI/dementia outcome.

There can also be error in the measurement of inflammatory markers. In this study, measurement error was minimized through the use of standardized protocols for blood collection and processing, and the use of standardized assays. Because we were most interested in typical inflammation levels rather than increased levels due to acute inflammation, sensitivity analyses excluded those with CRP > 10 mg/L, with similar results observed. Further, levels from multiple time points were used to reduce misclassification of chronically elevated inflammation. However, TNF- α was only measured from 1998-2000, and IL-6 was only available on a subset from 1988-1990.

The medication use data were limited due to lack of information about duration and frequency of use. However, the data were collected through direct examination of the medication containers at multiple phases over a 20 year period, including 5 and 10 years prior to the baseline phase. This enabled the separation of former users, which proved to be an important subgroup, different from never or current users in terms of characteristics and cognitive impairment risk.

Residual confounding is another limitation typical to observational studies, and might have affected the results reported in this dissertation, despite careful adjustment for measured confounders. Confounding due to indication, or indication bias, is a particular concern for the analyses of medication use with cognitive impairment, as the people who use medications usually do so for a reason. Thus, an observed association with medication use could actually reflect an underlying association with the reason for medication use. This is a limitation of all observational studies, yet reporting on these associations from observational studies is still important given that randomized controlled trials are limited by other factors including selected samples, relatively short follow-up periods, and ethical issues. Furthermore, one strength of this study was the extensive data collection and statistical adjustment for potentially confounding factors including sociodemographic variables, behavioral and lifestyle factors, biomedical measures, and medical histories. In addition, although limited by missing data on about 27% of the at-risk participants, *APOE* genotype was measured and evaluated as both a confounder and effect modifier of the main associations under investigation.

The study also has many strengths, in addition to those mentioned above in response to particular limitations. The large population-based cohort allowed for investigation of these associations among a broad spectrum of participants, including healthy agers, those with significant comorbidities, and those who were institutionalized. The longitudinal design allowed for prospective analyses to establish temporal associations with incident cognitive impairment during a 10-year period, which is longer than many previous studies. Furthermore, there are few large prospective cohort studies that have examined multiple inflammatory markers, and fewer that have measured them at multiple time points. In the EHLS cohort, 3 inflammatory markers were measured, with IL-6 and CRP measured at two time points 10 years apart, and additional earlier measures of CRP and IL-6 (on a subset) available from the BDES. The

results of this dissertation highlight the importance of prospectively measuring exposures over substantial periods of time in order to characterize the long-term exposure that may contribute to cognitive impairment development over several decades.

Future Studies

Future studies are needed to further determine the role of inflammation in the development of cognitive impairment. Ideally, the effect of sustained high CRP and IL-6 levels will be investigated in other cohorts, to determine whether the current results can be replicated. In addition, the statin by sex interaction reported from the EHLS cohort should encourage other studies to report whether similar sex differences are seen in their data. Future studies of NSAID and statin use will need to be performed in other prospective cohort studies with long-term medication use data that allow for the separation of former users, and sufficient lag time to avoid potential reverse causality or prescription bias. Such studies should collect detailed duration, frequency and dose data, particularly age at initiation. Additional data on the reason for medication use could be helpful in addressing indication bias. Randomized controlled trials (RCTs) would theoretically be the best design to examine medication use and cognitive impairment, except that the relevant exposure period is likely to be early enough in life that an RCT would be infeasible. However, if reliable surrogate endpoints for dementia could be developed, such an RCT might not need to be so long as to be infeasible.

Future useful studies in the EHLS cohort are possible, with some already planned. Additional follow-up will enable prospective analyses with the MCI/dementia outcome that was only available at the most recent phase. Cognitive decline in specific domains can also be examined with data from the cognitive battery at a second time point. Given that sustained high IL-6 levels in 1988-1990 and 1998-2000 showed the strongest association with cognitive impairment, but lacked statistical significance with a sample of 801, it would be beneficial to measure IL-6 levels from stored blood samples for the remaining members of the cohort. This would not only provide a larger sample for the 10-year sustained high IL-6

analysis, potentially resulting in a statistically significant association, but it would also enable comparison of those with sustained high IL-6 at all 3 time points (1988-1990, 1998-2000, and 2009-2010) to those with sustained low IL-6. With the currently available IL-6 data on only a subset in 1988-1990 there was a lack of statistical power to examine this latter comparison (only 44 participants had high IL-6 at all three points), although the point estimates suggested there may be more than 2-fold risk for this inflammatory profile.

The inflammatory response is complex, and it could be informative to measure an anti-inflammatory cytokine from stored blood samples, such as IL-10, in order to investigate whether the balance in pro- and anti-inflammatory cytokines might be more important in the development of cognitive impairment. The Leiden 85+ Study showed consistent significant associations between the TNF- α /IL-10 ratio and cognitive function in several domains among those with cardiovascular disease, indicating that pro-inflammatory profiles were associated with worse cognition compared to anti-inflammatory profiles.²⁸⁴ Another approach would be to examine the association between genes that regulate cytokines and cognitive impairment, and this will likely be the next step for many studies. A panel of single nucleotide polymorphisms (SNPs) thought to be important in cardiovascular disease was measured in the EHLS cohort, and may include some of the SNPs that regulate cytokines. Much remains unknown about inflammatory processes inside the brain, and smaller studies using brain imaging or measuring cytokines in the cerebrospinal fluid may provide much needed clarification that could be used to inform future research of systemic inflammation in larger population-based studies, for example regarding timing of exposure and outcome measures or revealing more appropriate markers of inflammation. Ideally such smaller studies would be done among unselected populations, such as case-control studies nested

within population-based cohorts, so results would be more generalizable than would results from clinic-based studies.

Finally, the Beaver Dam Offspring Study (BOSS) cohort is comprised of EHLS cohort adult offspring and utilizes very similar methods to the EHLS. In the younger BOSS cohort, systemic inflammation at younger ages can be more specifically investigated, which could be more strongly associated with cognitive impairment than inflammation measured at older ages, as has been shown for other cognitive risk factors, and would more clearly rule out the possibility of reverse causation (underlying cognitive pathology leading to increased systemic inflammation). In general, further follow-up in both the BOSS and EHLS cohorts will enable investigation of potential differences in associations by age, period, and birth cohort.

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APPENDIX

MCI/Dementia outcome

A goal of this dissertation was to develop a second cognitive outcome based on the clinical criteria for MCI and dementia. Extensive preliminary work was done during the proposal phase of this dissertation to compare various approaches. Feedback from the dissertation committee was considered in the choice of approach, and ultimately the decision was to use age-specific cutpoints (rather than a single set of cutpoints for all ages), determined based on score distributions in published normative data (rather than the distribution in the EHLS cohort), and to require subjective memory concerns for MCI and subjective reports of functional impairment for dementia as recommended in the clinical criteria (rather than waiving such criterion due to the lack of family or physician input, which is generally available in the clinical setting). A brief description of this new MCI/dementia outcome is given below.

The distributions of the 2009-2010 cognitive test scores are provided in Table A-1. Lower scores indicate better performance for the TMT-A and TMT-B, while they indicate worse performance on the VFT, AVLT, and DSST. Also note that at least 5% of participants scored 301 seconds on the TMT-B, which indicates they were unable to complete the test in the allotted 5 minutes (Table A-1). The frequencies of impaired performance, based on the age-specific cutpoints, are also shown in Table A-1. The numbers of participants classified as normal, MCI, and dementia, among those not meeting the definition for the other cognitive outcome (cognitive impairment) in 1998-2000, are shown in Table A-2. Finally, the proportions of participants classified as MCI and dementia, by age group, are displayed in Figure A-1.

Table A-1. Cognitive test score distributions and percent impaired (2009-2010) based on published norms, among those at risk in 1998-2000, EHLS

	N	Mean	SD	5 th %ile	Median	95 th %-ile	Impaired*
Test							n (%**)
TMT-A (sec)	1446	48.1	32.2	23	40	97	130 (9.0)
TMT-B (sec)	1398	126.9	67.0	56	106	301	150 (10.7)
VFT (words)	1467	32.8	11.8	14	32	52	129 (8.8)
AVLT (words)	1421	5.1	2.8	0	5	10	302 (21.3)
DSST (symbols)	1421	40.9	12.0	21	41	60	75 (5.3)

* Based on age-specific cut-points vary by age group, and in the case of the VFT, by education.

**Percentages are calculated out of the number of participants for whom data is available on a given test, and thus denominators vary by test, see first column of the table.

Table A-2. Frequency of cognitive impairment by MCI/dementia classification, among those at risk with follow-up data (n=1566), EHLS, 2009-2010

	Normal (n=1386)	MCI (n=102)	Dementia (n=78)
	n (%)	n (%)	n (%)
No	1340 (96.8)	88 (88.0)	17 (23.9)
Yes	44 (3.2)	12 (12.0)	54 (76.1)

*Cognitive impairment defined as MMSE < 24 or reported doctor-diagnosed AD.

Normal/MCI/Dementia based on new cognitive tests and memory questions.

Percentages are of those with non-missing data, sum of cells may not equal column total due to missing data for cognitive impairment definition.

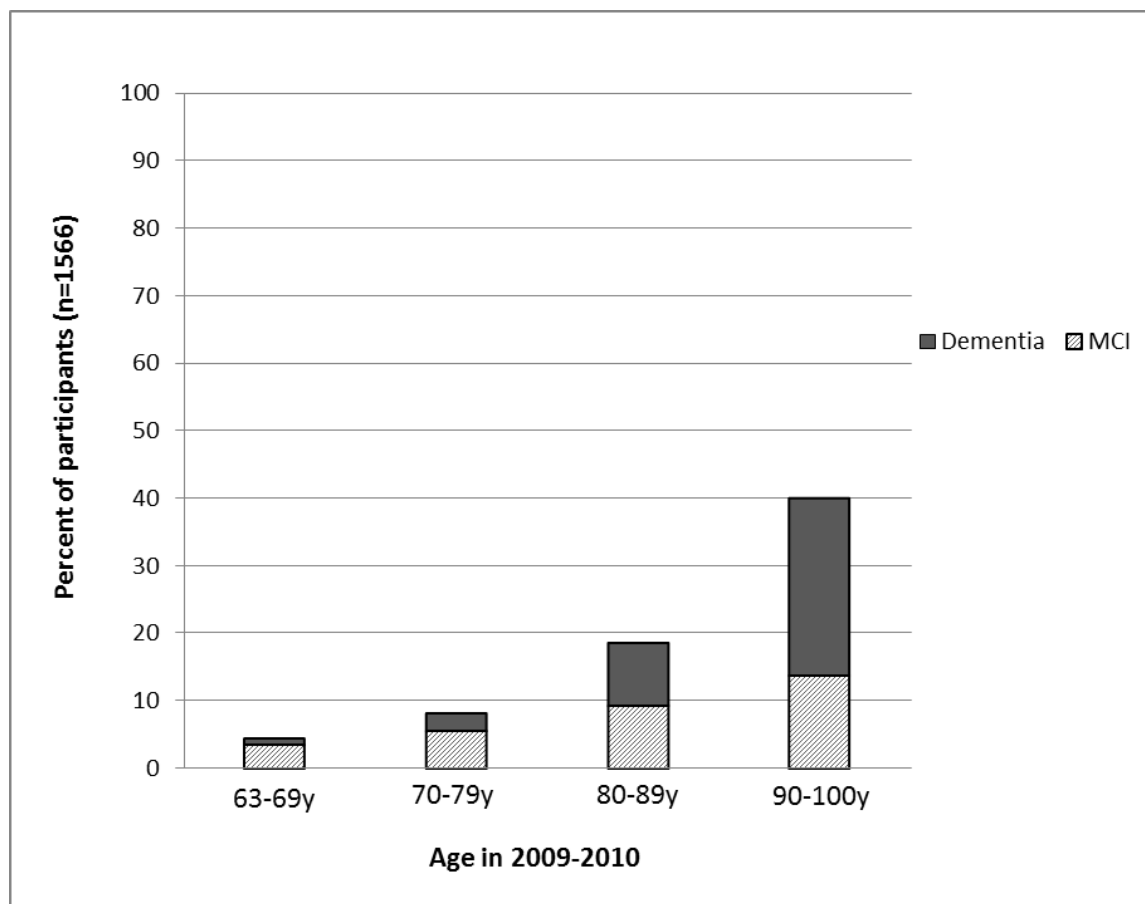


Figure A-1. Percentages of at-risk EHLS participants categorized as MCI or dementia, by age, among those with MCI/dementia data (2009-2010; n=1566)

Aim 1: Inflammatory markers

Evaluation of quadratic age term and proportional hazards assumptions

Table A-3. Cox proportional hazards models for 10-year incidence of cognitive impairment by baseline inflammatory marker levels, EHLS, 1998-2010

