PIRJO KOMULAINEN

The Association of Vascular and Neuroprotective Status Indicators with Cognitive Functioning

Population-Based Studies

Doctoral dissertation

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ABSTRACT

As an increasing number of individuals reach advanced age, cognitive impairment and dementia are growing public health problems and thus, there is a growing demand to identify modifiable risk factors for these disorders. Cognitive impairment and Alzheimer’s disease (AD), the most common cause of dementia, have recently been shown to share many risk factors with cardiovascular diseases. However, the specific role of various vascular risk factors for cognitive functioning is still unclear.

The aim of the thesis was to elucidate the role of novel cardiovascular risk indicators for cognition in the general population. The specific aims were to investigate the associations of metabolic syndrome, high sensitivity C-reactive protein, peripheral atherosclerosis as well as that of brain-derived neurotrophic factor with the risk of suffering cognitive impairment. The main outcome, cognitive function was assessed using both global and specific neuropsychological tests.

The presence of the metabolic syndrome increased the risk for poor memory during a 12-year follow-up period among elderly women. Of the single risk factors, only a low level of high density lipoprotein cholesterol was significantly associated with the risk of having a poor memory. Carotid intima-media thickness predicted an increased risk for poor memory and reduced cognitive speed and increased high sensitivity C-reactive protein predicted poor memory. No associations were seen between these atherosclerotic risk factors and global cognitive function as assessed by the Mini-Mental State Examination. A decreased brain-derived neurotrophic factor level was associated with impaired cognitive function in ageing women, but not in men.

These studies highlight the role of modifiable risk factors in the development of cognitive impairment and give additional support to current efforts of health care professionals to promote in the prevention and treatment of metabolic and cardiovascular disorders. In the future, high priority should be given to randomized controlled trials to reveal the impact of the risk factor modification on cognition and eventually dementia.
To my family
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<th>Abbreviation</th>
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<tr>
<td>AD</td>
<td>Alzheimer's disease</td>
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<tr>
<td>APOE</td>
<td>Apolipoprotein E</td>
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<td>BDNF</td>
<td>Brain-derived neurotrophic factor</td>
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<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CERAD</td>
<td>Consortium to Establish a Registry for Alzheimer's Disease</td>
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<td>CHD</td>
<td>Coronary heart disease</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>DR's EXTRA</td>
<td>Dose-Responses to Exercise Training</td>
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<td>HDL</td>
<td>High density lipoprotein</td>
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<td>hsCRP</td>
<td>High sensitivity C-reactive protein</td>
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<td>IFG</td>
<td>Impaired fasting glucose</td>
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<td>IGT</td>
<td>Impaired glucose tolerance</td>
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<td>IMT</td>
<td>Intima-media thickness</td>
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<td>LDL</td>
<td>Low density lipoprotein</td>
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<td>MCI</td>
<td>Mild cognitive impairment</td>
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<td>MMSE</td>
<td>Mini-Mental State Examination</td>
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<tr>
<td>MONICA</td>
<td>Monitoring of Trends and Determinants in Cardiovascular Disease</td>
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<tr>
<td>NGT</td>
<td>Normal glucose tolerance</td>
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<td>OGGT</td>
<td>Oral glucose tolerance test</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<td>WHO</td>
<td>World Health Organization</td>
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals I-IV:


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1 INTRODUCTION

Cognitive impairment, dementia, cardiovascular diseases and type 2 diabetes are emerging public health problems. During the next 50 years, the number of individuals with dementia is expected to display an approximately fourfold increase (1,2). Mild cognitive impairment (MCI) refers to a transitional, high risk state for dementia. In population-based studies, the prevalence of MCI is approximately 3% (3,4), though values as high as 19% have been reported (5). A recent review indicated that in individuals older than 65 years, the mean annual conversion rate from MCI to dementia is approximately 10% and this is higher in clinic-based studies (15%) compared to population-based studies (7.5%) (6). Poor performance on neuropsychological tests occurs several years before clinical diagnosis of dementia (7), and the neurodegenerative changes begin well before the clinical manifestations of dementia become apparent (8).

Recent studies have indicated that MCI and dementia share many risk factors with atherosclerotic cardiovascular disease and metabolic syndrome. The main proposed risk factors for cognitive impairment and dementia are presented in Figure 1. Importantly, many of them are potentially modifiable. However, the follow-up times in many of the previous studies have been relatively short, and the measurements for cognitive functioning have often not been detailed. Furthermore, there is still controversy about how cognitive impairment can be best assessed and defined, nor is there clear consensus about which screening instruments are best in the assessment of MCI.

While the roles of separate factors of metabolic syndrome have been recently studied, little is known about the role of metabolic syndrome in the development of cognitive impairment. Currently, very few data are available on cardiovascular status indicators possibly mediating the impact of an unhealthy lifestyle on cognition. The aim of the present thesis was to evaluate associations between vascular and neuroprotective status indicators with regard to the risk of cognitive impairment in ageing men and women. The associations between metabolic syndrome, atherosclerosis, inflammation and cognitive function were studied using specific tests for cognitive function in a 12-year
follow-up study in elderly women. Finally, the association of a neurotrophic factor with cognitive function was assessed in a representative sample of ageing men and women.
Figure 1. Proposed risk factors for cognitive impairment and dementia.
2 REVIEW OF LITERATURE

2.1 Cognitive function

2.1.1 Assessment of cognitive function

The memory system is complex and it requires the participation of many interconnected brain areas and nerve systems. It is known that several cognitive domains decline in ageing, but there is still a lack of normative data concerning cognitive decline in specific cognitive domains in ageing. Furthermore, the fact that same cognitive domains appear to be implicated in normal ageing and in early Alzheimer's disease (AD) suggests that the cognitive deficits observed preclinically are more quantitative, rather than qualitative. The schematic view of cognitive transition from normal cognition to MCI and to dementia is presented in Figure 2 (8).

Poor performance on standard neuropsychological tests such as memory and executive functions indicate a risk of progression to dementia (7-9). Traditionally, the Mini-Mental State Examination (MMSE) (10) has been used in screening global cognitive function. MMSE includes aspects of orientation, language, memory, concentration and constructional praxis, but is not sensitive enough to measure specific cognitive domains. In the detailed assessment of cognitive function, numerous of neuropsychological tests have been used to test memory and executive function which includes cognitive speed and language (11-14). Further test batteries, such as the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropsychological test battery (15), designed to assess basic cognitive functions affected in AD, are appropriate if one wishes to test memory, language, praxis and general intellectual status.
2.1.2 Definition of cognitive impairment and dementia

During the past years, several terms such as *cognitively impaired not demented*, *possible dementia prodrome*, *age-associated memory impairment* and *age-associated cognitive impairment* have been used to describe a phenomenon between normal ageing and mildly impaired cognitive function (16,17). The term "mild cognitive impairment" is generally used as a pathological condition to define persons in a transitional state between normal cognitive function and clinically probable AD (Figure 2) (7,17-19). Despite the rather wide variety in the concepts used to describe cognitive dysfunction (17), the terms have been recently refined to correspond more closely to MCI (3).

The recommendations for general MCI criteria are represented such that the person who is judged to be not normal but does not fulfil the criteria of dementia. Cognitive decline should be 1) self-reported and/or if possible, 2) reported by a close relative or friend, and 3) objective cognitive measures should be performed, and/or 4) evidence of

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**Figure 2.** Schematic view of cognitive transition from normal cognitive function to mild cognitive impairment (MCI) and dementia in terms of cognitive performance and pathological burden (adapted from Nestor et al. 2004) (8).
cognitive decline based on objective neuropsychological tests. The person with MCI has no notable difficulties in activities of daily living (18). Various clinical subtypes of MCI have been proposed, are suggested to represent different etiologies and to be risk states for different dementing disorders. The subtypes are: a) amnestic MCI; amnestic MCI - single domain and amnestic MCI -multiple domain, b) non-amnestic MCI; non-amnestic MCI -single domain and non-amnestic MCI -multiple domain (Figure 3) (17).

Although there are no generally accepted instruments to define the transition from normal cognitive function to MCI, commonly used neuropsychological tests and test batteries are recommended. In addition to specific neuropsychological tests, it is necessary to incorporate clinical features, combinations of measures, biomarkers and neuroimaging for a diagnostic accuracy as well as structured measurements of functional capacity (7,17-19). Abnormalities in cerebrospinal fluid biomarkers are useful in the prediction and diagnosis of early AD (20,21). Atrophy in mediotemporal lobe is suggested to be a significant predictor of conversion from normal ageing to MCI (22) as well as progression to AD (23) detected by magnetic resonance imaging. Magnetic resonance images of the hippocampus in ageing, MCI and AD are presented in Figure 4. The density of grey matter in individuals with MCI and AD can be registered by voxel-based morphometry imaging (Figure 5). Functional brain imaging offers potential insight into pathological features such as hypometabolism or hyperperfusion in MCI and AD (8). However, autopsy studies have revealed that many people with neuropathological changes of degenerative or vascular origin have no clinical signs of dementia, suggesting that the association between neuropathological lesions and clinical syndromes is more complex than previously thought. (24). More research is needed to clarify the added values of various clinical investigations in the diagnostic process.

The prevailing definitions of AD or dementia are based on the criteria of Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (25) and the National Institute of Neurological Disorders and Stroke-Alzheimer's Disease and Related Disorders (NINCDS-ADRDA) (26) working group. The essential features included in the DSM-IV are memory impairment and impairment in at least one additional cognitive domain, both of which interfere with social function or activities of
daily living. The NINCDS-ADRDA criteria for AD require that the diagnosis should be restricted to persons who have 1) clear evidence of progressive and significant deterioration of memory and other cognitive domains, 2) onset of the disease is insidious, typically after age 65 years and 3) lack of systemic or brain diseases that could account for the progressive memory and other cognitive deficits. In addition, the presence of psychiatric disorders must be excluded. Techniques to image neuropathological characteristics (Figures 4 and 5) and methods to detect pathological features e.g. in cerebrospinal fluid increase accuracy in definition of AD even in prodromal and preclinical phase (19).

Vascular cognitive impairment refers to individuals who have all types of mild to severe cognitive impairment associated with or presumed to be caused by cerebrovascular disease (27). Vascular dementia has been defined by the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (25), codes of International Classification of Diseases (ICD) (28) and specific criteria for ischaemic vascular dementia (29), probable and possible vascular dementia (30), and subcortical vascular dementia (31). The proposed criteria for vascular dementia are not interchangeable and the sensitivity and specificity of these diagnostic criteria are variable (32-34). Vascular cognitive impairment encompasses a heterogeneous group of disorders (Table 1). However, none of these criteria sets have been satisfactorily validated by prospective studies (27).
Figure 3. Subtypes of mild cognitive impairment (MCI) (modified from Petersen 2004) (17).
Figure 4. Magnetic resonance images of the hippocampus in ageing, mild cognitive impairment (MCI) and Alzheimer's disease (AD) (permission by M. de Leon, New York University School of Medicine, USA).

Figure 5. Loss of grey matter in individuals with mild cognitive impairment (red) and Alzheimer's disease (blue) using computational neuroanatomy method, voxel-based morphometry (adapted from Nestor et al. 2004, permission by P.S.) (8).
Table 1. Concept of sporadic vascular cognitive impairment according to different vascular pathologies (27)

<table>
<thead>
<tr>
<th>Type of vascular cognitive impairment</th>
<th>Characteristics</th>
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<tbody>
<tr>
<td>Multi-infarct dementia</td>
<td>Multiple large cortical infarcts are required in developing dementia. Not very common type in the elderly.</td>
</tr>
<tr>
<td>Post stroke dementia</td>
<td>Heterogeneity of the underlying vascular pathology is suggested, resulting from different vascular causes and changes in the brain, and degenerative pathology. Includes patients with multiple corticosubcortical infarcts, strategic infarcts, subcortical ischaemic vascular dementia and Alzheimer’s disease. Develops in up to a third of patients within a year of stroke. Is strongly associated with advancing age.</td>
</tr>
<tr>
<td>Subcortical ischaemic vascular dementia</td>
<td>Generally predictable outcome of vascular dementia, which incorporates small-vessel disease as the main vascular cause with lacunar infarct and ischaemic white-matter lesions as the primary type of brain lesion. The primary location of lesions is subcortical, and subcortical syndrome is the primary clinical manifestation. The onset is frequently insidious, and temporal relations between the cognitive syndrome, brain imaging features and evidence of cerebrovascular disease may not be clear.</td>
</tr>
<tr>
<td>Subcortical ischaemic vascular disease without dementia</td>
<td>White-matter lesions are observed on magnetic resonance imaging brain scans. The lesions can occur as early as 30 years of age, but their prevalence rises strikingly with age, by 70 years of age at least 70% of the population is affected. Lesions occur in the context of well-recognised vascular risk factors as well as novel and poorly recognised risk factors for white-matter lesions such as oxidative stress.</td>
</tr>
<tr>
<td>Mixed Alzheimer’s disease with cerebrovascular disease</td>
<td>Vascular and degenerative pathologies interact in terms of clinical expression of cognitive impairment. No clinical criteria for the diagnosis of Alzheimer’s disease with cerebrovascular disease are currently available. Is underestimated as a common cause of dementia, particularly in elderly people.</td>
</tr>
</tbody>
</table>
2.1.3 Epidemiology of cognitive impairment

The etiology of MCI, vascular dementia, and AD is heterogeneous and still partly unspecified. Cognitive impairment, dementia and its subtypes can be defined in various ways resulting in varying incidence and prevalence numbers in different studies. In population-based studies, the prevalence of MCI has ranged from 1% to 3%, even up to 19% (3-5) in individuals older than 65 years. In clinical studies, the annual rate of conversion from MCI to dementia ranges approximately 10% to 20% (35-37), but this number is lower in population-based studies (approximately 7.5%) (6). The incidence of MCI is estimated to be from 8 to 58 new patients (4,38,39) per thousand persons annually. The prevalence (40) and incidence (41) of dementia and AD increase with age and seem to be somewhat higher in women. The number of individuals with dementia is expected to increase nearly fourfold by the year 2050 (1,2).

MCI increases the risk of mortality by about 1.5 -fold to 3.0 -fold compared to those with normal cognitive function (37,42-44). In a Scandinavian cohort of very old individuals, the risk of mortality which resulted from dementia was approximately 30% for men and 50% for women (45).

2.1.4 Pathophysiology of cognitive impairment and dementia

AD and vascular dementia are two most common dementing diseases. AD is a progressive neurodegenerative disorder, characterized by neuronal loss, neuronal plaques and neurofibrillary tangles and decreased blood perfusion and metabolism in the brain. Hippocampus and entorhinal cortex are the first brain areas to be affected in the progressive neurodegenerative process leading to AD (Figure 6) (8). AD may be associated with large vessel atherosclerosis and amyloid angiopathy (46). Vascular amyloid angiopathy can cause cognitive impairment independent of plaque and tangle pathology (47), although its precise role in cognitive function is unclear. Histological evidence of senile plaque and neurofibrillary tangle formation can be seen in temporal lobe up to 40-50 years before the onset of dementia (48). Senile plaques are extracellular deposits, predominantly of beta-amyloid. Neurofibrillary tangles are intraneuronal inclusions, which are, in part, composed of abnormally phosphorylated tau protein (49).
After ischemic stroke, dementia may occur in as many as one out of four elderly individuals within a short term (50). Furthermore, the presence of silent brain infarcts, common even in healthy elderly people, is suggested to more than double the risk of dementia (51).

The definition of vascular dementia has been historically based on stroke and multi-infarct model and required memory impairment as an essential feature (27). However, executive dysfunction seems to be a typical cognitive disorder with a vascular foundation (52). Furthermore, mixed pathology is reported to be common; in AD there is evidence of considerable cerebrovascular pathology, including small-vessel disease and microinfarction, and there is evidence for substantial overlap between vascular dementia and AD (Table 1) (27). About half of patients with vascular cognitive impairment exhibit dementia (53).

Other dementias such as Lewy body dementia and frontal lobe dementia are more uncommon and exhibit somewhat dissimilar characteristics than that encountered with vascular dementia and AD (54). Moreover, there are rare causes of dementia (e.g. traumatic and infectious), which will not be referred to in the present thesis.

