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Cholesterol and late-life cognition. An epidemiological and clinical approach

Doctoral dissertation

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ABSTRACT - English

Alzheimer's disease (AD) and vascular dementia (VaD) are currently considered the most common dementia forms. They are usually approached separately, and much research is focused on identifying means to differentiate one from the other. However, evidence has accumulated during the past years that there is considerable overlap between AD and VaD in terms of clinical symptoms, neuropathology and risk factors, and that vascular factors are important not only for VaD but also for AD.

The aim of the present project is to investigate the relations between late-life cognition, dementia, AD, VaD and cholesterol metabolism in a more integrative way, by combining an epidemiological with a clinic-based approach, and by addressing both peripheral and brain cholesterol homeostasis. The epidemiological part is based on data on serum total cholesterol from a prospective 21-year follow-up cohort study in a Finnish population (Cardiovascular Risk Factors, Aging and Incidence of Dementia, CAIDE) and a retrospective 30-year follow-up cohort study in a multiethnic USA population (Kaiser Permanente of Northern California). Clinic-based studies were conducted at Karolinska University Hospital, Huddinge, Sweden, and focus on CSF markers of cholesterol homeostasis (total cholesterol, 24- and 27-hydroxycholesterol, apolipoprotein E) and the relations between serum markers of cholesterol homeostasis (total cholesterol, LDL, HDL, 24- and 27-hydroxycholesterol, lathosterol, and lanosterol) and brain volumes (white matter, gray matter, CSF, and total intracranial volume; parenchymal, white matter, gray matter and CSF fractions) in patients with subjective cognitive impairment (SCI), mild cognitive impairment (MCI) and AD.

The results of the project confirm the existence of a relation between cholesterol and AD as well as VaD. High serum total cholesterol at midlife consistently emerged as a risk factor for late-life dementia or cognitive impairment or poorer performance in cognitive tests. However, the significance of serum cholesterol at older ages turned out to be less straightforward. In epidemiological studies, late-life cholesterol did not appear to be related to cognition. Cholesterol levels decreased over time in most individuals, and especially in those who developed dementia or cognitive impairment. A decrease in cholesterol from midlife to late-life was significantly associated with increased risk of having a more impaired late-life cognitive status, even after adjusting for a wide range of confounders. This association was not significant in statin users. In clinical studies, patients with AD had lower serum levels of the cholesterol precursors lathosterol and lanosterol, and of 27-hydroxycholesterol compared to patients with MCI or SCI, suggestive of a possible disturbance in cholesterol homeostasis with decreased cholesterol synthesis. Also, lower total- and LDL-cholesterol were significantly related to lower brain volumes/higher CSF volumes in patients with AD, but not with MCI or SCI. On the contrary, in persons with SCI (but not AD or MCI) there was an inverse association between lathosterol, lanosterol and brain volumes (lower levels of cholesterol precursors related to higher brain volumes/lower CSF volumes). These patterns seem to indicate a central nervous system (CNS) - induced effect of disease on extracerebral cholesterol homeostasis.

A window into brain cholesterol metabolism was provided by CSF analyses. The relations found between 24-hydroxycholesterol (24OHC) and apolipoprotein E (apoE) levels in patients with AD and MCI (but not controls) indicate a role for the 24OHC-apoE system in the regulation and control of neurodegeneration, suggesting that the 24OHC-mediated secretion of apoE creates an overcapacity for transport of steroids from the CNS under conditions of neurodegeneration (when dying neuronal cells lead to increased levels of free cholesterol) but not under normal conditions. Patients with AD had the highest 24OHC levels in CSF and the lowest 24OHC levels in serum. Moreover, serum 24OHC was related to gray matter measurements. Thus, 24OHC levels in serum could function as a marker of the number of metabolically active neurons, while 24OHC in CSF may instead reflect neuronal damage and rate of neuronal loss.

These results point out the importance of shifting the focus from the extreme category of dementia as a late-life ´event´ to a life-long view of pathological processes influencing cognition. Cholesterol is among the modifiable risk factors that can be targeted by preventive strategies already at midlife. However, serum cholesterol levels may be influenced by the very disease(s) it contributes to; at older ages, circulating cholesterol may instead become a reflection of ongoing pathological processes in the brain. Further studies are needed to investigate the usefulness of markers of brain cholesterol metabolism in diagnosing the cause(s) of cognitive impairment and in monitoring disease progression.

National Library of Medicine Classification: WT 155, QU 95
Medical Subject Headings:Alzheimer Disease/prevention&control; Alzheimer Disease/epidemiology; Dementia, Vascular/epidemiology; Age Factors; Brain; Cholesterol; Homeostasis; Hydroxycholesterols; Risk Factors; Biological Markers; Apolipoprotein E; Lanosterol; Cerebrospinal Fluid; Longitudinal Studies

TIIVISTELMÄ - Suomi

Alzheimerin tautia (AT) ja verisuoniperäistä dementiaa (VD) pidetään nykyään yleisimpinä dementia muotoina. Niitä usein lähestytään erillisinä sairauksina ja paljon tutkimustyötä kohdistetaan niiden erottamiseen toisistaan. Viime vuosien tutkimukset ovat kuitenkin tuottaneet runsaasti näyttöä siitä, että AT:lla ja VD:llä on runsaasti päällekkäisyyttä taudinkuvan, neuropatologian (aivojen rakenteellisten muutosten) ja riskitekijöiden osalta, ja että verisuoniperäiset tekijät ovat tärkeitä VD:n lisäksi myös AT:ssa.

Tämän tutkimuksen tavoitteena on tutkia myöhemmän iän muistitoimintojen, dementian, AT:n, VD:n ja kolesteroliaineenvaihdunnan yhteyksiä kokonaisvaltaisella tavalla yhdistämällä epidemiologisen ja kliinisen lähestymistavan ja arvioimalla sekä ääreisverenkierron että aivojen kolesteroliaineenvaihduntaa. Tutkimuksen epidemiologinen osuus pohjautuu suomalaisen CAIDE-tutkimuksen (Kardiovaskulaariset riskitekijät, ikääntyminen ja terveys –tutkimus) 21 vuoden pituisen seuranta-aineiston kolesterolimittauksiin ja yhdysvaltalaiseen retrospektiiviseen monietniseen tutkimuskohorttiin (Kaise Permanente of Norhtern California). Kliiniset tutkimukset suoritettiin Karoliinisessa yliopistollisessa sairaalassa (Huddinge, Ruotsi) ja ne keskittyivät muistiongelmia potevien, lievästä muistitoimintojen häiriöstä (subjektiivinen kognitiivinen heikentyminen (SCI), lievä kognitiivinen heikentyminen (MCI)) ja AT:sta kärsivien potilaiden aivo-selkäydinnesteestä mitattujen kolesteroliaineenvaihdunnan merkkiaineiden (kokonaiskolesteroli, LDL, HDL, 24- ja 27-hydroxykolesteroli, lathosteroli, lanosteroli) ja aivojen tilavuusmittojen (valkean ja harmaan aineen tilavuudet, aivo-selkäydinnesteen tilavuus) suhteiden arviointiin.

Tutkimuksen tulokset vahvistavat kolesterolin ja AT:n sekä VD:n välistä yhteyttä. Kohonnut veren kolesterolipitoisuus keski-iässä on yhdenmukaisesti osoittautunut riskitekijäksi myöhemmän iän dementialle, kognitiiviselle heikentymiselle ja heikommalle suoriutumiselle kognitiivisissa testeissä. Vanhuusiän veren kolesterolipitoisuuden merkitys ei kuitenkaan ole niin selvä. Epidemiologisissa tutkimuksissa vanhuusiän kolesterolipitoisuus ei ole osoittautunut olevan yhteydessä kognitioon. Kolesterolipitoisuudet laskevat iän myötä valtaosalla ihmisistä ja etenkin niillä, joille kehittyy dementia tai muu kognitiivinen heikentyminen. Kolesterolipitoisuuden lasku keski-iän jälkeen oli merkitsevästi yhteydessä myöhemmän iän kognitiiviseen tasoon.

Kolesterolipitoisuuden lasku keski- ja vanhuusiän välissä oli merkitsevästi yhteydessä kohonneeseen riskiin heikentyneelle kognitiiviselle tasolle vanhuusiässä, silloinkin kun otettiin huomioon useita sekoittavia tekijöitä. Tämä yhteys ei ollut merkitsevä niillä, jotka käyttivät statiineja (kolesterolia alentavia lääkkeitä). Kliinisissä tutkimuksissa AT:a sairastavilla potilailla oli matalammat kolesterolin esiasteiden lathosterolin ja lanosterolin sekä myös 27-hydroxykolesterolin pitoisuudet verrattaessa MCI:tä tai SCI:tä sairastaviin potilaisiin, mikä saattaa viitata mahdolliseen kolesteroliaineenvaihdunnan häiriöön, johon liittyy heikentynyt kolesterolin tuotto. Lisäksi, AT sairastavilla matalammat kokonais- ja LDL-kolesterolipitoisuudet olivat yhteydessä pienempiin aivotilavuuksiin/suurempaan aivo-selkäydinnestetilavuuteen. Toisaalta SCI sairastavilla potilailla (mutta ei AT tai MCI sairastavilla) oli käänteinen yhteys lathosterolin, lanosterolin ja aivotilavuuksien välillä (matalammat kolesterolin esiasteiden pitoisuudet olivat yhteydessä suurempiin aivotilavuuksiin ja pienempiin aivo-selkäydinnestetilavuuksiin). Nämä tulokset viittaavat sairaudesta johtuvaan keskushermoston kykyyn indusoida muutoksia aivojen ulkopuolisessa kolesteroliaineenvaihdunnassa. Aivoselkäydinnesteen tutkimukset antoivat tietoa aivojen kolesteroliaineenvaihdunnasta. Yhteydet, joita havaittiin AT ja MCI sairastavien (mutta ei kontrollien) 24-hydroxykolesterolin ja apolipoproteiini E:n (apoE) tasoissa viittaavat siihen, että 24OHC-apoE järjestelmällä on rooli neurodegeneraation säätelyssä. Tämä saattaa viitata siihen, että 24OHC:n välittämä apoE:n eritys johtaa kohonneeseen kykyyn kuljettaa steroideja pois keskushermostosta neurodegeneraatiotilanteessa (kun aivosolujen kuolema johtaa kohonneisiin vapaan kolesterolin pitoisuuksiin), mutta ei normaalitilassa. AT sairastavilla potilailla oli korkeimmat 24OHC-pitoisuudet aivo-selkäydinnesteessä mutta alhaisemmat 24OHC-pitoisuudet seerumissa. Seerumin 24OHC-pitoisuudet olivat yhteydessä harmaan aineen tilavuuksiin. Täten seerumin 24OHC-pitoisuudet saattavat toimia aineenvaihdunnallisesti aktiivien aivosolujen merkkiaineena, kun taas aivoselkäydinnesteen 24OHC-pitoisuus saattaa heijastaa neuronien vaurioitumista ja tuhoa.

Nämä tulokset korostavat tärkeyttä muuttaa dementian luokittelua myöhäisiän äärimmäisestä tapahtumasta elinikäiseen kognitioon vaikuttavien patologisten prosessien jatkumoon. Kolesteroli on yksi muokattavista riskitekijöistä ja siihen voidaan puuttua ennaltaehkäisevin keinoin jo keski-iässä. Toisaalta, on pidettävä mielessä, että seerumin kolesterolipitoisuuksiin saattaa vaikuttaa itse sairaus, jonka syntyyn kolesteroli on myötävaikuttamassa ja myöhemmällä iällä veren kolesteroliarvo saattaa kuvastaa aivojen jo käynnissä olevia patologisia prosesseja. Lisätutkimuksia tarvitaan selvittämään kuinka aivojen kolesteroliaineenvaihdunnan merkkiaineet voivat edistää kognitiivisen heikentymisen diagnosointia ja taudin etenemisen seurantaa.

SAMMANFATTNING - Svenska

Alzheimer sjukdom (AD) och vaskulär demens (VaD) är de vanligaste demenstyperna. Oftast betraktas AD och VaD som helt skilda forskningsområden och många studier fokuserar på hur man kan skilja mellan dessa demenstyper. Det finns dock forskningsresultat som tyder på att AD och VaD kan ha gemensamma symtom, neuropatologiska förändringar och riskfaktorer, samt att vaskulära faktorer är viktiga för både AD och VaD.

Syftet med detta projekt är att analysera förhållanden mellan kognition hos äldre, demens, AD, VaD och kolesterol på ett integrativt sätt, genom att kombinera ett epidemiologiskt och kliniskt synsätt, samt genom att undersöka kolesterolmetabolism i både hjärnan och periferin. Den epidemiologiska delen fokuserar på totalkolesterol i blodet, med data från en prospektiv, befolkningsbaserad studie i Finland (Cardiovascular Risk Factors, Aging and Dementia, CAIDE) med 21 års uppföljningstid, samt data från en retrospektiv studie av en multietnisk amerikansk befolkning (Kaiser Permanente of Northern California) med 30 års uppföljningstid. De kliniska studierna genomfördes på Karolinska Universitetssjukhuset, Huddinge, Stockholm, och fokuserar på kolesterolmarkörer (totalkolesterol, 24- and 27-hydroxykolesterol, apolipoprotein E) i ryggmärgsvätskan (CSF) samt på förhållanden mellan kolesterolmarkörer i blodet (totalkolesterol, LDL, HDL, 24- and 27-hydroxykolesterol, lathosterol, lanosterol) och hjärnvolymer (vit substans, grå substans, CSF volymer; total intrakraniell volym; parenkym, vit substans och grå substans fraktioner) hos patienter med subjektiva kognitiva problem (SCI), lindrig kognitiv svikt (MCI) och AD.