	N at risk	% with follow-up	Cases	Cumulative incidence (%)	Model 1 (N=1947)	Model 2 (N=1904)	Statin non-users* (N=1513)
					HR (95% CI)	HR (95% CI)	HR (95% CI)
CRP							
Low	474	88.0	50	13.25	Reference	Reference	Reference
Med	904	86.1	61	8.69	0.61 (0.40, 0.91)	0.65 (0.42, 0.99)	0.76 (0.47, 1.22)
High	909	82.8	67	10.36	0.78 (0.52, 1.17)	0.71 (0.46, 1.09)	0.75 (0.46, 1.23)
CRP p-value					p=0.05	P=0.12	P=0.44
age ² p-value					P=0.05	P=0.05	P=0.14
PH assumption					0.19	0.19	0.24
IL-6							
Tertile 1	762	92.5	59	8.95	Reference	Reference	Reference
Tertile 2	762	86.0	63	10.88	0.90 (0.69, 1.54)	0.96 (0.65, 1.43)	0.97 (0.61, 1.52)
Tertile 3	763	77.1	56	11.60	1.03 (0.69, 1.54)	1.00 (0.65, 1.53)	1.00 (0.62, 1.62)
IL-6 p-value					P=0.75	P=0.98	P=0.99
age ² p-value					P= 0.06	P= 0.05	P=0.15
PH assumption					0.16	0.08	0.03
TNF-α							
Tertile 1	653	**	60	10.07	Reference	Reference	Reference
Tertile 2	643	**	50	8.67	0.73 (0.49, 1.10)	0.72 (0.48, 1.09)	0.64 (0.40, 1.02)
Tertile 3	655	**	68	12.33	0.84 (0.58, 1.23)	0.79 (0.54, 1.17)	0.68 (0.44, 1.07)
TNF p-value					P=0.32	P=0.26	P=0.12
age ² p-value					P= 0.06	P= 0.05	P=0.14
PH assumption					0.10	0.09	0.45

Model 1 covariates: baseline age, age², sex, and education. Model 2: Model 1 + smoking status, ever heavy drinking, SF-36 MCS, BMI, and stroke. Cumulative incidence using K-M method. *Includes only those reporting no statin use at baseline (Model 2 covariates). ** TNF- α was only measured in participants with some follow-up. PH assumption is the joint p-value for both Time*T2 and Time*T3. CRP/IL-6/TNF p-values are overall p-values.

The time-dependent terms $\text{Time} \times \text{Tertile 2} = 0$ and $\text{Time} \times \text{Tertile 3} = 0$ were tested together using the test statement in PHREG to obtain the joint p-values for the two terms together. The joint p-value was not significant in any model except for the sensitivity analyses among statin non-users for baseline IL-6. Among the whole at-risk sample, the joint p-value was $p=0.08$. To further investigate, I estimated the hazard ratios for IL-6 tertiles at 5 years and at 10 years among the whole at-risk sample, and found that they were less than 1 at 5 years and greater than 1 at 10 years. However, even with the interaction terms with time in the model, there was no significant association of between baseline IL-6 tertile and cognitive impairment (using the test statement: $\text{Time} \times \text{Tertile 2} = 0$, $\text{Time} \times \text{Tertile 3} = 0$, $\text{Tertile 2} = 0$, $\text{Tertile 3} = 0$, the p-value = 0.2664).

Table A-4. Cox proportional hazards models for 10-year incidence of cognitive impairment by previous 10-year pattern of inflammatory marker levels, EHLS, 1988-2010

	N at risk	% with follow-up	Incident cases	Cumulative incidence (%)	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Statin non-users* HR (95% CI)
CRP					<i>N=1941</i>	<i>N=1899</i>	<i>N=1470</i>
Low-Low	299	88.6	27	11.25	Reference	Reference	Reference
All Other	1529	86.2	123	10.40	0.88 (0.56, 1.39)	0.81 (0.50, 1.30)	0.88 (0.51, 1.52)
High-High	452	79.4	27	9.23	0.66 (0.37, 1.18)	0.52 (0.28, 0.99)	0.50 (0.24, 1.04)
CRP p-value					0.34	0.11	0.11
age ² p-value					0.04	0.03	0.13
PH assumption					0.08	0.16	0.24
IL-6					<i>N=801</i>	<i>N=779</i>	<i>N=604</i>
Low-Low	222	93.2	10	5.44	Reference	Reference	Reference
All Other	573	82.9	57	13.63	1.75 (0.85, 3.64)	1.82 (0.85, 3.88)	1.93 (0.77, 4.82)
High-High	164	72.6	14	16.51	2.34 (0.96, 5.72)	2.35 (0.90, 6.14)	3.35 (1.09, 10.3)
IL-6 p-value					0.17	0.20	0.11
age ² p-value					0.90	0.81	0.98
PH assumption					0.22	0.18	0.49

Model 1 includes age at E2, age², sex, and education. Model 2 includes Model 1 variables + smoking status, SF-36 Mental Component Score, ever heavy drinker, BMI, and stroke. Cumulative incidence using K-M method.

*Includes only those reporting no statin use at baseline, 5-y prior to baseline, and 10-y prior (Model 2 covariates). PH assumption is the joint p-value for both Time*T2 and Time*T3. CRP/IL-6/TNF p-values are overall p-values.

Table A-5. Logistic regression models for the association of 20-year inflammatory marker patterns with cognitive impairment, EHLS, 1988-2010.

	N at risk	# of cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Statin non-users* OR (95% CI)
CRP			<i>N=1365</i>	<i>N=1341</i>	<i>N=563</i>
Low-Low-Low	129	10	Reference	Reference	Reference
All Other	1081	59	0.58 (0.27, 1.22)	0.51 (0.23, 1.15)	0.79 (0.20, 3.15)
High-High-High	164	11	0.66 (0.25, 1.74)	0.41 (0.14, 1.22)	0.33 (0.05, 2.23)
CRP p-value			0.35	0.22	0.44
age ² p-value			0.86	0.75	0.75
IL-6			<i>N=554</i>	<i>N=543</i>	<i>N=242</i>
Low-Low-Low	132	4	Reference	Reference	Reference
All Other	378	31	1.83 (0.60, 5.55)	1.68 (0.54, 5.23)	0.83 (0.14, 5.02)
High-High-High	44	4	2.59 (0.57, 11.69)	2.10 (0.41, 10.83)	2.36 (0.16, 35.07)
IL-6 p-value			0.44	0.61	0.64
age ² p-value			0.40	0.49	0.34

Model 1 includes age at E2, age², sex, and education. Model 2 covariates: Model 1 + smoking status, ever heavy drinking, SF-36 MCS, BMI, and stroke. *Includes only those reporting no statin use at each of 5 time points (Model 2 covariates). CRP/IL-6 p-values are overall p-values.

Although the quadratic age term was significant in some models, it did not substantially affect the estimated associations, and was not retained in the models reported in Manuscript #1 (Chapter 4).

Other than the baseline IL-6 association discussed above, there was no indication that the proportional hazards assumption was violated in the other associations examined.

Alternative Cox models: Age as the time scale

For the main analyses that used Cox models, I used time on study as the time scale. Here, I have reported the results using age as the time scale (for those models that used Cox regression). I used the same covariates for the Model 2s as determined in the modeling process with time on study as the time scale. Left truncation was achieved by using age in 1998-2000 (cognitive baseline) as the age at entry (with a negligible offset term of -0.0001). The end age was the age in 2003-2005, if impaired or censored at EHLS3, or otherwise, age in 2009-2010.

Results were largely similar with age as the time scale. The association for 10-year IL-6 profile was somewhat attenuated, and non-significant with either method (HR = 1.70 for High-High vs. Low-Low with age as the time scale, vs. HR = 2.35 with time on study).

Table A-6. Cox proportional hazards models for 10-year incidence of cognitive impairment by baseline inflammatory marker levels, EHLS, 1998-2010

	N at risk	% with follow-up	Cases	Cumulative incidence (%)	Model 1 (N=1947)	Model 2 (N=1904)	Statin non-users* (N=1513)
					HR (95% CI)	HR (95% CI)	HR (95% CI)
CRP							
Low	474	88.0	50	13.25	Reference	Reference	Reference
Med	904	86.1	61	8.69	0.70 (0.48, 1.02)	0.73 (0.50, 1.08)	0.84 (0.54, 1.30)
High	909	82.8	67	10.36	0.88 (0.61, 1.27)	0.77 (0.52, 1.15)	0.79 (0.50, 1.23)
CRP p-value					0.16	0.26	0.56
IL-6							
Tertile 1	762	92.5	59	8.95	Reference	Reference	Reference
Tertile 2	762	86.0	63	10.88	0.85 (0.60, 1.22)	0.92 (0.64, 1.34)	0.92 (0.60, 1.40)
Tertile 3	763	77.1	56	11.60	0.98 (0.68, 1.42)	0.92 (0.62, 1.37)	0.95 (0.60, 1.49)
IL-6 p-value					0.63	0.90	0.93
TNF-α							
Tertile 1	653	**	60	10.07	Reference	Reference	Reference
Tertile 2	643	**	50	8.67	0.81 (0.55, 1.18)	0.79 (0.54, 1.17)	0.73 (0.48, 1.13)
Tertile 3	655	**	68	12.33	0.80 (0.56, 1.14)	0.77 (0.54, 1.10)	0.70 (0.46, 1.04)
TNF p-value					0.39	0.30	0.17

AGE is the time scale, with an offset for starting age. Model 1 covariates: sex and education. Model 2 covariates: Model 1 + smoking status, ever heavy drinking, SF-36 MCS, BMI, and stroke. *Includes only those reporting no statin use at baseline (Model 2 covariates). ** TNF- α was only measured among those with some follow-up. CRP/IL-6/TNF p-values are overall p-values.

Table A-7. Cox proportional hazards models for 10-year incidence of cognitive impairment by previous 10-year pattern of inflammatory marker levels, EHLS, 1988-2010

	N at risk	% with follow-up	Incident cases	Cumulative incidence (%)	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Statin non-users* HR (95% CI)
CRP					<i>N=1941</i>	<i>N=1899</i>	<i>N=1470</i>
Low-Low	299	88.6	27	11.25	Reference	Reference	Reference
All Other	1529	86.2	123	10.40	0.96 (0.63, 1.46)	0.84 (0.54, 1.30)	0.91 (0.55, 1.50)
High-High	452	79.4	27	9.23	0.73 (0.42, 1.24)	0.55 (0.31, 0.98)	0.51 (0.26, 1.01)
CRP p-value					0.39	0.10	0.08
IL-6					<i>N=801</i>	<i>N=779</i>	<i>N=604</i>
Low-Low	222	93.2	10	5.44	Reference	Reference	Reference
All Other	573	82.9	57	13.63	1.34 (0.67, 2.69)	1.36 (0.66, 2.79)	1.34 (0.57, 3.17)
High-High	164	72.6	14	16.51	1.83 (0.80, 4.21)	1.70 (0.70, 4.12)	2.09 (0.76, 5.77)
IL-6 p-value					0.35	0.50	0.30

AGE is the time scale, with an offset for starting age. Model 1 includes sex and education. Model 2 includes Model 1 variables + smoking status, SF-36 Mental Component Score, ever heavy drinker, BMI, and stroke.

*Includes only those reporting no statin use at baseline, 5-y prior to baseline, and 10-y prior (Model 2 covariates). CRP/IL-6 p-values are overall p-values.

Comparison of those included and not included in the 10-year IL-6 categorical analyses

One possible explanation for the opposite associations noted for the 10-year IL-6 and 10-year CRP analyses could be due to differences in the samples, as a large number of participants were not included in the IL-6 analyses (n=1941 in the CRP analyses, n=801 in the IL-6 analyses). In order to determine whether this might be the case, participant characteristics were compared between those included in the IL-6 analyses and those not included (because they did not have IL-6 levels available in 1988-1990), among those included in the 10-year CRP analyses. The comparison indicated that the two groups were not substantially different, although the difference in mean age was statistically significant, those included in the 10-year IL-6 analyses were only 1 year older on average than those not included. Differences in the samples are not a likely explanation for the substantially different 10-year CRP and IL-6 results.