Ageing predisposes an individual to atherosclerosis and its consequences (55). Increased oxidative stress and accumulation of oxidatively modified molecules such as proteins, nucleic acids and lipids, promote dysfunction of various metabolic and signalling pathways (56). In addition, energy deficits may occur in brain neurons due to an alteration in the cerebral vascular function. In unsuccessful brain ageing, molecular damage to neurons and inflammatory processes result in synaptic dysfunction and neuronal degeneration and death, without replacement of the lost neurons (56). In successful brain ageing, the neurons and glial cells adapt to the adversities of ageing by increasing their ability to cope with stress, and by compensating for lost or damaged cells by producing new brain cells and remodelling neuronal circuits (56). One possible explanation may be related to neurotrophic factors.
2.2 Risk factors for cognitive impairment

2.2.1 Hypertension

There is increasingly strong evidence that hypertension is a risk factor for cognitive impairment and dementia. Elevated blood pressure at midlife increases the risk of dementia and its main subtypes, AD and vascular dementia (57-59) and cognitive impairment (60-62) later in life. Increased blood pressure at midlife has been associated with brain atrophy and increased number of neuritic plaques in neocortex and hippocampus, as well as with increased number of neurofibrillary tangles in hippocampus at death in elderly men (63).

Even high blood pressure in old age has been reported to increase the risk of subsequent dementia/AD (64). However, results from studies where the blood pressure measurement was conducted later in life and/or with shorter follow-up times, have yielded inconsistent results (65). The divergent findings may be explained due to the timing of blood pressure measurement in relationship to age and the clinical onset of cognitive impairment (66). Blood pressure may become decreased many years before the diagnosis of dementia and be a part of the disease process (64,65).

Systolic blood pressure and pulse pressure increase with age mainly due to reduced elasticity of the large arteries. Atherosclerosis results from structural abnormalities in arteries, and together with endothelial dysfunction, leads to arterial rigidity, which in
turn increases systolic blood pressure (67). High blood pressure is a major risk factor for stroke and white-matter lesions, which in turn may promote the clinical expression of AD and dementia (65). Thus, atherosclerosis may be involved in the pathogenesis and progression of both vascular dementia and AD (65). There is increasing evidence from epidemiological and randomized trials that antihypertensive treatment may prevent cognitive impairment and dementia (65).

2.2.2 Lipids and lipoproteins
Midlife high serum total cholesterol level was associated with MCI (60) and AD in the elderly in population-based studies (57,59). In an autopsy study, the premortem presence of hypercholesterolemia has been shown to be an early risk factor for AD amyloid pathology in human brain (68). Similarly for blood pressure, studies with shorter follow-up times or where the cholesterol measurements have been conducted later in life, have reported no association (69,70) or a negative association between cholesterol and risk of dementia (71). It has been reported that cholesterol values may start to decline a decade before the clinical diagnoses of dementia and may be a part of the AD process (72,73). The exact mechanisms relating cholesterol to AD are not known, but aberrations of cholesterol homeostasis have been linked with the amyloid precursor protein metabolism, beta-amyloid production (74) and several other features of AD pathology such as phosphorylation of tau protein and the production of neurofibrillary tangles (74).

There is little data available on the association between low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol and cognitive function (69,75). A low level of HDL cholesterol was associated with cognitive impairment and dementia (76-78), but inconsistent findings exist (79). HDL cholesterol is critical for the maturation of synapses and the maintenance of synaptic plasticity (80,81), and high HDL cholesterol levels may protect against hippocampus atrophy in elderly people (82), as well as preventing inflammation (83) and the aggregation and polymerization of beta-amyloid protein (84). A low level of HDL cholesterol is a known risk factor for atherosclerosis (85), which in turn may lead to impaired cognitive function. In the follow-up studies, plasma triglycerides have either not been associated with cognitive
performance (69,86), and dementia (71) or else the association has been weak (87). Epidemiological studies with different study designs and populations have shown a 40-70% reduction in the risk of AD associated with statin use (88-90). On the other hand, in two randomized placebo-controlled trials, no benefits for statin use against cognitive decline were found (91,92).

2.2.3 Overweight and obesity
Obesity at midlife has been associated with an increased risk of dementia and AD in later life (93,94). Overweight among elderly women has also reported to be predictive for subsequent AD (95). In an exercise and diet intervention study, loss of intra-abdominal fat was associated with improved memory in ageing people who were at an increased risk for developing type 2 diabetes (96). On the other hand, weight loss in late life is suggested to precede a clinical diagnosis of dementia (97,98). There is little data available about the association between obesity and MCI. Since it is a risk factor for cardiovascular disease, obesity may have an indirect effect on cognitive function. Adipose tissue releases cytokines and many bioactive mediators, such as leptin, adiponectin, interleukin-6 and tumor necrosis factor-alpha, that influence body weight homeostasis, insulin resistance, diabetes, alteration in lipids, blood pressure, blood coagulation, fibrinolysis and inflammation and leads to endothelial dysfunction and atherosclerosis (99), which in turn is associated with cognitive function.

2.2.4 Glucose intolerance
Glucose intolerance and type 1 and type 2 diabetes have been linked with cognitive impairment (100). In prospective studies, the presence of diabetes has increased the risk for cognitive impairment (101-103), and dementia (104,105). These associations have been found also in cross-sectional studies (106-108). There is less data available on the association between pre-diabetic conditions including impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) and cognitive function. In follow-up studies, IGT associated with mildly impaired cognitive function in men and women (109) stroke-related dementia in men (110), and AD (106) or such an association was not found (111).
There are several plausible mechanisms which could mediate the association between diabetes, cognitive impairment and dementia (Figure 7). Acute and chronic hyperinsulinemia have opposite effects on the neural substrate of memory: acute hyperinsulinemia may facilitate memory whereas chronic hyperinsulinemia and insulin resistance are to be deleterious to memory (112). Hyperinsulinemia and insulin resistance are well established risk factors for vascular diseases which in turn have implications for vascular dementia and AD (112). Insulin receptors are distributed unevenly throughout the brain, being present on synapses and modulating neurotransmitter function (113). The overlapping distributions of insulin receptors, insulin and insulin-sensitive glucose transporters are consistent with the possibility of insulin-stimulated glucose uptake in specific brain regions, such as the hippocampus and hypothalamus (113). Alterations in insulin metabolism have effects on central nervous system, through their involvement in synaptic plasticity and amyloid and tau metabolism (100). Moderate hyperinsulinemia can elevate inflammatory markers and beta-amyloid concentration in the brain, and may increase the risk for AD (114). Type 2 diabetes is associated with atrophy in hippocampus (115), and is a risk factor for silent and symptomatic brain infarcts (100,116,117), and in these ways it can lead to cognitive impairment.

2.2.5 Metabolic syndrome
The metabolic syndrome is characterized by the clustering of obesity, glucose intolerance, hypertension, low HDL cholesterol level, and high triglycerides, each of which is a known risk factor for atherosclerotic cardiovascular events (85). The pathophysiological feature is insulin resistance (118), which in turn is associated with obesity (119). Obesity may induce systemic oxidative stress and increased oxidative stress is, at least partly, the underlying cause of dysregulation of adipocytokines and development of metabolic syndrome (119). Obesity as well as hypertension and type 2 diabetes have been suggested to be essential in the pathogenesis of vascular dementia and AD (57,64,104,112). In the diabetic condition, oxidative stress impairs glucose uptake in muscle and fat (120,121) and decreases insulin secretion from pancreatic beta cells (122). Increased oxidative stress also underpins the pathophysiology of
hypertension (67) and atherosclerosis (123) and affects directly the condition of vascular wall cells. In a 1-year randomized controlled trial, weight loss reduced metabolic syndrome in middle-aged men and women (124).

In prospective studies, the association between the clustering of vascular risk factors and dementia was found in men (125), with AD in women (78) and cognitive impairment in men and women, especially in those with a high level of inflammation (126). Metabolic syndrome also has been associated with dementia and cognitive impairment in cross-sectional studies (127-129). However, in very old age the presence of the metabolic syndrome was associated with decelerated cognitive decline (130). The current data are limited, because the measurements used to assess cognitive function were non-specific, especially for MCI, populations were heterogeneous and definitions of metabolic syndrome have been inconsistent.

**Figure 7.** Suggested pathogenesis of cognitive impairment in metabolic syndrome and type 2 diabetes (modified from Biessels 2005) (131).
2.2.6 Atherosclerosis

In the elderly, there is cumulative evidence pointing to a link between vascular risk factors and atherosclerosis and AD (132,133). Some investigators have proposed that AD occurs as a secondary event related to atherosclerosis of extracranial (134) or intracranial (135) vessels and is linked to brain hypoperfusion (136) or discrete brain infarction (137). One tenable hypothesis is that atherosclerosis and AD are independent, but convergent disease processes (132). The earliest atherosclerotic changes include increased endothelial permeability to lipoproteins and this is suggested to precede the formation of atherosclerotic lesions (138). Little data is available on the association between endothelial dysfunction and cognitive function (139).

Several epidemiological studies suggest that cardiovascular and cerebrovascular diseases are associated with the subsequent development of AD (137,140). Vascular damage in the brain may create the kind of conditions, possibly due to inflammation, which predispose to neurodegeneration (75). On the other hand, inflammatory processes increase the presence of vascular injury, such as those caused by hypertension, atherosclerosis and hypercholesterolemia (141,142). Alternatively, cerebrovascular disease may simply increase the severity of dementia or hasten the age at onset (75,117,143). Atherosclerosis is suggested to be a progressive disease which acts throughout the lifespan and which is characterized by the accumulation of lipids within arterial walls. Specific arterial sites such as bifurcations, are prone to local plaque formation (138).

The role of peripheral atherosclerosis, which can be quantitated by high resolution B-mode carotid artery ultrasonography as intima-media thickness (IMT), as a detrimental factor for cognitive function is still unclear. The few longitudinal studies that have evaluated the association between carotid IMT and cognitive function have yielded conflicting results (144-146). Some cross-sectional studies have found an inverse association between carotid IMT, and global cognitive function (147) as well as memory (148,149) and executive function (144). An association has been found between the increasing prevalence of plaques in the carotid arteries and poor performance on various neuropsychological tests in men, though not in women (150). Carotid IMT and plaques may represent partly different ethiopathological conditions.
and different phases of the atherosclerotic process (151), and thus, the effects on cognition may differ.

Microglial activation is suggested to be an early event in the pathogenesis of AD in individuals with mild and early disease (152). Immunohistochemical analysis of advanced carotid atherosclerotic plaques has revealed the presence of amyloid precursor protein, beta-amyloid, platelets and activated macrophages that surround intimal microvessels (153). Furthermore, pathological angiogenesis is hypothesized to have a role in the pathogenesis of AD (154). Abnormalities in the brain microcirculation in AD have been detected, and it has been suggested that these precede the parenchymal amyloid deposit, and further promote the development of neurodegenerative diseases (155,156).

2.2.7 Inflammation

The serum level of high sensitivity C-reactive protein (hsCRP) is a marker of systemic low-grade inflammation, and is associated with increased risk of stroke (133) and cardiovascular disease (157). The inflammatory response may contribute to neuronal dysfunction, and ultimately lead to neuronal death, although the precise sequence of events is unclear. Inflammatory markers such as interleukin-1, interleukin-6, tumor necrosis factor -alpha and hsCRP have been suggested to be involved in the pathogenesis of AD (133,158-160), and other types of dementia (161), and to be an early hallmark in the AD pathogenesis (449 Tarkowski,E. 2003; }. Limited data of the role of inflammatory biomarkers in MCI are available (18).

Increased serum or plasma concentrations of hsCRP has been associated with impaired cognition (164-166) and an increased risk of vascular dementia (167) and AD in follow-up studies (168-170) though other studies have failed to confirm these associations (171,172). Elevated serum levels of hsCRP were associated with an increased incidence of AD in the subsequent 25 years, independent of cardiovascular risk factors (169). In large cross-sectional studies, hsCRP associated positively with measures of adiposity such as body mass index (BMI), waist circumference and waist-to-hip ratio (173,174), which in turn may have an effect on neuronal degeneration (126,165,169). Weight loss may be one way to lower elevated hsCRP levels (175).
2.2.8 Physical inactivity
There is some evidence, based on follow-up studies, that regular physical activity has a protective effect against cognitive impairment (176-178) and dementia (94,179,180), and poor cardiorespiratory fitness precedes the cognitive decline (181). In a 1-year randomized controlled trial, persons with AD undertook a simple exercise program, and had a slower decline in daily activities than those with AD and routine medical care (182). Physical activity makes a beneficial contribution to vascular and metabolic risk factors (99,183-189) and atherosclerotic cardiovascular disease (190,191), which in turn may have a crucial role in the prevention and treatment of cognitive impairment and cognitive decline.

2.2.9 Unhealthy nutrition
In follow-up studies, unsaturated fat intake at midlife protected against the risk for cognitive decline, whereas a moderate intake of saturated fats increased the risk (192). A Mediterranean diet had a beneficial effect on cardiovascular risk factors (193) and was associated with a reduced risk for AD (194). In the meta-analyses, fish consumption (195) and fruit and vegetable consumption (196) have had a protective effect on ischemic (195,196) and haemorrhagic (196) stroke. However, a systematic review suggested that, the results were inconclusive with respect to vitamin B₆, or B₁₂ or folic acid supplementation, alone or in combination as a means of maintaining cognitive function (197). Caffeine may stimulate the secretion of acetylcholine, which in turn prevents beta-amyloid-induced neurotoxicity in cerebellar neurons (198), or may increase the antioxidant capacity in plasma. There is limited data about the effects of dietary modifications and cognitive function.

2.2.10 Inappropriate alcohol consumption
Increasing alcohol consumption has been associated with unfavourable metabolic abnormalities such as increased blood pressure, uric acid and mean fasting glucose (199). Long-term heavy alcohol drinking (200) or binge drinking, consumption of a large amount of alcohol in one session (201), has been suggested to lead to dementia. On the contrary, moderate alcohol drinking in middle-age and old age was favourably
related to cognitive function in several cognitive domains in late-life (202) and it appears to decrease the risk of MCI approximately twofold compared to never drinkers (203). Further, patients with MCI who were moderate drinkers had a lower rate of progression to dementia than the non-drinkers (204). No associations were found between any levels of drinking and the incidence of MCI (204). Mild to moderate use of alcohol may decrease the risk for dementia/AD compared to non-use of alcohol (205). Alcohol by itself may have favourable effects on HDL cholesterol level, antioxidant activity, nitric oxide production, endothelial function and platelet aggregation (207). More research is needed to clarify the association between the consequences of alcohol consumption and cognitive function.

2.2.11 Smoking
Smoking may increase the risk for cognitive impairment and dementia (208-211) compared to never-smoking. Some earlier case-control studies did suggest that smoking may be protective for AD, but this may have been biased by a "healthy survivor" effect (212). In voxel-based morphometry, decreased regional gray matter density has been determined in smokers compared to never-smokers (213). Furthermore, smoking is associated with an increased incidence of cardiovascular disease including coronary heart disease and cerebrovascular disease, elevated oxidative stress (212), and all of these would be predicted to impair cognitive function.

2.2.12 Hormonal changes
Several epidemiological studies have suggested that women using hormonal replacing therapy have a lower risk of cognitive impairment and dementia (214). However, randomized controlled trials have not verified these results (215). In a meta-analysis, the use of hormone replacement therapy failed to protect against coronary heart disease (216). There is little data available on the association between hormonal changes and cognitive function in men (217).
2.2.13 Socioeconomic and psychosocial risk factors

The association between education and lower risk of dementia has been reported in several studies. Higher levels of education seem to protect against cognition impairment and delay the clinical manifestation of dementia (218). In addition to physical activity, other leisure-time activities like social activities and mental stimulation in the elderly may also protect against dementia (219,220). These leisure-time activities are often interrelated and being socially active may encourage an individual to be also physically active (221). Education and leisure activities may be important in increasing cognitive reserve, and thereby in the maintenance of cognitive function and the prevention of a cognitive decline (222,223). Depressive symptoms may associate with a pre-clinical phase of AD, but the causal relation with AD is controversial (224). In patients with MCI, depression has increased the risk of developing AD (225). On the other hand, psychosocial factors (226), socio-economic inequalities (227) and depression (228) are suggested to be associated with cardiovascular risk factors and diseases, and that kind of interaction may strengthen the link to cognitive impairment.