Resultaten bekräftar sambandet mellan kolesterol och både AD och VaD. Hög kolesterol i medelåldern framstod som en riskfaktor för demens eller kognitiv svikt i alla studier. Betydelsen av kolesterolnivån senare i livet blev dock långt ifrån entydig. I de epidemiologiska studierna var kolesterol tillsynes inte relaterad till kognition. Kolesterolnivåerna minskade efter medelåldern, särskilt hos de personer som senare fick demens eller kognitiv svikt. En sådan minskning var relaterad till ökad risk för demens eller kognitiv svikt, även efter att man tog hänsyn till flera andra faktorer. Detta samband var inte signifikant hos personer som behandlades med statiner. I kliniska studier hade patienter med AD lägre nivåer av kolesterolprekursorerna lathosterol och lanosterol, samt av 27-hydroxykolesterol i jämförelse med patienter med MCI eller SCI, vilket tyder på en möjlig störning i kolesterolhomeostasen (med minskad kolesterolsyntes). Lägre total- och LDL-kolesterol var relaterade till lägre hjärnvolymer hos patienter med AD, men inte med SCI eller MCI. Tvärtom fanns det ett omvänt samband mellan lathosterol, lanosterol och hjärnvolymer (lägre kolesterolprekursorer relaterade till högre hjärnvolymer) hos patienter med SCI (men inte AD eller MCI). Dessa mönster tyder på att sjukdomsrelaterade förändringar i centrala nervsystemet möjligen kan påverka kolesterol i periferin.
CSF analyser skapade ett ´fönster´ mot kolesterol i hjärnan. Sambanden mellan 24-hydroxykolesterol (24OHC) och apolipoprotein E (apoE) hos patienter med AD och MCI (men inte kontrollgruppen) visar att 24OHC-apoE systemet är inblandat i regleringen och kontrollen av neurodegeneration. Detta antyder också att den 24OHC-relaterade produktionen av apoE skapar en ökad kapacitet för kolesteroltransport från centrala nervsystemet under neurodegenerationsprocessen (när döende neuron leder till ökad kolesterolnivå), men inte i ett normalt tillstånd. Patienter med AD hade de högsta 24OHC-nivåerna i CSF och de lägsta i blodet. Dessutom var 24OHC i blodet relaterad till grå substansmätningar. 24OHC i blodet kan således fungera som en markör av antalet aktiva neuron, medan 24OHC i CSF istället kan spegla neuronala skador och neuronförlustens hastighet.

Dessa resultat betonar att det är viktigt att byta fokus från demens (ett extremt tillstånd) som en isolerad ´händelse´ hos äldre, till ett livslångt perspektiv på olika patologiska processer som påverkar kognition. Kolesterol är en av de modifierbara riskfaktorerna som förebyggande strategier kan riktas mot redan i medelåldern. Kolesterolnivåerna kan dock påverkas av själva sjukdomarna som de bidrar till; hos äldre personer kan kolesterol i blodet istället spegla pågående patologiska processer i hjärnan. Fler studier behövs för att undersöka hur markörer av hjärnkolesterol kan användas för att identifiera orsakerna till kognitiv svikt och för att övervaka sjukdomsförloppet.

"If we knew what it was we were doing, it would not be called research, would it?"

- Albert Einstein -

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Kuopio, August 2009

Alina Solomon

ABBREVIATIONS

24OHC	24-hydroxycholesterol
27OHC	27-hydroxycholesterol
AD	Alzheimer´s disease
APOE	Apolipoprotein E (gene)
ApoE	Apoipoprotein E (protein)
APP	Amyloid precursor protein
Aβ	β-amyloid
BBB	Blood-brain barrier
BMI	Body mass index
BP	Blood pressure
CAIDE	Cardiovascular risk factors, Aging and Incidence of DEmentia study
CSF	Cerebrospinal fluid
DSM	Diagnostic and Statistical Manual of Mental Disorders
GM	Gray matter
HDL	High density lipoproteins
HR	Hazard ratio
ICD	International Statistical Classification of Diseases and Related Health Problems
LDL	Low-density lipoproteins
MCI	Mild cognitive impairment
MMSE	Mini Mental State Examination
NFT	Neurofibrillary tangles
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer´s Disease and Related Disorders Association
NINDS-AIREN	National Institute of Neurological Disorders and Stroke - Association Internationale pour la Recherche et l´Enseignement en Neurosciences
NP	Neuritic plaques
OR	Odds ratio
P-Tau	Phospho-tau
RCT	Randomized controlled trial
SCI	Subjective cognitive impairment
TC	Serum total cholesterol
T-Tau	Total-tau
VaD	Vascular dementias
WHO-CINDI	World Health Organization-Countrywide Integrated Noncommunicable Diseases Intervention
WHO-MONICA	World Health Organization-Multinational MONItoring of trends and determinants in CArdiovascular disease
WML	White matter lesions

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to by their Roman numerals in the text.

I. Solomon A, Kåreholt I, Ngandu T, Winblad B, Nissinen A, Tuomilehto J, Soininen H, Kivipelto M. Serum cholesterol changes after midlife and late-life cognition: twenty-one-year follow-up study. Neurology 2007;68(10):751-6

II. Solomon A, Kåreholt I, Ngandu T, Wolozin B, MacDonald SWS, Winblad B, Nissinen A, Tuomilehto J, Soininen H, Kivipelto M. Serum total cholesterol, statins and cognition in non-demented elderly. Neurobiol Aging 2009;30(6):1006-9

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CONTENTS

1. INTRODUCTION

The present set of studies focuses on late-life cognition, dementia, Alzheimer´s disease (AD), and vascular dementia (VaD), providing some insight into their relationship with cholesterol. In current clinical research, workers are not expected to inquire upon the boundaries of their objects of study, which are given in ´operational´ definitions (such as DSM or ICD). To briefly iterate the conventional, AD is a progressive neurodegenerative disease and the main cause of dementia (Qiu 2007, Ferri 2005), closely followed by its diagnostic competitor, vascular dementia (VaD) (Qiu 2007, Fratiglioni 2000). These diagnoses are established in a two-step procedure, identification of dementia (where a decline from a previous level of functioning, with significant social or occupational impairment is an essential feature) and identification of cause (neurodegenerative or vascular). Yet, seen in the broader context of an association between modifiable vascular risk factors and AD, and between neurodegenerative and vascular pathophysiological processes in the brain, the very association between cholesterol, cognition, AD and VaD raises important questions about basic issues such as ´AD´, ´VaD´, ´dementia´, ´neurodegeneration´, or ´abnormal cholesterol levels´.

Since the relation of lipids with vascular pathology and the relation of protein aggregation with neurodegeneration have long been regarded as separate matters of investigation, disturbances in cholesterol metabolism are a more recent addition to the AD equation. This addition has caused a significant degree of controversy, concentrated on two main points: (1) on a molecular level, the weight of the cholesterol contribution to a protein-dominated (and amyloid-dominated) AD pathophysiology, and (2) on a clinical level, the relationship between the categories of ´vascular´ and ´neurodegenerative´ in diagnostic criteria for AD and VaD. Moreover, the emergence of cholesterol as a modifiable element involved in AD or VaD is one of the factors emphasizing the limitations of the two-step diagnostic approach, which can only identify the problem in its extreme form, dementia, when intervention may be too late. The focus on the extreme category of dementia, with disregard to subtler manifestations of diseases, has also produced a debate on whether cholesterol should be considered a risk factor or a risk marker for disease, and much conflicting data on the meaning of ´abnormal cholesterol levels´.

Apart from the problems inherent to current dementia-related research, cholesterol itself presents many unsolved difficulties. The human body contains two main pools of cholesterol, cerebral and extracerebral, which are very different from one another. Moreover, cholesterol is distributed in diverse compartments within these pools. Cholesterol research has been

governed by the link with atherosclerosis for decades, leading to considerable knowledge about the extracerebral pool and its components, while most details about brain cholesterol metabolism and the cerebral-extracerebral cholesterol interactions are still unknown.
The present project investigates cognition, dementia, AD, VaD and cholesterol in a more integrative way, by combining an epidemiological with a clinic-based approach, and by addressing both peripheral and brain cholesterol homeostasis. Since the results raise questions about the very definitions of the objects of inquiry, and since little attention is usually paid to how various concepts, categories or classifications emerge or to what impact such processes have on subsequent directions in research and clinical practice, the Introduction provides a minimum of historical deconstruction.

1.1. History matters

Two metaphors are usually associated with nosology: the clinician as cataloguing species in an exotic garden (´naturalist´view); and the clinician as a sculptor carving shapes out of formless matter, i.e. creating ´clinical forms´ (´creationist´ view). Each approach has its own problems, as the garden approach must explain why the naturalist happens to be there in the first place, while the creationist view demands an understanding of the sculptor´s ´inner vision´ (Berrios 1990a). Clinicians tend to be more interested in clinical cartography than in challenging the alleged immutability of disease (Berrios 1990a). A useful example is a question asked about AD (McMenemey 1970), which persists (more or less covertly) to the present day: ´How far are we justified in departing from the criteria originally laid down by the person who first described the malady?`. The answer is difficult to find, since the ´original´ AD cases have limited resemblance to those defined by current criteria, and it is not possible to pinpoint the exact moment when AD was first ´discovered´. One solution has been to assume that Alois Alzheimer got his discovery partially wrong, i.e. he considered it a pre-senile rarity, and did not exactly mind about arteriosclerotic changes in Auguste D´s brain.
However, there has been a shift towards the creationist approach, illustrated by a counter-question (Berrios 1990a): ´Should we worry about upsetting the boundaries of any disease?´. In this view, the ´final description´ of a disease or symptom results from the interaction between biological signals (originating in an affected brain site) and the layers of psychosocial codes involved in the process of disease or symptom formation (Berrios 1996). It is thus important to examine scientific matters in their own historical context. Yet anachronistic reading is not easy to avoid, since in the late 19th century and early 20th century

even crucial concepts such as dementia, neuron, neurofibril or plaque were not fully crystallized and had different meanings for different persons.

1.1.1. Dementia

The history of the word ´dementia´ must not be confused with that of the concepts or behaviours involved. By the 19th century, two main definitions of dementia were recognized (legal and clinical), both having psychosocial incompetence as their central concept. The clinical definition was by no means focused on cognition, but included also delusions and hallucinations. Irreversibility and old age were not characteristics of the condition (when it occurred in elderly it was called ´senile dementia´; states of psychological dilapidation in young people could be called ´dementia praecox´) (Berrios 1996; Kraepelin 1896, 1910). Dementia was considered a terminal state to all sorts of mental, neurological and physical conditions. This concept was gradually fragmented during the 19th century. In the beginning of the 20th century, senile and arteriosclerotic dementias had remained as main dementia groups after the pruning down of several clinical states (for example by redefinition as independent conditions, i.e. Korsakoff´s syndrome, myxoedema; hidden under a different name, i.e. dementia praecox as schizophrenia; the concept of ´pseudodementia´ was created to deal with cases of ´dementia´ that recovered) (Berrios 1996).

Among the main factors that contributed to these changes were:

1) The development of the anatomo-clinical model

The anatomo-clinical model, according to which signs and symptoms were ´signals´ emitted by biological lesions in the body, was introduced during early 19th century (Berrios 1994). The view that dementia was both a clinical and neuropathological category was already established in 1822, but it was unclear what type of lesions were involved, and whether they affected the same areas of the brain (Berrios 1990a). Senile dementia in subjects without previous mental illness became an important research topic as it somewhat simplified the problem of determining the cause of brain lesions. Brain changes were initially described in terms of gross anatomy (colour, consistency, weight etc), but inadequate brain preservation, fixation and staining techniques hindered conclusions. By the beginning of the 20th century more than 100 techniques had become available, with about 15 for visualising neurofibrils

(Berrios 1990a). Researchers had to agree not only on shapes or colours, but also on the meaning of what they saw, which was not at all clear during Alois Alzheimer´s time.

The cell theory (cells as the fundamental units of life, formed through scission of pre-existing cells) had already been accepted and cells were also seen as the basic elements of pathological processes (Virchow 1858). The nervous system, from being ´fancied...an unusually simple whole, from the unity of which resulted the unity of the body in general´ (Virchow 1858), was ´split´ into several components, and the presence of non-nervous elements was recognized. Virchow, for example, described neuro-glia, which he thought of as connective cells, a ´glue´ holding nervous elements together (Virchow, 1858). Between 1880 and the First World War, two views competed for supremacy: the neuron theory (neurons as independent units, never touching or touching only sporadically), and the reticular theory (brain cells as forming a syncitium, a network). In 1906 (the year of Alzheimer´s famous report), Cajal and Golgi received the Nobel Prize ´in recognition of their work on the structure of the nervous system´ and the neuronist-reticulist debate is obvious in their Nobel Lectures. Alzheimer´s position is however unclear, although Nissl, his lifelong friend, was a reticulist (Berrios 1990a).

2) The ´temporalisation´ of mental disorders and the view that senility was an exaggerated and/or pathological form of ageing

The view that certain diseases seem to be related to particular stages of life is very old, but until the 19th century there was no linear, evolutionary or temporalised model of life. The concept of linear time was introduced in biology only during late 18th century, and the creation of the ´late-onset mental disease´-concept took place during the 19th century, facilitated by evolution theory, degeneration theory (initially a mixture of moral, theological and biological reasoning, appalling for today´s reader (Hoff 2008)), developmental psychology, as well as by historicism (history as the only fundamental frame within which humans and their creations can be understood) (Berrios 1999).

Before ´temporalisation´, the concept of mental illness was monolithic (more or less fixed form whose elements – symptoms – were considered specific for the disease in question, instead of remaining the same irrespective of the disease in which they appeared), atemporal (becoming ill changed one´s essence, disease was a trait rather than a state), and with a symbolic relation to the body (i.e. placing melancholia in the heart). There could thus be no full recovery (in today´s sense) – only lucid intervals, no ´acute´ or ´chronic´ diseases; importantly, cross-sectional diagnostic assessment was considered enough.

After ´temporalisation´, mental illness became a state, a process happening in time and necessitating information on course and outcome. The concept of ´period of life´ was redefined ontogenetically, anatomically and physiologically; the relation of certain diseases to biological stages of life was now the result of specific pathological changes (Berrios 1996).

3) The reaffirmation of the intellectual (cognitive) functions

The choice of cognitive impairment as the hallmark of dementia (cognitive paradigm) was guided by both clinical and ideological factors. As institutionalized patients were often cognitively incompetent, and cognition was considered as the defining feature of the human species, dementia became first and foremost pathology of the ´intellect´ (Berrios 1990b).

During the 19th century, measurements were gradually introduced in psychology and medicine. The second half of the century marked the beginning of programmatic experimental research on higher mental processes. Since memory was the cognitive function whose measurement was best developed (Ebbinghaus 1885), memory deficits became the central feature of dementia (and still are today). The cognitive paradigm had become established by the First World War, and as a consequence other symptoms (i.e. hallucinations, delusions, mood and behavioural disorders) were left out and explained as epiphenomena, as unrelated to the central mechanisms of dementia (Berrios 1996). After the 1980s, the cognitive paradigm began to broaden, and the importance of mapping non-cognitive symptoms, particularly in evaluating early and advanced dementia stages, is more emphasized today.