Table A-8. Baseline characteristics by inclusion in the 10-year IL-6 analyses, among those in the 10-year CRP analyses (n=1941)

Characteristic	Not included in IL-6 (N=1140)	Included in IL-6 (N=801)	p-value
	N (%)	N (%)	
Male sex	478 (41.9)	316 (39.5)	0.43
Education			0.17
<12y	165 (14.5)	102 (12.7)	
12y	549 (48.2)	420 (52.4)	
13-15	207 (18.2)	132 (16.5)	
≥16y	219 (19.2)	147 (18.4)	
Smoking status			0.91
Never	530 (46.9)	376 (47.5)	
Past	480 (42.5)	334 (42.2)	
Current	119 (10.5)	82 (10.4)	
Ever heavy drinking	271 (24.0)	184 (23.1)	0.83
Exercise			0.98
0	622 (55.1)	450 (56.8)	
1-2 times/week	171 (15.2)	116 (14.7)	
3 times/week	336 (29.8)	226 (28.5)	
Hypertension	623 (54.8)	479 (59.9)	0.08

Table A-8 (continued). Baseline characteristics by inclusion in the 10-year IL-6 analyses, among those in the 10-year CRP analyses (n=1941)

Characteristic	Not included in IL-6 (N=1140)	Included in IL-6 (N=801)	p-value
	N (%)	N (%)	
Diabetes	127 (11.3)	92 (11.6)	0.87
MI	72 (6.3)	72 (9.0)	0.06
Angina	100 (8.9)	68 (8.6)	0.62
Stroke	16 (1.4)	16 (2.0)	0.49
Statin use	235 (20.8)	163 (20.6)	0.82
Arthritis	472 (41.8)	369 (46.6)	0.14
Self-reported Health			0.26
Excellent	258 (22.9)	152 (19.2)	
Good	745 (66.1)	537 (67.8)	
Fair/Poor	125 (11.1)	103 (13.0)	
C-reactive protein			0.60
Low	251 (22.0)	162 (20.2)	
Medium	453 (39.7)	322 (40.2)	
High	436 (38.3)	317 (39.6)	
Interleukin-6			0.21
Tertile 1	432 (37.9)	272 (34.0)	
Tertile 2	364 (31.9)	287 (35.8)	
Tertile 3	344 (30.2)	242 (30.2)	
Tumor necrosis factor- α			0.17
Tertile 1	394 (34.6)	255 (31.8)	
Tertile 2	386 (33.9)	255 (31.8)	
Tertile 3	360 (31.6)	291 (36.3)	
	Mean (sd)	Mean (sd)	
Age	66.6 (8.3)	67.5 (8.8)	0.03
BMI	30.3 (5.9)	30.1 (5.8)	0.80
Non HDL cholesterol	163.2 (38.0)	162.8 (40.4)	0.78
HDL cholesterol	50.5 (16.0)	51.7 (17.5)	0.16
Cholesterol	213.6 (37.6)	214.1 (41.0)	0.89
SF-36 Mental Score	55.7 (7.0)	55.4 (7.5)	0.36
CRP (mg/L)	4.1 (5.9)	4.2 (8.2)	0.60
IL-6 (pg/mL)	2.4 (3.8)	2.4 (3.4)	0.98
	Median (25 th and 75 th percentile)		
CRP (mg/L)	2.22 (1.10, 4.40)	2.34 (1.17, 4.63)	---
IL-6 (pg/mL)	1.52 (0.95, 2.56)	1.59 (1.00, 2.72)	---

* Note: Age- and sex-adjusted p-values for categorical variables are based on Cochran-Mantel-Haenszel statistics for general association, for continuous variables, from LSMeans in PROC GLM. Percentages are of those with non-missing data, and thus may not equal the number with the characteristic divided by the column total.

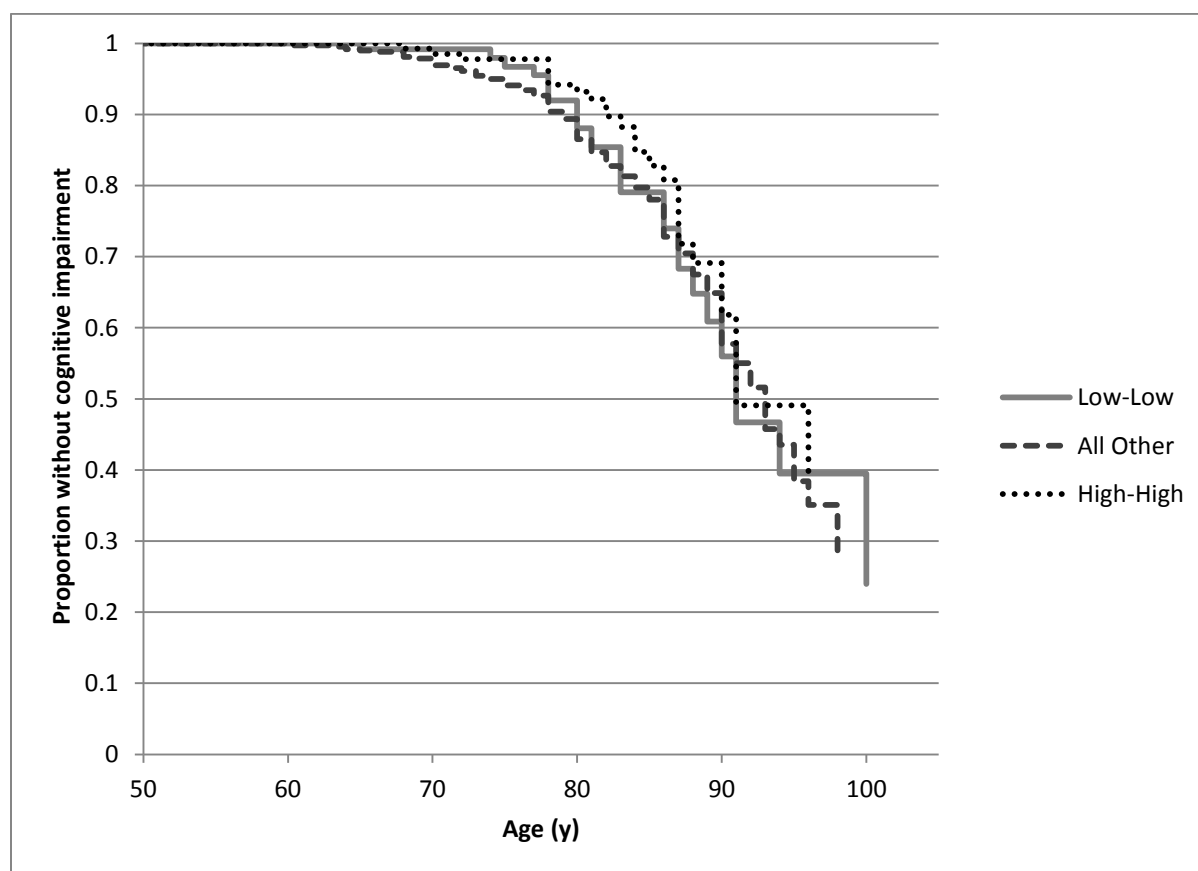
Cognitive impairment survival plots by 10-year inflammatory profiles

Figure A-2. Proportion without cognitive impairment by 10-year CRP profile (based on estimated survival curves).

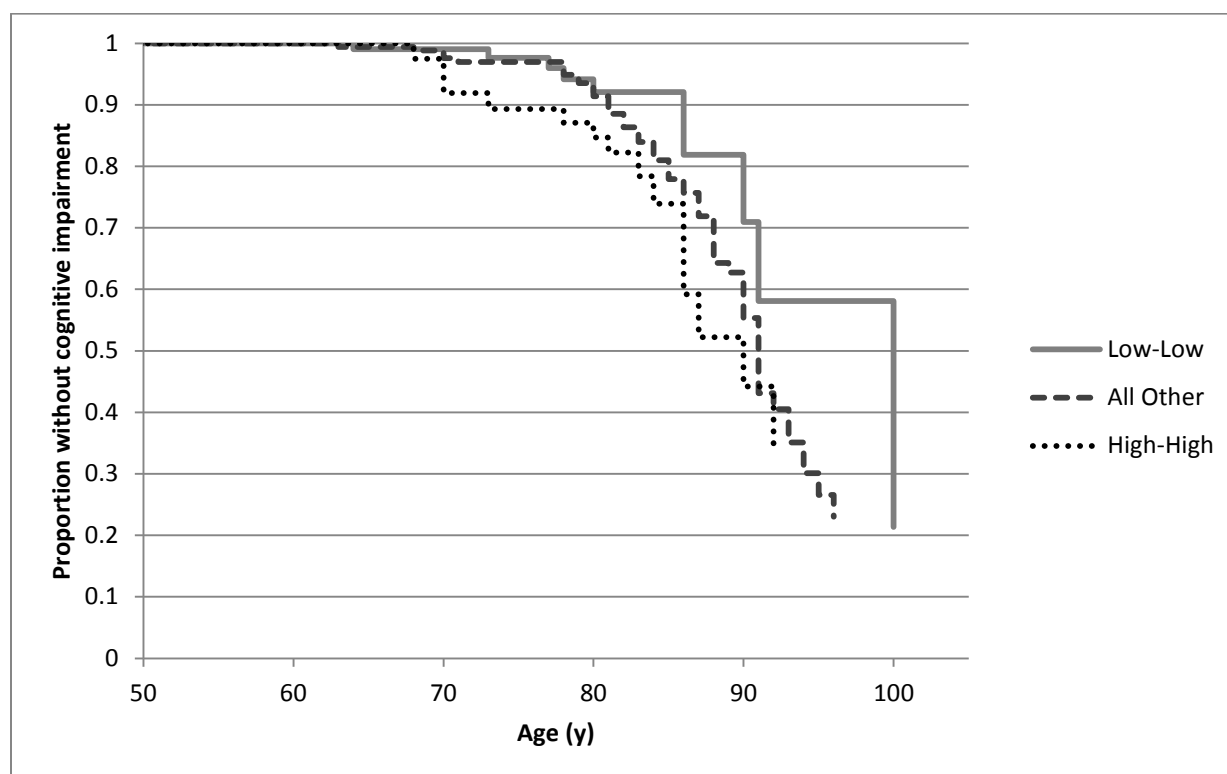


Figure A-3. Proportion without cognitive impairment by 10-year IL-6 profile (based on estimated survival curves).

These plots were generated in Excel 2010 with output from PROC PHREG (SAS), with entry age set to baseline age (1998-2000) and end age set to age in 2003-2005 (if impaired or censored at EHLS3) or age in 2009-2010.

Continuous 20-y change in inflammatory markers

In order to compare results to a previous study,²⁸⁰ change in inflammatory marker levels were also modeled as continuous variables on the $\log_2$ scale. This is achieved by taking the ratio of the level in 2009-2010 to the level in 1988-1990 (e.g., $\log_2$ (IL-6 in 2009-2010 / IL-6 in 1988-1990)).

Below are shown the distributions of multiplicative change between 1988-1990 and 2009-2010 in marker levels, on the $\log_2$ scale, where a value of 1 indicates a multiplicative increase of 2, a value of 2 represents a multiplicative increase of 4, etc. First is the distribution among the whole at-risk sample, then among only those who were non-users of statins at each of the 5 phases.

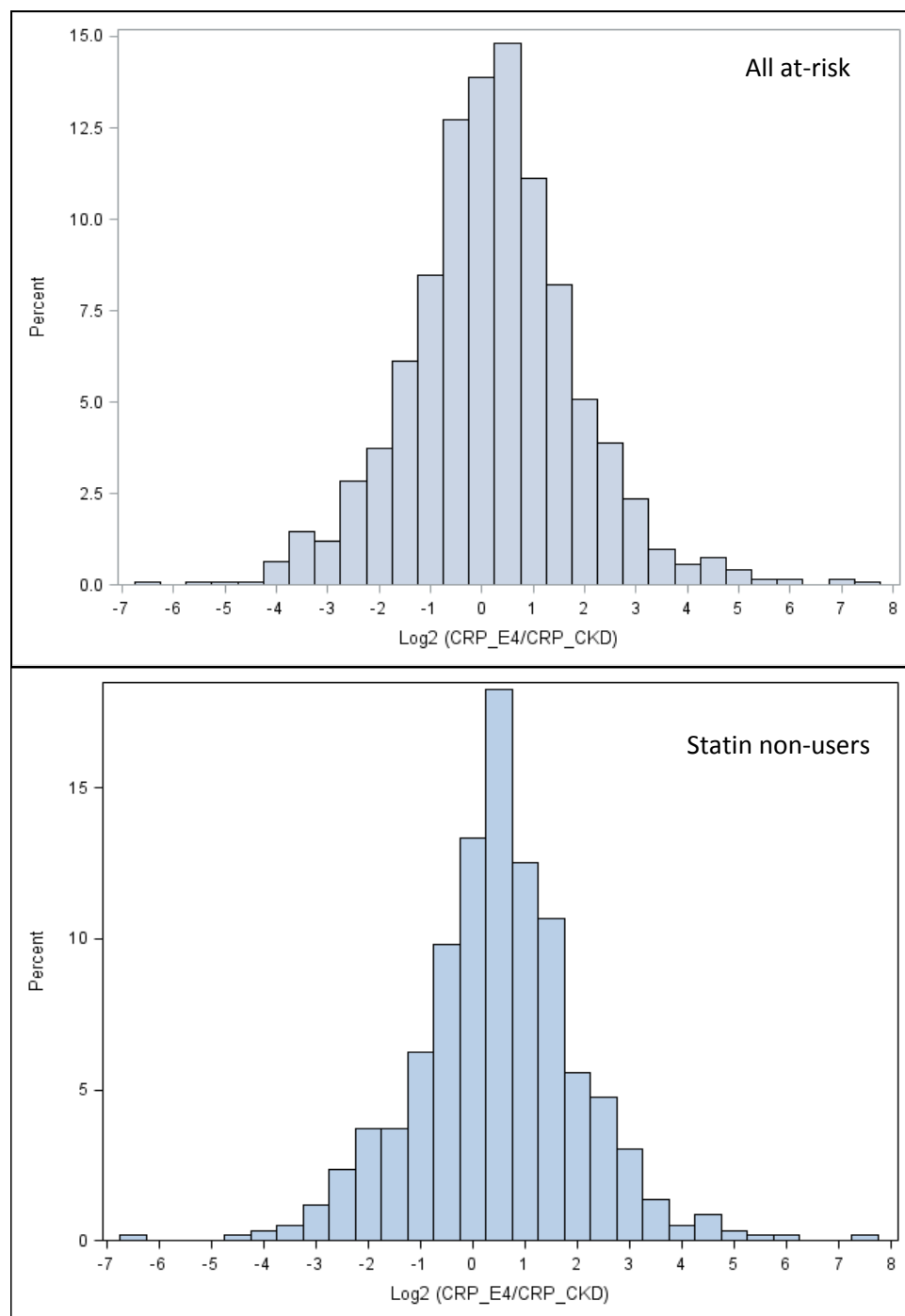


Figure A-4. Distributions of multiplicative change between 1988-1990 and 2009-2010 in marker levels, on the log₂ scale.