2.2.14 Genetic risk factors

Human apolipoprotein E (APOE) has three alleles (ε2, ε3, ε4), with the APOE ε4 allele representing a major genetic risk factor for AD (229,230). The mechanisms relating APOE ε4 allele to AD are still partly unknown. APOE is an important cholesterol transporter, and it has been found in extracellular amyloid deposits and intracellular neurofibrillary tangles in the brain. The APOE ε4 allele is known to strengthen all of the biochemical disturbances which are characteristics of AD, such as beta amyloid deposition, tangle formation, neuronal death, oxidative stress, synaptic plasticity and dysfunction of lipid homeostasis and cholinergic signalling (231). In contrast, the APOE ε2 allele may be protective (233). In the meta-analysis, a role for the APOE genotype in the pathogenesis of some cases of ischemic cardiovascular disease has been postulated (232). In the meta-analysis, a role for the APOE genotype in the pathogenesis of some cases of ischemic cardiovascular disease has been postulated (233). Also several other risk genes for cognitive impairment and AD have been suggested (234), but their impact and role need still to be verified.
2.2.15 Reduced neuroprotective function

The mechanisms that maintain the integrity of nerve cell circuits and facilitate responses to environmental demands include the production of neurotrophic factors (56,235,236). Brain-derived neurotrophic factor (BDNF), a member of the neurotrophins's superfamily, modulates synaptic transmission and plasticity and has a key role in regulating growth, survival, and maintenance of neurons (236-240). BDNF and its receptor tropomyosin-related kinase B (TrkB) are expressed at high levels in hippocampus (236,241), an area that is crucial in memory, learning (242) and the development of AD (8).

While circulating concentrations of BDNF (243,244) and its receptor TrkB (245,246) has decreased, hippocampal BDNF mRNA levels have remained unchanged with increasing age (245,246). Low circulating (247,248) and post-mortem parietal cortex levels of BDNF have been found in patients with MCI (249) and AD (249-251). Furthermore, low BDNF levels have been observed in individuals with type 2 diabetes, (252) the metabolic syndrome, (253) acute coronary syndromes, (254) depression (255) and with physical inactivity, (256-258) all of which have been linked with cognitive impairment and AD (94,104,132).

There is little data available on the link between circulating BDNF and MCI (249) and AD (248,250,251,259) in humans. No data are available of the association between decreased BDNF and cognitive function in general population.

2.3 Summary of the review of literature

As more and more individuals reach advanced age, the occurrence of cognitive impairment and dementia will increase substantially. There were approximately 25 million individuals with dementia in 2000, and by the year 2050, this number is estimated to increase to over 110 million. Dementia seems to be more common in females; this is partly because of females have a longer life expectancy than men, but also other factors (e.g. hormonal, lifestyle-related) may be involved. Evidence has been accumulating during the recent years indicating that cognitive impairment and dementia share many risk factors with cardiovascular disease. However, there is still a lack of data which would clarify the role of vascular risk factors in the development of
cognitive impairment and the mechanisms underlying these associations. Obesity and other cardiovascular risk factors are suggested to be interrelated with lifestyle factors such as physical activity, fitness and nutrition, and thereby with cognition, but the data are limited. Representative long-term studies examining several biological and lifestyle-related risk factors and having data on various cognitive domains are needed to better understand these associations and to identify those individuals who are potentially at increased risk for dementia. Furthermore, randomized controlled trials are required to investigate to what extent risk factor modification can effect cognitive functioning.
3 AIMS OF THE STUDY

The aim of the present thesis was to evaluate associations between vascular and neuroprotective status indicators with regard to the risk of cognitive impairment in ageing men and women. The associations between metabolic syndrome, atherosclerosis, inflammation and cognitive function were studied using specific tests for cognitive function in a 12-year follow-up study in elderly women. The association of a neurotrophic factor with cognitive function was assessed in a representative sample of ageing men and women.

The specific aims of the present study were to investigate:

1) The association of metabolic syndrome and its major components on cognitive function in elderly women in a 12-year follow-up. (Study I)

2) The association of peripheral atherosclerosis and cognitive function in elderly women in a 12-year follow-up. (Study II)

3) The association between serum high sensitivity C-reactive protein and cognitive function in elderly women in a 12-year follow-up. (Study III)

4) The association between brain-derived neurotrophic factor and cognitive function in ageing men and women in a cross-sectional setting. (Study IV)
4 METHODS

4.1 Study Population and design

Studies I-III
The subjects were derived from a population-based, randomly selected sample of 299 women 50 to 60 years of age, initially examined as part of a large population-based risk factor survey, the Finnish part of the WHO MONICA (Monitoring Trends and Determinants in Cardiovascular Disease), in 1982 (Figure 8) (260). The original study sample was stratified separately for men and women by ten year age groups to obtain even age and gender specific groups. The subjects lived in the province of Kuopio, one of the monitoring areas of the Finnish part of the WHO MONICA project. For the baseline examinations of the present study in 1991-1992, we only evaluated women 60 to 70 years of age. Of the 260 women who were invited, a total of 202 women completed the examinations between October 1991 and March 1992. Because 32 women had died (n=27) or could not be contacted (n=5), 170 women 70 to 80 years of age were available for the 12-year follow-up study in 2003. Of 170 women who were invited in the follow-up study, 57 did not participate (Figure 8). All of the 113 women who participated in the study completed the examinations between March 2003 and June 2003 (Figure 8). After excluding the women with missing data on any variable of interest at baseline or after 12 years of follow-up, the final study populations consisted of 91-101 women with complete data (Table 2). A detailed evaluation of cognitive function was performed in 2003. Otherwise, the same methods were used to assess study variables in study years 1991-1992 and 2003. The women who were participated in both study years were included in the analysis. The study protocol was approved by the Ethics Committee of the University of Kuopio. All participants provided a written informed consent.
Figure 8. Formation of study population in the Studies I-III.
Study IV
The subjects were participants of the Dose-Responses to Exercise Training (DR’s EXTRA) Study, which is an ongoing 4-year randomized controlled trial on the effects of regular physical exercise and diet on endothelial function, atherosclerosis and cognition (Figure 9). The study population was a representative random sample of 1500 men and 1500 women 55-74 years of age living in the city of Kuopio in Eastern Finland. Of the initially invited individuals, 81 had died, moved elsewhere or had a serious chronic disease and therefore were not in a position to participate in the study. Furthermore, 210 did not respond to the postal invitation and 647 stated that they were not interested participating in the study. Accordingly, 2062 men and women were willing to participate in the study and 1410 subjects participated in all four baseline examinations between April 2005 and November 2006. Reasons for not participating in the visits were cardiovascular disease (n=33), musculoskeletal disease (n=27), cancer (n=14), dementia (n=8), other diseases (n=75), death (n=33), removal from Kuopio (n=59), loss of motivation (n=207), and unknown reasons (n=196) (Figure 9). After excluding the subjects with missing data on BDNF (n=16) or cognitive function (n=1) and those with type 1 diabetes (n=4), the final study sample consisted of 1389 individuals (684 men, 705 women). The study protocol was approved by the Ethics Committee of the Kuopio University and University Hospital. All participants provided a written informed consent.
Figure 9. Formation of study population in the Study IV.

<table>
<thead>
<tr>
<th>Original subject source of men and women aged 55-74 years in 2002</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n=16 010</strong></td>
</tr>
<tr>
<td>Random sample, invited to the study in 2002</td>
</tr>
<tr>
<td><strong>n=3000</strong></td>
</tr>
<tr>
<td>Willing to participate</td>
</tr>
<tr>
<td>Run-in phase in 2003-2005</td>
</tr>
<tr>
<td><strong>n=2062</strong></td>
</tr>
<tr>
<td>Invited to the baseline examinations in 2005</td>
</tr>
<tr>
<td><strong>n=1829</strong></td>
</tr>
<tr>
<td>Participated in the baseline examinations in 2005-2006</td>
</tr>
<tr>
<td><strong>n=1479</strong></td>
</tr>
<tr>
<td>Randomization</td>
</tr>
</tbody>
</table>

- Death: 20
- Severe disease: 48
- Moved elsewhere: 13
- Not interested: 647
- No response: 210

- Death: 9
- CVD: 3
- Cancer: 2
- Dementia: 3
- Musculoskeletal disease: 4
- Other disease: 27
- Loss of motivation: 59
- Moved elsewhere: 20
- Unknown reasons: 103
- Unreachable: 3

- Death: 24
- CVD: 12
- Cancer: 12
- Dementia: 4
- Musculoskeletal disease: 15
- Other disease: 29
- Loss of motivation: 126
- Moved elsewhere: 38
- Unknown reasons: 84
- Unreachable: 6

- CVD: 18
- Dementia: 1
- Musculoskeletal disease: 8
- Other disease: 19
- Loss of motivation: 22
- Moved elsewhere: 1

Reference  Aerobic Exercise  Resistance Exercise  Dietary  Aerobic +Dietary  Resistance +Dietary
<table>
<thead>
<tr>
<th>Study</th>
<th>Study Population</th>
<th>Exposure</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Women 101</td>
<td>Metabolic syndrome</td>
<td>Risk of poor cognition during 12 years&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>Women 91</td>
<td>Atherosclerosis</td>
<td>Risk of poor cognition during 12 years&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>Women 97</td>
<td>Inflammation</td>
<td>Risk of poor cognition during 12 years&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>Men and Women 1389</td>
<td>Brain-derived neurotrophic factor level</td>
<td>Cognitive function at baseline of the DR's EXTRA Study&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Metabolic syndrome at baseline and cognitive function at 12-year follow-up,

<sup>2</sup> Carotid intima-media thickness at baseline and cognitive function at 12-year follow-up and

<sup>3</sup> Serum high sensitivity C-reactive protein at baseline and cognitive function at 12-year follow-up in a randomly selected cohort based on the Finnish part of the WHO MONICA in 1982.

<sup>4</sup> DR's EXTRA Study; a randomized controlled 4-year trial.
EXAMINATION PROTOCOL

Studies I-III

Baseline visits
Examinations were carried out over four days, at least eight days apart. The examination protocol consisted of a wide variety of examinations including bone mineral density, functional capacity, global cognitive function, depression, biochemistry, anthropometry, carotid atherosclerosis, nutrition, and questionnaires about physical activity, health status, lifestyle and demographic factors.

The study visits and the measurements used in Studies I-III are presented here. The women brought the completed questionnaire of background information which has been mailed two weeks before the survey to the first visit. A study nurse checked the questionnaire at that time. Global cognitive function and depression were assessed. In the second visit, the women provided blood samples for laboratory determinations. The women were instructed to fast 12 hours before these measurements and to abstain from drinking alcohol and using analgesics (except paracetamol) for one week. In the third visit, a trained nurse measured carotid artery IMT, body weight and height, waist and hip circumference and blood pressure.

12-year follow-up visits
Examinations were carried out over two days, at least eight days apart. The examination protocol consisted of a wide variety of examinations including functional capacity, global and detailed cognitive function, depression, biochemistry, anthropometry, carotid atherosclerosis, nutrition, and questionnaires of physical activity, health status, lifestyle and demographic factors. The invitation letter contained general information about the study. Those who did not respond to the invitation letter were phoned to determine the reason for non-participation.

In the first visit, the women gave blood samples for laboratory determinations. Before these measurements the women were instructed to fast for 12 hours and to abstain from drinking alcohol, using analgesics (except paracetamol) for one week. At the same visit, carotid artery ultrasonography was performed, and a self-administered questionnaire of
background information was given to be completed at home. On the second visit, body weight and height, waist and hip circumference and blood pressure were measured. Cognitive function was assessed using a detailed test battery including a screening test of global cognitive function. A nurse received and checked the self-administered questionnaire on the background information.

**Study IV**

Examinations were carried out over three days, approximately seven days apart. The examination protocol consisted of a wide variety of examinations including cardiorespiratory fitness, carotid atherosclerosis, endothelial function, muscle and adipose biopsy, cognitive function, depression, functional capacity, biochemistry, anthropometry, blood pressure, nutrition, and questionnaires about physical activity and background information. The invitation letter contained general information of the study, instructions about the laboratory measurements and instructions how to complete the self-administered questionnaire of background information. Those who did not respond to the invitation letter were phoned to determine the reason for non-participation.

The study visits and the measurements used in the Study IV were as follows: on the first visit, the subjects gave blood samples for laboratory determinations. Before these measurements the subjects were instructed to fast for 12 hours and to abstain from drinking alcohol for three days, from smoking for 12 hours and from intense physical activity for one day. During the same visit, the subjects returned the self-administered questionnaire of background information, and anthropometry and blood pressure were measured. On the second visit, a cognitive test battery including a depression scale was performed, and the questionnaire about background information was checked and completed. On the third visit, the randomization into a reference group and five intervention groups was performed. After these visits, a 2-hour Oral Glucose Tolerance Test (OGTT) was performed in those who met the specific criteria (see 4.6).
4.2 Assessment of Cognition

4.2.1 Studies I-III
Global cognitive function was assessed by the MMSE score (10) at baseline and at 12-year follow-up (Table 3). At follow-up, cognitive function was assessed in more detail using measures of memory and cognitive speed (Table 3). Memory was assessed using the Word Recall Test (261,262) and a prospective memory task (263). Cognitive speed was assessed with the first and second chart of the Stroop Test (264) and with the Letter-digit Substitution Test (265).