1.1.2. Alzheimer´s disease

After 1863, the neuropathological features of ´senile dementia´ became cortical atrophy, enlarged ventricles and tissue ´softening´ (ascertained as vascular in nature) (Berrios 1990a). Belijahow (1889) described nerve cell death in senile dementia; Blocq and Marinesco (1892) reported ´amas ronds´ (round heaps) in the brain of an elderly epileptic patient, considered the first mention of plaques as neuropathological structures; Redlich (1898) reported ´miliare Sklerose´ (military sclerosis) in senile dementia, referring to it as ´plaques´ (the first use of the term) (Goedert, 2008). A paper by Fischer from 1907 provided the first description of what is now known as neuritic plaques. Damage of neurofibrils in patients with senile dementia was shown as early as 1904, and in June 1906, five months before Alzheimer´s report, Fuller had specifically remarked on the presence of neurofibrillar bundles in senile dementia (Berrios 1990a).

From Alzheimer´s famous report in November 1906, ´Uber eine eigenartige Erkrankung der Hirnrinde´, ´eigenartige´ has been translated as either ´characteristic´ or ´peculiar´ (Alzheimer 1907). Analysis of the context and semantics of ´eigenartig´ during that period of time suggests that Alzheimer is most likely to have meant ´peculiar, odd´ (Berrios 1990a). Since the brain changes and clinical syndrome that he described were not new, what attracted his attention as an experienced clinician and pathologist may have been the unfamiliarity of their association in a younger patient. Alzheimer actually insisted on the fact that he did not think it was a new disease, but an atypical form of senile dementia (Alzheimer 1911, Perusini 1911). By 1910 about eight cases had been published, although at least two of them seem to have been reported twice (Berrios 1990a). Nevertheless, in the 8th edition of his Textbook of Psychiatry (1910) Kraepelin decided to baptize it ´Alzheimer´s disease´.

1.1.3. `Alzheimer´s other dementias`: vascular dementias

The work of Alzheimer has been more or less reduced to the ´discovery of his disease´, and this inaccuracy often forms the basis for leaving out vascular dementias. Alzheimer has actually written more on arteriosclerotic brain changes than on ´Alzheimer´s disease´ (the articles from 1895, 1897, 1898, 1902, and 1913) (Libon 2006).

When Lobstein described the histopathology of arterioscleriosis in 1833, little did he imagine that gradual strangulation of blood supply to the brain was to become the favourite mechanism of ageing and senile dementia (Berrios 1996). By the 1840s, a number of vascular lesions, including lacunar infarcts, etat crible, and interstitial atrophy of the brain had already been described (Libon 2006, Roman 2002). A part of Alzheimer´s work focused on distinguishing between dementia paralytica (now syphilis) and arteriosclerotic dementia, an important problem at that time. Already in 1898 Alzheimer discussed ´dementia praesenilis´ as well (it is however unclear what cases his description is based on), and in the same paper questioned the view of senile dementia as caused by arteriosclerosis, discussing the possible contribution of vascular and neuronal causes to the observed brain changes: ´In our discussion of dementia senilis, we had to admit to some doubt as to whether or not the arteriosclerosis of the brain vessels may be considered the only cause of senile brain degeneration, or whether perhaps primary atrophic alterations of the nerve cells had to be taken into account.´ (Alzheimer 1898). The 1898 paper includes many references to the work of Binswanger and has been credited by some translators with coining ´Binswanger´s disease´. Alzheimer´s views on brain arteriosclerosis are also evident in his 1902 paper ´Mental disturbances of

arteriosclerotic origins´, where he presents his own and Binswanger´s work and emphasizes the heterogeneity of vascular brain pathology: ´...vessels may be affected by arteriosclerosis in different locations, and the resulting clinical conditions bear so little resemblance to each other that it is difficult to give a summary of the symptoms of cerebral arteriosclerosis.´ (Alzheimer 1902). He describes several types of ´arteriosclerotic brain atrophy´ (later vascular dementias): ´nervous´ arteriosclerosis (with very mild symptoms of ´mental and physical fatigue, memory impairment, headache, attacks of vertigo´); ´severe progressive arteriosclerotic brain degeneration´ (with marked clinical fluctuations and frequent ´attacks´); ´Binswanger´s encephalitis subcorticalis chronica´; ´perivascular sclerosis´; and ´dementia post apoplexiam´ (´not caused by stroke as such but by arteriosclerotic areas in the hemispheres´). He also argued the case for the importance of studying vascular brain disease: ´I hope to have proved that arteriosclerotic brain atrophy is a disorder which can definitely be distinguished from other brain diseases.´

Despite these (and other) reports, arteriosclerosis remained the main cause of senility long into the 20th century. As late as 1941, Cajal gave his book `The world seen at eighty´ the subtitle ´Impressions of an arteriopath´ (Impressiones de un arteriosclerotico). The neurodegenerative-vascular dichotomy of today is thus a later 20th century creation.

1.1.4. 20th century classifications

There are currently several (similar but not identical) classifications and diagnostic criteria for dementia, AD and VaD (i.e. DSM, ICD, NINCDS–ADRDA, NINDS-AIREN). A brief outline of their conceptual evolution (with DSM as example) can shed some light on how present problems occurred. A major shift happened between DSM II and III, from mental illnesses conceptualized as broad, etiologically defined entities that were continuous with normality, to symptom-based, categorical diseases (Mayes 2005).

In between the time of Alzheimer and Kraepelin, and the 1970s there is the little analysed period that conceptualized dementia in psychodynamic terms. Already in 1933 an autopsy study had reported a surprisingly loose correlation between clinical dementia and brain pathology (Ballenger 2006). Gradually, psychosocial factors started to be emphasized over brain pathology in dementia aetiology (lack of correlation accounted for by differing ability among individuals to compensate for organic lesions). The publication of DSM I (1952) and DSM II (1968) reflected these changes (Grob 1991). In DSM I, the category of chronic brain disorders included (among other conditions): chronic brain syndrome (CBS) with cerebral

arteriosclerosis, CBS with circulatory disturbance other than cerebral arteriosclerosis, CBS with senile brain disease, CBS with other disturbances of growth, metabolism and nutrition (Alzheimer´s disease is listed as a presenile disorder with ´characteristic´ brain pathology), CBS with disease of unknown or uncertain cause (Huntington and Pick diseases among others). CBS was a basic syndrome consisting of impairment in orientation, memory, all intellectual functions, judgement, and lability and shallowness of affect; it could be mild, moderate or severe, depending on the severity of brain lesions (but the severity of the overall psychosocial impairment depended more on personality and social factors). DSM I also repeatedly emphasized the difficulty of clinical differentiation between CBS with arteriosclerosis, and with senile or presenile brain disease (´both underlying pathological changes may be present simultaneously´). DSM II (based on the mental disorders section of ICD 8) showed a similar conceptual ground, even if CBS was renamed as organic brain syndrome.

In DSM III (1980), the continuum of functionality was made invisible by limiting the focus to the extreme syndrome of dementia, where ´the essential feature is a loss of intellectual abilities of sufficient severity to interfere with social or occupational functioning´. The concept of senile dementia was separated from arteriosclerosis and united with Alzheimer´s disease (following the 1960s studies of Blessed, Thomlinson and Roth): ´Primary Degenerative Dementia of the Alzheimer type is the most common Dementia´ (DSM III). Criticisms of ´arteriosclerotic dementia´ or chronic brain ischemia as an explanation for mental decline contributed to the development of the concept of ´multi-infarct dementia´ (MID) (Hachinski 1974), but in DSM III vascular dementia and MID became identical and the heterogeneity of cerebrovascular disease was lost. Moreover, vascular disease was listed as ´much less common than Primary Degenerative Dementia´. These general lines are still present in DSM-IV-TR (2000) and ICD 10 (1993), although ICD 10 lists ´subcortical vascular dementia´, ´mixed cortical and subcortical vascular dementia´ and ´other vascular dementia´ together with MID. A vicious circle has thus been created: the definition of dementia is based on memory and relies heavily on AD, AD diagnosis has to exclude VaD, but in order to diagnose VaD memory impairment must be present.

Evidence has been accumulating during recent years that there is a considerable overlap between AD and VaD in terms of clinical symptoms, neuropathology and risk factors, and that neurodegenerative and vascular pathophysiological processes interact (Wahlund 2009). A return to the continuum approach to diagnostic criteria is currently being advocated (Hachinski 2008). However, DSM III produced a thorough and irreversible change in mental

health. For the first time, because it was based on ´clear-cut´ categories, a classification could serve as common language not only for clinicians, but also for researchers and payers providing financial reimbursement (i.e. insurance companies, managed health care operations, governments), as well as for organizations financing research (i.e. pharmaceutical companies, governments) (Mayes 2005). One important factor contributing to the maintenance of the categorical approach to disease classification is the requirement that medication be shown effective for specific diseases before receiving approval for patient treatment. The solution to these problems remains to be determined. A new set of criteria for AD has recently been proposed (Dubois 2007), as well as harmonization criteria for vascular cognitive impairment (which is intended to replace VaD) (Hachinski 2006).

1.1.5. Old age cognition

The various domains of cognitive functioning are theoretical constructs rather than directly measurable physical entities. Systematic attempts to define such domains, as well as the search for ways to quantify them, started in the 19th century. Psychology was establishing itself as a science and one of the earliest successes was the study of childhood psychology through intelligence testing of school children. By the 1920s and 1930s, this area of expertise was expanding to include persons at the other end of life (Hirshbein 2002). Because the same methods and assumptions from the work with children were used for elderly, a symmetrical picture of human development resulted, with a rising curve of child development and a declining one of old age. The methods have improved significantly to the present day (i.e. interpreting test scores according to age and education, developing tests that were more appropriate for elderly), and the cognitive symmetry between childhood and old age has been nuanced, but the view that old age means cognitive decline still persists (Ory 2003, Craik and Bialystok 2006, Grady 2008). Chronological age is a good predictor of cognitive abilities, language or sensorimotor coordination at younger ages, but it loses its precision at older ages. A second index of time (correlated with chronological age) becomes more important as people grow older, namely the subjective sense of remaining time until death. Goals, preferences, and cognitive processes seem to change systematically as time horizons change, and across many dimensions older and younger people behave remarkably similarly when time horizons are equated (Carstensen 2006).

1.2. Present matters: cholesterol, AD and VaD

The idea of a relation between cholesterol and VaD is not new (i.e. Mumenthaler 1975). The role of lowering plasma cholesterol levels in prophylaxis and therapy probably began to be discussed once cholesterol was linked to atherosclerosis. However, as the AD/VaD dichotomy was establishing itself, plasma lipids were initially thought to be useful in differentiating AD from VaD (especially MID) (i.e. Muckle 1985, Erkinjuntti 1988).

The main studies suggesting a link between AD and cholesterol were published in the beginning of the 1990s. The APOE ε4 allele of the cholesterol transporter apolipoprotein E (apoE) was reported as a major risk factor for AD (Corder 1993, Poirier 1993), and abundant senile plaques were described in the brains of non-demented patients with coronary artery disease (Sparks 1990). Since then, many common risk and protective factors for both AD and VaD have been reported in various studies (Table 1.1).

Table 1.1. Main proposed risk and protective factors common for stroke and dementia.

Non-modifiable risk factors	Modifiable factors	
	Risk factors	**Protective factors**
Advanced age Genetic factors (e.g. APOE ε4) Family history	Cerebrovascular disease/Stroke Cardiovascular diseases Hypertension Hypercholesterolemia Obesity Diabetes Smoking Homocysteine Psychosocial stress/Depression	High education Physical activity Active lifestyle Alcohol consumption Antioxidants Fish oils Antihypertensives Statins

1.2.1. Genetic connections: APOE

The APOE-AD association has been confirmed in several studies worldwide, in both sporadic and late-onset familial AD cases. To date, the APOE ε4 allele is the only genetic risk factor for AD of established general significance. APOE ε4 is a susceptibility gene, being neither necessary nor sufficient for AD development. The risk of AD increases, and the age of onset decreases with the number of ε4 alleles, in a dose-dependent manner.

ApoE has a central role in lipid metabolism, and the ε4 allele is associated with increased plasma total and LDL cholesterol levels, atherosclerosis, and coronary heart disease. However, the mechanisms relating different apoE isoforms to AD pathophysiology are not completely understood. Within the brain apoE has many functions, being involved in

cholesterol and phospholipid redistribution during development, regeneration after brain injury, and synaptic plasticity; deposition and clearance of β-amyloid (Aβ); inflammatory processes; aggregation of tau; neurotransmission; neuronal survival and sprouting (Hooijmans 2008). Some of these functions do not seem to involve cholesterol.

The APOE ε4 allele seems to be a risk factor for stroke and VaD as well (Schmidt 2002), although conflicting results have been reported in some studies (Tonk 2007, Kim 2008). Problems with outcome definitions due to the heterogeneity of both stroke and VaD are a likely cause of differences in results.

Recent epidemiological studies suggest that APOE ε4 carriers may be more vulnerable to environmental factors (e.g. physical inactivity, saturated fat intake, alcohol drinking, diabetes, high BP, low B12/folate) and could thus benefit more from lifestyle changes and pharmacological interventions (Anttila 2004, Rovio 2005, Laitinen 2006, Kivipelto 2008).

1.2.2. Epidemiological connections

The main longitudinal population-based studies investigating the association between cholesterol and dementia/cognition are summarized in Table 1.2 (midlife cholesterol) and Table 1.3 (late-life cholesterol). Of the long-term follow-up studies on plasma total cholesterol at midlife, the Finnish Cohort of the Seven Countries Study, the Cardiovascular Risk Factors, Aging and Incidence of Dementia (CAIDE) Study (Finland) and the Kaiser Permanente Medical Care Program of Northern California (USA) reported high cholesterol as a risk factor for subsequent dementia, AD or MCI. In the Honolulu-Asia Aging Study (HAAS), clustered cardiovascular risk factors (including cholesterol) were associated with increased risk of dementia, VaD or AD with cerebrovascular disease (but not AD). No significant associations were reported in the Israeli Ischemic Heart Disease Study. Differences between populations and between outcome definitions in these studies could explain the differences in results. Importantly, midlife total cholesterol has also been linked to AD-type brain changes in autopsy studies (Launer 2001, Pappolla 2003).

The Framingham study has over 40 years of follow-up and cholesterol measurements from several occasions starting at midlife. However, the approach taken in the analyses (averaging total cholesterol levels over 30 years) does not allow conclusions on the relation between midlife cholesterol and subsequent dementia/cognitive functioning.