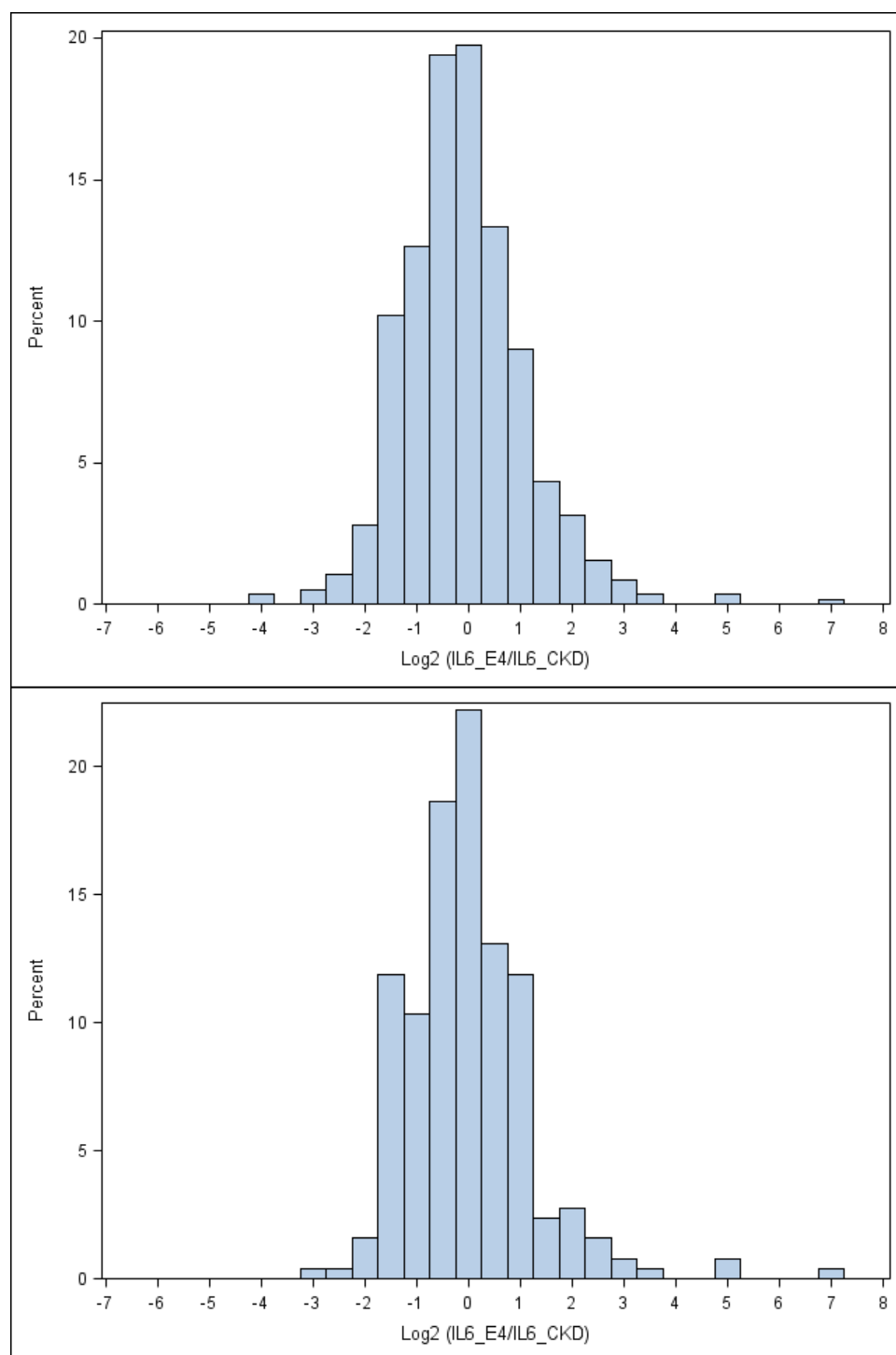


Figure A-5. Distributions of multiplicative change between 1988-1990 and 2009-2010 in marker levels, on the log₂ scale.

The highlights from these results were reported in the text of Manuscript #1 (Chapter 4). Here the complete results are shown in table format.

Table A-9. Associations of inflammatory marker 20-year change with incident cognitive impairment in 2009-2010.						
Exposure	Model 1		Model 2		Statin non-users (Model 2)	
	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)
IL-6 change (per 2-fold)	577	1.37 (1.03, 1.82)	560	1.40 (1.04, 1.89)	250	1.55 (0.93, 2.60)
CRP change (per 2-fold)	1429	1.05 (0.91, 1.21)	1393	1.06 (0.92, 1.23)	584	1.32 (1.06, 1.65)

* Model 1 covariates: baseline age, sex, and education. Model 2 covariates: Model 1 + smoking status, ever heavy drinking, SF-36 MCS, BMI, and stroke.

Table A-10. Associations of inflammatory marker 20-year change with MCI/dementia in 2009-2010.						
Exposure	Model 1		Model 2		Statin non-users (Model 2)	
	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)
IL-6 change (per 2-fold)	576	1.03 (0.82, 1.29)	559	1.03 (0.81, 1.32)	251	1.16 (0.74, 1.81)
CRP change (per 2-fold)	1430	1.03 (0.93, 1.15)	1391	1.04 (0.93, 1.16)	585	1.24 (1.03, 1.49)

* Model 1 covariates: baseline age, sex, and education. Model 2 covariates: Model 1 + smoking status, ever heavy drinking, BMI, hypertension, diabetes, myocardial infarction, SF-36 MCS, and arthritis.

Comparison of those with high-high CRP to those with low-low CRP

The categorical 10-year CRP survival analysis indicated that those with high-high CRP were about half as likely to develop cognitive impairment. The survival plots indicate that those in the low-low or all other 10-year CRP groups had similar cognitive impairment trajectories, and the high-high group had lower incidence. Given that the IL-6 results and the continuous 20-year change in CRP analyses showed the opposite pattern, suggesting higher inflammation is associated with increased cognitive impairment, the high-high CRP group was characterized to determine whether there was something odd about those with this exposure pattern that might explain the unexpected inverse association. The high-high CRP group was substantially less healthy than the low-low group, with more current smokers and heavy drinkers, less frequent exercise, and higher prevalence of hypertension and diabetes. They were less likely to be male, had lower education levels, and self-reported worse health. They also had higher levels of the other inflammatory markers, although adjustment for 10-year IL-6 exposure category did not alter the 10-year CRP results. Given these characteristics, which are often associated with increased risk of cognitive impairment, it appears that those with high-high CRP are likely to be hardy survivors.

Table A-11. Comparison of baseline characteristics between those with low-low CRP and those with high-high CRP (1988-1990 to 1998-2000; n=624)

Characteristic	Low-Low (N=265)	High-High (N=359)	p-value
	N (%)	N (%)	
Male sex	131 (49.4)	113 (31.5)	<0.0001
Education			0.004
<12y	26 (9.8)	52 (14.5)	
12y	119 (44.9)	180 (50.1)	
13-15	45 (17.0)	73 (20.3)	
≥16y	75 (28.3)	54 (15.0)	
Smoking status			<0.0001
Never	146 (55.5)	153 (42.7)	
Past	93 (35.4)	153 (42.7)	
Current	24 (9.1)	52 (14.5)	
Ever heavy drinking	46 (17.5)	79 (22.0)	0.0003

Table A-11 (continued). Comparison of baseline characteristics between those with low-low CRP and those with high-high CRP (1988-1990 to 1998-2000; n=624)

Characteristic	Low-Low (N=265)	High-High (N=359)	p-value
	N (%)	N (%)	
Exercise			0.0001
0	120 (45.6)	231 (64.5)	
1-2 times/week	46 (17.5)	42 (11.7)	
3 times/week	97 (36.9)	85 (23.7)	
Hypertension	108 (40.8)	243 (67.9)	<0.0001
Diabetes	16 (6.1)	66 (18.4)	<0.0001
MI	16 (6.0)	29 (8.1)	0.16
Angina	22 (8.4)	32 (8.9)	0.42
Stroke	3 (1.1)	6 (1.7)	0.51
Statin use	45 (17.1)	78 (21.8)	0.06
Arthritis	103 (39.2)	185 (51.7)	0.007
Self-reported Health			<0.0001
Excellent	84 (31.9)	42 (11.7)	
Good	161 (61.2)	249 (69.6)	
Fair/Poor	18 (6.8)	67 (18.7)	
Interleukin-6			<0.0001
Tertile 1	158 (59.6)	56 (15.6)	
Tertile 2	80 (30.2)	109 (30.4)	
Tertile 3	27 (10.2)	194 (54.0)	
Tumor necrosis factor- α			<0.0001
Tertile 1	113 (42.6)	104 (29.0)	
Tertile 2	91 (34.3)	115 (32.0)	
Tertile 3	61 (23.0)	140 (39.0)	
	Mean (sd)	Mean (sd)	
Age	66.8 (8.8)	67.1 (8.4)	0.90
BMI	26.7 (4.4)	33.3 (7.0)	<0.0001
Non HDL cholesterol	152.6 (35.0)	166.1 (39.4)	<0.0001
HDL cholesterol	55.6 (18.0)	49.9 (16.2)	<0.0001
Cholesterol	207.6 (35.1)	216.1 (39.3)	0.07
SF-36 Mental Score	56.0 (6.4)	55.0 (8.3)	0.14
CRP (mg/L)	0.58 (0.22)	9.97 (11.7)	<0.0001
IL-6 (pg/mL)	1.6 (3.3)	3.4 (4.1)	<0.0001
	Median (25 th and 75 th percentile)		
CRP (mg/L)	0.57 (0.42, 0.76)	6.71 (4.59, 10.87)	--
IL-6 (pg/mL)	1.00 (0.62, 1.47)	2.37 (1.53, 3.72)	--

* Note: Age- and sex-adjusted p-values for categorical variables are based on Cochran-Mantel-Haenszel statistics for general association, for continuous variables, from LSMeans in PROC GLM. Percentages are of those with non-missing data, and thus may not equal the number with the characteristic divided by the column total.

Evaluation of potential cases among those lost to follow-up

Table A-12. Frequency of cognitive impairment by age group and inclusion in analyses, with estimated cognitive impairment among those with missing cognitive follow-up, among those at risk in 1998-2000.								
With cognitive follow-up							Missing cognitive follow-up (not in analyses)	
	With exposure data (in analyses)		Missing exposure data (not in analyses)		Total			
	n=1948		n=105		n=2053			
	Total N	Impaired N (% of Total N)	Total N	Impaired N (% of Total N)	Total N	Impaired N (% of Total N)	Total N	<i>Estimated Impaired N (% of Total N)</i>
Age, y								
53-59	512	7 (1.4)	26	1 (3.9)	538	8 (1.5)	29	0 (1.5)
60-69	734	42 (5.7)	38	1 (2.6)	772	43 (5.6)	70	4 (5.6)
70-79	541	84 (15.5)	29	6 (20.7)	570	90 (15.8)	133	21 (15.8)
80-97	161	45 (28.0)	12	2 (16.7)	173	47 (27.2)	137	37 (27.2)
*For those with missing cognitive follow-up, estimated number of cases based on the age-specific frequency of impairment in the 2053 with cognitive impairment status at 2003-2005 or 2009-2010.								

In Table A-12, the frequency of cognitive impairment is shown by age group, among those with cognitive follow-up data (n=2053). The frequency is shown separately for those included in the analyses (i.e., have at least baseline CRP data; n=1948) and those not included in any analyses (have no baseline inflammation data; n = 105). For those missing cognitive follow-up data in 2003-2005 and 2009-2010 (n=369), the number of cognitive impairment cases was estimated, based on the age-specific frequency of impairment among the 2053 with known status. This is a conservative estimate, as it assumes that those lost to follow-up had the same risk of cognitive impairment as those with cognitive follow-up data, while it is actually more likely that the incidence of cognitive impairment would be greater among those lost to follow-up, had they survived and participated.