4.2.2 Study IV
Cognitive assessments were performed using the CERAD neuropsychological test battery (Table 4) (15), which is designed to assess basic cognitive functions such as memory, language, praxis and general intellectual status affected in AD. Norms are available for the use of CERAD in the Finnish population (266). Trained study nurses performed the tests during the second study visit.
**Table 3.** Individual cognitive tests carried out in Studies I-III

<table>
<thead>
<tr>
<th>Test</th>
<th>Object</th>
<th>Instruction and score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mini-Mental State Examination</td>
<td>To measure global cognitive function including orientation, language, memory, concentration and constructional praxis.</td>
<td>The maximum score is 30.</td>
</tr>
<tr>
<td>Word Recall Test</td>
<td>To measure immediate memory.</td>
<td>Three different word lists, including 10 words matched for word frequency are presented from tape. The score of immediate recall was the total number of correct words in three word lists. The maximum score is 30.</td>
</tr>
<tr>
<td>Prospective Memory Task</td>
<td>To measure prospective memory.</td>
<td>At the beginning of the test session, the subject was asked to remind the investigator that she must sign a paper at the end of the test session. The score was categorized as remembering without reminder (score=4), with one reminder (score=3), with two reminders (score=2) or not remembering (score=1).</td>
</tr>
<tr>
<td>Stroop Test</td>
<td>To measure cognitive speed.</td>
<td>The first and second chart of the 40 item Stroop Test using time needed to read the normal text for colour names and naming of colour dots on a sheet of paper. Higher scores indicate poorer cognitive speed.</td>
</tr>
<tr>
<td>Letter-digit Substitution Test</td>
<td>To measure cognitive speed.</td>
<td>The subject is given an example of letters and corresponding numbers. The subject is asked to copy numbers in blank cells below letters as shown in an example during 60 seconds. The maximum score is number of correctly copied numbers during 60 seconds. Lower scores indicate poorer cognitive speed.</td>
</tr>
<tr>
<td>Test</td>
<td>Object</td>
<td>Instruction and score</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Verbal Fluency Test</td>
<td>To measure semantic memory, verbal production and language.</td>
<td>The subjects were asked to name as many animals (animal category) as possible in 1 minute. The score is the total number of different animals named.</td>
</tr>
<tr>
<td>Modified Boston Naming Test</td>
<td>To measure visual naming.</td>
<td>The subjects were asked to name 15 objects. The items are stratified into three groups of five items each, the names having high, medium, and low frequency of occurrence in the Finnish language. The maximum score is 15.</td>
</tr>
<tr>
<td>Mini-Mental State Examination</td>
<td>To measure global cognitive function including orientation, language, memory, concentration and constructional praxis.</td>
<td>The maximum score is 30.</td>
</tr>
<tr>
<td>Word List Memory</td>
<td>To measure ability to remember newly learned information.</td>
<td>The subjects are presented with 10 unrelated items to remember on printed cards. The subjects are instructed to read aloud each word as it is presented. Immediately following presentation of each 10 words, the subject is asked to recall as many items as possible. On each of three learning trials, the 10 words are presented in a different order. The maximum score on each trial is 10. The maximum total score is 30.</td>
</tr>
<tr>
<td>Constructional Praxis</td>
<td>To measure visuo-spatial and constructional ability.</td>
<td>Four line drawings of figures of increasing complexity (circle, diamond, overlapping rectangles and cube) are presented to the subject for copying. At the end of the session, the subject is asked to make a mental note of the objects, because later they will be asked to draw them without model. The maximum score is 11. For each subject, a saving score can be calculated and is presented as a percentage reflecting the delayed ability to draw the objects without model ( \frac{\text{copy from object/delayed drawing}}{100} ).</td>
</tr>
<tr>
<td>Test</td>
<td>Description</td>
<td>Methodology</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Word List Recall</td>
<td>To measure delayed memory.</td>
<td>After 5 minutes for the Word List Memory task the subject is asked to remind as many words as possible. A maximum of 90 seconds is allowed. A maximum number of correct responses is 10. For each subject, a saving score can be calculated and is presented as a percentage reflecting the relative amount of verbal information retained over the delay interval ([\text{delayed words/Trial 3 words from Word list memory}] \times 100].</td>
</tr>
<tr>
<td>Word List Recognition</td>
<td>To measure correctly recognized words presented in the Word List Memory task.</td>
<td>The words are presented among 10 distracting words. The number of correctly identified distracting words is also counted. The maximum score for each is 10.</td>
</tr>
<tr>
<td>Clock Drawing</td>
<td>To measure executive function.</td>
<td>The subject is asked to draw a clock with all the numbers, and set the hands at ten past eleven. The maximum score is 6.</td>
</tr>
</tbody>
</table>

CERAD; The Consortium to Establish a Registry for Alzheimer’s Disease neuropsychological test battery (15).
4.3 Assessment of blood pressure
Blood pressure was recorded using a mercury sphygmomanometer from the right arm in a sitting position after a five minutes rest. Two independent consecutive measurements of systolic and diastolic blood pressure were taken and the mean of the measurements was used in the analyses.

4.4 Assessment of serum lipids and lipoproteins
All venous blood samples were taken after a 12 hour fast by 10.00 a.m. without stasis. Serum total cholesterol (CHOD-PAP, Roche Diagnostics GmbH, Mannheim, Germany (Studies I-III) and Cholesterol, Thermo Electron Corporation, Finland (Study IV)) and triglycerides (GPO-PAP, Thermo Clinical Labsystems Oy, Finland (Studies I-III) and Triglycerides, Thermo Electron Corporation, Finland (Study IV)) were measured by enzymatic photometric methods. LDL cholesterol was calculated according to the Friedewald formula (267) (Studies I-III) and measured by an enzymatic photometric method (Study IV). HDL cholesterol was measured by an enzymatic photometric method (CHOL CHOD-PAP, Roche Diagnostics GmbH) in supernatant after precipitation with dextran sulphate and MgCl\(_2\) (Studies I-III) and using a direct enzymatic photometric method (Thermo Electron Corporation, Finland) (Study IV). KONE Pro clinical chemistry analyzer (KONE, Finland) (Studies I-III) and KONELAB 20XTi clinical chemistry analyzer (KONE, Finland) (Study IV) was used.

4.5 Anthropometric measurements
Anthropometric measurements were performed based on WHO MONICA protocol (268), with the subject in light indoor clothing without shoes. Waist circumference (to the nearest 0.5 cm) was measured on bare skin, at the mid-distance between the bottom of the rib cage and the top of the iliac crest. Hip circumference (to the nearest 0.5 cm) was measured at the level of the trochanter majors. Subjects stood with their feet 12 cm apart with their weight equally distributed on each leg. The mean of two measurements of the circumferences was used in the analyses. In the 84 participants, the intra-class correlation for two study nurses between the mean of two waist assays was 0.995 (P<0.0001) and between mean of two hip assays 0.981 (P<0.0001) during the same
study visit in 2003 (Studies I-III). The coefficient of variation for these measures was
0.7% for waist and 0.5% for hip. Body weight with a 0.1 kg precision was measured
using a digital scale. Body height was measured with a 0.1 cm precision using a metal-
scaled height meter. BMI was calculated as weight divided by height squared (kg/m²).

4.6 Assessment of glucose tolerance and definition of diabetes

After a 12 hour fast, the hexokinase method (Glucose, Thermo Clinical Labsystems Oy,
Espoo, Finland) was used for blood glucose analyses (Studies I-III) or plasma glucose
analyses (Study IV). In Study IV, oral post-challenge glucose tolerance test (OGTT)
with a 75 g glucose load was carried out in the morning by 10.00 a.m. Subjects with no
previously diagnosed type 2 diabetes underwent the OGTT. The blood samples were
taken in the fasting state, and at 30, 60 and 120 minutes after the glucose load, the
maximum deviation allowed was ±3 minutes. In addition to a 12 hour fast, the subjects
were instructed to abstain from alcohol intake for three days, heavy physical activity for
one day and smoking for 12 hours before the examination. They were asked to arrive
the study center by bus/ car.

Subjects were classified into 4 groups according to their glucose tolerance status using
the WHO criteria (269). Those with normal glucose tolerance (NGT) had a fasting
plasma glucose level <6.1 mmol/l and a 2-hour post-challenge glucose level <7.8
mmol/l. Those with IFG had a fasting plasma glucose level between 6.1 mmol/l and 6.9
mmol/l and a 2-hour post-challenge glucose level <7.8 mmol/l. Those with IGT had a
fasting plasma glucose level <7.0 mmol/l and a 2-hour post-challenge glucose level
between 7.8 and 11.1 mmol/l. Subjects were classified as having type 2 diabetes if they
reported having diabetes diagnosed by a physician or had a fasting plasma glucose level
≥7.0 mmol/l or a 2-hour post-challenge glucose level ≥11.1 mmol/l. The subjects with
type 1 diabetes or type 2 diabetes, or who refused to participate in the OGTT (n=201)
were classified as having NGT if the fasting plasma glucose level was <6.1 mmol/l or
IFG if the fasting plasma glucose level was 6.1 to 6.9 mmol/l or type 2 diabetes if they
reported having type 2 diabetes diagnosed by a physician or their fasting plasma glucose
level was ≥7.0 mmol/l.
4.7 Definition of metabolic syndrome

The metabolic syndrome was defined by the National Cholesterol Education Program criteria (270), summing up the category scores (0=low risk, 1=increased risk) for blood pressure, blood/plasma glucose, HDL cholesterol, triglycerides and waist circumference (Table 5). The existence of at least three risk factors was defined as the metabolic syndrome (270) (Table 5).

Table 5. Individual components of the metabolic syndrome defined by NCEP

<table>
<thead>
<tr>
<th>Component</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure</td>
<td>$\geq 130/85$ mm Hg and/or use of antihypertensive medication</td>
</tr>
<tr>
<td>Blood/Plasma glucose</td>
<td>$\geq 6.1$ mmol/l</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>$&lt;1.29$ mmol/l in women or $&lt;1.03$ mmol/l in men</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>$\geq 1.7$ mmol/l</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>$&gt;88$ cm in women or $&gt;102$ cm in men</td>
</tr>
</tbody>
</table>

NCEP; the National Cholesterol Education Program criteria (270).

4.8 Assessment of carotid atherosclerosis

Carotid artery atherosclerosis was assessed noninvasively by B-mode ultrasonography (185,271). Certified sonographers measured carotid artery IMT, while being scanned the subject was in the supine position, with the head turned 45° from the side. An ultrasound device with a high-resolution 10-MHz transducer was used, adhering to a standardized and pretested protocol. At each examination, the sonographer used three different standardized scanning angles including lateral (45° from vertical axis), anterior (20-30°) and posterior (50-60°) projection. The scannings were recorded on super VHS videotape. The same sonographer read all the scannings in both study years. The sonographer measured carotid IMT of the far wall of the right and left carotid arteries. The carotid IMT measurement extended to the longest wall region where the lumen-intima and media-adventitia boundaries could be clearly identified by an automated edge-detection program on the basis of the active contour (272). We used the mean of
left and right carotid bifurcation IMT in the statistical analyses, because arterial bifurcations are prone to local plaque formation (138).

4.9 Assessment of low-grade inflammation
Serum hsCRP concentrations were measured by a commercial immunoassay (IMMULITE 2000 High-Sensitivity CRP, Diagnostic Products Corp., Los Angeles, CA, USA) using the IMMULITE 2000 Analyzer (Diagnostic Products Corp.). In the 97 participants, the intra-class correlation between two hsCRP assays during the same study visit was >0.999 (P<0.0001) at baseline and >0.997 (P<0.0001) at 12-year follow-up. The coefficient of variation was 1.7% at baseline and 3.6% at 12-year follow-up. Blood samples for hsCRP were taken at baseline and 12-year follow-up and were stored at -80°C until analysis in 2005.

4.10 Assessment of lifestyle factors
Alcohol consumption (drinks/ previous week) (Studies I-IV), smoking (no vs. yes) (Studies I-III) and (never/ former/ current) (Study IV) and physical activity which lasts at least 30 minutes per session and causes breathlessness and sweating (sessions/ week) (Studies I-III) were assessed using a self-administered questionnaire.

4.11 Assessment of diseases, medications and socioeconomic status
Studies I-III
The women completed a self-administered questionnaire on diseases diagnosed by a physician in the previous year (no vs. yes) and medications in a previous week (no vs. yes). A subject was considered to have prevalent cardiovascular disease (CVD) if she reported coronary heart disease (CHD), cardiac insufficiency and ischemic or haemorrhagic cerebrovascular stroke (Studies I and III) or CHD and cardiac insufficiency (Study II). A subject was considered to have prevalent CHD if she reported to have angina pectoris or myocardial infarction. The use of hormone replacement therapy (no vs. yes) and education (years) were enquired at baseline. Depressive symptoms were assessed using the Zung self-report 20-item scale (273). The
item scores were summed up with a maximum score of 80 points, >50 points indicating depressive symptoms (273).

**Study IV**
The subjects completed a self-administered questionnaire on diseases diagnosed by a physician (no vs. yes) and medications. A subject was considered to have prevalent CVD if he/she reported CHD, (ischemic or haemorrhagic) cerebrovascular stroke, transient ischemic attack, cardiac insufficiency, arrhythmia or lower extremity peripheral artery disease. A subject was considered to have prevalent CHD if he/she reported having angina pectoris, myocardial infarction, bypass coronary artery surgery or percutaneous transluminal coronary artery angioplasty. The use of hormone replacement therapy (no vs. yes) and education (years) were ascertained. Depressive symptoms were assessed using the 20-item scale, the Center of Epidemiological Studies Depression Scale (CES-D) (274). The item scores were summed up with a maximum score of 60 points, ≥16 points indicating depressive symptoms (274).

4.12 **Assessment of brain-derived neurotrophic factor**
Blood samples were drawn by 10.00 a.m. into glass tubes containing EDTA, which were spun immediately at 3000 x g for 10 min. Plasma was isolated and stored at -80 °C until analyzed. Plasma concentrations of BDNF were measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA). After thawing, samples were centrifuged at 10000 x g for 10 min at 4 °C for complete platelet removal. Samples were analyzed in duplicate and mean concentrations were calculated. A coefficient of variability in duplicates ≤10% was considered acceptable. The interassay coefficient of variability was lower than 13%.

4.13 **Statistical methods**
In Studies I-III, the scores of memory and cognitive speed were converted to standardized z-scores. In standardized z-scoring, the individual values of the variable are scaled so that the mean is zero and standard deviation is 1. Z-scoring has no effect on the distribution of the variable or its association with other variables. The z-values
for the Letter-digit Substitution Test were reversed. A sum of z-scores from the Word Recall Test and the prospective memory test was computed to describe memory. Similarly, the sum of z-scores from the Letter-digit Substitution Test and the Stroop Test was used to describe cognitive speed. The MMSE score of ≥25 was defined as good general cognition. This cut-off point was used in the analyses. The confounding variables were chosen for the analyses based on their known association with dependent and independent variables as well as to avoid co-linearity. In all studies, the level of significance was set at P<0.05. The analyses were conducted using the SPSS for Windows, Release 11.5 (SPSS Inc., Chicago, IL).

4.13.1 Study I
For statistical analyses, z-scores were dichotomised at median (0.115 for memory and -0.266 for cognitive speed), and a "good" memory was defined as a score above the median and a "good" cognitive speed was defined as a score below the median. Differences in characteristics of women with or without metabolic syndrome were analysed using Independent-Samples t-test or $\chi^2$-test as appropriate. Analysis of covariance was used to test for an association between the metabolic risk factors at baseline and memory, cognitive speed and the MMSE score at 12-year follow-up. The associations of metabolic syndrome and single metabolic risk factors at baseline and at 12-year follow-up with the risk of having poor memory, poor cognitive speed, and low MMSE score were analysed using multiple logistic regression analysis. Data were first adjusted for age, education and depression, and further for hormone replacement therapy, BMI and prevalent cardiovascular disease, including coronary heart disease, cardiac insufficiency and stroke.

4.13.2 Study II
Differences in the basic characteristics at baseline and at the 12-year follow-up were analyzed using Independent-Samples t-test or $\chi^2$-test, and changes between study years by using Paired-Samples t-test or McNemar-test as appropriate. Correlation coefficients were calculated. The association between IMT and cognitive functions were analyzed separately with linear regression analysis. The association across IMT tertiles and
memory or cognitive speed was tested using analysis of covariance, and a linear trend
between these variables was tested using multivariate linear regression analysis. Since
the distribution of MMSE was skewed, even after logarithmic transformation, the
association between IMT and MMSE was studied using logistic regression analysis.
The women who had a stroke were excluded from the analyses. The analyses were
adjusted for age, education, depression, type 2 diabetes, plasma LDL cholesterol,
systolic blood pressure, prevalent cardiovascular disease (coronary heart disease,
cardiac insufficiency), the use of hormone replacement therapy, physical activity,
alcohol consumption and smoking.

4.13.3 Study III
Serum hsCRP concentration was categorized as low (<1.0 mg/L), average (1.0 to 3.0
mg/L), and high (>3.0 mg/L) according to the statement for healthcare professionals
from the Centers for Disease Control and Prevention and the American Heart
Association (157). Differences in the characteristics of women with low, average or
high hsCRP levels were assessed using analysis of variance or $\chi^2$-test as appropriate.
The associations of hsCRP with memory and cognitive speed were analysed with linear
regression analyses. The differences in the means of memory among the hsCRP tertiles
were tested using analysis of covariance, and the linear trend across the tertiles was
derived from linear regression analysis. Analyses were first adjusted for age, education,
depression (model 1), and then additionally for the use of hormone replacement therapy,
smoking and LDL cholesterol and BMI (model 2). However, we re-ran the analyses
controlling also for fasting blood glucose (correlated significantly with LDL
cholesterol) and systolic blood pressure (correlated significantly with hormone
replacement therapy) at baseline. Since the distribution of hsCRP was skewed at
baseline and 12-year follow-up, logarithmic transformation was used. Since the
distribution of MMSE was skewed, even after logarithmic transformation, logistic
regression analysis was used.
4.13.4 Study IV

Since the distribution of BDNF was skewed, log-transformation was used. The distributions of individual tests of the CERAD test battery were skewed even after log-transformation.