The association between cholesterol and dementia/cognition later in life and closer to dementia onset is less clear, as different studies have led to conflicting results (Table 1.3).

Table 1.2. High midlife total cholesterol as risk factor for subsequent dementia/cognitive impairment

Study	Population and follow-up time	Dementia	AD	VaD	AD with CVD	MCI/Cognition
Finnish Cohort of the Seven Countries Study						
Notkola et al. 1998	N=444 men (47 dementia, 27 AD) Baseline age: 40–59 years Follow-up: 15–25 years		Yes			
Honolulu-Asia Aging Study						
Kalmijn et al. 2000	N=3734 men (251 dementia, 82 AD, 73 VaD, 32 AD with CVD) Mean baseline age: 53 years Mean follow-up: 25 years	Yes (clustered CV risk factors) No (cholesterol alone)	No	Yes (clustered CV risk factors) No (cholesterol alone)	Yes (clustered CV risk factors) No (cholesterol alone)	
CAIDE Study						
Kivipelto et al. 2001a	N=1449 (57 dementia, 48 AD) Mean baseline age: 50 years Mean follow-up: 21 years		Yes			
Kivipelto et al. 2001b	N=1352 (82 MCI) Mean baseline age: 50 years Mean follow-up: 21 years					Yes (MCI risk)
Israeli Ischemic Heart Disease Study						
Beeri et al. 2004	N=1892 men (309 dementia) Baseline age: 44.5 years Follow-up: 36 years	No				
Kaiser Permanente Medical Care Program of Northern California						
Whitmer et al. 2005	N=8845 (721 dementia) Baseline age: 40–44 years Mean follow-up: 27 years	Yes				
Framingham Study – midlife TC data available, but average TC levels over time used in analyses						
Tan et al. 2003	N=1026 (77 AD) TC averaged over 30 years Follow-up: 10 years		No			
Elias et al 2005	N=1894 (no dementia) TC averaged over 16-18 years Follow-up: 4-6 years					Lower TC associated with poorer cognitive test performance

Table 1.3. Late-life total cholesterol in relation to subsequent dementia/cognitive impairment

Study	Population and follow-up time	Dementia	AD	VaD	Cognition
Yoshitake et al, 1995 (Japan)	N=828 (103 dementia, 42AD, 50VaD) Mean age 73.6 years, follow-up 7 years		No	No	
Hyman et al, 1996 (USA)	N=1899 Age 65+, follow-up 4-7 years				Higher TC protective against cognitive impairment
Kalmijn et al, 1996 (Netherlands)	N=353 men Mean age 74.6 years, follow-up 3 years				No
Kuusisto et al, 1997 (Finland)	N=980 (46 AD) Age range 66-75 years, follow-up **3.5** years		Lower TC risk factor		
Wada et al, 1997 (Japan)	N=93 Mean age 79.4 years, follow-up 3 years				Higher TC protective against cognitive decline
Romas et al, 1999 (USA)	N=987 (126 AD) Mean age 73 years, follow-up **2.5** years		Lower TC risk factor		
Slooter et al, 2000 (Netherlands)	N=6435 (395 dementia) Mean age 69.5 years, follow-up **5.8** years	No	No	No	
Karlamangla et al, 2004 (USA)	N=267 Mean age 74 years, follow-up 4.5 years				No
Reitz et al, 2004 (USA)	N=1168 (119 AD, 54 VaD) Mean age 78.4 years, follow-up 4.8 years		Higher TC protective	No	
Solfrizzi et al, 2004 (Italy)	N=2963 (139 MCI) Mean age 71.8 years, follow-up 3.5 years				Higher TC protective against MCI
Li et al, 2005 (USA)	N=2141 (152 AD) Mean age 74.9 years, follow-up **5.6** years	No	No		
Mielke et al, 2005 (Sweden)	N=382 (19 AD, 23 VaD) Age 70 years, follow-up 18 years	Higher TC protective			
Reitz et al, 2005 (USA)	N=1147 Mean age 76.3 years, follow-up 7 years				No association with changes in cognitive functions
Vanhanen et al, 2006 (Finland)	N=959 (45 AD) Mean age 73 years, follow-up 3.5 years		Higher TC protective		
Reitz et al, 2008 (USA)	N=854 (324 MCI) Mean age 76 years, follow-up: about 5 years				No (MCI)

Interestingly, shorter-term follow-up studies of older individuals report either no association, or results that are opposite to those of long-term follow-up studies with midlife as a start point. These contradictions likely indicate that the concept of an adverse lipid profile does not remain constant with age. Several cross-sectional and longitudinal studies have shown that total cholesterol levels tend to increase with age in young- or middle-aged adults, but later decrease as individuals get older (Abbott 1997). This pattern can be partly explained by physiological ageing, unintentional or voluntary changes in lifestyle (i.e. cardiovascular prevention programs meant to lower cholesterol levels) or selective mortality. However, there is another explanation that must be considered in shorter-term follow-up studies of older populations. The relationship between cholesterol and health status seems to be bidirectional, as lower cholesterol levels are frequently observed in association with clinical and subclinical diseases (Ferrara 1997), and a decrease in cholesterol levels, although beneficial for other age groups, may reflect early or occult disease or a gradual decline in the overall health of older persons. Chronic diseases with a long preclinical phase (such as AD or even cerebrovascular disease) pose inherent difficulties in risk factor identification. Since disease onset cannot be pinpointed, chances are that true risk relationships (factors increasing the probability of getting the disease) and reverse causality (the effects of the disease itself on various factors) get confused. At late-life, any apparently normal population will be a mixture of individuals without the disease and individuals who have the disease, although clinically silent and undiagnosed. Therefore, midlife is more suitable as a start point when looking for risk factors, since the disease is not so likely to already be present.

It is thus important to investigate not only cholesterol at specific time points, but also the pattern of change in cholesterol levels from midlife to late-life in relation to cognition/dementia.

1.2.3. Cholesterol homeostasis behind the blood-brain barrier

Epidemiological studies have focused mainly on circulating total cholesterol levels, but plasma cholesterol only represents the tip of the iceberg. As the blood-brain barrier (BBB) separates the brain from the circulation, brain cholesterol homeostasis is tightly regulated locally and is mostly independent from the periphery (Björkhem and Meaney 2004). The human brain is the most cholesterol-rich organ, accounting for only 2% of the total body mass, but containing 25% of the total cholesterol in the body. The major pool of brain cholesterol (about 70-80%) is in myelin. Another pool is represented by neurons and glial

cells. Since about 90% of brain cells are glial cells, neurons contribute to only a small fraction of the total brain cholesterol (Vance 2005). Most cellular cholesterol is found in the plasma membrane, particularly in lipid rafts. These membrane microdomains, critical for normal functioning of the brain, are found in both neurons and glial cells and their distribution within the cell membrane depends upon the cell type (Korade 2008). Cholesterol is also part of the membranes of intracellular organelles performing specialized tasks.

Cellular cholesterol homeostasis is maintained by the balance of synthesis, transport, storage and degradation, with important differences in the brain compared to the rest of the body. Synthesis in the developing CNS is relatively high, but declines to a very low level in the adult due to efficient recycling. Brain cholesterol has thus an extremely long half-life, estimated to at least 5 years (Björkhem and Meaney 2004), which is remarkable as the brain has a high metabolic rate (9-fold greater than the average metabolic rate of the individual). Oligodendrocytes have a clear role in myelin formation, but the roles of neurons and astrocytes in cholesterol synthesis are poorly understood (Pfrieger 2003, Korade 2008). Neurons, specializing in generation of electrical activity, may reduce or even abandon cholesterol production after birth, outsourcing it to astrocytes (since synthesis is too energy-consuming) (Pfrieger 2003). However, only specific types of neurons seem to depend on external cholesterol. Brain cholesterol homeostasis is not uniform, but the cholesterol content and expression level of specific enzymes show strong regional variation (Pfrieger 2003). For example, hippocampal and cortical neurons show different intrinsic concentrations of cholesterol (Korade 2008).

The main cholesterol transporter in the brain is apoE, compared to the periphery where apoB and apoAI are predominant (Blain and Poirier 2004). Brain apoE is synthesized primarily by glial cells (especially astrocytes) (Vance 2005). Astrocytes secrete cholesterol bound to apoE in the form of HDL-like particles, which are internalized by neurons (Björkhem and Meaney 2004). Other proteins involved in transporting cholesterol in lipoproteins in the circulation are also present in the CNS (for example apoAI, D and J, several members of the LDL receptor family, and membrane transporters of the ABC family) (Vance 2005). Their relative importance for the transport and recycling of cholesterol within CNS is still unknown, and there likely is considerable redundancy in these systems.

1.2.4. Brain-periphery interactions: 24- and 27-hydroxycholesterol

Oxysterols are mono-oxygenate derivatives of cholesterol (or cholesterol precursors) that are important as intermediates and end products in cholesterol excretion pathways. The introduction of a hydroxyl group in the cholesterol molecule makes it more polar, drastically reduces its half-life and directs it to excretion or to oxidation to water-soluble bile acids. The physical properties of oxysterols allow them to pass lipophilic membranes and to be redistributed in the body at a much faster rate than cholesterol (Lange 1995). Oxysterols are typically found in conjunction with cholesterol in almost all biological locations, but at significantly lower concentrations (10-100 thousand-fold less). They may be formed by spontaneous or enzyme-mediated processes and tend to be unevenly distributed across different tissues.

24-hydroxycholesterol (24OHC) is formed almost exclusively in the brain, where it is present in greater amounts than in any other organ. It is produced by cholesterol 24-hydroxylase (CYP46), an enzyme present in certain types of neurons (Björkhem 2006). Analysis of RNA isolated from different regions of the human brain indicated that the cholesterol 24-hydroxylase mRNA is broadly distributed, with somewhat higher levels in zones rich in gray matter (i.e. putamen, cerebral cortex, caudate nucleus, amygdala) and lower levels in regions rich in white matter (i.e. corpus callosum) (Leoni 2005). There is a daily flux of about 6-7 mg of 24OHC from the brain into the circulation, and the majority of this efflux occurs as direct transport across the BBB (Lutjohann 1996, Xie 2003). It was estimated that about 1% of the 24OHC produced in the brain is transported to the circulation through the CSF.
Despite efficient recycling, a small excess of cholesterol needs to be exported from the brain into the circulation to maintain steady state. Two such mechanisms are currently known, the most important of which involves conversion of cholesterol to 24OHC (Björkhem 2006). There is also some excretion of apoE-bound cholesterol via the CSF. The precise sites and mechanisms of cholesterol elimination remain to be established (Björkhem and Meaney 2004). 24OHC is also involved in the cholesterol trafficking between astrocytes and neurons, as it can act as a signalling molecule inducing apoE-mediated cholesterol efflux from astrocytes, and it has a direct effect on apoE transcription, protein synthesis and secretion (Vaya 2007).

27-hydroxycholesterol (27OHC) is formed from cholesterol in most extrahepatic organs by sterol 27-hydroxylase, a cytochrome P450 species located in the inner membrane of mitochondria. Production of 27OHC represents an important mechanism for the daily elimination of cholesterol by the body. There is also a flux of 27OHC from the circulation into the brain (Heverin 2005, Leoni 2005).

1.2.5. Cholesterol, neurodegenerative and vascular pathology: some mechanisms

1.2.5.1. Interactions between AD-type and vascular pathology

That the two types of pathologies can interact with each other was clearly shown by findings from the Nun Study (Snowdon 1997). Elderly nuns could carry a heavy load of typical AD changes without having cognitive impairment as long as they did not have, in addition, cerebrovascular lesions (CVLs). CVLs have thus the potential to tip the balance so that persons with AD pathology express a dementia syndrome. For the same degree of dementia severity, AD patients with CVLs can present a lower burden of degenerative lesions than ´pure´ AD cases. Also, in many individuals a combination of minor AD-type and vascular pathologies may cause dementia, when these minor pathologies would not have done so individually, which indicates their synergistic effect. A high proportion of individuals fulfilling the neuropathological criteria for AD also have significant CVLs lesions, just like VaD cases diagnosed by current criteria can have considerable AD pathology at autopsy.

Cerebrovascular structure is profoundly altered in AD. AD patients show atherosclerosis in both intracranial and extracranial vessels (Hofman 1997, Roher 2003). Moreover, atherosclerosis has been associated with increased frequency of neuritic plaques (Honig 2005). One possible mechanism is through cerebral infarcts which influence β-amyloid processing, although additional mechanisms may exist.

Persons with AD can have cerebral amyloid angiopathy (CAA), which has been associated with microbleeds. In addition, the number of cerebral microvessels is reduced, endothelial cells are flattened, smooth muscle cells are modified and there is basement membrane pathology, with β-amyloid deposits in the microvascular wall (Girouard and Iadecola, 2006). Also, CAA is not uncommon in VaD, where it is related to cortical microinfarcts (Haglund 2006). APOE ε4 allele is associated with increased vascular Aβ deposition (Kumar-Singh 2008). While CAA related to capillaries and smaller arterioles (a type more closely linked

with AD) is related to APOE ε4, larger vessel CAA seems to be associated with APOE ε2 (Kumar-Singh 2008).

Cerebrovascular dysfunction is also present in persons with AD. Resting cerebral blood flow (CBF) is reduced and the increase in CBF produced by activation is attenuated, and β-amyloid seems to be one of the factors involved in CBF dysregulation. Most of the deleterious effects of β-amyloid were initially attributed to the form deposited in plaques, but more recent data indicate that soluble β-amyloid oligomers may actually be the culprit. β-amyloid has long been known to have neurotoxic and proinflammatory properties. However, a growing body of evidence indicates that it also has profound effects on blood vessels, causing vasoconstriction and attenuating acetylcholine-induced vasodilatation. Neurons, glia and vascular cells act as an integrated unit which maintains the homeostasis of the brain's microenvironment, and β-amyloid-induced dysfunction of this unit can result in vascular dysregulation. In turn, ischemia may upregulate APP and β-amyloid cleavage. Vascular dysfunction can affect β-amyloid trafficking across the BBB, reducing the rate of β-amyloid clearance from the brain (Girouard and Iadecola, 2006). These two processes reinforce each other, and vascular dysregulation becomes more pronounced as the disease progresses. This connection between ischaemia and Aβ is a possible explanation for the fact that co-occurrence of ischaemic lesions and AD pathology aggravates dementia. It remains to be established whether neurofibrillary tangles also contribute to vascular dysregulation and, if so, the mechanism through which they exert their effect.
Resting CBF reductions observed in AD may not be sufficient to produce acute ischaemic injury. However, cerebral protein synthesis (which is crucial for learning, memory, and for normal cognitive functioning), is susceptible to reductions in CBF (Girouard and Iadecola, 2006). Also, owing to an altered cerebrovascular autoregulation, reduced arterial pressure (for example in various cardiovascular conditions) might result in reductions in CBF severe enough to cause ischaemic cell injury. Recurrent ischemia in susceptible territories such as deep white matter could explain the frequent occurrence of WML in patients with AD.