Table A-13. Observed and estimated numbers of cognitive impairment cases, by age group and inclusion in analyses, among those at risk of cognitive impairment in 1998-2000.						
	Included in analyses, have exposure and follow-up data		Not included in analyses, missing exposure or outcome data		Estimated cases if all had complete exposure and follow-up data	
	Total	Cases	Total	Cases*	Total	Cases*
	n=1948	n=178	n=474	n=72	n=2422	n=250
Age, y						
53-59	512	7	55	1	567	8
60-69	734	42	108	5	842	47
70-79	541	84	162	27	703	111
80-97	161	45	149	39	310	84
*For those with missing cognitive follow-up, estimated number of cases based on the age-specific frequency of impairment in the 2053 with cognitive impairment status at 2003-2005 or 2009-2010.						

Table A-13 builds from Table A-12. Table A-13 shows the number of cases included in any inflammatory marker analysis, and also the number of cases that were likely to have occurred among those not included in the analyses due to missing inflammation exposure or cognitive impairment outcome data (n=474, combined from the 105 with known cognitive status but missing inflammation, and the 369 missing cognitive follow-up, with number of cases estimated from the age-specific rates in the whole sample in Table A-12). Although cognitive impairment was a relatively rare outcome overall, it was much more frequent in the older age groups. Given that most of those not included in the analyses due to missing outcome data were in the older age groups, it is likely that a substantial number of cases would have occurred among this subset during the 10-year follow-up. This quantity of potential cases among those lost to follow-up is enough to substantially alter the estimated associations, depending on exposure status. Because the proportions lost to follow-up were greater in the higher inflammation groups, incidence may have been underestimated to a greater extent. Overall, the inverse CRP association could reflect greater losses to follow-up among the higher inflammation group.

Aim 2: NSAID use*Primary NSAID results*

This first set of NSAID results (below) are the complete results from the primary analyses. An abbreviated version of these results was presented in Manuscript #2 (Chapter 5). The complete set of results presented here includes current use (y/), history of use (never/former/current; “status”), and duration results for any NSAID (aspirin and non-aspirin NSAID combined), aspirin, non-aspirin NSAID, and the questionnaire aspirin item (e.g., not including aspirin-containing medications from the inventory list; “regular aspirin use”). These results also include sensitivity analyses for the significant association between aspirin use duration at baseline with incident cognitive impairment. Cox models have time on study as the time scale and variables are not time-dependent.

Table A-14. Cox models for the association of baseline NSAID use with incident cognitive impairment in 2003-2005 or 2009-2010.

	At risk (n)	Follow- up (%)	Cases (n)	Cumulative incidence (%)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
NSAID use in 1998-2000					<i>N=2013</i>	<i>N=1998</i>
No	668	82.5	47	9.56	Reference	Reference
Yes	1691	86.5	137	10.66	1.10 (0.77, 1.57)	1.08 (0.75, 1.57)
					P=0.60	P=0.68
NSAID status in 1998-2000					<i>N=2005</i>	<i>N=1990</i>
Never user	369	84.0	19	6.69	Reference	Reference
Former user	288	80.9	26	12.68	1.62 (0.86, 3.04)	1.43 (0.75, 2.73)
Current user	1691	86.5	137	10.66	1.46 (0.88, 2.42)	1.35 (0.80, 2.29)
					P=0.28	P=0.49
Duration of use (in 1998-2000)					<i>N=1742</i>	<i>N=1730</i>
Never user	369	84.0	19	6.69	Reference	Reference
Current, not 5y prior	471	86.8	37	10.06	1.42 (0.79, 2.56)	1.36 (0.74, 2.48)
Current and 5y prior	640	87.5	48	9.68	1.50 (0.85, 2.64)	1.48 (0.83, 2.64)
Current, 5y and 10y prior	540	85.9	50	12.65	1.50 (0.86, 2.64)	1.32 (0.73, 2.41)
					P=.50	P=0.62

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 is additionally adjusted for baseline smoking status, ever heavy drinking, hypertension, SF-36 Mental Component Score, arthritis, self-rated health. p-values are overall p-values for the class variable.

Table A-15. Cox models for the association of aspirin-containing medication use with incident cognitive impairment in 2003-2005 or 2009-2010.

	At risk (n)	Follow- up (%)	Cases (n)	Cumulative incidence (%)	Model 1	Model 2
					HR (95% CI)	HR (95% CI)
Aspirin use in 1998-2000					<i>N=2011</i>	<i>N=1998</i>
No	1107	85.0	75	8.94	Reference	Reference
Yes	1250	85.7	109	11.65	1.15 (0.84, 1.58)	1.20 (0.87, 1.67)
					P=0.39	P=0.27
Aspirin status in 1998-2000					<i>N=1995</i>	<i>N=1982</i>
Never user	753	86.5	42	7.13	Reference	Reference
Former user	333	82.3	30	12.67	1.46 (0.88, 2.43)	1.46 (0.87, 2.44)
Current user	1250	85.7	109	11.65	1.36 (0.93, 1.99)	1.43 (0.96, 2.12)
					P=0.22	P=0.18
Duration of use (in 1998-2000)					<i>N=1699</i>	<i>N=1689</i>
Never user	753	86.5	42	7.13	Reference	Reference
Current, not 5y prior	486	87.2	48	12.60	1.67 (1.07, 2.62)	1.83 (1.15, 2.89)
Current and 5y prior	440	85.2	33	10.36	1.18 (0.72, 1.92)	1.30 (0.79, 2.15)
Current, 5y and 10y prior	294	85.0	26	11.91	1.25 (0.73, 2.12)	1.16 (0.66, 2.04)
					P=0.15	P=0.07

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 also includes baseline smoking status, ever heavy drinking, myocardial infarction, stroke, SF-36 Mental Component score, self-rated health. p-values are overall p-values for the class variable.

Table A-16. Cox models for the association of non-aspirin NSAID-containing medication use history in 1998-2000 with incident cognitive impairment in 2003-2005 or 2009-2010.

	At risk (n)	Follow- up (%)	Cases (n)	Cumulative incidence (%)	Model 1	Model 2
					HR (95% CI)	HR (95% CI)
Non-aspirin NSAID use					<i>N=2014</i>	<i>N=2000</i>
No	1587	83.9	128	10.95	Reference	Reference
Yes	774	88.2	56	9.19	0.94 (0.67, 1.32)	0.93 (0.65, 1.32)
					P=0.73	P=0.67
Non-aspirin NSAID use status					<i>N=1987</i>	<i>N=1973</i>
Never user	1212	84.6	97	10.77	Reference	Reference
Former user	337	83.1	27	10.84	1.09 (0.69, 1.72)	1.01 (0.62, 1.64)
Current user	774	88.2	56	9.19	0.97 (0.68, 1.38)	0.92 (0.63, 1.36)
					P=0.90	P=0.90
Duration of use					<i>N=1696</i>	<i>N=1684</i>
Never user	1212	84.6	97	10.77	Reference	Reference
Current, not 5y prior	407	88.5	30	9.18	0.94 (0.60, 1.46)	0.98 (0.62, 1.56)
Current and 5y prior	223	88.3	16	9.21	1.13 (0.64, 2.01)	1.00 (0.54, 1.85)
Current, 5y and 10y prior	131	87.8	10	10.04	0.98 (0.49, 1.97)	0.95 (0.45, 1.97)
					P=0.96	P=1.00

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 also includes baseline smoking status, ever heavy drinking, stroke, SF-36 Mental Component score, self-rated health, and arthritis. p-values are overall p-values for the class variable.

Table A-17. Cox models for the association of baseline regular aspirin use with incident cognitive impairment in 2003-2005 or 2009-2010.

	At risk (n)	Follow- up (%)	Cases (n)	Cumulative incidence (%)	Model 1	Model 2
					HR (95% CI)	HR (95% CI)
Regular aspirin use in 1998-2000					<i>N=2011</i>	<i>N=1997</i>
No	1412	85.8	99	9.09	Reference	Reference
Yes	945	84.8	85	12.42	1.04 (0.76, 1.42)	1.05 (0.76, 1.47)
					P=0.82	P=0.76
Regular aspirin use status in 1998-2000					<i>N=1992</i>	<i>N=1978</i>
Never user	1091	87.5	68	7.79	Reference	Reference
Former user	296	80.1	27	13.36	1.34 (0.83, 2.18)	1.18 (0.72, 1.95)
Current user	945	84.8	85	12.42	1.15 (0.81, 1.63)	1.14 (0.79, 1.64)
					P=0.46	P=0.72
Duration of regular aspirin use (in 1998-2000)					<i>N=1739</i>	<i>N=1730</i>
Never user	1091	87.5	68	7.79	Reference	Reference
Current, not 5y prior	486	86.6	42	11.67	1.22 (0.81, 1.85)	1.27 (0.83, 1.96)
Current and 5y prior	204	83.3	21	14.66	1.20 (0.70, 2.04)	1.26 (0.73, 2.19)
Current, 5y and 10y	232	83.6	21	12.50	1.03 (0.60, 1.76)	0.87 (0.48, 1.58)
					P=0.77	P=0.50

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 further adjusted for baseline smoking status, ever heavy drinking, hypertension, myocardial infarction, stroke, SF-36 Mental Component score, self-rated health. p-values are overall p-values for the class variable.

Table A-18. Associations of NSAID use status in 1998-2000 with MCI/dementia in 2009-2010, among those at risk of cognitive impairment in 1998-2000.

	At risk (n)	Follow- up (%)	Cases (n)	MCI/Dementia (%)	Model 1 OR (95% CI) <i>N=1535</i>	Model 2 OR (95% CI) <i>N=1526</i>
NSAID use						
No	668	63.0	43	10.21	Reference	Reference
Yes	1691	65.9	133	11.93	1.13 (0.77, 1.65) P=0.54	1.02 (0.69, 1.52) P=0.91
NSAID use status						
Never user	369	65.6	16	6.61	Reference	Reference
Former user	288	60.4	25	14.37	2.01 (1.02, 3.97)	1.57 (0.78, 3.17)
Current user	1691	65.9	133	11.93	1.68 (0.97, 2.92) P=0.11	1.35 (0.77, 2.39) P=0.44
Duration of use						
Never user	369	65.6	16	6.61	Reference	Reference
Current, not 5y prior	471	69.2	41	12.58	1.77 (0.95, 3.32)	1.50 (0.79, 2.86)
Current and 5y prior	640	69.1	48	10.86	1.68 (0.92, 3.08)	1.39 (0.74, 2.61)
Current, 5y and 10y	540	60.4	43	13.19	1.70 (0.91, 3.16) P=0.30	1.12 (0.58, 2.17) P=0.50

*Model 1 adjusted for baseline age, age²(ns), sex, and education. Model 2 is further adjusted for baseline smoking status, SF-36 Mental Component score, arthritis, and self-rated health. p-values are overall p-values for the class variable.

Table A-19. Associations of aspirin-containing medication use status in 1998-2000 with MCI/dementia in 2009-2010, among those at risk of cognitive impairment in 1998-2000.

	At risk (n)	Follow- up (%)	Cases (n)	MCI/Dementia (%)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Aspirin use					<i>N=1533</i>	<i>N=1524</i>
No	1107	65.7	74	10.18	Reference	Reference
Yes	1250	64.6	102	12.64	1.08 (0.77, 1.51)	1.14 (0.81, 1.61)
					P=0.65	P=0.46
Aspirin use status					<i>N=1522</i>	<i>N=1513</i>
Never user	753	68.7	48	9.28	Reference	Reference
Former user	333	59.8	24	12.06	1.14 (0.66, 1.95)	1.08 (0.62, 1.89)
Current user	1250	64.6	102	12.64	1.14 (0.78, 1.67)	1.18 (0.79, 1.76)
					P=0.79	P=0.71
Duration of use					<i>N=1308</i>	<i>N=1301</i>
Never user	753	68.7	48	9.28	Reference	Reference
Current, not 5y prior	486	70.0	46	13.53	1.26 (0.80, 1.99)	1.38 (0.86, 2.21)
Current and 5y prior	440	64.3	32	11.31	1.04 (0.63, 1.71)	1.08 (0.65, 1.82)
Current, 5y and 10y	294	57.5	23	13.61	1.13 (0.64, 1.99)	1.04 (0.57, 1.88)
					P=0.77	P=0.57

*Model 1 adjusted for baseline age, age²(ns), sex, and education. Model 2 is further adjusted for baseline smoking status, SF-36 Mental Component score, arthritis, and self-rated health. p-values are overall p-values for the class variable.

Table A-20. Associations of non-aspirin NSAID-containing medication use status in 1998-2000 with MCI/dementia in 2009-2010, among those at risk of cognitive impairment in 1998-2000.