Differences in the characteristics between men and women were analysed using Independent-Samples t-test or \( \chi^2 \)-test as appropriate. P-value for difference between men and women is derived from log transformed data as appropriate. Based on recent norms in a Finnish population, the individual tests of the CERAD were dichotomized at the lowest decile (266). The associations of BDNF with the individual tests of the CERAD were analysed separately in men and women using logistic regression analysis because of the skewed distributions of the tests even after log-transformation. Data are presented as odds ratios, Nagelkerke R-squares (ability of the model to explain the dependent variable), Wald's test quantity (explain whether odds ratios vary significantly from 1) and P-values. The data were adjusted for age, education, depression, impaired glucose metabolism (IFG, IGT, type 2 diabetes), cardiovascular disease (coronary heart disease, stroke, transient ischemic attack, cardiac insufficiency, arrhythmias, lower extremity peripheral artery disease), antihypertensive medication, lipid lowering medication, the use of sex hormones in women, smoking, and alcohol consumption. In further analyses, the data were adjusted also for waist circumference.
5 RESULTS

5.1 Characteristics of the study population

5.1.1 Studies I–III

Basic and clinical characteristics of the 113 women at baseline and at 12-year follow-up are presented in Table 6. For further analyses, the participants were categorized according to whether or not they had the metabolic syndrome (Study I), with or without data of carotid intima-media thickness (Study II) and levels of high sensitivity CRP (Study III) at baseline and at follow-up. The mean duration of formal education was 8.3 years. At baseline, none of the women had diabetes, and during the 12-years the prevalence of type 2 diabetes increased from 0 to 12 cases. Of the women, 27%, used hormone replacement therapy.

The non-participants (n=89) were older (65.6 vs. 63.9 years, P<0.001) and less educated (7.0 vs. 8.5 years, P=0.004) and had lower MMSE score (27.5 vs. 28.6 points, P=0.002), higher weight (72.4 vs. 67.9 kg, P=0.003) and BMI (29.2 vs. 27.4, P=0.004), greater waist circumference (88.0 vs. 83.4 cm, P=0.002) and greater hip circumference (102.0 vs. 98.8 cm, P=0.025), lower HDL cholesterol (1.5 vs. 1.6 mmol/l, P=0.015), higher triglycerides (1.6 vs. 1.2 mmol/l, P=0.008), systolic blood pressure (161.3 vs. 155.5 mm Hg, P=0.052) and diastolic blood pressure (91.9 vs. 88.5 mm Hg, P=0.023), as well as more type 2 diabetes (5 vs. 0%, P=0.023) and metabolic risk factors (2.1 vs. 1.6, P<0.001) at baseline than the participants.
<table>
<thead>
<tr>
<th></th>
<th>At baseline</th>
<th>At follow-up</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>63.9 (3.1)</td>
<td>75.3 (3.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Memory, z-value*</td>
<td>0.061 (2.71)</td>
<td>0.061 (2.71)</td>
<td></td>
</tr>
<tr>
<td>Cognitive speed, z-value *</td>
<td>-0.059 (2.33)</td>
<td>-0.059 (2.33)</td>
<td></td>
</tr>
<tr>
<td>MMSE**, points†</td>
<td>28.6 (1.8)</td>
<td>25.8 (2.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>67.9 (10.7)</td>
<td>68.4 (12.1)</td>
<td>0.354</td>
</tr>
<tr>
<td>Height, cm</td>
<td>157.6 (5.3)</td>
<td>155.5 (5.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>83.4 (10.6)</td>
<td>91.8 (11.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip circumference, cm</td>
<td>98.8 (10.3)</td>
<td>102.9 (8.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index**</td>
<td>27.4 (4.4)</td>
<td>28.1 (4.9)</td>
<td>0.002</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>4.8 (0.6)</td>
<td>5.3 (1.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum total cholesterol, mmol/l</td>
<td>6.2 (1.0)</td>
<td>5.3 (0.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum LDL** cholesterol, mmol/l</td>
<td>4.1 (0.9)</td>
<td>3.5 (0.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum HDL** cholesterol, mmol/l</td>
<td>1.6 (0.3)</td>
<td>1.3 (0.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/l</td>
<td>1.21 (0.4)</td>
<td>1.17 (0.5)</td>
<td>0.399</td>
</tr>
<tr>
<td>Serum high sensitivity C-reactive protein, mg/l ‡</td>
<td>2.7 (7.24)</td>
<td>2.4 (3.35)</td>
<td>0.643</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>155.5 (21.9)</td>
<td>141.7 (19.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>88.5 (10.1)</td>
<td>71.9 (9.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of stroke, %</td>
<td>4</td>
<td>8</td>
<td>0.063</td>
</tr>
<tr>
<td>Prevalent cardiovascular disease**, %</td>
<td>19</td>
<td>29</td>
<td>0.008</td>
</tr>
<tr>
<td>Prevalent depressive symptoms, %</td>
<td>1</td>
<td>3</td>
<td>0.625</td>
</tr>
<tr>
<td>Use of antihypertensive medication, %</td>
<td>24</td>
<td>55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Use of lipid lowering medication, %</td>
<td>6</td>
<td>34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical activity, ≥2 times/week, %</td>
<td>63</td>
<td>88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td>7</td>
<td>4</td>
<td>0.219</td>
</tr>
<tr>
<td>Alcohol consumption, drinks/week</td>
<td>0.6 (1.3)</td>
<td>0.7 (1.6)</td>
<td>0.685</td>
</tr>
<tr>
<td>Carotid artery intima-media thickness, mm §</td>
<td>1.03 (0.3)</td>
<td>1.25 (0.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Metabolic risk factors, NCEP**, sum</td>
<td>1.5 (0.9)</td>
<td>2.4 (1.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Metabolic syndrome, NCEP**, %</td>
<td>13</td>
<td>48</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means (± SD) or percentages (%) and Paired-Samples t-test, Wilcoxon-test, Sci-square-test or McNemar-test was used as appropriate.

* Data were available for 109 women, † 104 women, ‡ 107 women and P-value for difference is derived from log transformed data, and § 59 women.

**MMSE, Mini-Mental State Examination; Body mass index, calculated as weight divided by height squared (kg/m²); LDL, Low-density lipoprotein; HDL, High-density lipoprotein; Cardiovascular disease includes coronary heart disease (angina pectoris, myocardial infarction), cardiac insufficiency; NCEP, the National Cholesterol Education Program criteria (270).
5.1.2 Study IV

Basic and clinical characteristics of the participants who attended all three baseline visits are presented in Table 7. The women had higher plasma BDNF levels (1735.9 (± SD 1466.2) vs. 1494.7 (± SD 1448.0) pg/ml, P<0.001) compared to men. Data for BDNF levels were available for 687 men and 707 women.

The non-participants (those who dropped out during baseline examinations n=69) were older (69.7 vs. 66.5 years, P<0.001), less educated (8.9 vs. 11.2 years, P<0.001), had lower MMSE score (26.0 vs. 27.5 points, P<0.001), height (163.2 vs. 166.7 cm, P=0.020), and serum HDL cholesterol level (1.5 vs. 1.7 mmol/l, P=0.003), greater weight (81.9 vs. 77.1 kg, P=0.049), waist circumference (102.2 vs. 93.9 cm, P<0.001), hip circumference (107.4 vs. 100.9 cm, P<0.001) and BMI (30.7 vs. 27.7, P<0.001), higher plasma glucose level (6.3 vs. 5.8 mmol/l, P=0.003) and serum triglyceride level (1.6 vs. 1.3 mmol/l, P=0.028) and more metabolic risk factors (2.4 vs. 1.9, P=0.001), depressive symptoms (34.6 vs. 13.2%, P<0.001), coronary heart disease (32.3 vs. 14.7%, P<0.001) and stroke (13.1 vs. 4.1%, P=0.001), worse smoking status (never 40.3 vs. 54.6%, former 35.5 vs. 34.7%, current 24.2 vs. 10.7%, P=0.003) and glucose tolerance (normal glucose tolerance 52.2 vs. 65.8%, impaired fasting glucose 24.6 vs. 10.4%, impaired glucose tolerance 1.4 vs. 10.8% and diabetes mellitus 21.7 vs. 13.0%, P<0.001) and they were less likely to use hormone replacement therapy (10.0 vs. 26.4%, P=0.004) than the participants. In individual tests of the CERAD battery, the non-participants performed worse in Verbal Fluency Test (20.8 vs. 24.0 words/minute, P=0.001), Word Naming Test (12.4 vs. 13.2 words/ out of 15 words, P=0.006), MMSE Score (26.0 vs. 27.5 points, P<0.001), Word List Learning Score (19.6 vs. 21.8 words/ out of 30 words, P<0.001), Constructional Praxis Score (9.1 vs. 9.6 points, P=0.001), Word List Recall Score (6.3 vs. 7.2 words/ out of 10 words, P=0.001), Word List Saving Score (80.3 vs. 85.5%, P=0.020), and Clock Drawing Test (5.0 vs. 5.3 points, P=0.042) than the participants.
Table 7. Basic and clinical characteristics of the 1410 men and women

<table>
<thead>
<tr>
<th></th>
<th>Men (n=696)</th>
<th>Women (n=714)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>66.3 (5.4)</td>
<td>66.6 (5.3)</td>
<td>0.259</td>
</tr>
<tr>
<td>Education, years</td>
<td>11.1 (3.9)</td>
<td>11.3 (3.8)</td>
<td>0.122</td>
</tr>
<tr>
<td>MMSE*, points</td>
<td>27.6 (2.0)</td>
<td>27.5 (2.0)</td>
<td>0.349</td>
</tr>
<tr>
<td>Height, cm</td>
<td>173.6 (6.1)</td>
<td>159.9 (5.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>83.6 (13.7)</td>
<td>70.7 (13.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index*</td>
<td>27.7 (4.0)</td>
<td>27.6 (5.0)</td>
<td>0.811</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>99.0 (11.2)</td>
<td>88.9 (13.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip circumference, cm</td>
<td>99.8 (8.0)</td>
<td>102.0 (9.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose tolerance status, %</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normal glucose tolerance</td>
<td>59</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
<td>14</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>11</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus*</td>
<td>16</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/l</td>
<td>6.0 (1.1)</td>
<td>5.7 (0.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum total cholesterol, mmol/l</td>
<td>4.9 (0.9)</td>
<td>5.2 (0.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum LDL* cholesterol, mmol/l</td>
<td>3.2 (0.8)</td>
<td>3.2 (0.8)</td>
<td>0.238</td>
</tr>
<tr>
<td>Serum HDL* cholesterol, mmol/l</td>
<td>1.5 (0.4)</td>
<td>1.9 (0.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/l</td>
<td>1.4 (0.8)</td>
<td>1.3 (0.6)</td>
<td>0.087</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>145.3 (18.8)</td>
<td>150.2 (21.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>84.2 (9.3)</td>
<td>82.4 (9.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Metabolic risk factors, NCEP*, sum</td>
<td>1.9 (1.1)</td>
<td>1.9 (1.1)</td>
<td>0.387</td>
</tr>
<tr>
<td>Alcohol consumption, drinks/ week</td>
<td>6.4 (8.2)</td>
<td>2.2 (4.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Never</td>
<td>35</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>51</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>14</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>History of stroke, %</td>
<td>5</td>
<td>4</td>
<td>0.525</td>
</tr>
<tr>
<td>Prevalent hypertension, %</td>
<td>45</td>
<td>47</td>
<td>0.463</td>
</tr>
<tr>
<td>Prevalent cardiovascular disease*, %</td>
<td>54</td>
<td>46</td>
<td>0.007</td>
</tr>
<tr>
<td>Prevalent depressive symptoms, %</td>
<td>11</td>
<td>15</td>
<td>0.020</td>
</tr>
<tr>
<td>Use of sex hormones, %</td>
<td>4</td>
<td>49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Use of antihypertensive medication, %</td>
<td>35</td>
<td>35</td>
<td>0.810</td>
</tr>
<tr>
<td>Use of lipid lowering medication, %</td>
<td>41</td>
<td>43</td>
<td>0.610</td>
</tr>
</tbody>
</table>

Values are means (± SD) or percentages (%) and Independent-Samples t-test, Mann-Whitney U-test or Sci-square-test was used as appropriate. *MMSE, Mini-Mental State Examination; Body mass index, calculated as weight divided by height squared (kg/m²); Diabetes mellitus, type 2 diabetes including 4 individuals with type 1 diabetes; LDL, Low-density lipoprotein; HDL, High density lipoprotein; NCEP, the National Cholesterol Education Program criteria; Cardiovascular disease includes coronary heart disease, stroke, transient ischemic attack, cardiac insufficiency, arrhythmia, lower extremity peripheral artery disease.
5.2 Metabolic syndrome and cognitive function (Study I)

At baseline, 58% of the women had one, 27% had two, 9% had three and 4% had four metabolic risk factors. There was no statistically significant difference in numbers of smokers (P=0.286) or users of hormone replacement therapy (P=0.073) between women with the metabolic syndrome and those without it. The women with or without the metabolic syndrome did not differ in terms of age, education, depression, the MMSE score, alcohol consumption, or prevalence of cardiovascular disease.

At 12-year follow-up, 23% of the women had one, 27% had two, 36% had three, 11% had four, and 2% had five metabolic risk factors. Women with the metabolic syndrome were less likely to be physically active (P=0.019) and tended to have a poorer memory (P=0.083) than those without it. The prevalence of metabolic syndrome increased from 13% at baseline to 49% at follow-up (P<0.001).

In longitudinal analyses, the presence of the metabolic syndrome at baseline associated with poor memory at 12-year follow-up (Figure 10A). The number of metabolic risk factors at baseline associated directly with memory at 12-year follow-up adjusted for baseline age, education and depression (Figure 11). Further adjustment for hormone replacement therapy did not alter the association (P=0.038 for trend). Women with the metabolic syndrome at baseline had a 4.27 (95% confidence interval 1.02 to 17.90; P=0.047) times higher risk of having poor memory at the follow-up than women without it after these adjustments. Further adjustment for hormone replacement therapy did not have any effect on the relationship (odds ratio 4.12, 95% confidence interval 0.97 to 17.53; P=0.056). Furthermore, adjustment for lipid lowering medication or the MMSE score did not change the results (result not shown). The presence of the metabolic syndrome at baseline was not significantly related with poor cognitive speed at follow-up (odds ratio 1.92, 95% confidence interval 0.54 to 6.82, P=0.319) or with the MMSE score (odds ratio 0.19, 95% confidence interval 0.02 to 1.58, P=0.123).

In the cross-sectional analyses at 12-year follow-up, women with the metabolic syndrome had a 2.47 (95% confidence interval 1.05 to 5.85; P=0.039) times higher risk of having poor memory than those without it after adjustment for age, education and depression. Further adjustment for hormone replacement therapy did not alter the association (odds ratio 2.45, 95% confidence interval 1.03 to 5.80, P=0.042).
syndrome was not significantly associated with poor cognitive speed (odds ratio 0.96, 95% confidence interval 0.41 to 2.22, \( P=0.921 \)) or the MMSE score (odds ratio 1.79, 95% confidence interval 0.67 to 4.78, \( P=0.245 \)).

With respect to the single metabolic risk factors, a low HDL cholesterol level at baseline was associated with poor memory at 12-year follow-up after adjustment for age, education and depression (Figure 10B). When all components of the metabolic syndrome were entered simultaneously as continuous variables into a logistic regression model with these covariates, only HDL cholesterol was independently associated with memory. The risk of having poor memory increased by 46.5% (95% confidence interval 15% to 66%, \( P=0.008 \)) with every 1 SD decrease in HDL cholesterol level. The magnitude of the association between HDL cholesterol and memory was unchanged after further adjustment for hormone replacement therapy, BMI and cardiovascular disease (result not shown). An increased waist circumference (odds ratio 2.00, 95% confidence interval 0.77 to 5.22, \( P=0.154 \)) and higher triglyceride levels (odds ratio 2.78, 95% confidence interval 0.73 to 10.62, \( P=0.136 \)) at baseline tended to be associated with poor memory at follow-up.
Figure 10. Memory (z-score) at 12-year follow-up in 101 elderly women with (n=88) or without (n=13) the metabolic syndrome and with lower (n=15) or higher (n=86) serum HDL cholesterol values at baseline adjusted for age, education and depression.