Cholinergic mechanisms also play a role in the modulation of regional blood flow in the brain. The cholinergic system and CBF appear to be linked in a reciprocal manner: CBF changes may affect central cholinergic neurons, and unimpaired cholinergic function is necessary to regulate regional CBF. Cholinergic deficits have been shown in both AD and

VaD, and the choline acetyltransferase (ChAT) activity deficit is more pronounced in patients diagnosed with mixed dementia (AD and VaD) (Roman and Kalaria, 2006).
It is also interesting that soluble β-amyloid and tau proteins have been identified by immunocytochemical methods in patients with VaD, even when conventional histopathological analysis did not reveal classical AD pathology (Kalaria 2002). It can be thus hypothesized that the β-amyloid-driven neurodegenerative process may predominate in some cases, while in others ischemia may be the main pathogenic process. However, the more the disease progresses and the pathological changes interact and accumulate, the harder it becomes to disentangle the degenerative and vascular components and to identify the ´true cause´.

1.2.5.2. The roles of cholesterol

The most obvious connection between increased plasma cholesterol and dementia is through vascular mechanisms. Cholesterol is involved in atherosclerosis, and several cardio- and cerebrovascular conditions have been reported to increase the risk of both AD and VaD (Jefferson and Benjamin 2009). Increased plasma total cholesterol (especially at midlife) has also been linked to AD-type pathology in autopsy studies (Launer 2001, Pappolla 2003). In addition, several population-based imaging studies using MRI have documented a positive correlation between the number of cortical infarcts and WML with plasma cholesterol levels (Breteler 1994, Murray 2005, Amarenco 2006).
Cholesterol-rich diets in rabbits or mice were associated with increased cerebral accumulation of β-amyloid (Sparks 1994, Refolo 2000, Shie 2002) and tau hyperphosphorylation (Rahman 2005). Interestingly, in an APP/PS1 mouse model of AD pathology, the effects of cholesterol-rich diet on vascular parameters (i.e. relative cerebral blood volume) were observed before effects on Aβ load were noted (Hooijmans 2009).
Less data is available on LDL and HDL. Higher LDL was found to increase the risk of VaD or dementia with stroke (Moroney 1999, Reitz 2004), and was also associated with WML (Amarenco 2006). A dose-response relation between plasma HDL and number of neuritic plaques (NP) and neurofibrillary tangles (NFT) has been reported (Launer 2001), while low HDL levels were associated with lower hippocampal volumes in some (Wolf 2004) but not all (den Heijer 2005) studies. HDL is particularly interesting, since only HDL-like lipoproteins are found in the CSF (Michikawa 2006) and CSF HDL levels correlate with plasma HDL levels (there is no such correlation between total cholesterol and LDL in plasma and CSF).

Findings on brain cholesterol levels seem to depend on the particular pool in question. An increase in brain cholesterol levels (Hartmann 2007), as well as decreased cholesterol in the white matter of AD patients (Michikawa 2006), have been reported. The temporal cortex from AD patients showed loss of lipid rafts, while hippocampi showed reduced cholesterol content in the rafts (Korade 2008). Changes in the asymmetric transbilayer distribution of cholesterol in neuronal plasma membranes have been suggested as more important in Aβ production than the total or bulk cholesterol (Wood 2002).

The relationship between cholesterol and Aβ seems to be bidirectional. Cholesterol is a major component of lipid rafts, which are involved in modulating cellular structures and cellular function, including APP processing. It has been hypothesized that APP is located in two different compartments in the membrane, and that non-amyloidogenic α-cleavage occurs outside the lipid rafts, while APP within rafts is processes by β- and γ-secretases, leading to the formation of Aβ (Hartmann 2007). Very little is known about a physiological function of Aβ besides its pathological AD-related aspects, but there is evidence that APP processing, and especially Aβ, influence lipid metabolism. Aβ can inhibit cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA), although it is not clear how this regulation is mediated (Grimm 2007). A negative feed-back cycle can thus be hypothesized: when increased cholesterol increases Aβ production, Aβ acts to inhibit cholesterol synthesis. The difficulty consists in determining where and how this balance becomes disrupted. There is evidence that increased cholesterol could be a determinant for switching APP processing towards the amyloidogenic pathway (Xiong 2008), and some studies have shown that soluble oligomers of Aβ impair cholesterol synthesis and finally reduce cholesterol levels in neurons (Michikawa 2006). Besides their involvement in Aβ production, lipid rafts seem to be involved in Aβ aggregation and toxicity, but may also be involved in Aβ clearance (Cordy 2006).

The extracellular accumulation of Aβ in AD is well-known (with cholesterol and apoE co-localized in the core of amyloid plaques) (Hooijmans 2008), but the importance of intraneuronal Aβ accumulation was recognized more recently. Extracellular Aβ contributes to the intracellular pool of Aβ, although the mechanisms of Aβ uptake by neurons are poorly understood. A model has been proposed in which free Aβ and Aβ bound to apoE and other ligands enter the neurons by different mechanisms. The complex Aβ-apoE is internalized by LDL receptor related protein and related receptors, while uptake of Aβ not related to apoE is raft-mediated and requires cholesterol (Saavedra 2007). The proportion of each pool of Aβ is

determined by Aβ levels in the extracellular space and by the efficiency of Aβ binding to apoE (which is isoform-dependent). At very high Aβ levels, the model predicts that the pool of free Aβ becomes more significant than the pool of Aβ-apoE (Saavedra 2007).

To complicate the matter further, it has been suggested that the ratio between free and total cellular cholesterol is important in APP processing (Björkhem and Meaney 2004), but the exact role of free and esterified cholesterol is unclear. One study showed that an increase in cholesterol esters can regulate the generation of Aβ, and acyl-coenzyme A cholesterol acyltransferase (ACAT) inhibitors can downregulate Aβ production (Puglielli 2003).

Apart from plasma membrane cholesterol, mitochondrial cholesterol is beginning to receive attention in relation to dementia. Mitochondria are cholesterol-poor organelles, including about 0.5-3% of the content found in other cellular membranes. However, mitochondrial cholesterol fulfills vital physiological functions (Garcia-Ruiz 2009), and its accumulation may be important in pathophysiological processes leading to dementia.

A connection between cholesterol and tauopathy is suggested by Niemann-Pick disease models, where there is cholesterol accumulation with development of NFT that are essentially identical to those observed in AD. It has been reported that the state of tau phosphorylation is modulated not so much by total cellular cholesterol levels as by cholesterol levels in specific compartments such as lipid rafts, with reduced cholesterol promoting phosphorylation (Michikawa 2006).

Moreover, the failure of fine tuning of brain cholesterol homeostasis seems to affect several chemical neurotransmitters, receptor and transporter systems, including the cholinergic system (Koudinov 2005).

1.2.5.3. 24- and 27-hydroxycholesterol

Plasma 24OHC levels seem to be increased in the early stages of AD, possibly reflecting ongoing demyelinization, but they are reduced in advanced AD, probably as a consequence of the loss of neurons containing CYP46 (Björkhem 2006). Decreased plasma levels of 24OHC and 27OHC have been reported in patients with AD or VaD compared to non-demented controls (Kölsch 2004). Plasma 24OHC has also been linked to hippocampal size in middle-aged normal individuals (Koschack 2007).

Increased 27OHC levels were found in CSF in patients with advanced AD, who are believed to have BBB disturbances (Björkhem 2006). Autopsy studies in AD patients have shown that the levels of 24OHC were decreased in most brain areas, while the levels of 27OHC were

increased (Heverin 2004); also, altered patterns of expression of CYP46 and CYP27A1 (the enzyme involved in 27OHC synthesis) were observed (Bogdanovic 2001, Brown 2004).
It has been shown that 24OHC is an efficient inhibitor of the formation of Aβ peptides under *in vitro* conditions, while 27OHC has a much lower capacity to inhibit the reactions. A stimulatory effect of 24OHC on α-secretase activity was observed *in vitro*; this effect was antagonized by 27OHC (Björkhem 2009). It is thus possible that 27OHC ´translates´ peripheral high total cholesterol into changes in the brain (Mateos 2009, Björkhem 2009).

1.2.6. Effects of statins on cholesterol, β-amyloid and brain vascular lesions

Statins are HMG-CoA reductase inhibitors, interfering with cholesterol synthesis at its rate-limiting step, and reducing the production of both cholesterol and its isoprenoid intermediates. In addition, statins have several cholesterol-independent (pleiotropic) effects: reduction of endothelial dysfunction, atherosclerotic plaque stabilization, antiinflammatoy and antioxidant effects, effects on coagulation, immunomodulation, and reduced cell proliferation (Alegret and Silvestre 2006).

1.2.6.1. On plasma and brain cholesterol

In plasma, statin treatment decreases LDL and may slightly increase HDL. APOE ε2 carriers show greater reductions in LDL and APOE ε4 carriers are the least responsive, but the latter may have a greater reduction in cardiovascular outcomes (Nieminen 2008). The effects of statins on the human brain are however more difficult to study. Depending on their solubility in lipid solvents or water, statins can be more lipophilic or hydrophilic, and this characteristic influences their crossing of the BBB. Also, the BBB may be affected in dementia, which may influence drug levels in the brain. It is currently unclear whether statins need to enter the brain and act directly on disease processes or whether a more indirect action on the brain should be desirable.
Results from in vitro and in vivo animal studies are not easy to translate into a human context, especially as the statin dosages in such studies are usually much higher than therapeutic dosages in patients. Data on the effects of statins on brain cholesterol in mice, rats or guinea pigs are conflicting, indicating either a decrease (Eckert 2001, Refolo 2001, Petanceska 2002, Johnson-Anuna 2005, Burns 2006) or no change in total cholesterol levels (Fassbender 2001, Lutjohann 2004, Thelen 2006, Cibickova 2009). These studies investigated statins with different degrees of lipophilicity, but it is difficult to identify a pattern: lovastatin decreased

brain cholesterol in mice in two studies (Eckert 2001, Burns 2006) but no effect was reported by another study (Johnson-Anuna 2005); pravastatin was effective in one study (Johnson-Anuna 2005) and had no effect in other two (Lutjohann 2004, Thelen 2006); simvastatin did not influence total cholesterol (Fassbender 2001, Lutjohann 2004, Burns 2006, Thelen 2006, Cibickova 2009), with one exception (Johnson-Anuna 2005); and atorvastatin decreased (Petanceska 2002) or did not affect brain cholesterol levels (Burns 2006, Cibickova 2009). Moreover, lovastatin and simvastatin (but not pravastatin) were reported to lower levels of unesterified cholesterol in synaptic plasma membrane (SPM), and slightly enhance cholesterol ester levels, although total SPM cholesterol levels remained unaffected (Kirsch 2003). Several studies have reported that, despite a lack of effect on total cholesterol levels, statins can decrease brain cholesterol synthesis (Fassbender 2001, Lutjohann 2004, Thelen 2006, Cibickova 2009) and may or may not influence 24OHC and HMGCo-A reductase levels (Lutjohann 2004, Thelen 2006, Cibickova 2009).

Because statins block cholesterol synthesis, disruption of lipid raft integrity by these drugs has been investigated, again with conflicting results. In vitro studies of neuronal structures and immune cells reported a disruption in lipid raft integrity after treatment, but other studies show that statins do not alter intracellular cholesterol levels or raft integrity (Zipp 2007).

The transbilayer distribution of cholesterol is asymmetrical, with the cytofacial leaflet of the membrane including the highest amount of cholesterol. Statins have been reported to modify cholesterol transbilayer distribution, but the pattern is not yet clear: in one study, the more hydrophilic pravastatin only affected the exofacial leaflet (decreasing cholesterol levels), lovastatin reduced cholesterol in both leaflets, and simvastatin-induced cholesterol depletion occurred predominantly in the cytofacial leaflet (Kirsch 2003); in another study, simvastatin, lovastatin and atorvastatin caused cholesterol to translocate from the cytofacial to the exofacial leaflet (Burns 2006).

1.2.6.2. On β-amyloid

Several studies have reported that statin treatment lowers Aβ production (Buxbaum 2001, Fassbender 2001, Refolo 2001, Petanceska 2002, Burns 2006), but conflicting results exist as well (Park 2003, Cibickova 2009). β-secretase is affected by statins (Parsons 2007). The effect on Aβ could be cholesterol-dependent, and in addition statin inhibition of protein isoprenylation can result in decreased Aβ secretion (Ostrowski 2007). It has been suggested that two pools of Aβ exist and appear to function independently of each other, with the intracellular pool regulated by isoprenoids and the secreted pool regulated by cellular

cholesterol levels (Reid 2007). Statins also reduce Aβ-mediated microglial neurotoxicity in vitro independently of cholesterol lowering, through isoprenoid depletion (Cordle 2005). Other pleiotropic effects may be important as well.

Several clinical studies have investigated the effects of statins on Aβ production and cholesterol metabolism in various populations, from adult healthy controls to patients with hypercholesterolemia or AD (Höglund and Blennow 2007). While in vitro studies interpret reduced Aβ levels in cell media as reduced Aβ production, studies in humans measure Aβ in blood and CSF, where reduced levels are considered a pathological finding. It is not clear whether statin treatment should be expected to lead to lowered or increased Aβ, or how the changes should be interpreted. With two exceptions, most clinical studies reported no effect of statins on Aβ levels in blood or CSF (Höglund and Blennow 2007). However, it seems that statin treatment can reduce cholesterol synthesis in the CNS.

1.2.6.3. On vascular brain lesions

The potential role of statins in dementia of vascular origin has been less explored so far. Such actions of statins may be partly independent of their cholesterol-lowering effect. For example, a recent study investigated the effect of atorvastatin and pitavastatin on a rat model of vascular dementia (Koladiya 2008). Both atorvastatin and pitavastatin attenuated L-Methionine induced endothelial dysfunction associated memory deficits. Statins also reversed L-Methionine induced rise in brain oxidative stress and serum cholesterol (Koladiya 2008). Acute neurovascular unit protection by simvastatin in transient cerebral ischemia in rats has also been reported (Nagaraja 2006).