	At risk (n)	Follow-up (%)	Cases (n)	MCI/Dementia (%)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Non-aspirin NSAID use					<i>N=1536</i>	<i>N=1527</i>
No	1587	63.9	117	11.54	Reference	Reference
Yes	774	67.6	59	11.28	1.04 (0.74, 1.48)	0.87 (0.60, 1.27)
					P=0.81	0.48
Non-aspirin NSAID status					<i>N=1519</i>	<i>N=1510</i>
Never user	1212	64.8	85	10.83	Reference	Reference
Former user	337	62.9	29	13.68	1.35 (0.84, 2.18)	0.99 (0.59, 1.65)
Current user	774	67.6	59	11.28	1.13 (0.78, 1.64)	0.87 (0.58, 1.30)
					P=0.44	P=0.78
Duration of use					<i>N=1299</i>	<i>N=1291</i>
Never user	1212	64.8	85	10.83	Reference	Reference
Current, not 5y prior	407	67.8	33	11.96	1.15 (0.73, 1.80)	0.93 (0.58, 1.51)
Current and 5y prior	223	68.6	19	12.42	1.52 (0.87, 2.67)	1.05 (0.57, 1.95)
Current, 5y and 10y prior	131	65.6	7	8.14	0.71 (0.31, 1.65)	0.44 (0.18, 1.06)
					P=0.35	P=0.31

*Model 1 adjusted for baseline age, age²(ns), sex, and education. Model 2 is further adjusted for baseline smoking status, SF-36 Mental Component score, arthritis, and self-rated health. p-values are overall p-values for the class variable.

Table A-21. Associations of regular aspirin use history in 1998-2000 with MCI/dementia in 2009-2010, among those at risk of cognitive impairment in 1998-2000.

	At risk (n)	Follow-up (%)	Cases (n)	MCI/Dementia (%)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Regular aspirin use					<i>N=1533</i>	<i>N=1524</i>
No	1412	67.6	102	10.68	Reference	Reference
Yes	945	61.3	74	12.78	0.96 (0.68, 1.34)	0.94 (0.66, 1.33)
					P=0.80	P=0.71
Regular aspirin use status					<i>N=1519</i>	<i>N=1510</i>
Never user	1091	70.4	74	9.64	Reference	Reference
Former user	296	58.4	25	14.45	1.38 (0.83, 2.30)	1.20 (0.71, 2.03)
Current user	945	61.3	74	12.78	1.04 (0.73, 1.50)	0.99 (0.68, 1.45)
					P=0.45	P=0.76
Duration of use					<i>N=1335</i>	<i>N=1329</i>
Never user	1091	70.4	74	9.64	Reference	Reference
Current, not 5y prior	486	65.8	41	12.81	1.15 (0.75, 1.76)	1.17 (0.75, 1.82)
Current and 5y prior	204	59.8	15	12.30	0.90 (0.48, 1.68)	0.85 (0.44, 1.64)
Current, 5y and 10y prior	232	54.3	18	14.29	1.09 (0.60, 1.97)	0.93 (0.50, 1.73)
					P=0.87	P=0.79

*Model 1 adjusted for baseline age, age²(ns), sex, and education. Model 2 is further adjusted for baseline smoking status, SF-36 Mental Component score, arthritis, and self-rated health. p-values are overall p-values for the class variable.

Table A-22. Cognitive impairment in 2009-2010, by NSAID use history in 2009-2010, among those at risk of cognitive impairment in 1998-2000 with cognitive follow-up in 2009-2010.

	N	Incident cases (n)	Cognitive impairment (%)
NSAID status in 2009-2010			
Never users	76	2	2.63
Former users	360	29	8.06
Current users	1078	68	6.31
Phase began reporting persistent use (current users only)			
2009-2010, not 5y prior	122	10	8.20
5y prior	145	12	8.28
10y prior	197	12	6.09
15y prior	296	16	5.41
20y prior	240	12	5.00

*N is those with exposure data and cognitive follow-up. There were relatively few that had exposure data and no cognitive f/u, so N at risk and % f/u are not included in the table.

Among those at risk of cognitive impairment at baseline, there were 1514 participants with sufficient NSAID use data to categorize never/former/current use status in 2009-2010 and with cognitive impairment follow-up in 2009-2010. Of these, 76 participants (2 cases) were not using NSAIDs at any of the 5 examination phases, and 240 participants (12 cases) were using at all 5 of the examination phases. Thus, exposure to any NSAID is too ubiquitous in this study population to estimate any association with never users as the reference group.

Table A-23. Logistic regression models for the association of aspirin use history in 2009-2010 with cognitive impairment in 2009-2010

	N	Incident cases (n)	Cognitive impairment (%)	Model 1 (OR; 95% CI)	Model 2 (OR; 95% CI)
Aspirin use in 2009-2010				<i>N=1527</i>	<i>N=1497</i>
No	601	43	7.15	Reference	Reference
Yes	927	61	6.58	0.79 (0.51, 1.22)	0.77 (0.50, 1.21)
				P=0.30	P=0.26
Aspirin history status in 2009-2010				<i>N=1511</i>	<i>N=1490</i>
Never users	271	13	4.80	Reference	Reference
Former users	314	30	9.55	1.76 (0.87, 3.60)	1.64 (0.79, 3.40)
Current users	927	61	6.58	1.11 (0.58, 2.12)	1.05 (0.54, 2.05)
				P=0.13	P=0.20
Duration of use in 2009-2010 (former users excluded)					
Phase began reporting persistent use				<i>N=1169</i>	<i>N=1168</i>
Never users	271	13	4.80	Reference	Reference
2009-2010	338	15	4.44	0.86 (0.39, 1.90)	0.86 (0.38, 1.94)
10y prior	229	20	8.73	1.72 (0.80, 3.70)	1.72 (0.79, 3.76)
15y prior	195	14	7.18	1.14 (0.50, 2.61)	1.25 (0.54, 2.92)
20y prior	137	9	6.57	0.85 (0.33, 2.16)	0.65 (0.24, 1.79)
				P=0.32	P=0.21

*Among those at risk of cognitive impairment in 1998-2000 with cognitive follow-up in 2009-2010. Model 1 adjusted for baseline age, age², sex, and education. Model 2 further adjusted for baseline hypertension, stroke, and self-rated health. p-values are overall p-values for the class variable.

Table A-24. Logistic regression models for the association of non-aspirin NSAID use history in 2009-2010 with cognitive impairment in 2009-2010

	N	Incident cases (n)	Cognitive impairment (%)	Model 1 (OR; 95% CI)	Model 2 (OR; 95% CI)
Non-aspirin NSAID use in 2009-2010				<i>N=1527</i>	<i>N=1495</i>
No	1211	84	6.94	Reference	Reference
Yes	317	20	6.31	1.26 (0.73, 2.16)	1.14 (0.65, 2.02)
				P=0.40	P=0.65
Non-aspirin NSAID use status in 2009-2010				<i>N=1496</i>	<i>N=1479</i>
Never users	675	46	6.81	Reference	Reference
Former users	505	34	6.73	1.06 (0.65, 1.72)	0.89 (0.52, 1.50)
Current users	317	20	6.31	1.31 (0.73, 2.34)	1.09 (0.59, 2.03)
				P=0.66	P=0.80
Duration of use in 2009-2010 (former users excluded)					
Phase began reporting persistent use				<i>N=981</i>	<i>N=979</i>
Never users	675	46	6.81	Reference	Reference
2009-2010	133	9	6.77	1.29 (0.59, 2.81)	1.17 (0.53, 2.60)
10y, 15y, or 20y prior	174	10	5.75	1.27 (0.58, 2.75)	1.13 (0.49, 2.60)
				P=0.73	P=0.91

* Among those at risk of cognitive impairment in 1998-2000 with cognitive follow-up in 2009-2010. Model 1 adjusted for baseline age, age², sex, and education. Model 2 further adjusted for baseline hypertension, arthritis, and self-rated health. p-values are overall p-values for the class variable.

Table A-25. Logistic regression models for the association of regular aspirin use history in 2009-2010 with cognitive impairment in 2009-2010

	N	Incident cases (n)	Cognitive impairment (%)	Model 1 (OR; 95% CI)	Model 2 (OR; 95% CI)
Regular aspirin use in 2009-2010				<i>N=1529</i>	<i>N=1500</i>
No	660	54	8.2	Reference	Reference
Yes	870	39	4.5	0.46 (0.29, 0.73) P=0.0008	0.46 (0.29, 0.73) P=0.0009
Regular aspirin status in 2009-2010				<i>N=1495</i>	<i>N=1476</i>
Never users	351	16	4.56	Reference	Reference
Former users	275	38	13.82	2.71 (1.42, 5.17)	2.29 (1.19, 4.44)
Current users	870	39	4.48	0.76 (0.41, 1.43) p<0.0001	0.70 (0.37, 1.33) p<0.0001
Duration of use in 2009-2010 (former users excluded)					
Phase began reporting persistent use				<i>N=1165</i>	<i>N=1160</i>
Never users	351	16	4.56	Reference	Reference
2009-2010 or 5y prior	455	18	3.96	0.84 (0.41, 1.72)	0.79 (0.38, 1.63)
10y, 15y, or 20y prior	360	19	5.28	0.77 (0.37, 1.61) P=0.78	0.71 (0.33, 1.49) P=0.65

* Among those at risk of cognitive impairment in 1998-2000 with cognitive follow-up in 2009-2010. Model 1 adjusted for baseline age, age², sex, and education. Model 2 further adjusted for baseline hypertension and self-rated health. p-values are overall p-values for the class variable.

Table A-26. Logistic regression models for the association of aspirin use history in 2009-2010 with MCI/dementia in 2009-2010

	N	Cases (n)	MCI/Dementia (%)	Model 1 (OR; 95% CI)	Model 2 (OR; 95% CI)
Aspirin use in 2009-2010				<i>N=1532</i>	<i>N=1496</i>
No	602	71	11.79	Reference	Reference
Yes	931	97	10.42	0.78 (0.56, 1.10) P=0.16	0.79 (0.56, 1.13) P=0.20
Aspirin history status in 2009-2010				<i>N=1516</i>	<i>N=1489</i>
Never users	272	24	8.82	Reference	Reference
Former users	314	46	14.65	1.49 (0.87, 2.57)	1.54 (0.89, 2.69)
Current users	931	97	10.42	1.00 (0.61, 1.62) P=0.11	1.02 (0.62, 1.68) P=0.11
Duration of use in 2009-2010 (former users excluded)					
Phase began reporting persistent use				<i>N=1174</i>	<i>N=1169</i>
Never users	272	24	8.82	Reference	Reference
2009-2010	339	31	9.14	0.95 (0.54, 1.70)	0.96 (0.53, 1.74)
10y prior	232	29	12.50	1.19 (0.65, 2.16)	1.28 (0.69, 2.35)
15y prior	195	17	8.72	0.77 (0.39, 1.51)	0.83 (0.41, 1.65)
20y prior	137	17	12.41	1.00 (0.50, 2.02) P=0.78	0.90 (0.44, 1.86) P=0.73

* Among those at risk of cognitive impairment in 1998-2000 with cognitive follow-up in 2009-2010. Model 1 adjusted for baseline age, age², sex, and education. Model 2 further adjusted for baseline arthritis and SF-36 Mental Component Score. p-values are overall p-values for the class variable.

Table A-27. Logistic regression models for the association of non-aspirin NSAID use history in 2009-2010 with MCI/dementia in 2009-2010

	N	Cases (n)	MCI/Demen tia (%)	Model 1 (OR; 95% CI)	Model 2 (OR; 95% CI)
Non-aspirin NSAID use in 2009-2010				<i>N</i> =1532	<i>N</i> =1495
No	1215	138	11.36	Reference	Reference
Yes	318	30	9.43	0.98 (0.64, 1.51) P=0.93	0.80 (0.51, 1.27) P=0.35
Non-aspirin NSAID use status in 2009-2010				<i>N</i> =1501	<i>N</i> =1479
Never users	680	67	9.85	Reference	Reference
Former users	504	66	13.10	1.43 (0.98, 2.09)	1.10 (0.73, 1.66)
Current users	318	30	9.43	1.18 (0.73, 1.88) P=0.18	0.84 (0.51, 1.40) P=0.58
Duration of use in 2009-2010 (former users excluded)					
Phase began reporting persistent use				<i>N</i> =987	<i>N</i> =982
Never users	680	67	9.85	Reference	Reference
2009-2010	133	15	11.28	1.38 (0.74, 2.57)	0.98 (0.51, 1.90)
10y, 15y, or 20y prior	175	14	8.00	1.02 (0.54, 1.92) P=0.58	0.69 (0.35, 1.36) P=0.55

* Among those at risk of cognitive impairment in 1998-2000 with cognitive follow-up in 2009-2010. Model 1 adjusted for baseline age, age², sex, and education. Model 2 further adjusted for baseline arthritis, SF-36 Mental Component Score, ever heavy drinking, and self-rated health. p-values are overall p-values for the class variable.