Figure 11. Longitudinal association between number of metabolic risk factors (0-1 risk factors, n=61; 2 risk factors, n=27; ≥3 risk factors, n=13) at baseline and memory at 12-year follow-up adjusted for age, education and depression.
5.3 Atherosclerosis and cognitive function (Study II)

The mean carotid IMT was 1.02 mm at baseline and 1.25 mm at follow-up (mean increase 0.23 mm, P<0.001). At baseline, carotid IMT correlated with prevalent hypertension (r=0.285, P=0.052). During the follow-up, the prevalences of hypertension (P<0.001) and cardiovascular disease increased (P<0.007) while the prevalence of hypercholesterolemia decreased (P<0.011). At 12-year follow-up, carotid IMT correlated with prevalent hypertension (r=0.313, P=0.032) and the use of drug treatment for hypertension (r=0.336, P=0.021).

In cross-sectional analyses at 12-year follow-up, increased carotid IMT was associated with poor memory (Figure 12). On average, memory decreased by -0.275 standard deviations (SD) for every 0.1 mm increase in carotid IMT (Table 8). The association remained statistically significant after adjustment for all confounders (Table 8). The risk for poor memory increased with increasing carotid IMT tertiles after all adjustments (P=0.076 for linear trend). There was no association between carotid IMT and cognitive speed (Table 8) or MMSE score at 12-year follow-up (odds ratio 2.6, 95% confidence interval 0.4 to 15.3).
Figure 12. Cross-sectional association between carotid intima-media thickness (IMT) and memory at 12-year follow-up in 91 women.

Table 8. Cross-sectional associations between increased carotid intima-media thickness (IMT) and risk of poor memory or cognitive speed at 12-year follow-up in 91 women

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>95% CI</td>
<td>P-value</td>
<td>β</td>
</tr>
<tr>
<td>Memory</td>
<td>-2.751</td>
<td>-4.58- -0.92</td>
<td>0.004</td>
<td>-2.054</td>
</tr>
<tr>
<td>Cognitive speed</td>
<td>1.177</td>
<td>-0.48-2.84</td>
<td>0.162</td>
<td>0.779</td>
</tr>
</tbody>
</table>

Data are from linear regression analyses. β denotes standardized regression coefficient and 95% CI denotes 95% confidence interval.
Model 1: Unadjusted
Model 2: adjustments for age, education, depression, type 2 diabetes, low-density lipoprotein cholesterol, systolic blood pressure, cardiovascular disease (coronary heart disease, cardiac insufficiency), hormone replacement therapy, physical activity, alcohol consumption and smoking at the time of carotid IMT measures.
Memory; sum of z-scores from the Word Recall Test and the prospective memory test.
Cognitive speed; sum of z-scores from the Letter-digit Substitution Test and the Stroop Test.
In the longitudinal analyses, increased carotid IMT was associated with poor memory (Figure 13). Increased carotid IMT at baseline predicted poor memory and cognitive speed at 12-year follow-up (Table 9). On average, memory decreased by -0.500 SD and cognitive speed by 0.256 SD for every 0.1 mm increase in carotid IMT. The magnitude of the association between carotid IMT and memory or cognitive speed was unchanged after adjustment for all confounders (Table 9). Further adjustment for baseline MMSE score did not change the associations (P=0.001 for memory and P=0.021 for cognitive speed). In this case also, the risk for poor memory (P=0.023 for linear trend) and cognitive speed (P=0.070 for linear trend) increased with increasing carotid IMT tertiles after all adjustments. With respect to memory, the difference between the lowest and the highest tertile was borderline significant (P=0.062) and the difference between the second and the highest tertile was significant (P=0.010) after all adjustments. There was no association between carotid IMT and the MMSE score (odds ratio 4.6, 95% confidence interval 0.1 to 150.4). There was no association between carotid IMT at baseline and the change of the MMSE score (standardized regression coefficient β -1.808, 95% confidence interval -4.82 to 1.20, P=0.230) after all adjustments including baseline MMSE.
Figure 13. Longitudinal association between carotid intima-media thickness (IMT) at baseline and memory at 12-year follow-up in 47 women.

Table 9. Longitudinal associations between increased carotid intima-media thickness (IMT) at baseline and risk of poor memory or cognitive speed at 12-year follow-up in 47 women

<table>
<thead>
<tr>
<th></th>
<th>Mode 1</th>
<th></th>
<th>Model 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta ) 95% CI P-value</td>
<td>( \beta ) 95% CI P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memory</td>
<td>-5.004 -7.74- -2.27 0.001</td>
<td>-4.851 -7.81- -1.89 0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cognitive speed</td>
<td>2.562 1.19-4.94 0.035</td>
<td>3.059 0.21-5.91 0.036</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are from linear regression analyses. \( \beta \) denotes standardized regression coefficient and 95% CI denotes 95% confidence interval.
Model 1: Unadjusted
Model 2: adjustments for age, education, depression, low-density lipoprotein cholesterol, systolic blood pressure, cardiovascular disease (coronary heart disease, cardiac insufficiency), hormone replacement therapy, physical activity, alcohol consumption and smoking at the time of carotid IMT measures.
Memory; sum of z-scores from the Word Recall Test and the prospective memory test.
Cognitive speed; sum of z-scores from the Letter-digit Substitution Test and the Stroop Test.
5.4 Inflammation and cognitive function (Study III)

At baseline, the median serum hsCRP was 1.12 mg/L, ranging from 0.01 to 70.14 mg/L (interquartile range 0.53 to 2.42). A total of 46 of the women had hsCRP <1.0 mg/L (low), 33 had 1.0-3.0 mg/L (average) and in 18 the level was >3.0 mg/L (high) at baseline. Women, with high serum hsCRP had more depressive symptoms (P<0.001 between groups), higher fasting blood glucose (P=0.040 between groups), higher systolic blood pressure levels (P=0.027 between groups), BMI (P=0.020 between groups) and waist circumference (P=0.073 between groups), and they were less likely to use hormone replacement therapy (P=0.065 between groups) than those with low hsCRP. There was no difference in the MMSE score across the CRP groups at baseline (P=0.980 between groups).

At 12-year follow-up, the median serum hsCRP was 1.02 mg/L, ranging from 0.01 to 22.11 mg/L (interquartile range 0.36 to 2.66). The median hsCRP (P=0.506) or number of women in the three hsCRP groups (P=0.708) did not differ at baseline and at 12-year follow-up. At 12-year follow-up, hsCRP was associated with BMI (26.3 ± 4.3 in low, 29.5 ± 3.9 in average, and 29.5 ± 5.6 in high hsCRP group, P=0.010 between groups) and waist circumference (87.9 ± 9.7 cm in low, 95.9 ± 10.6 cm in average, and 95.2 ± 12.3 cm in high hsCRP group, P=0.002 between groups).

In longitudinal analyses, higher baseline hsCRP was associated with poorer memory at 12-year follow-up (Table 10). Additional adjustment for blood glucose and systolic blood pressure slightly weakened the association (standardized regression coefficient \( \beta = -0.758 \), 95% confidence interval -1.585 to 0.070, P=0.072). Memory at 12-year follow-up worsened linearly with increasing hsCRP at baseline (Figure 14). There was no association between hsCRP at baseline and cognitive speed (Table 10) or the MMSE score (odds ratio 0.56, 95% confidence interval 0.256 to 1.232, P=0.150) at 12-year follow-up.
Table 10. Longitudinal association between baseline serum high sensitivity C-reactive protein (hsCRP) concentration and memory and cognitive speed at follow-up

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>95% CI</td>
</tr>
<tr>
<td>Memory</td>
<td>-0.842</td>
<td>-1.602 - -0.083</td>
</tr>
<tr>
<td>Cognitive speed</td>
<td>-0.199</td>
<td>-0.893 - 0.469</td>
</tr>
</tbody>
</table>

Data are from linear regression analyses. β denotes standardized regression coefficient and 95% CI denotes 95% confidence interval.

Model 1. adjusted for age, education and depression.
Model 2. adjusted for variables in model 1 and the use of hormone replacement therapy, smoking, serum low-density lipoprotein cholesterol and body mass index at the time of hsCRP measurements.

Memory; sum of z-scores from the Word Recall Test and the prospective memory test.
Cognitive speed; sum of z-scores from the Letter-digit Substitution Test and the Stroop Test.

Figure 14. Memory (sum of z-scores from the Word Recall Test and the prospective memory test) at 12-year follow-up in women with low (<1.0 mg/L, n=46), average (1.0-3.0 mg/L, n=33), or high (>3.0 mg/L, n=18) serum high sensitivity C-reactive protein (hsCRP) concentrations at baseline adjusted for age, education, depression, the use of hormone replacement therapy, smoking, serum low-density lipoprotein cholesterol and body mass index at the time of hsCRP measurements.
In cross-sectional analyses at 12-year follow-up, higher hsCRP was associated with poorer memory either without adjustment or after adjustment for age, education and depression (standardized regression coefficient $\beta = -0.757$, 95% confidence interval -1.491 to -0.023, $P=0.043$) and further adjustment for the use of hormone replacement therapy, smoking, serum LDL cholesterol and BMI ($\beta = -0.709$, 95% confidence interval -1.461 to 0.043, $P=0.064$). Additional adjustment for blood glucose and systolic blood pressure weakened slightly the association ($\beta = -0.688$, CI 95% -1.446 to 0.070, $P=0.075$). HsCRP was not associated with cognitive speed ($\beta = -0.049$, CI 95% -0.728 to 0.630, $P=0.886$) or the MMSE score (odds ratio 0.91, 95% confidence interval 0.452 to 1.851, $P=0.803$) at 12-year follow-up.

5.5 Brain-derived neurotrophic factor and cognitive function (Study IV)

Characteristics of subjects
The characteristics of the subjects are presented in Table 11. The age range was from 57 to 79 years. Women had a higher mean plasma BDNF level than men (1720.9 vs. 1495.0 pg/ml, $P<0.001$). There were no differences in men or women in BDNF levels between those ≤65 years of age and those who were older or between individuals with depression and those without it. In men, mean plasma BDNF level was lower in those with BMI ≥ 25 compared to those with BMI <25 (1455.4 pg/ml vs. 1617.8 pg/ml, $P=0.027$).

Women performed better in Word List Memory Score ($P<0.001$), Word List Recall Score ($P<0.001$), Word List Saving Score ($P=0.020$), Word List Recognition Score ($P<0.001$) and Clock Drawing Score ($P=0.006$), but worse in Naming Test ($P<0.001$) compared to men (Table 12). The norms used in the DR's EXTRA Study and in Finnish population are presented in Table 13.
## Table 11. Basic and clinical characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Women (n=705)</th>
<th>Men (n=684)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td>66.6 (5.3)</td>
<td>66.3 (5.4)</td>
<td>0.303</td>
</tr>
<tr>
<td><strong>Education, years</strong></td>
<td>11.3 (3.8)</td>
<td>11.1 (3.9)</td>
<td>0.137</td>
</tr>
<tr>
<td><em><em>MMSE</em>, points</em>*</td>
<td>27.5 (2.0)</td>
<td>27.6 (1.9)</td>
<td>0.396</td>
</tr>
<tr>
<td><strong>Body mass index</strong>*</td>
<td>27.6 (5.0)</td>
<td>27.6 (4.0)</td>
<td>0.999</td>
</tr>
<tr>
<td><strong>Waist circumference, cm</strong></td>
<td>88.9 (13.0)</td>
<td>98.8 (11.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Fasting plasma glucose, mmol/l</strong></td>
<td>5.7 (0.8)</td>
<td>6.0 (1.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em><em>Serum LDL</em> cholesterol, mmol/l</em>*</td>
<td>3.2 (0.8)</td>
<td>3.2 (0.8)</td>
<td>0.245</td>
</tr>
<tr>
<td><em><em>Serum HDL</em> cholesterol, mmol/l</em>*</td>
<td>1.9 (0.5)</td>
<td>1.5 (0.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Serum triglycerides, mmol/l</strong></td>
<td>1.3 (0.6)</td>
<td>1.4 (0.8)</td>
<td>0.105</td>
</tr>
<tr>
<td><strong>Systolic blood pressure, mm Hg</strong></td>
<td>150.1 (21.1)</td>
<td>145.3 (18.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure, mm Hg</strong></td>
<td>82.4 (9.2)</td>
<td>84.2 (9.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Glucose tolerance status, %</strong></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normal glucose tolerance</td>
<td>73</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
<td>7</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>10</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>10</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td><strong>Prevalent hypertension, %</strong></td>
<td>47</td>
<td>45</td>
<td>0.441</td>
</tr>
<tr>
<td><em><em>Prevalent cardiovascular diseases</em>, %</em>*</td>
<td>33</td>
<td>40</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Prevalent depressive symptoms, %</strong></td>
<td>16</td>
<td>11</td>
<td>0.013</td>
</tr>
<tr>
<td><strong>History of stroke, %</strong></td>
<td>4</td>
<td>5</td>
<td>0.350</td>
</tr>
<tr>
<td><strong>Use of sex hormones, %</strong></td>
<td>49</td>
<td>4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Use of antihypertensive medication, %</strong></td>
<td>43</td>
<td>41</td>
<td>0.580</td>
</tr>
<tr>
<td><strong>Use of lipid lowering medication, %</strong></td>
<td>35</td>
<td>35</td>
<td>0.807</td>
</tr>
<tr>
<td><strong>Alcohol consumption, drinks/week</strong></td>
<td>2.2 (4.3)</td>
<td>6.4 (8.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Smoking status, %</strong></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Never</td>
<td>74</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>19</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>7</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

Values are means (± SD) from Independent-Samples t-test or Mann-Whitney U-test or percentages from \( \chi^2 \)-test.

* MMSE, Mini-Mental State Examination; Body mass index, calculated by dividing weight by height squared (kg/m\(^2\)); LDL, low-density lipoprotein; HDL, high-density lipoprotein. Cardiovascular diseases include coronary heart disease, stroke, transient ischemic attack, cardiac insufficiency, arrhythmia, and lower extremity peripheral artery disease.
Table 12. Characteristics of the subjects in individual tests of the CERAD test battery

<table>
<thead>
<tr>
<th>Test</th>
<th>Women (n=705)</th>
<th>Men (n=684)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal Fluency, words/ minute</td>
<td>24.0 (6.0)</td>
<td>24.1 (6.6)</td>
<td>0.721</td>
</tr>
<tr>
<td>Naming, words/ out of 15 words</td>
<td>12.8 (2.0)</td>
<td>13.6 (1.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MMSE, points</td>
<td>27.5 (2.0)</td>
<td>27.6 (1.9)</td>
<td>0.396</td>
</tr>
<tr>
<td>Word List Memory, words/ out of 30 words</td>
<td>22.6 (3.5)</td>
<td>21.0 (3.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Constructional Praxis, points</td>
<td>9.3 (1.1)</td>
<td>9.6 (1.1)</td>
<td>0.827</td>
</tr>
<tr>
<td>Word List Recall, words/ out of 10 words</td>
<td>7.5 (1.8)</td>
<td>6.8 (1.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Word List Saving Score, %</td>
<td>86.4 (15.3)</td>
<td>84.5 (16.7)</td>
<td>0.020</td>
</tr>
<tr>
<td>Word List Recognition Score, %</td>
<td>97.0 (6.0)</td>
<td>95.6 (6.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Constructional Praxis Saving Score, %</td>
<td>90.7 (14.0)</td>
<td>92.0 (11.9)</td>
<td>0.121</td>
</tr>
<tr>
<td>Clock Drawing, points</td>
<td>5.4 (1.0)</td>
<td>5.3 (1.0)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Values are means (± SD) and Mann-Whitney U-test was used unless otherwise indicated.