In a study on patients with cerebral small vessel disease, pravastatin was found to improve acetazolamide-induced cerebral vasoreactivity; the effect was more pronounced in patients with prominent impairment of cerebral vasomotor function (Sterzer 2001). Statins have also been shown to reduce the risk of ischaemic stroke (Endres 1998, White 2000, Heart Protection Study 2002).

1.2.7. Statins and prevention of dementia/cognitive impairment

The main longitudinal studies related to statins or other lipid-lowering agents (LLAs) and prevention of dementia/cognitive impairment are summarized in Tables 1.4, 1.5 and 1.6. An initial wave of mostly cross-sectional studies resulted in a very positive attitude towards the role of statins in dementia prevention (Rockwood 2006). The current attitude continues to be

positive, but it is nevertheless more nuanced after many studies that are heterogeneous in design, populations, outcomes, and drug exposure evaluation. It is obvious that the established effects of statins on cardiovascular outcomes raise major ethical questions regarding population-based trials in dementia prevention. The data come thus mainly from studies with other types of designs (such as case-control or prospective cohort). Dementia and AD are the main outcome in 13 such studies, of which 8 are positive and 5 indicate a lack of effect. VaD was considered as separate outcome only in three studies, and one study indicated a positive effect (Table 1.4). Other outcomes (such as cognition) were considered in 9 additional studies, the majority of which indicate positive effects of statins (Table 1.5).

Currently available literature offers several important lessons with respect to planning and interpreting studies on statins and dementia prevention. For example, the conventional retrospective case-control study has a particular potential to give the appearance of a protective effect, because the relevant case exposure period is usually truncated at an arbitrary ´reference´ time prior to symptom onset or time of diagnosis, giving controls a greater probability of exposure (Li 2004). This problem was solved in nested case-control studies by limiting the exposure period for controls at a reference time period similar to cases. Also, exposure to statins is not randomized outside clinical trials, but is determined by a wide range of health-related factors. Case-control studies are more likely to suffer from prescription bias than prospective cohort studies. Several issues related to indication bias have been discussed by currently available papers, such as the ´healthy user effect´, which can occur when physicians are more likely to prescribe a new drug to patients who are better educated, more health-conscious and with better access to health care. On the other hand, physicians may less readily prescribe statins to persons with dementia or cognitive impairment, due to concerns about adherence, complications or priorities in health care resource allocation. It has been shown that dementia status is a predictor of underutilization of other medicines for the prevention of vascular disease (Moroney 1999). Indication bias has been managed in several ways in the studies on LLAs, by allowing for a lag period before the dementia diagnosis, by using a propensity score as a measure of a person’s likelihood of being prescribed LLAs, or by focusing on the risk of a precursor syndrome (MCI or cognitive impairment, no dementia - CIND) instead of dementia risk. The optimal length of the lag period before dementia diagnosis is however difficult to establish, as the onset of dementia or cognitive impairment and the onset of the underlying disease(s) are hard, if not impossible to establish.

Table 1.4. Summary of studies on lipid-lowering agents (LLAs) in dementia/AD prevention

Study (publication year)	Design and population	Outcomes	Treatment evaluation	Results
Jick et al. (2000)	Nested case-control (GP patients) UK-based General Practice Research Database 284 cases, 1080 controls Age ≥50 (85.6% 70-89 years old) Follow-up: mean 5.5 years	Dementia	Research database Past/current use (at least 1 prescription 180 days before index date)	Statins: protective effect (current use), independent of hyperlipidemia Other LLAs: no effect
Rockwood et al. (2002)	Case-control (community and institutions) Canadian Study of Health and Aging (CSHA) 492 cases (326 AD), 823 controls Age ≥65 years Follow-up: up to 5 years	Dementia AD	Self-reported	Protective effect of statins and other LLAs in subjects younger than 80 years
Zamrini et al. (2004)	Nested case-control (patients) Veteran Affairs Medical Center (VAMC), US 309 cases, 3088 controls Mean age 73 years; males only; multiethnic Follow-up: up to 4 years	AD	VAMC prescription files Past/current use (prescription filled 6 months before index date) Duration of use	Statins: protective effect (past use), especially among subjects with cardio/cerebrovascular conditions
Reitz et al. (2004)	Cohort (community) Medicare recipients, Manhattan, US 1168 subjects (119 AD, 54 VaD) Mean age 78 years; multiethnic Follow-up: mean 4.8 years	AD VaD	Self-reported	No effect
Li et al. (2004)	Cohort (community) Adult Changes in Thought Study (Seattle, US) 2356 subjects (312 dementia, 168 AD) Mean age 75 years; multiethnic Follow-up: up to 8 years	Dementia AD	Pharmacy database (duration, cumulative& average daily dose); use defined as ≥2 consecutive fills in 6 months	No effect of either statins or other LLAs Protective effect of statins if inappropriately analyzed as case-control study
Zandi et al. (2005)	Cohort (community and institutions) Cache County Study, Utah, US 3308 subjects, 185 dementia (104 AD) Mean age: about 73-75 years Follow-up: 3 years	Dementia AD	Direct visual inspection of containers; physician and nursing home records Duration of use	No effect of either statins or other LLAs
Rea et al. (2005)	Cohort (community) Cardiovascular Health Study, US 2798 subjects (480 dementia, 245 AD, 62 VaD, 151	Dementia AD VaD	Self-reported Past/current use	No effect for either statins (irrespective of lipophilicity) or other LLAs

	mixed); mean age 75 years; multiethnic Follow-up: 5 years	Mixed		
Li et al. (2006)	Cohort (community) Adult Changes in Thought Study (Seattle, US) 2356 subjects (380 dementia, 204 AD) Age: ≥65 years Follow-up: up to 8 years	AD	Pharmacy database Use: at least 3 prescriptions before dementia onset	Protective effect of statins more likely before the age of 80 years
Wolozin et al. (2007)	Retrospective linkage, Decision Support System (DSS) database, US Veterans Affairs medical system 1290071 patients: 841963 statins; 394739 cardiovascular and 53369 warfarin comparator Age: ≥65 years Follow-up: 2 years	Dementia	DSS database Use: repeat prescription refills during 7 months (gaps no longer than 6 weeks)	Strong protective effect for simvastatin, modest protective effect for atorvastatin
Arvanitakis et al. (2008)	Cohort Religious Order Study, US 929 subjects, 191 AD; autopsy data: 262 subjects Mean age 75 years Follow-up: up to 12 years	AD Cognition Neuropathology	Direct visual inspection of containers	Statins: no effect on AD/cognition, irrespective of lipophilicity; users of more lipophilic statins less likely to have amyloid; no relation to tangles or amyloid load
Smeeth et al. (2008)	Cohort (GP patients) The Health Improvement Network (THIN), UK 129288 statin users&matched sample of 600241 non-users; 5172 dementia (725 AD) Age ≥40 years Median follow-up: 4.4 years	Dementia AD Non-AD	THIN database	Protective effect of statins against dementia in general, AD and non-AD dementias
Haag et al. (2008)	Cohort (community) The Rotterdam Study, Netherlands 6992 subjects (582 AD) Mean age 69 years Follow-up: mean 9 years	AD	Pharmacy databases (units delivered, prescribed daily no. of units, date of delivery, drug dosage, duration of prescription)	Protective effect of statins, independent of lipophilicity
Sparks et al. (2008)	Elective statin use in a RCT cohort Alzheimer´s Disease Anti-inflammatory Prevention Trial (ADAPT) 2233 subjects (25 incident AD) Mean age: 74 years Follow-up: up to 4 years	AD	LLA use recorded at each scheduled RCT visit	Elective statin use associated with reduced AD risk

Table 1.5. Summary of studies on lipid-lowering agents (LLAs) and other AD-related outcomes

Study (publication year)	Design and population	Outcomes	Treatment evaluation	Results
Yaffe et al. (2002)	Observational study of 1037 post-menopausal women enrolled in the Heart and Estrogen/progestin Replacement Study Mean age 71 years Duration: 4 years	Cognition	Self-reported	Positive effects of statins
Bernick et al. (2005)	Cohort (community) Cardiovascular Health Study, US 3334 subjects Mean age: 73-75 years; multiethnic Follow-up: average 7 years	Cognition MRI (WM, atrophy measures)	Self-reported Untreated/intermittent use /continuous use (≥4 years continuous use)	Statin use associated with slight reduction in rate of cognitive decline (not related to baseline lipid levels)
Parale et al. (2006)	Before and after comparison study with controls 97 subjects (49 atorvastatin, 48 placebo) Age ≥40 years Duration: 6 months	Cognition	Atorvastatin 10 mg/day vs placebo	Positive effects
Li et al. (2007)	Cohort (community) Adult Changes in Thought Study (Seattle, US) 110 subjects Mean age 74 years; mainly Caucasian Follow-up: about 7 years	Neuropathology	Pharmacy database (estimated duration, cumulative& average daily dose); use: at least 3 prescriptions	Statin use related to reduced NFT burden. Typical AD pathology (Braak ≥IV, CERAD ≥moderate) less common in statin users.
Rockwood et al. (2007)	Case-control (community) Canadian Study of Health and Aging (CSHA) 347 cases, 693 controls Age: ≥65 years Follow-up: 5 years	CIND	Self-reported	Statins: protective effect in subjects younger than 80 years. No effect for other LLAs.
Szwast et al. (2007)	Cohort (community) Indianapolis Ibadan Dementia Project 1146 subjects, 32 dementia Mean age 77 years; African-American Follow-up: 3 years	Cognition Dementia	Self-reported Baseline use/use over time	Baseline statin use associated with less cognitive decline Less clear effect for use over time
Reitz et al. (2008)	Cohort (community) Medicare recipients (northern Manhattan) 854 subjects (324 MCI) Mean age 76 years; multiethnic Follow-up: about 5 years	MCI	Self-reported	No effect of statins

Cramer et al. (2008)	Cohort (community) Sacramento Area Latino Study on Aging (SALSA) 1674 subjects, 130 dementia/CIND Mean age 70 years; Mexican Americans Follow-up: 5 years	Dementia/CIND	Direct inspection of containers	Protective effect of statins

Table 1.6. RCTs with statins for prevention of dementia/cognitive impairment

Study (publication year)	Population	Outcomes	Treatment	Results
Muldoon et al. (2000)	RCT (hypercholesterolemic adults) 209 subjects Mean age: 46 years Duration: 6 months	Cognition	Lovastatin 20 mg vs placebo	Lack of improvement between baseline and post-treatment assessments
HPS Collaborative Group (2002)	RCT (subjects with substantial 5-year risk of death from coronary heart disease) Heart Protection Study (HPS) 20536 subjects (62 incident dementia) Age: 40-80 years Duration: mean 5 years	Cognition Dementia (tertiary outcomes)	Simvastatin 40 mg vs placebo	No effect
Shepherd et al. (2002)	RCT, Pravastatin in elderly individuals at risk of vascular disease (PROSPER) 5804 subjects, mean age 75 years Duration: mean 3.2 years	Cognition (tertiary outcome)	Pravastatin 40 mg vs placebo	No effect
Muldoon et al. (2004)	RCT (hypercholesterolemic adults) 283 subjects Mean age 54 years Duration: 6 months	Cognition	Simvastatin 10mg or 40mg vs placebo	Lack of improvement between baseline and post-treatment assessments
Carlsson et al. (2008)	RCT, 57 middle-aged asymptomatic children of AD patients Duration: 4 months	Cognition	Simvastatin 40 mg vs placebo	Positive effects on cognition

Another issue is the reliability of drug exposure evaluation. Interview data can be affected by recall bias, while pharmacy or other systematic databases are more reliable. Self-reported use can lead to exposure misclassification if only baseline use is considered, since persons starting therapy during follow-up are classified as non-users. In this respect, the frequency of ascertainment of statin use is important. Some studies were able to make a more detailed evaluation of LLAs exposure and included information on current versus past use, or continuous versus intermittent use, although the definitions of these categories, as well as the results, vary from study to study. It is also difficult to determine whether ´current use´ functions more as a marker of good health and ´past use´ (treatment discontinuation) as a marker of poor health, than merely as drug exposure parameters. Duration of LLAs use, dosage or even change of drug type during treatment were considered as well in some studies, but power issues (small number of exposed persons and persons who developed dementia/AD) limited extensive analyses of such information in relation to the outcome(s) and in different groups of subjects. No clear pattern of LLAs use has emerged as particularly beneficial.

Exposure evaluation can become more difficult due to changes in drug availability. One example comes from UK, where in June 2004 statins became available for sale in pharmacies without prescription (Smeeth 2008). The UK study reported nevertheless a beneficial effect of statins in dementia prevention even after restricting the analyses to the interval before 2004.
However, even when exposure evaluation is reliable, the interval between drug exposure and disease onset in dementia studies remains a problem. Dementia (and especially, but not exclusively, AD) has a preclinical phase in which the underlying disease(s) begin to manifest themselves as cognitive impairment, and the duration of this phase, as well as the exact onset of the disease(s), cannot be pinpointed with current diagnostic methods and criteria. Many studies include older populations (with individuals who may have clinically silent AD at the start of the study), and have rather short follow-up times (<6 years). The Adult Changes in Thought Study, with up to 8 years follow-up and LLAs exposure evaluation from pharmacy database, reported initially no significant effect of statins or other LLAs in dementia/AD prevention (Li 2004), but later analyses indicated that a protective effect of statins would be more likely before the age of 80 years (Li 2006). The autopsy of 110 subjects from the same study suggested that statin use may be related to reduced NFT burden; also, typical AD pathology (Braak ≥IV, CERAD ≥moderate) seemed to be less common in statin users (Li 2007). The Rotterdam study, with a mean follow-up of 9 years and drug information also

from pharmacy database, reported a protective effect of statins in AD prevention (Haag 2009). Results from the Religious Orders Study (follow-up up to 12 years) did not show any effect of statins on AD or cognition, and no relation of statins to tangles or amyloid load (Arvanitakis 2008).