Table A-28. Logistic regression models for the association of regular aspirin use history in 2009-2010 with MCI/dementia in 2009-2010

	N	Incident cases (n)	MCI/dementia (%)	Model 1 (OR; 95% CI)	Model 2 (OR; 95% CI)
Regular aspirin use in 2009-2010				<i>N=1532</i>	<i>N=1498</i>
No	662	85	12.8	Reference	Reference
Yes	871	73	8.4	0.58 (0.41, 0.82) P=0.002	0.58 (0.41, 0.83) P=0.003
Regular aspirin status in 2009-2010				<i>N=1498</i>	<i>N=1474</i>
Never users	351	28	7.98	Reference	Reference
Former users	277	51	18.41	2.11 (1.27, 3.51)	2.11 (1.26, 3.54)
Current users	871	73	8.38	0.91 (0.57, 1.46) P=0.0002	0.91 (0.56, 1.47) P=0.0002
Duration of use in 2009-2010 (former users excluded)					
Phase began reporting persistent use				<i>N=1166</i>	<i>N=1160</i>
Never users	351	28	7.98	Reference	Reference
2009-2010 or 5y prior	455	37	8.13	1.02 (0.60, 1.73)	1.03 (0.60, 1.78)
10y, 15y, or 20y prior	361	35	9.70	0.97 (0.56, 1.68) P=0.98	0.94 (0.53, 1.65) P=0.94

*Model 1 adjusted for baseline age, age²(ns), sex, and education. Model 2 is further adjusted for baseline SF-36 Mental Component score and arthritis. *N is those with exposure data and cognitive follow-up. There were relatively few that had exposure data and no cognitive f/u (excluded from table), so N at risk and % f/u were not included in the table. p-values are overall p-values for the class variable.

The sensitivity analyses below are for the significant association between aspirin use duration in 1998-2000 and incident cognitive impairment during the subsequent 10 years.

Table A-29. Cox model for the association of duration of aspirin-containing medication use in 1998-2000 with cognitive impairment, EXCLUDING stroke at baseline or during follow-up.

	Model 1	Model 2
	HR (95% CI)	HR (95% CI)
Duration of aspirin use	<i>N=1609</i>	<i>N=1601</i>
Never user	Reference	Reference
Current, not 5y prior	1.64 (1.03, 2.61)	1.76 (1.09, 2.84)
Current and 5y prior	1.26 (0.76, 2.07)	1.41 (0.85, 2.35)
Current, 5y and 10y prior	1.10 (0.62, 1.97)	1.16 (0.64, 2.12)
	P=0.21	P=0.13

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 also includes baseline smoking status, ever heavy drinking, myocardial infarction, ~~stroke~~, SF-36 Mental Component score, self-rated health (poor/fair, good, vs. excellent). p-values are overall p-values for the class variable.

Table A-30. Cox model for the association of duration of aspirin-containing medication use in 1998-2000 with cognitive impairment, EXCLUDING stroke or TIA at baseline or during follow-up.

	Model 1	Model 2
	HR (95% CI)	HR (95% CI)
Duration of aspirin use	<i>N=1520</i>	<i>N=1513</i>
Never user	Reference	Reference
Current, not 5y prior	1.53 (0.94, 2.49)	1.64 (0.99, 2.71)
Current and 5y prior	1.21 (0.72, 2.02)	1.38 (0.81, 2.34)
Current, 5y and 10y prior	1.15 (0.64, 2.06)	1.23 (0.67, 2.25)
	P=0.40	P=0.27

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 also includes baseline smoking status, ever heavy drinking, myocardial infarction, ~~stroke~~, SF-36 Mental Component score, self-rated health (poor/fair, good, vs. excellent). p-values are overall p-values for the class variable.

Table A-31. Cox model for the association of duration of aspirin-containing medication use in 1998-2000 with cognitive impairment, EXCLUDING baseline myocardial infarction, angina, or stroke.

	Model 1	Model 2
	HR (95% CI)	HR (95% CI)
Duration of aspirin use	<i>N</i> =1469	<i>N</i> =1460
Never user	Reference	Reference
Current, not 5y prior	1.89 (1.18, 3.03)	2.00 (1.23, 3.24)
Current and 5y prior	0.92 (0.52, 1.62)	1.00 (0.56, 1.77)
Current, 5y and 10y prior	1.24 (0.66, 2.35)	1.19 (0.62, 2.28)
	P=0.02	P=0.02

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 also includes baseline smoking status, ever heavy drinking, myocardial infarction, stroke, SF-36 Mental Component score, self-rated health (poor/fair, good, vs. excellent). p-values are overall p-values for the class variable.

Demonstration of inverse associations with cross-sectional analyses

Table A-32. Logistic regression models for the cross-sectional associations of NSAID use with prevalent cognitive impairment in 1998-2000

	OR (95% CI)
Any NSAID use	N=2567
No	Reference
Yes	0.60 (0.44, 0.82)
Aspirin use	N=2565
No	Reference
Yes	0.70 (0.51, 0.95)
Non-aspirin NSAID	N=2569
No	Reference
Yes	0.63 (0.44, 0.91)

Adjusted for age, age² (n.s), sex, and education.

Table A-33. Logistic regression models for the cross-sectional associations of NSAID use with prevalent cognitive impairment in 2009-2010

	OR (95% CI)
Any NSAID use	N=1620
No	Reference
Yes	0.71 (0.47, 1.09)
Aspirin use	N=1627
No	Reference
Yes	0.74 (0.50, 1.11)
Non-aspirin NSAID	N=1627
No	Reference
Yes	1.08 (0.64, 1.81)

Adjusted for age, age² (n.s.), sex, and education. Includes those impaired in 1998-2000.

Table A-34. Logistic regression models for the associations of any NSAID use at various phases with MCI/dementia in 2009-2010.

	Excluding prevalent cognitive impairment	Including prevalent cognitive impairment
	OR (95% CI)	OR (95% CI)
NSAID use in 1998-2000	N=1535	N=1623
No	Reference	Reference
Yes	1.13 (0.77, 1.65)	1.06 (0.74, 1.53)
NSAID use in 2003-2005	N=1493	N=1577
No	Reference	Reference
Yes	0.85 (0.57, 1.26)	0.82 (0.56, 1.21)
NSAID use in 2009-2010	N=1524	N=1625
No	Reference	Reference
Yes	0.90 (0.62, 1.29)	0.78 (0.56, 1.11)

Adjusted for baseline age, age² (n.s.), sex, and education.

These results demonstrate that methodological differences could explain the inverse associations reported cross-sectional or case-control studies. The observed associations are more inverse with shorter lag time between exposure and outcome, and with inclusion of prevalent cases.

Alternative Cox models: Time-Dependent NSAID use

The following set of results includes NSAID use as a time-dependent variable, current use (yes/no). The primary approach incorporated a 5-year lag, so NSAID use was from the phase prior to the outcome, i.e., for events in 2003-2005, exposure used the value from baseline NSAID use status (y/n) and for events in 2009-2010, NSAID use status in 2003-2005 was used to assign exposure value. Additional models estimated the association with no lag or a 10 year lag. I adjusted the Model 2's for the covariates that were selected in the modeling process for the non-time-dependent Cox models. There were no significant violations of the proportional hazards assumption, and the quadratic age term was significant.

Table A-35. Time-dependent Cox models for the association of any NSAID use with incident cognitive impairment, with various lag periods.

	Model 1 N=2052	Model 2 N=1999
	HR (95% CI)	HR (95% CI)
No lag period		
No	Reference	Reference
Yes	0.68 (0.48, 0.95)	0.65 (0.46, 0.93)
5 year lag		
No	Reference	Reference
Yes	0.96 (0.67, 1.38)	0.92 (0.63, 1.33)
10 year lag		
No	Reference	Reference
Yes	1.11 (0.79, 1.55)	1.07 (0.75, 1.53)

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 adjusted for Model 1 factors + baseline smoking status, ever heavy drinking, SF-36 MCS, self-reported health, arthritis, and hypertension.

Table A-36. Time-dependent Cox models for the association of type-specific NSAID use with incident cognitive impairment, with 10 year lag period.

	Model 1 N=2052	Model 2 N=2000
	HR (95% CI)	HR (95% CI)
Aspirin		
No	Reference	Reference
Yes	1.15 (0.84, 1.58)	1.14 (0.82, 1.57)
Non-aspirin NSAID		
No	Reference	Reference
Yes	0.88 (0.62, 1.26)	0.87 (0.60, 1.27)

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 adjusted for Model 1 factors + baseline smoking status, ever heavy drinking, SF-36 MCS, self-reported health, stroke, MI (aspirin only) arthritis (non-aspirin NSAID only).

For aspirin and non-aspirin NSAID use, time-dependent models with no lag or 5 year lag were not possible, as type-specific NSAID use data were not available from the 2003-2005 period.

Alternative Cox models: Age as the time scale

For the main analyses that used Cox models, I used time on study as the time scale. Here, I have reported the results using age as the time scale (for those models that used Cox regression). I used the same covariates for the Model 2s as determined in the modeling process with time on study as the time scale. Left truncation was achieved by using age in 1998-2000 (cognitive baseline) as the age at entry (with a negligible offset term of -0.0001). The end age was the age in 2003-2005, if impaired or censored at EHLS3, or otherwise, age in 2009-2010.

Table A-37. Cox models for the association of baseline NSAID use with incident cognitive impairment, with age as the time scale.

	At risk (n)	Follow- up (%)	Cases (n)	Cumulative incidence (%)	Model 1	Model 2
					HR (95% CI)	HR (95% CI)
NSAID use in 1998-2000					<i>N=2013</i>	<i>N=1998</i>
No	668	82.5	47	9.56	Reference	Reference
Yes	1691	86.5	137	10.66	1.04 (0.74, 1.45)	1.01 (0.71, 1.44)
					P=0.83	P=0.95
NSAID status in 1998-2000					<i>N=2005</i>	<i>N=1990</i>
Never user	369	84.0	19	6.69	Reference	Reference
Former user	288	80.9	26	12.68	1.30 (0.80, 2.11)	1.29 (0.70, 2.38)
Current user	1691	86.5	137	10.66	1.45 (0.80, 2.62)	1.20 (0.73, 1.99)
					P=0.46	P=0.70
Duration of use (in 1998-2000)					<i>N=1742</i>	<i>N=1730</i>
Never user	369	84.0	19	6.69	Reference	Reference
Current, not 5y prior	471	86.8	37	10.06	1.25 (0.72, 2.19)	1.19 (0.67, 2.11)
Current and 5y prior	640	87.5	48	9.68	1.37 (0.80, 2.34)	1.32 (0.76, 2.30)
Current, 5y and 10y prior	540	85.9	50	12.65	1.33 (0.78, 2.26)	1.17 (0.66, 2.07)
					P=0.70	P=0.79

*Model 1 adjusted for sex, and education. Model 2 is additionally adjusted for baseline smoking status, ever heavy drinking, hypertension, SF-36 Mental Component Score, arthritis, self-rated health. p-values are overall p-values for the class variable.

Table A-38. Cox models for the association of aspirin-containing medication use with incident cognitive impairment, with age as the time scale.

	At risk (n)	Follow- up (%)	Cases (n)	Cumulative incidence (%)	Model 1	Model 2
					HR (95% CI)	HR (95% CI)
Aspirin use in 1998-2000					n=2011	n=1998
No	1107	85.0	75	8.94	Reference	Reference
Yes	1250	85.7	109	11.65	1.09 (0.81, 1.46)	1.13 (0.83, 1.53)
					P=0.59	P=0.45
Aspirin status in 1998-2000					n=1995	n=1982
Never user	753	86.5	42	7.13	Reference	Reference
Former user	333	82.3	30	12.67	1.34 (0.83, 2.14)	1.32 (0.82, 2.13)
Current user	1250	85.7	109	11.65	1.24 (0.87, 1.78)	1.29 (0.89, 1.87)
					P=0.40	P=0.35
Duration of use (in 1998-2000)					n=1699	n=1689
Never user	753	86.5	42	7.13	Reference	Reference
Current, not 5y prior	486	87.2	48	12.60	1.51 (0.99, 2.29)	1.64 (1.07, 2.52)
Current and 5y prior	440	85.2	33	10.36	1.10 (0.69, 1.74)	1.18 (0.74, 1.88)
Current, 5y and 10y prior	294	85.0	26	11.91	1.13 (0.69, 1.86)	1.08 (0.63, 1.83)
					0.24	P=0.12

*Model 1 adjusted for sex, and education. Model 2 also includes baseline smoking status, ever heavy drinking, myocardial infarction, stroke, SF-36 Mental Component score, self-rated health. p-values are overall p-values for the class variable.

Table A-39. Cox models for the association of non-aspirin NSAID-containing medication use history in 1998-2000 with incident cognitive impairment, with age as the time scale.