Table 13. Norms for use of the CERAD test battery based on Finnish population and in the DR's EXTRA Study

<table>
<thead>
<tr>
<th>Test</th>
<th>Finnish population</th>
<th>DR's EXTRA Study*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal Fluency, words/ minute</td>
<td>&lt;14</td>
<td>&lt;17</td>
</tr>
<tr>
<td>Naming, words/ out of 15 words</td>
<td>&lt;10</td>
<td>&lt;11</td>
</tr>
<tr>
<td>MMSE, points</td>
<td>&lt;25</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Word List Memory, words/ out of 30 words</td>
<td>&lt;15</td>
<td>&lt;17</td>
</tr>
<tr>
<td>Constructional Praxis, points</td>
<td>&lt;7</td>
<td>&lt;9</td>
</tr>
<tr>
<td>Word List Recall, words/ out of 10 words</td>
<td>-</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Word List Saving Score, %</td>
<td>&lt;61</td>
<td>&lt;63</td>
</tr>
<tr>
<td>Word List Recognition Score, %</td>
<td>&lt;86</td>
<td>&lt;90</td>
</tr>
<tr>
<td>Constructional Praxis Saving Score, %</td>
<td>&lt;25</td>
<td>&lt;75</td>
</tr>
<tr>
<td>Clock Drawing, points</td>
<td>&lt;4</td>
<td>&lt;4</td>
</tr>
</tbody>
</table>

*Based on recent data from a Finnish population (266), the individual tests of the CERAD test battery were dichotomized at the lowest decile in the DR's EXTRA Study.
Plasma BDNF and cognitive function

Women in the lowest 10% of the CERAD scores had lower BDNF levels compared to women in the upper 90%. A significant difference was observed on Naming Test (P=0.005), MMSE Score (P=0.016), Word List Recall Score (P=0.018), Word List Saving Score (P=0.006) and Word List Recognition Score (P=0.018). In men, there were no such differences in any cognition test (P-values ranged from 0.086 to 0.803).

Decreased plasma BDNF levels were associated with impaired cognitive function in women (Table 14). In women, the risk for a low score in Naming Test increased by 53%, MMSE by 60%, Word List Memory by 51%, Word List Recall by 52%, Word List Saving by 51% and Word List Recognition by 60% with one standard deviation (SD) decrease in plasma BDNF. A similar pattern was observed also for other cognitive tests (Verbal Fluency, Constructional Praxis, Clock Drawing), but the associations between BDNF were not significant. Data were adjusted for age, education, depression, impaired glucose metabolism, cardiovascular disease, antihypertensive medication, lipid lowering medication, use of sex hormones, smoking and alcohol consumption. Additional adjustment for waist circumference did not change the magnitude of the associations. The magnitude of the association between BDNF and cognitive function was unchanged across unadjusted, age adjusted or fully adjusted analyses.

In the multivariate analyses, the use of sex hormones did not change the magnitude of the association between BDNF and cognitive function in women. Individuals who reported use of sex hormones performed better (P<0.05) in the tests of general cognitive function (MMSE), memory (Word List Recall), language (Naming) and executive function (Constructional Praxis). No statistically significant difference was found in other CERAD tests.
Table 14. Odds ratios (OR) for having low scores in tests of cognitive function with 1 SD decrease in plasma brain-derived neurotrophic factor (BDNF) levels in women and men

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
<th>Nagelkerke</th>
<th>Wald quantity</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
<th>Nagelkerke</th>
<th>Wald quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal Fluency, words/ minute</td>
<td>1.13</td>
<td>0.88-1.44</td>
<td>0.348</td>
<td>0.08</td>
<td>0.88</td>
<td>0.369</td>
<td>0.12</td>
<td>0.12</td>
<td>0.08</td>
<td>0.88</td>
</tr>
<tr>
<td>Naming, words/ out of 15 words</td>
<td>1.53</td>
<td>1.22-1.92</td>
<td>&lt;0.001</td>
<td>0.19</td>
<td>13.31</td>
<td>0.369</td>
<td>0.12</td>
<td>0.12</td>
<td>0.08</td>
<td>0.88</td>
</tr>
<tr>
<td>MMSE, points</td>
<td>1.60</td>
<td>1.19-2.15</td>
<td>0.002</td>
<td>0.19</td>
<td>9.52</td>
<td>0.369</td>
<td>0.12</td>
<td>0.12</td>
<td>0.08</td>
<td>0.88</td>
</tr>
<tr>
<td>Word List Memory, words/ out of 30 words</td>
<td>1.51</td>
<td>1.05-2.17</td>
<td>0.026</td>
<td>0.21</td>
<td>4.98</td>
<td>0.369</td>
<td>0.12</td>
<td>0.12</td>
<td>0.08</td>
<td>0.88</td>
</tr>
<tr>
<td>Constructional Praxis, points</td>
<td>1.14</td>
<td>0.92-1.42</td>
<td>0.222</td>
<td>0.14</td>
<td>1.49</td>
<td>0.369</td>
<td>0.12</td>
<td>0.12</td>
<td>0.08</td>
<td>0.88</td>
</tr>
<tr>
<td>Word List Recall, words/ out of 10 words</td>
<td>1.52</td>
<td>1.12-2.06</td>
<td>0.008</td>
<td>0.18</td>
<td>7.08</td>
<td>0.369</td>
<td>0.12</td>
<td>0.12</td>
<td>0.08</td>
<td>0.88</td>
</tr>
<tr>
<td>Word List Saving Score, %</td>
<td>1.51</td>
<td>1.14-2.01</td>
<td>0.004</td>
<td>0.12</td>
<td>8.11</td>
<td>0.369</td>
<td>0.12</td>
<td>0.12</td>
<td>0.08</td>
<td>0.88</td>
</tr>
<tr>
<td>Word List Recognition Score, %</td>
<td>1.60</td>
<td>1.17-2.18</td>
<td>0.003</td>
<td>0.13</td>
<td>8.62</td>
<td>0.369</td>
<td>0.12</td>
<td>0.12</td>
<td>0.08</td>
<td>0.88</td>
</tr>
<tr>
<td>Constructional Praxis Saving Score, %</td>
<td>1.15</td>
<td>0.88-1.50</td>
<td>0.310</td>
<td>0.15</td>
<td>1.03</td>
<td>0.369</td>
<td>0.12</td>
<td>0.12</td>
<td>0.08</td>
<td>0.88</td>
</tr>
<tr>
<td>Clock Drawing, points</td>
<td>1.06</td>
<td>0.76-1.48</td>
<td>0.721</td>
<td>0.04</td>
<td>0.13</td>
<td>0.369</td>
<td>0.12</td>
<td>0.12</td>
<td>0.08</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Data are derived from logistic regression analysis adjusted for age, education, depression, impaired glucose metabolism, cardiovascular disease (coronary heart disease, stroke, transient ischemic attack, cardiac insufficiency, arrhythmia, lower extremity peripheral artery disease), use of antihypertensive and lipid lowering medication, use of sex hormones in women, smoking and alcohol consumption.
6 DISCUSSION

6.1 Summary of main findings
The main findings are summarised as follows:

* Metabolic syndrome was associated with the risk of having poor memory in elderly women. With respect to the single risk factors, low level of HDL cholesterol was independently associated with the risk of having poor memory.

* Peripheral atherosclerosis, measured ultrasonographically as carotid intima-media thickness, was associated with the risk of having poor memory and poor cognitive speed in elderly women.

* High serum concentrations of high sensitivity C-reactive protein were associated with the risk of having poor memory in elderly women.

* Decreased plasma brain-derived neurotrophic factor level was associated with impaired memory and general cognitive function in ageing women. The kind of association was not found in men.

* No associations were found between cardiovascular risk factors and the Mini-Mental State Examination score in elderly women.
6.2 Methodological aspects

6.2.1 Study population and design

In Studies I-III, the population-based cohort of elderly women provided an opportunity to investigate the association between well-known cardiovascular risk factors and cognitive function over a long follow-up period. The present study made it possible to confirm the limited data between vascular risk factors and cognitive impairment. AD and dementia are more common in women than in men (40,41), and therefore it was justified to focus this research on elderly women. Even though the sample size was limited, our data were obtained from in-person measurements at the study centre. In many large studies, telephone interviews have been used in data collection, which may reduce their reliability. To evaluate cognitive function, we used a screening test for global cognitive function and a detailed test battery for memory and cognitive speed at follow-up. Thus, the prospective design remained partial. Since approximately 15% of the women had died, 15% had severe health problems, and 10% had other reasons for non-participation. The participants were quite aged, and though they were from the province of Kuopio, some of them would have had to travel a distance as much as 100 kilometers from the research laboratory, which may partly explain the participation rate and selection of the study population. In many studies, the weakness is that cognitively impaired individuals are excluded at baseline, and many studies do not include data on individuals lost to follow-up (11). Individuals with cognitive decline are less likely to participate in clinical studies (275). In the present study, the participants were younger, better educated, healthier, and had better global cognitive function and a metabolic risk factor profile at baseline than the non-participants. Therefore the present results may be underestimated the prevalence in the entire population. In Studies I-III, it is not possible to make any inference of causality.

In Study IV, there was an opportunity to investigate the association between neurotrophic factor level and cognitive function at baseline of a large ongoing randomized controlled trial. The study population was a representative population sample of ageing men and women living in the city of Kuopio. The only exclusion criteria for the intervention study were conditions that would have prevented safe engagement in exercise training, malignant diseases and other conditions preventing co-
operation, as judged by the research physicians. Otherwise, those participants who had some diseases which might have affected cognitive function were not excluded. On the period, the individuals confirmed their willingness to participate in the study and baseline measurements, the most common reasons for not participating in the study were loss of motivation and unknown reasons, whereas during baseline measurements, the participants dropped-out for medical reasons (Figure 9). In epidemiological studies, the participation rate has decreased during the past 30 years, especially in recent years, and is approximately 50% to 70% at this moment (276). The participation rate in the present study was nearly 70%, evidence of rather high compliance when taking into consideration the age of the participants and the substantial demands of the study. We believe that the study population is representative and also that those who participated in baseline measurements were motivated. We collected data on those who dropped-out during baseline visits. Subsequently, this randomized controlled intervention study focused on exercise and nutrition which makes it possible to confirm our cross-sectional observations and to investigate dose-response relationship between lifestyle factors and cognitive function in specific age and gender groups. At the time, the study design does not make it possible to infer causality or more generalization of the results.

6.2.2 Assessment of cognition
The schedule of the cognitive measures was designed by experienced neuropsychologists. Before the study visits, the participants were informed about the measurements in a letter and trained study nurses performed the measures with standard protocols. Global cognitive function was measured using the MMSE score in all studies. The MMSE score is a well-known screening instrument that measures orientation, language, memory, concentration and constructional praxis (10), but it is within the normal range in most people with MCI (7). In addition to global cognitive function, a detailed evaluation of cognitive function was performed at the follow-up in Studies I-III and in Study IV. Poor performance on neuropsychological tests such as delayed recall, naming and executive functions are evidence of a risk for progression to dementia (7-9). In the present studies, appropriate combinations of neuropsychological tests were used to identify those with incipient cognitive impairment. Furthermore, in all studies,
depressive symptoms were evaluated and data were carefully controlled for potential confounders, which is important especially in an elderly population where many factors may affect cognitive performance. We had no resources to use neuroimaging, and we had some missing data concerning cognition and other variables of interest.

*In Studies I-III, memory and cognitive speed were only evaluated at follow-up, therefore it was not possible to examine the change in these functions that occurred during the follow-up. Thus, we could not exclude the possibility that some of the women had had mildly impaired cognition at baseline. However, at that time their global cognitive function had been good, only two of the women scored <25 at baseline, and even additional adjustment for baseline MMSE did not change the magnitude of the associations. There were no associations between the predisposing factors and global cognitive function, whereas the associations were perceived using more specific neuropsychological tests. We used a sum score of the two tests to characterize memory and cognitive speed, which may be more reliable than the use of only a single test. Since there were no specific cut-off points in the scores of memory and cognitive speed, we used the variables as continuous when appropriate. There is very little longitudinal population-based data available about the association between cardiovascular risk factors and detailed evaluated cognitive function examined in Studies I-III. Thus, we believe that the present results highlight the role of these modifiable risk factors in the development of MCI.*

*In Study IV, we used the CERAD test battery which is considered to measure rather extensively cognitive domains that are prone to deficits in MCI and dementia (15). In all of the tests, we calculated specific cut-off points based on recent norms in a Finnish population (266). Age, gender and education are closely related to cognitive function, and they were taken into consideration when the cut-off points were calculated. The present study possibly revealed important information of development of cognitive impairment in ageing women. Even though CERAD is a broad and sensitive cognitive test battery, all cognitive processes that may or may not be affected by BDNF cannot be found out using this test battery. Cognitive function is a topical issue at the moment, and some of the participants were somewhat nervous at the beginning of the test session. The personal informative discussion before the start of the test session seemed to lessen
this stress. Early diagnosis of cognitive impairment is shown to decrease symptoms of anxiety and to elicit relief after receiving diagnostic accuracy (277).

6.2.3 Assessment of risk factors

The schedule for the study visits and the measurements used were carefully designed. The instruments for study measures were calibrated, and the study personnel was trained before the study started (Studies I-IV). Body weight and height, waist and hip circumference as well as blood pressure are straightforward measures, and widely used in clinical studies. In anthropometric measurements, the differences between the study nurses may be greater than expected. However, we duplicated waist and hip circumference measures with two study nurses in 84 participants during the follow-up visit (Studies I-III). The intra-class correlation for the two nurses was 0.995 between waist measurements and 0.981 between hip measurements during the same study visit. The coefficient of variation was 0.7% for waist circumference and 0.5% for hip circumference. The anthropometric measurements were based on the MONICA protocol (268), and we used the mean of two measurements in statistical analyses. Ageing is associated with a redistribution of both fat and lean tissue within the body, and intra-abdominal fat can be estimated by anthropometric measurements (278) easily and inexpensively. The measures of blood pressure were based on the general guidelines for the Finnish health care. In the statistical analyses, we used the mean of two consecutive measurements. The measurement of blood pressure is responsive to external factors, therefore the importance of standardized circumstances and meters used must be emphasized. In the present studies, differences between the study nurses and repeatability of the meters used were not tested, which may slightly limit the validity of the studies. Increased blood pressure and obesity in middle-age have been linked previously to cognitive impairment.

The participants were informed about the laboratory measurements by letter. All laboratory assessments followed standard protocols and all blood samples were taken by experienced laboratory technicians and were stored as appropriate. Intra-class correlation between two hsCRP assays during the same study visit was >0.999 at baseline and >0.997 at 12-year follow-up. The coefficient of variation was 1.7% at
baseline and 3.6% at 12-year follow-up (Study III). We feel that the laboratory assessments and determinations were conducted in a reliable manner. High sensitivity CRP is a sensitive marker of systemic low-grade inflammation (157), and increased concentrations of hsCRP have been associated with impaired cognition (164,165,167). Furthermore, inflammation has been linked to the pathogenesis of cardiovascular disease (157,279), obesity and insulin resistance (280), which in turn have been associated with the risk of cognitive impairment (93,112). Serum BDNF levels measured by ELISA is suggested to represent a mixture of the proBDNF and mature BDNF forms (251). Therefore, we could not assess the association of each form of BDNF with cognition. However, mature BDNF is suggested to decrease more sharply than proBDNF in the early phase of Alzheimer's disease (249). Research into the association between biomarkers and cognitive function provides important supplemental information about the prediction and diagnosis of MCI and dementia. In Study IV, glucose tolerance was tested using the OGTT (269), which is a reliable method for identifying individuals in a pre-diabetic condition. The participants were categorized into four groups (normal fasting glucose, impaired fasting glucose, impaired glucose tolerance and type 2 diabetes) according to their glucose tolerance status using the WHO criteria (269). In many studies, the definition of diabetes has been based on self-reported medical history or fasting blood glucose concentrations. This means that individuals in a pre-diabetic condition or having undiagnosed diabetes have been overlooked. Glucose intolerance has been shown to disturb cognitive function and evoke functional and structural damage of the brain as well as an increase in the risk of vascular injuries (100,115).