It is currently unclear when LLAs treatment should be started and what duration it should have in order to observe an effect on dementia incidence. It is also unclear whether there is a difference between types of LLAs, i.e. more lipophilic versus more hydrophilic statins. Currently available data do not indicate different effects in dementia prevention (Rea 2005, Arvanitakis 2008, Haag 2009), although users of more lipophilic statins were reported in one study to be less likely to have amyloid (Arvanitakis 2008). Another study indicated a strong protective effect for simvastatin, and modest protective effect for atorvastatin (Wolozin 2007). The major strength of this study is the statistical power provided by a very large number of subjects (over one million) and a well-structured database, but indication bias cannot be excluded since the majority of subjects received simvastatin. Regarding LLAs other than statins, current data do not indicate any effect in dementia prevention (Tables 1.4, 1.5).
Statins are approved for use in patients with hyperlipidemic disorders. However, such patients typically have several other conditions such as diabetes, hypertension, and cardio/cerebrovascular diseases, which are also known to increase the risk of dementia/AD. Different efforts to minimize confounding were made in all studies. Interestingly, some results seem to indicate that statins may be more effective in preventing dementia in persons with cardio/cerebrovascular conditions (Zamrini 2004).

The RCTs of statins in prevention of cognitive decline or dementia are summarized in Table 1.6. The largest are HPS and PROSPER, which reported no effects (HPS Collaborative Group 2002, Shepherd 2002). However, the add-on design (with cognition/dementia as tertiary outcomes in studies focused on prevention of cardiovascular events) and the possible lack of power (too few dementia cases) question these results. HPS included only a telephone interview of cognitive status (TICS) at the end of the study, and PROSPER had only a Mini-Mental State Examination (MMSE) at the last clinical visit, without any information on baseline cognitive performance. Two smaller and shorter RCTs (Muldoon 2000, Muldoon 2004) generated a debate about the safety of statin treatment by reporting ´minor decrements in cognitive functioning´. However, these represented not an absolute decline in cognitive performance, but lack of improvement between baseline and post-treatment assessments

(Muldoon 2004), observed over 6 months in a population of generally healthy hypercholesterolemic middle-aged adults. The National Lipid Association Statin Safety Assessment Task Force has concluded that statin treatment is safe with respect to cognition (McKenney 2006). Another small RCT has recently reported positive effects of statins on cognition in middle-aged asymptomatic children of AD patients (Carlsson 2008).

1.2.8. Statins in patients with dementia

Only AD has so far been considered as an outcome in RCTs with statins. Four RCTs have investigated the effects of statin treatment in persons who already have AD. The first study involved a 26-week treatment with 80 mg simvastatin versus placebo in 44 patients with mild and moderate AD (MMSE 12 to 26). Slower progression of cognitive decline as measured with MMSE (but not ADAS-Cog) was reported (Simons 2002). Some beneficial effects were also reported for atorvastatin (80 mg versus placebo) in the Alzheimer´s Cholesterol-Lowering Treatment (ADCLT) study, a 1-year RCT of 63 patients with mild to moderate AD (MMSE 12-28) (Sparks 2005). Secondary assessment indicated that the ADCLT subjects who had the greatest benefit from atorvastatin therapy in terms of their 6-month ADAS-cog score were those with higher cholesterol levels at trial entry, those with the APOEε4 allele, and those less affected by AD at trial entry (ie, with higher entry MMSE scores) (Sparks 2006). Interestingly, a pilot ADCLT substudy showed a nonsignificant reduction in total hippocampal volume with atorvastatin therapy, driven by a highly significant reduction in right hippocampal volume (Sparks 2008a). A similar finding was reported from the beta-amyloid immunization (AN1792) treatment trial in AD (Fox 2005), where active immunization was associated with significant clinical benefit, reduced Aβ load, and reduced hippocampal volume. The authors suggested that removal of Aβ and/or other protein constituents from the tissue might have caused a ´fluid shift´ out of the tissue, resulting in shrinkage. An alternative explanation was proposed in the ADCLT substudy, based on findings of reduced brain tissue density in AD patients compared with age-matched normal controls: neuronal loss in the hippocampus may be accompanied by increased fluid balance (reduced density) in an attempt to retain the previous volume at the expense of function; accordingly, as the hippocampus shrinks, it approaches a more normal density for the remaining neuronal complement, and cognitive function improves (Sparks 2008a).

The Atorvastatin/Donepezil in Alzheimer´s Disease Study (LEADe) is an 80-week multicenter RCT of atorvastatin 80 mg versus placebo in patients with mild to moderate AD (MMSE 13-25) receiving background therapy of donepezil 10 mg daily (Jones 2008). 641 subjects (mean age 74 years) were enrolled. More detailed results are expected, but it seems that there was a lack of significance of the positive signal of benefit on the ADAS-cog in the treatment of AD with atorvastatin (Sparks 2009). It has also been reported that, in analyses of the data from the LEADe study when considering only individuals evaluated for APOE genotype, atorvastatin produced a significant benefit on the ADAS-cog compared with placebo (Sparks 2008b). This suggests that a subset of individuals with AD benefit from statin therapy, but the magnitude of the difference produced in the subset of study subjects was insufficient to make the positive signal significant in the entire LEADe cohort (Sparks 2009).
Cholesterol Lowering Agent to Slow Progression of AD (CLASP) study is another large multicenter RCT, investigating the effects of 18-months simvastatin treatment (20mg/day for 6 weeks then 40mg/day) versus placebo. 406 subjects with mild to moderate AD (mean age 74 years, mean MMSE 20) were randomized and detailed results are expected, although preliminary results seem to indicate a benefit-neutral effect of simvastatin in AD treatment (Sano 2008, Sparks 2009).

Interestingly, some recent observational studies have reported a slower cognitive decline in memory clinic patients with AD treated with LLAs (Masse 2005); lower risk of deterioration with statin use in AD patients from the community (Ellul 2007); delay of cognitive decline with statin use in subjects with AD from the Dementia Progression Study of the Cache County Study on Memory, Health and Aging (Rosenberg 2008); and reduced risk of hospitalization with dementia among statin users (Horsdal 2008). However, a post-hoc analysis conducted on data pooled from three RCTs of galantamine in patients with AD showed no significant effects of statin treatment (Winblad 2007).

2. AIMS OF THE PROJECT

The general aim of the project was to investigate the relation between cholesterol metabolism and late-life cognition, using a combination of epidemiological and clinic-based approaches.

The epidemiological approach implies certain limitations of the extent to which a person's health status can be paraclinically investigated. However, it offers the essential opportunity to formulate a population-based frame for adequately interpreting results from the evaluation of the highly selected group of patients who are usually referred to specialized memory clinics. The extensive evaluation of each patient's status in a clinical setting facilitates the investigation of cholesterol metabolism both in the periphery and the brain, their relationship, and the association they have with cognition.

The specific aims of the present set of studies were:

1) To analyse changes in serum total cholesterol from midlife to late-life and their association with late-life cognition, with focus on dementia/AD in a Finnish population (Study I).

2) To investigate the relationship between serum total cholesterol, lipid-lowering drugs and cognitive functions in non-demented elderly from a Finnish population (Study II).

3) To evaluate midlife serum total cholesterol as a risk factor for both Alzheimer's disease (AD) and vascular dementia (VaD) in a multiethnic US population (Study III).

4) To assess the relations between cholesterol-related (levels of apoE, oxysterols) and AD-related markers (tau, Aβ42) in the CSF of memory clinic patients (Study IV).

5) To study plasma markers of brain and peripheral cholesterol homeostasis in relation to brain volumes in memory clinic patients (Study V).

3. SUBJECTS AND METHODS

3.1. Epidemiological approach

The epidemiological approach combines a prospective long-term cohort study in a Finnish population (Cardiovascular Risk Factors, Aging and Dementia, CAIDE) with a retrospective long-term cohort study in a multiethnic US population (Kaiser Permanente). Baseline evaluations are at midlife in both cases. Studies I and II (based on CAIDE data) are designed to take into account the whole continuum of late-life cognitive functioning; regarding dementia syndromes, CAIDE focuses however mainly on Alzheimer-type dementia. Study III (based on Kaiser Permanente data) considers only dementia syndromes, including both Alzheimer and vascular dementia types.

3.1.1. CAIDE

The origins of the Cardiovascular Risk Factors, Aging and Incidence of Dementia (CAIDE) study can be traced back to the Seven Countries Study and 1960s international mortality statistics showing particularly high cholesterol levels in Finland and the highest rate of cardiovascular mortality in Finnish men (Keys 1980, Puska 1995). The North Karelia Project (one of the first community-based cardiovascular disease prevention projects in the world) was started in 1972 with the aim of shifting the risk factor profile of the entire North Karelian population through community-based intervention (Puska 1995). Positive results determined an extension of the prevention program to the rest of Finland, and prevention strategies became a part of national public health policy. The levels of cardiovascular risk factors (including cholesterol) are systematically monitored in Finland since 1972, through the National FinRisk Study involving surveys carried out every 5 years using independent, random and representative population samples from different parts of Finland (Vartiainen 2000). The study protocol is standardized according to international recommendations (the 1982-1992 cohorts were part of the WHO MONICA and WHO CINDI projects) and has been described in detail elsewhere (Vartiainen 2000).

Participants in the CAIDE study were derived from the 1972, 1977, 1982 and 1987 cohorts. Participation rates in these baseline surveys were high, ranging from 83% to 93% (Puska 1995). A random sample of 2000 persons (still alive at the end of 1997, aged 65-79 years and

living in the region of Kuopio and Joensuu towns) were invited for a first re-examination carried out in 1998. Altogether 1449 persons (72.5%) participated; 900 (62.1%) were women, and 549 (37.9%) were men. The mean age (SD) was 50.4 (6.0) years at the baseline (midlife) examination and 71.3 (4.0) years at the 1998 re-examination. The mean duration of follow-up was 20.9 years. The study was approved by the local ethics committee (University of Kuopio and Kuopio University Hospital), and written informed consent was obtained from all participants.

3.1.1.1. Midlife (baseline) examination

The surveys of 1982 and 1987 followed the WHO MONICA protocols, and the methods used in 1972 and 1977 were comparable (WHO MONICA Project Principal Investigators, 1998). In brief, the baseline survey comprised a self-administered questionnaire on sociodemographic characteristics, health-related behaviours and medical history, including cerebrovascular and cardiovascular events and conditions diagnosed by a physician. Venous blood specimens were taken to determine serum total cholesterol levels. Serum total cholesterol level was determined in 1972 and 1977 from frozen serum samples by using the Liebermann–Burchard method; in 1982 and 1987, total cholesterol level in fresh serum samples was measured by using an enzymatic method (CHOD-PAP Monotest, Boehringer, Mannheim, Germany). The enzymatic assay yielded values that were 2.4% lower than those measured by the Liebermann–Burchard method; thus, we corrected the values for total cholesterol level from the 1972 and 1977 surveys accordingly. All cholesterol levels were determined at the same central laboratory, and the laboratory data were standardized against those of national and international reference laboratories.

3.1.1.2. Late-life examination (1998)

Survey methods in 1998 were identical to those applied in the previous surveys. Information on each subject’s medical status was updated and the history of cardio/cerebrovascular events/conditions (i.e. myocardial infarction, stroke or diabetes mellitus) (yes/no) was inquired, as well as cholesterol-lowering drugs usage. Statins began to be used in Finland in mid-1990s, so the number of persons receiving them was still small in 1998.

Blood leukocyte samples were analyzed to determine APOE genotype. To extract DNA, a standard phenol-chloroform technique was used; APOE genotypes were analyzed by polymerase chain reaction and HhaI digestion, as described previously (Tsukamoto 1993).

Late-life examination – cognitive functioning

Cognitive functioning was investigated in three phases:

1) Screening phase: all participants were evaluated using the following cognitive tests:

- Mini Mental State Examination (MMSE) as a measure of global cognition (Folstein 1975)
- Immediate word recall tests for episodic memory (Nyberg 1997, Heun 1998); the mean number of correct words was calculated from three different lists of 10 words each
- Category fluency test for semantic memory (Borkowski 1967); the score was the number of correct animal names generated in 60 seconds
- Purdue Peg Board test and letter-digit substitution test for psychomotor speed (Wechsler 1944, Tiffin 1968); the sum of normalized scores in these tests was calculated
- Stroop test for executive functioning (Stroop 1935); the difference was calculated between time used for naming the colour of the ink used to write the name of another colour, and time used for naming colours of dots
- Prospective memory task (Einstein 1997)
- Subjective memory rating (Bennett-Levy 1980).

2) Clinical phase: candidates were subjects with 24 points or less on MMSE. Participants underwent a thorough cardiovascular and neurological examination by a physician and a detailed neuropsychological evaluation by a neuropsychologist. A review board consisting of the study physician, the study neuropsychologist and a senior neurologist ascertained the preliminary diagnoses based on all available information. Subjects judged to have possible dementia were invited to attend phase 3 of the examination.

3) Differential diagnostic phase included brain magnetic resonance imaging (MRI), blood tests, chest radiograph, electrocardiogram, and cerebrospinal fluid (CSF) analysis. All data

accumulated from the screening and clinical phases were carefully re-assessed by the review board before the final diagnosis was established.

Late-life examination – MCI and dementia diagnoses

Dementia diagnosis was based on DSM-IV criteria, and probable and possible AD were diagnosed according to NINCDS-ADRDA criteria (McKhann 1984). Patients with AD displayed generalised and/or medial temporal lobe atrophy, and none had significant vascular pathology on MRI. Isolated, minor lacunae or moderate white matter changes were not considered as exclusion criteria for AD. AD patients scored four or less on the Hachinski Ischemia Scale (Hachinski 1975). VaD diagnosis was based on the NINDS-AIREN criteria (Roman 1993). Consensus criteria were used to diagnose other dementia types: frontotemporal (Neary 1998), dementia with Lewy bodies (McKeith 1996), and alcohol-related dementia (Oslin 1998). Dementia diagnoses for non-participants were derived from patient records of the local hospitals and primary health care centres. No records were available for 24 of the 551 non-participants.

Participants were classified as having mild cognitive impairment (MCI) using a modified version of the Mayo Clinic AD Research Criteria (Petersen 1999). These included: 1) memory complaint by patient, family or physician; 2) normal activities of daily living; 3) normal global cognitive function as judged by physician; 4) objective impairment of memory or other areas of cognitive functioning as evidenced by scores >1.5 SD below age-appropriate mean; 5) Clinical Dementia Rating (CDR) score of 0.5; 6) absence of dementia.

3.1.1.3. Statistical analyses - Studies I and II

Study I – cholesterol changes and cognition (diagnoses)

Analyses included subjects with all levels of cognitive functioning (control, MCI, dementia). As cognition is a continuum, too sharp distinctions between the control, MCI, and dementia categories were avoided in this study. The three cognitive outcomes were thus considered to have an ordinal nature (irrespective of definition, MCI is a higher degree of cognitive impairment compared with control, and dementia is a higher degree of cognitive impairment

compared with MCI), and ordinal logistic regressions were used for investigating the cholesterol-cognition relationship (results presented as odds ratios (ORs) with 95% CIs).