	At risk (n)	Follow- up (%)	Cases (n)	Cumulative incidence (%)	Model 1	Model 2
					HR (95% CI)	HR (95% CI)
Non-aspirin NSAID use					<i>N=2014</i>	<i>N=2000</i>
No	1587	83.9	128	10.95	Reference	Reference
Yes	774	88.2	56	9.19	0.94 (0.68, 1.29)	0.88 (0.63, 1.23)
					P=0.69	P=0.44
Non-aspirin NSAID status					<i>N=1987</i>	<i>N=1973</i>
Never user	1212	84.6	97	10.77	Reference	Reference
Former user	337	83.1	27	10.84	1.09 (0.71, 1.68)	1.02 (0.65, 1.60)
Current user	774	88.2	56	9.19	0.96 (0.69, 1.35)	0.88 (0.62, 1.26)
					P=0.87	P=0.74
Duration of use					<i>N=1696</i>	<i>N=1684</i>
Never user	1212	84.6	97	10.77	Reference	Reference
Current, not 5y prior	407	88.5	30	9.18	0.93 (0.62, 1.41)	0.95 (0.62, 1.47)
Current and 5y prior	223	88.3	16	9.21	1.12 (0.65, 1.92)	0.94 (0.53, 1.67)
Current, 5y and 10y prior	131	87.8	10	10.04	1.03 (0.53, 1.98)	1.00 (0.50, 2.00)
					P=0.95	P=0.99

*Model 1 adjusted for sex, and education. Model 2 also includes baseline smoking status, ever heavy drinking, stroke, SF-36 Mental Component score, self-rated health, and arthritis. p-values are overall p-values for the class variable.

Table A-40. Cox models for the association of baseline regular aspirin use with incident cognitive impairment, with age as the time scale.

	At risk (n)	Follow- up (%)	Cases (n)	Cumulative incidence (%)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Regular aspirin use in 1998-2000					<i>N=2011</i>	<i>N=1997</i>
No	1412	85.8	99	9.09	Reference	Reference
Yes	945	84.8	85	12.42	0.98 (0.73, 1.31)	1.00 (0.73, 1.37)
					P=0.87	P=0.99
Regular aspirin use status in 1998-2000					<i>N=1992</i>	<i>N=1978</i>
Never user	1091	87.5	68	7.79	Reference	Reference
Former user	296	80.1	27	13.36	1.22 (0.78, 1.91)	1.10 (0.69, 1.74)
Current user	945	84.8	85	12.42	1.06 (0.76, 1.46)	1.06 (0.75, 1.49)
					P=0.69	P=0.90
Duration of regular aspirin use (in 1998-2000)					<i>N=1739</i>	<i>N=1730</i>
Never user	1091	87.5	68	7.79	Reference	Reference
Current, not 5y prior	486	86.6	42	11.67	1.16 (0.79, 1.71)	1.20 (0.80, 1.80)
Current and 5y prior	204	83.3	21	14.66	1.07 (0.65, 1.76)	1.12 (0.67, 1.86)
Current, 5y and 10y prior	232	83.6	21	12.50	0.93 (0.57, 1.54)	0.84 (0.48, 1.46)
					P=0.83	P=0.60

*Model 1 adjusted for sex, and education. Model 2 further adjusted for baseline smoking status, ever heavy drinking, hypertension, myocardial infarction, stroke, SF-36 Mental Component score, self-rated health. p-values are overall p-values for the class variable.

Aim 2: Statin use

Table A-41. Prevalence of statin use by examination phase, among the 2422 at risk of cognitive impairment at 1998-2000.					
	1988-1990	1993-1995	1998-2000	2003-2005	2009-2010
Statins	N (%)	N (%)	N (%)	N (%)	N (%)
No	2410 (99.5)	2265 (96.0)	1893 (80.2)	1252 (63.8)	808 (52.6)
Yes	11 (0.5)	95 (4.0)	468 (19.8)	710 (36.2)	727 (47.4)
Total	2421	2360	2361	1962	1535

Demonstration of inverse association with cross-sectional analyses

Similar to several other prospective epidemiological studies, the less robust cross-sectional analysis yielded a slightly more inverse association (A-38, below) than the overall prospective analyses (HR current vs. never = 0.98; 95% CI = 0.67, 1.43, Table 6-2, Chapter 6).

Table A-42. Logistic regression models for the cross-sectional association of statin use with prevalent cognitive impairment in 1998-2000

	All	Women	Men
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Statin use	<i>N=2497</i>	<i>N=1439</i>	<i>N=1058</i>
Never	Reference	Reference	Reference
Current	0.82 (0.52, 1.27)	0.45 (0.19, 1.08)	1.07 (0.63, 1.83)
Models adjusted for baseline age, age ² , sex, and education. The statin x sex interaction term has p= 0.0642.			

Alternative Cox models: Time-Dependent statin use

The following set of results includes statin use as a time-dependent variable, current use (yes/no). The primary approach incorporated a 5-year lag, so statin use was from the phase prior to the outcome, i.e., for events in 2003-2005, exposure used the value from baseline statin use status (y/n) and for events in 2009-2010, NSAID use status in 2003-2005 was used to assign exposure value. The models were also set up with no lag or a 10-year lag. I adjusted the Model 2's for the covariates that were selected in the modeling process for the non-time-dependent Cox models. There were no significant violations of the proportional hazards assumption, and the quadratic age term was significant.

Table A-43. Cox regression models for the association of time-dependent statin use with incident cognitive impairment (no lag).

	Model 1		Model 2	
	<i>N</i>	HR (95% CI)	<i>N</i>	HR (95% CI)
Statin use				
All participants	2052	0.63 (0.45, 0.90)	1944	0.65 (0.44, 0.95)
Women only	1224	0.74 (0.47, 1.17)	1167	0.70 (0.42, 1.17)
Men only	828	0.51 (0.30, 0.88)	777	0.58 (0.32, 1.04)

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 is adjusted for Model 1 + baseline smoking status, heavy drinking history, SF-36 MCS, self-rated health, HDL cholesterol, nonHDL cholesterol, stroke, MI, and angina. Sex x statin interaction term was not significant, but stratified results presented to compare with the 5-year lag stratified results.

Table A-44. Cox regression models for the association of time-dependent statin use with incident cognitive impairment (5-year lag).

	Model 1		Model 2	
	<i>N</i>	HR (95% CI)	<i>N</i>	HR (95% CI)
Statin use				
All participants	2052	0.88 (0.62, 1.26)	1944	1.08 (0.74, 1.59)
Women only	1224	1.19 (0.76, 1.87)	1167	1.33 (0.81, 2.18)
Men only	828	0.54 (0.30, 0.99)	777	0.80 (0.42, 1.52)

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 is adjusted for Model 1 + baseline smoking status, heavy drinking history, SF-36 MCS, self-rated health, HDL cholesterol, nonHDL cholesterol, stroke, MI, and angina. Sex x statin interaction term had p=0.02 in Model 1, p=0.07 in Model 2.

Table A-45. Cox regression models for the association of time-dependent statin use with incident cognitive impairment (10-year lag).

	Model 1		Model 2	
	<i>N</i>	HR (95% CI)	<i>N</i>	HR (95% CI)
Statin use				
All participants	2052	1.28 (0.81, 2.02)	1944	1.71 (1.05, 2.78)
Women only	1224	1.91 (1.10, 3.31)	1167	2.49 (1.37, 4.53)
Men only	828	0.55 (0.22, 1.35)	777	0.97 (0.38, 2.50)

*Model 1 adjusted for baseline age, age², sex, and education. Model 2 is adjusted for Model 1 + baseline smoking status, heavy drinking history, SF-36 MCS, self-rated health, HDL cholesterol, nonHDL cholesterol, stroke, MI, and angina. Sex x statin interaction term had p=0.02 in Model 1 and Model 2.

Alternative Cox models: Age as the time scale (statin use)

For the main analyses that used Cox models, I used time on study as the time scale. Here, I have reported the results using age as the time scale (for those models that used Cox regression). I used the same covariates for the Model 2s as determined in the modeling process with time on study as the time scale. Left truncation was achieved by using age in 1998-2000 (cognitive baseline) as the age at entry (with a negligible offset term of -0.0001). The end age was the age in 2003-2005, if impaired or censored at EHLS3, or otherwise, age in 2009-2010.

Table A-46. Cox regression models for the association of baseline statin use with incident cognitive impairment, with age as the time scale.

	At risk (n)	Follow-up (%)	Cases (n)	Cumulative incidence (%)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Statin status in 1998-2000						
All participants					N=1971	N=1902
Never users	1835	84.7	141	10.2	Reference	Reference
Current users	468	89.1	39	11.0	1.03 (0.72, 1.48) P=0.85	1.41 (0.95, 2.10) P=0.09
Women only					N=1164	N=1133
Never users	1100	85.9	76	9.1	Reference	Reference
Current users	245	89.8	30	15.4	1.56 (1.01, 2.40) P=0.04	1.96 (1.19, 3.22) P=0.008
Men only					N=807	N=769
Never users	735	83.0	65	11.8	Reference	Reference
Current users	223	88.3	9	5.7	0.47 (0.23, 0.94) P=0.03	0.83 (0.39, 1.78) P=0.64
Duration of use in 1998-2000						
All participants					N=1959	N=1890
Never users	1835	84.7	141	10.2	Reference	Reference
New users	377	89.4	30	10.4	0.97 (0.65, 1.44)	1.26 (0.82, 1.95)
Continuing users	77	88.3	6	10.4	1.07 (0.47, 2.43) P=0.97	1.67 (0.72, 3.90) P=0.33

Model 1 adjusted for baseline age, age², sex, and education. Model 2 includes Model 1+ baseline smoking status, ever heavy drinking, HDL cholesterol, nonHDL cholesterol, stroke, SF-36 Mental Component Score, self-rated health, myocardial infarction, and angina. There were only 16 "former" users in 1998-2000, excluded from analyses due to small sample. There was a significant sex x statin use status interaction (p = 0.03 Model 1; p = 0.0068 for Model 2), but no interaction was tested in for the duration variable, given too few continuing users. p-values are overall p-values for the class variable.

Aim 3: APOE genotype

Table A-47. Characteristics in 1998-2000, by APOE ε4 carrier status, EHLS (n=2422)					
Characteristic	Missing (n=649)	Non-carrier (n=1517)	Carrier (n=256)	Unadjusted p-values	Age- and sex- adjusted p-values
	N (%)	N (%)	N (%)		
Male sex	268 (41.3)	610 (40.2)	112 (43.8)	<0.549	0.219 (adjusted for age only)
Age, y					
53-59	93 (14.3)	397 (26.2)	77 (30.1)	<0.0001	<0.0001 (adjusted for sex only)
60-69	166 (25.6)	573 (37.8)	103 (40.2)		
70-79	219 (33.7)	426 (28.1)	58 (22.7)		
80-97	171 (26.4)	121 (8.0)	18 (7.0)		
Education, y					
< 12	149 (23.0)	206 (13.6)	26 (10.2)	<0.0001	0.084
12	297 (45.8)	766 (50.5)	134 (52.3)		
13-15	109 (16.8)	256 (16.9)	50 (19.5)		
≥ 16	94 (14.5)	288 (19.0)	46 (18.0)		
Obese (BMI ≥ 30)	252 (38.8)	714 (47.1)	116 (45.3)	<0.0001	0.42
Smoking status					
Never	284 (43.8)	714 (47.1)	113 (44.1)	<0.0001	0.090
Past	264 (40.7)	638 (42.1)	109 (42.6)		
Current	66 (10.2)	145 (9.6)	28 (10.9)		
Exercise weekly	213 (32.8)	645 (42.5)	125 (48.8)	<0.0001	0.050
Diabetes	95 (14.6)	178 (11.7)	28 (10.9)	<0.0001	0.297
Hypertension	404 (62.3)	856 (56.4)	150 (58.6)	<0.0001	0.182
Hearing Impaired	376 (57.9)	681 (44.9)	108 (42.2)	<0.0001	0.955
Any CVD	134 (20.7)	197 (13.0)	34 (13.3)	<0.0001	0.190
MI	75 (11.6)	106 (7.0)	28 (10.9)	<0.0001	0.048
Stroke	61 (9.4)	70 (4.6)	13 (5.1)	<0.0001	0.048
NSAID use	244 (37.6)	694 (45.8)	118 (46.1)	<0.0001	0.196
Statin use	113 (17.4)	326 (21.5)	29 (11.3)	<0.0001	0.001
Aspirin use	95 (14.6)	247 (16.3)	43 (16.8)	<0.0001	0.900
Self-rated health					
Excellent	80 (12.3)	310 (20.4)	65 (25.4)	<0.0001	<0.0001
Good	379 (58.4)	1006 (66.3)	161 (62.9)		
Fair	135 (20.8)	165 (10.9)	19 (7.4)		
Poor	18 (2.8)	15 (1.0)	5 (2.0)		
	Mean (sd)	Mean (sd)	Mean (sd)		
Age, mean (y)	72.4 (10.2)	67.0 (8.5)	65.7 (8.4)		
Mean IMT, mean (mm)	0.93 (0.25)	0.86 (0.22)	0.83 (0.19)		
Note: Percentages are of the total number of participants in the group (e.g., 649, 1517, or 256) and may not sum to 100% due to missing data (e.g., for Health, they sum to 94.3%, as 5.7% were missing self-reported health)					

Those missing *APOE* data were less healthy; they were older, less likely to exercise, more likely to have diabetes, hypertension, hearing impairment, CVD, MI history, stroke history, rate their health as fair or poor, and have larger IMT. Those who were carriers of the $\epsilon 4$ allele appeared somewhat healthier than the non-carriers; they were younger, more likely to exercise weekly and reported better overall health, but had about the same prevalence of chronic diseases and were less likely to use statins.