Diseases, medications, lifestyle factors and socio-economic status were assessed by a self-administered questionnaire, which is a practical and inexpensive method to implement. Data collection by questionnaires requires good design and well-defined instructions on how the questions are to be answered. To avoid misunderstandings and missing data it is useful to check the questionnaires as soon as they are received. In the present studies, the questionnaires were checked and completed when received, however there were some still missing data, which may partly have reduced the power in the statistical analyses. On the other hand, in population-based studies it is impossible
to obtain truly comprehensive data and as such, overestimation or underestimation is typical when lifestyle factors such as physical activity and nutrition are assessed by a self-administered questionnaire. For Study II, the data that evaluated strokes between baseline measurements and follow-up were obtained from the national hospital discharge register that covers the whole population in Finland and this is a reliable source of cardiovascular outcomes. Ischemic stroke increases the risk for dementia (50), although it may be reversible in a substantial proportion of patients within one year post-stroke (281). On the other hand, in self-report the subjects may be confused, for example differentiating between stroke and transient ischemic attack.

The metabolic syndrome was defined by the National Cholesterol Education Program criteria (270). These criteria are simple to use in clinical evaluation and do not emphasize any of the features in the risk factor score as opposed to the other well known criteria (85). Clustering of metabolic risk factors is shown to be a greater risk for cognitive decline than its individual components. Individuals with metabolic syndrome are at an increased risk for vascular diseases such as type 2 diabetes and stroke and thereby for cognitive impairment. However, the specific cut-off values which are used in the most common criteria may not be suitable in the oldest old individuals. In late life, the presence of the metabolic syndrome may decelerate cognitive impairment (130), but more evidence is needed to support this assumption.

Peripheral atherosclerosis was measured noninvasively as carotid artery intima-media thickness as previously reported (185,271). Certified sonographers performed the scannings according to a certain protocol and one study nurse read all the scannings. In Study II, no quality control measurements were performed, because this would have required an additional study visit. However, the women were so aged and one more visit might represented an additional unwelcome stress. The quality control measurements were performed in another study and they expressed high reliability (185). Carotid IMT has been considered to be a useful surrogate marker of atherosclerosis (282,283), and to be associated with an increased risk of coronary and cerebral events (284,285). Carotid IMT has a better sensitivity in detecting pre-clinical atherosclerosis compared to angiography (282). For the statistical analyses, we used the mean carotid IMT of left and right bifurcation, a location known to be prone to atherosclerosis, since it may be
subjected to alterations in blood flow such as decreased shear stress and increased
turbulence in this area (138).

6.3 Results

6.3.1 Metabolic syndrome and cognitive function (Study I)

Metabolic disorders such as type 2 diabetes and metabolic syndrome are increasing in a
dramatic manner throughout the world. The results of the present study are in
accordance with a recent study examining the prevalence of metabolic syndrome among
Finnish men and women over a decade (286). The present results when viewed in
combination with previous findings (78,126,129) emphasize the role of metabolic
syndrome in cognitive impairment. In the oldest old, opposing results have been found
(130), likewise concerning the associations between cognition and high levels of blood
pressure (65), cholesterol (71), glucose (130) and body weight (97,98). In the present
study, the follow-up time was rather long and we used specific tests to evaluate the
association between metabolic syndrome and cognitive function. Impairment in
memory and executive functions typically precedes dementia, therefore the detailed
evaluation of cognitive function was a useful way of identifying those at increased risk
to convert dementia. Metabolic syndrome did not associate with the MMSE.

Insulin resistance, the pathophysiological feature of metabolic syndrome, and
diabetes are deleterious to ageing-related cognitive function, especially memory. In the
present study, the prevalence of type 2 diabetes increased 12% during the follow-up
period, which may partly explain the association between metabolic syndrome and
impaired memory. Type 2 diabetes, as well as associated diseases such as obesity and
hypertension, have been suggested as pathogenetic mechanisms in AD and vascular
dementia due to their interaction with inflammation and processing and deposition of
beta-amyloid protein, a hallmark of AD (112,114). However, in late life, moderate
overweight has been indicative of good cognitive status (287). In the present study,
traditional risk factors such as blood pressure, blood glucose levels or waist
circumference were not significantly associated with memory functions suggesting that
metabolic syndrome may give some additive value in predicting the development of
memory decline compared to the separate conventional risk factors. More research will
be needed to clarify the relative importance and putative interactions between single
metabolic risk factors for cognitive impairment. On the other hand, some criticism has
been raised against the definition of the metabolic syndrome, because some risk factors
associated with insulin resistance, such as physical inactivity, are not included in the
definition of the syndrome (288).

Low serum levels of HDL cholesterol associated independently with poor memory 12
years later. Our results are in agreement with recent cross-sectional findings reporting
that lower HDL cholesterol levels are associated with impaired memory (289). It has
been suggested that high HDL cholesterol levels may protect against hippocampal
atrophy in elderly people (82). HDL is the main carrier of cholesterol into the brain and
the availability of cholesterol improves synaptic growth and regeneration (81). As the
predominant lipoprotein in the human brain, HDL cholesterol can also prevent
inflammation (83) as well as inhibiting the aggregation and polymerisation of beta-
amyloid protein (84). Furthermore, low levels of HDL cholesterol are a known risk
factor for cardiovascular disease (290), which in turn may lead to dementia. Further
investigations in representative population samples should be conducted to study the
additive values of metabolic syndrome beyond single risk factors and the relative
importance of each risk factor for memory and cognition.

6.3.2 Atherosclerosis and cognitive function (Study II)
The present study is one of the first to report an association between increased IMT and
poor memory and poor cognitive speed among relatively healthy elderly women over a
long follow-up period. Previous findings have been mainly cross-sectional (144,147-
150), only a few have been population-based (144,150). The inconsistences between the
findings may be partly related to differences in the study populations, follow-up times,
and cognitive measures used. The development of atherosclerosis in carotid arteries is
suggested to start with an increased IMT in the bifurcation, possibly due to alterations in
the blood flow in this area (138,283). Thus, the bifurcation is the preferred site to
measure pre-clinical atherosclerosis. Carotid IMT and carotid stenosis may represent a
different phase of the atherosclerotic process (151), and thus, the effects on cognition
may differ in a given population.
Memory, executive functioning and perceptual speed may be impaired for several years before the clinical diagnosis of AD (291). The same cognitive domains appear to be implicated in normal ageing, but there is a lack of data of normative rates for age-related cognitive decline. There is a considerable overlap in the risk factors, symptomatology, and pathophysiology of vascular dementia and AD (292), and thus, it may not be possible to make a sharp distinction between degenerative and vascular diseases, especially in elderly individuals. In view of these issues, we focused both on memory performance and cognitive speed/executive functions on specific neuropsychological tests without using any proposed diagnostic concepts, whose relevance in the general population is still unclear. In the present study, increased IMT was associated not only with poor cognitive speed but also with memory, but not with general cognitive functioning. This supports the view that sensitive measures of cognition are important when evaluating the effects of atherosclerosis on cognition (13).

Vascular risk factors and diseases have been linked with an increased risk for cognitive decline, but the exact mechanisms underlying the association are still obscure. Carotid IMT has been suggested as being a marker of medial hypertrophy but it is not synonymous with atherosclerosis (151). Carotid IMT has also been suggested as a predictive measure of target organ damage including microangiopathy in patients with essential hypertension (293). Thus, our findings indicate that the atherosclerotic process and end organ diseases of the arteries may be important in mediating the association between vascular risk factors and cognition. Atherosclerosis may impair cerebral blood flow and metabolism, and ultimately lead to neuronal dysfunction and cell death (294). Atherosclerosis also increases the risk of strokes and white matter lesions that may contribute to cognitive impairment. In addition, other vascular outcomes such as aberrations in the capillary ultrastructure in the brain and endothelial dysfunction may partly explain the observed associations. It will be crucial to study these issues in the future, especially in view of the fact that clarification of the vascular component in cognitive impairment may be useful in terms of prevention. Furthermore, in addition to traditional vascular risk factors, socio-economic inequality may be an important factor in the link between IMT and cognitive function (295).
The predictive role of cognitive impairment for subsequent dementia and loss of independence has been reported in a number of studies (296,297), and those individuals exhibiting cognitive impairment may represent an ideal group for targeted interventions. The observed relation between IMT and cognitive impairment may have implications for the prevention of cognitive impairment and dementia as vascular risk factors can be treated. Non-invasive assessment of arteries and the use of sensitive neuropsychological tests may become important tools in detecting those individuals with a high risk of suffering cognitive impairment, which, importantly, might be preventable by treatment aimed at achieving a reduction in the progression of atherosclerosis.

6.3.3 Inflammation and cognitive function (Study III)

Inflammation is suggested to be one of the key events in the pathogenesis of atherosclerosis and AD. The inflammatory response may contribute to neuronal dysfunction and lead to neuronal death in the brain. The present results are in accordance with previous studies indicating that increased low-grade inflammation is associated with cognitive impairment. Increased serum hsCRP concentrations have been associated with poor memory (164), poor global cognitive performance (165,166,168), vascular dementia (167) and AD (169,170) in previous population-based studies. In some studies no such association has been found (171,172). In the previous studies, the age-range of the participants has varied between 30 and 85 years, the follow-up time was generally rather short and very few studies have used a detailed evaluation of cognitive function, which may partly explain the dissimilarities between the studies, and represent limitations to attempts to generalization. In the present 12-year follow-up, we applied also a detailed evaluation of memory and cognitive speed, the domains which are known to be impaired before any clinical diagnosis of dementia (8). However, due to the lack of baseline data of these functions, we were not able to analyse the change in specific cognitive domains. HsCRP was not associated with global cognitive function.

The present data may broaden the aspects on the association between inflammatory biomarkers and MCI. Since there are no generally accepted instruments to define the transition between normal cognitive function and MCI, in addition to commonly used neuropsychological tests, other tools such as biomarkers are recommended if one
wishes to obtain an accurate diagnosis (7, 17, 18). Limited data are available of the association between the role of inflammatory biomarkers and MCI (18). Cognitive impairment seems to have a common vascular etiology (60, 64, 104), and atherosclerosis may represent a chronic inflammatory process (184). On the other hand, inflammation increases cardiovascular risk (157, 279), which in turn is associated with cognition. In the present study, adjustment for several vascular factors only slightly modified the association between hsCRP and cognition, which indicates that the association between high hsCRP and poor memory is not totally mediated through vascular factors. Thus, inflammatory markers such as hsCRP may represent an additional fact for inclusion in the global assessment of cardiovascular risk and cognitive impairment.

### 6.3.4 Brain-derived neurotrophic factor and cognitive function (Study IV)

Our study indicated that decreased plasma BDNF level was associated with impaired cognitive function in non-demented ageing women, but not in men. Furthermore, we observed that women have higher BDNF levels than men. To our knowledge, the present study is the first one examining the association between circulating BDNF level and cognitive function in an elderly general population.

We observed that decreased BDNF levels were associated with impaired global cognitive function as well as impairment in specific components of memory. However the associations were not seen in tests measuring executive function. Low serum BDNF levels have been previously associated with MMSE scores in demented persons (247, 248), but no previous data are available on its associations between specific cognitive domain in non-demented population. Decreased levels of BDNF mRNA in post-mortem hippocampus (259), temporal cortex (251, 259) and parietal cortex (250), were seen in Alzheimer's disease. Downregulation of BDNF, including proBDNF (249, 251), is suggested to be an event underlying early Alzheimer's disease (249). Both the number of BDNF immunopositive neurons and the intensity of immunostaining within cell bodies in hippocampus and temporal cortex appear to be decreased in Alzheimer's disease (251, 259).

BDNF plays a key role in modulating synaptic transmission and plasticity of neurons (236-240). Synaptic plasticity is thought to be the activity-dependent cellular
mechanism for learning and memory, called long-term potentiation (LTP) (298,299). BDNF has been hypothesized to be an important factor in LTP (298-300), especially for the development of the late phase of LTP (241), a form of synaptic plasticity believed to underlie long-term memory (301). BDNF is important for consolidation of LTP especially in hippocampal region CA1 (298,299) an area which is vulnerable to neuronal pathology (302). BDNF has been demonstrated to modulate LTP indirectly by inhibiting synaptic fatigue (298). In BDNF knockout mice, synaptic fatigue (303) and impairment in LTP were enhanced (241,303). On the other hand, induction of LTP may increase BDNF mRNA levels in hippocampus (241). The findings from experimental studies indicating that BDNF modulates hippocampal LTP imply that BDNF may play a role in the regulation of learning and memory (299) and provide a biological basis for our finding.

The gender difference for the association between plasma BDNF levels and cognitive function may be explained by sex hormones. The youngest women were 58 years of age and thus, almost all women were posmenopausal. Approximately half of the women used sex hormones. In multivariate analyses women who used sex hormones had better cognitive function. However, the use of sex hormones did not modify the association between BDNF and cognition. Altogether 28 men had prostate cancer and 11 of them had used sex hormones. Thus, these small numbers suggest that prostate cancer or usage of sex hormones in males were not major confounders for the association between BDNF and cognition.

Estrogen receptors are found in hippocampus and in frontal lobes (304), areas which play a crucial role in regulating learning and memory functions (242). Estrogens have neurotrophic effects by protecting against beta-amyloid toxicity (305-307), an important etiological factor in Alzheimer's disease. Estradiol can induce BDNF expression and the effects of estradiol in hippocampus may be mediated by BDNF (308). In some animal experiments, estrogen treatment has increased BDNF expression in hippocampus (309-311). However, other studies have shown no effect of estrogen treatment on BDNF mRNA in hippocampus (312) or cortical regions (309,312,313).

Progesterone is also suggested to be neuroprotective by increasing the expression of BDNF mRNA and protein (314). Progesterone was shown to reduce tau
hyperphosphorylation, when administered either alone or in combination with estrogen (307), but had no effect on beta-amyloid accumulation (307). On the other hand, progesterone was shown to decrease BDNF levels (310) and to reverse the increase in BDNF levels initiated by estrogen treatment (315).

Androgen receptors are also found in brain regions that are important for learning and memory (316). Low testosterone levels were detected in patients with mild cognitive impairment or Alzheimer's disease (217). On the other hand, testosterone has been suggested to have neurotrophic and neuroprotective effects and to protect against beta amyloid deposition and phosphorylated tau proteins (217).

However, the interrelationship between sex hormones and cognitive function remains unclear. There is an important gender difference in the rate of hormonal changes with ageing: in menopausal women estradiol falls to nearly undetectable levels, whereas in healthy men testosterone production declines slowly (316). Hormone replacement therapy may enhance cognitive function if the timing for treatment is optimal, i.e. on postmenopause or soon after it but further studies are needed (304,317,318).

Further long-term human studies in general population are needed to confirm these novel results. In addition, randomized controlled trials are needed to provide further evidence for the effects of life-style factors such as exercise and diet on BDNF and cognitive function in ageing people.
7 CONCLUSIONS AND FUTURE IMPLICATIONS

* Metabolic syndrome and of the single risk factors low HDL cholesterol may be significant contributors to poor memory. To what extent improving the metabolic profile may prevent or postpone cognitive impairment remains to be shown in adequately planned and conducted randomized controlled trials in various intervention modalities.

* Increased carotid IMT, used as a surrogate measure of atherosclerosis, appears to predict an increased risk for cognitive impairment, particularly poor memory and cognitive speed, in elderly women. These findings add evidence that preventive actions against atherosclerotic progression may be important also with regard to cognitive impairment.

* High serum hsCRP concentration was associated with an increased risk of poor memory, thus supporting the role of chronic low-grade inflammation in the processes leading to cognitive impairment.

* The present study indicated that decreased plasma BDNF level is associated with impaired memory and general cognitive function in ageing women. While the present study may have identified a potentially important determinant of cognitive function in women, our cross-sectional findings need to be confirmed.

* The investigated vascular status indicators were associated with specific cognitive tests, but not with crude global cognitive performance (measured with MMSE), highlighting the importance of sensitive cognitive measures in this kind of studies.

* These cohort studies highlight the role of modifiable risk factors in the development of cognitive impairment and provide additional support to the current efforts of health care professionals to effectively prevent these metabolic and cardiovascular disorders.
8 REFERENCES


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