Changes in serum total cholesterol levels from midlife to late-life were divided into five categories: the increase group was separated from the decrease group, and the latter was divided into four groups so that each of the five groups would have a relatively similar size. Because no exact guidelines are available in the literature, we used 0.5, 1, and 2 mmol/L as cutoffs for a clear and simple categorization. The following five categories were thus created:

- increase (307 subjects)
- decrease 0 to 0.5 mmol/L (217 subjects) – reference group
- decrease 0.5 to 1 mmol/L (224 subjects)
- decrease 1 to 2 mmol/L (342 subjects)
- decrease >2 mmol/L (231 subjects)

Midlife serum total cholesterol values were classified as high (≥6.4 mmol/L) or normal (<6.4 mmol/L). This cutoff was chosen according to previous recommendations (Pyörälä 1994). A lower cutoff was not used because cholesterol levels in this population were high (≥6.4 mmol/L in 58% of the subjects). Changes in body mass index (BMI) from midlife to late life were categorized into increase (69.4% of the subjects) and decrease (30.4% of the subjects). History of cardiovascular/cerebrovascular conditions was considered positive if the subjects had been diagnosed with myocardial infarction or stroke or diabetes.

Statistical analyses were done using Stata 9.1 and SPSS software for Windows, version 13.0. $\chi 2$ tests were used to compare APOE ε4 allele frequency and history of cardiovascular/cerebrovascular conditions between controls, subjects with MCI, and dementia. All ordinal regression analyses were controlled for age at baseline, follow-up time, sex, education, midlife cholesterol, and BMI changes; and additionally for the presence of at least one APOE ε4 allele and history of cardiovascular/cerebrovascular conditions. Ordinal logistic regression was also utilized to explore the possibility of an interaction between APOE and cholesterol changes in relation to late-life cognition. A similar analysis was performed for sex and cholesterol changes. The level of significance was $p<0.05$ in all analyses.

Study II – cholesterol changes and cognitive test performance in persons without dementia

This study focused on CAIDE participants who did not have dementia in 1998. The outcome was the subjects´ performance in several cognitive domains: global cognition, episodic memory, category fluency, executive functioning and psychomotor speed (as defined in 3.1.1.2.1). Test scores were used as dependent variables in multiple linear regressions (distributions were normalized by logarithmic transformations) and results were shown as relative differences in test performances, and p-values.

Categorization of cholesterol changes was based on previous analyses (Study I):

- increase (325 subjects)
- decrease 0–0.5 mmol/L (classified as little or no change; 234 subjects) – reference group
- decrease > 0.5 mmol/L (823 subjects)

Midlife cholesterol levels were categorized according to the ATP III guidelines (NCEP, 2002): 1) <200 mg/dL (5.18 mmol/L); 2) 200–239 mg/dL (5.18–6.19 mmol/L); and 3) ≥240 mg/dL (6.2 mmol/L). They were classified as low, intermediate (reference group) and high respectively (instead of ATP III terminology), because at the time of the midlife evaluation cholesterol levels were particularly high in Eastern Finland (Keys, 1980).

Statistical analyses were performed using Stata 9.1. Multiple linear regressions were adjusted for age, follow-up time, sex, education, presence of at least one APOE $\varepsilon 4$ allele, late-life history of myocardial infarction/stroke/diabetes, late-life depressive symptoms (Beck scale, Beck 1961) and lipid-lowering drugs. BMI, BP or changes in BMI and BP (increase/decrease) were also considered. Interaction terms were entered in all models for exploring the possibility of an interaction between APOE and cholesterol in relation to cognition. A similar analysis was done for sex and cholesterol. The level of significance was $p < 0.05$ in all analyses.

3.1.2. Kaiser Permanente

Kaiser Permanente is an integrated managed care organization, based in Oakland, California. It is one of the oldest and largest health maintenance organizations in the United States (founded in 1945; operates in nine states and Washington DC as of 2006). The Division of

Research (DOR), established in 1961, piloted a computer-based medical record system which resulted in the accumulation of a large volume of medical data that can be used for research purposes.

Kaiser Permanente of Northern California covers more than one fourth of the population in the geographic areas served. Members are representative of the sociodemographics of the local population (Krieger 1992). Member retention is high, particularly for persons aged 40 and above. A recent analysis found a termination rate (including deaths) of 21% at four years of follow-up for persons 40 years of age and up, with much lower rates for persons who had been members for at least two years.

3.1.2.1. Midlife (baseline) examination - the Multiphasic Health Checkup

A health examination, called the Multiphasic Health Checkup (MHC), is the primary source of data for studies of health measurements and different disease outcomes, including dementia. Study III is a retrospective cohort study of members of the Kaiser Permanente Medical Care Program of Northern California who participated in voluntary periodic MHCs in San Francisco and Oakland, CA, between 1964 and 1973 when they were 40-45 years old. If members attended more than one MHC during this interval, data from the first visit were considered. The present cohort includes 9844 persons who were still members of the health plan in 1994 when computerized outpatient diagnoses of dementia became available.

At the MHC, participants were interviewed and information on demographics, lifestyle, and medical history was collected including questions on medical conditions and medication use (Collen 1978). Systolic and diastolic blood pressure, weight, and height were measured according to standard procedures (Collen and Davis 1969) and body mass index was calculated (kg/m^2). Blood was drawn for total serum cholesterol and levels were measured with an Auto-analyzer (Technicon Co., White Plains, NY) from 1964 to 1968, with an Autochemist (AGA Corp, Stockholm, Sweden) from 1969 to 1972, and with an Auto-Analyzer (model SMA-12, Technicon, CO) in 1973 (Collen 1978, Iribarren 2000). The participants were considered to have hypertension if they had one of the following: self-report of physician diagnosed hypertension, use of antihypertensive medication, systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg. Diabetes was defined by self report of physician diagnosed diabetes, use of insulin or oral hypoglycemic agents, a fasting glucose (last food eaten in ≥8 hours) of ≥140 mg/dl, or a non-fasting (last food eaten in ≤4

hours) glucose of ≥200 mg/dl. If the participants had no mention of a self report of hypertension or diabetes, no mention of medications taken for these diseases, and no laboratory or hematologic evidence of these diseases, it was assumed that the participants did not have these risk factors at midlife. Stroke was recorded from hospital discharge diagnoses (ICD-9 codes for ischaemic stroke, 433-438, hemorrhagic stroke, 430-432) from 1985 through the end of the study, June 2007. The MHC study was approved by the Internal Review Board of Kaiser Permanente.

3.1.2.2. Late-life evaluation - ascertainment of dementia diagnoses

Dementia diagnoses were ascertained through electronic medical records from a database that contains diagnoses from all outpatient encounters at Kaiser Permanente medical centers and clinics. The form is completed by the treating clinician. Diagnoses considered in this study included AD (ICD 9 CM code 331.0) and VaD (ICD 9 CM code 290.4) from visits to Neurology. Diagnoses were ascertained from January 1994 to June 2007 when the MHC participants would have been 61 to 88 years of age. Mortality information was available on our cohort through the end of 2005 using the California Automated Mortality Linkage System, which has a sensitivity of 0.97 compared to the National Death Index (Arellano 1984). From January 1, 2005, to December 31, 2006, mortality information was available using a weighted linkage system incorporating matches by social security number, name, date of birth, and home address to Social Security Death Data. From January 1, 2007, to June 2007, mortality information was not yet available.

3.1.2.3. Statistical analyses - Study III

Cholesterol levels were grouped into three categories, according to the 2002 Adult Treatment Panel (ATP) III guidelines:

- Desirable: <200 mg/dl (<5.2 mmol/l) - reference category
- Borderline: 200-239 mg/dl (5.2-6.2 mmol/l)
- High: ≥240 mg/dl (≥6.2 mmol/l)

For a more detailed evaluation of the relation between cholesterol and AD, analyses were also done using four cholesterol categories (quartiles):

- Q1: <198 mg/dl (<5.1 mmol/l) - reference category
- Q2: 198-220 mg/dl (5.1-5.7 mmol/l)
- Q3: 221-248 mg/dl (5.7-6.4 mmol/l)
- Q4: 249-500 mg/dl (6.4-12.9 mmol/l)

All analyses were done using SAS version 8.0 (SAS Institute, Cary, NC). $\chi 2$ tests and F tests were used to determine if demographic and clinical characteristics at the time of the MHC were significantly different by presence of AD or VaD.

Cox proportional hazards-age as time scale were used to investigate the relationship between midlife cholesterol and AD or VaD. Participants were censored according to age at dementia diagnosis, age at date of death, age at date of end of Kaiser membership, or age at end of follow-up, June 1, 2007. Models were adjusted for age (as time scale), sex, education (categorized as high school, trade school, college 1-2 years, college 3-4 years and postgraduate, with grade school as reference), race/ethnic group (self-reported, it was entered in analyses as black, Asian, or other, with Caucasian as reference group), midlife BMI (continuous variable), diabetes and hypertension (yes/no). For AD, the model was additionally controlled for late-life stroke (yes/no). To determine whether the association between midlife cholesterol and risk of AD or VaD varied by sex or race/ethnic group, we entered interaction terms to the fully adjusted models. The level of significance was $p < 0.05$ in all analyses.

3.2. Clinic-based approach

3.2.1. Karolinska University Hospital Memory Clinic – general characteristics

Clinic-based studies (Study IV and V) were conducted at the Division of Geriatric Medicine, Karolinska University Hospital, Huddinge, Sweden. The division examines/treats about 600 patients/year in the outpatient memory clinic and about 400 patients/year in the inpatient ward. The division has total responsibility for early-onset dementias (<65 years) in the area, and national responsibility for familial cases/genetic analyses (i.e. the Swedish mutation and the Arctic mutation were detected here).

Routine evaluations of patients consist of medical examination, blood tests (including APOE genotype, cholesterol, vitamin B12 and folate, homocystein etc.) and CSF analyses (including T-Tau, P-Tau, and Aβ42), detailed neuropsychological evaluation, EEG, and neuroimaging (MRI/CT, SPECT). Dementia and AD are diagnosed according to DSM-IV and NINCDS-ADRDA (McKhann 1984) criteria. CSF biomarkers are also taken into account when establishing the diagnosis (Andersson 2007). Criteria for identifying MCI are: (1) not demented; (2) has self and/or informant report of cognitive decline and impairment on objective cognitive tasks; (3) has preserved basic ADL/minimal impairment in complex instrumental functions (Winblad 2004). Persons with cognitive complaints but without impairment on objective cognitive tasks are classified as having subjective cognitive impairment (SCI).

Patients at the Karolinska University Hospital Memory Clinic are younger and present with milder cognitive symptoms compared to more traditional geriatric psychiatry clinics (Andersson 2007). They also have a wider range of diagnoses and aetiologies of cognitive impairment, such as depression, anxiety, post-traumatic syndromes, whiplash sequelae, normal pressure hydrocephalus, obstructive sleep apnoea, or hyper/hypothyroidism. In addition, an important proportion of the patients show normal cognitive functioning on neuropsychological testing. In 2005, 435 of the outpatients at the Memory Clinic were referred to extensive dementia investigation (188 males, 247 females; mean age±SD was 63.0±10.5 years; mean MMSE score was 27.0±3.0). The distribution of the diagnoses is shown in Figure 3.1. The majority of patients (76%) did not have dementia but SCI or MCI. Only 15% of the diagnoses were represented by AD and 2% by VaD.

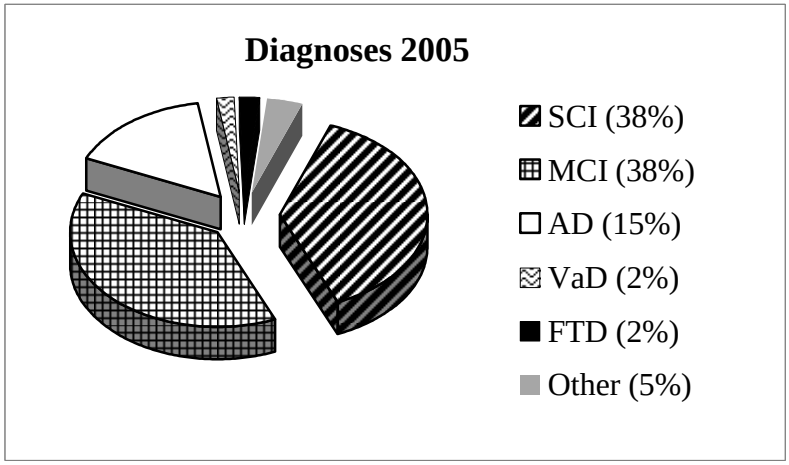

Figure 3.1. Distribution of clinical diagnoses in 2005

3.2.2. Patients and methods

Studies IV and V take into account various degrees of cognitive impairment, with a focus on Alzheimer disease. Patients were referred to the Memory clinic beginning with 2002 from primary care centres for investigation of suspected dementia. They were all living independently in the community (they were not in need of formal care or aid from the community). The local Ethics committee at Karolinska University Hospital Huddinge approved both studies. All patients (or nearest relatives) and controls gave their informed consent to participate in the study, which was conducted according to the provisions of the Helsinki Declaration.

3.2.2.1. Study IV – CSF markers of cholesterol metabolism

Study IV is a small pilot study including 20 MCI patients and 17 AD patients with available CSF samples. The control group (43 persons) consisted of patients referred to the Neurology Clinic at Karolinska University Hospital for headache of uncertain cause. They underwent a comprehensive clinical and laboratory evaluation to rule out any signs of organic CNS disease.

CSF was collected for diagnostic purpose by lumbar puncture in polypropylene tubes, gently mixed to avoid gradient effects and centrifuged at 2000×*g* for 10 min. Aliquots were stored at −80^{0}C until biochemical analysis. Tau was determined using a sandwich enzyme-linked immunosorbent assay (ELISA) constructed to measure Tau (both normal Tau and P-Tau) (Blennow 1995). P-Tau (P-Thr181) was determined using a sandwich ELISA, with monoclonal antibody (MAb) HT7 (recognizing all forms of Tau) used as capturing antibody and biotinylated MAb AT270 (specific to PThr181 P-Tau) used as a detection antibody (Vanmechelen 2000). Aβ42 was determined using a specific sandwich ELISA (Andreasen 1999). All kits were purchased by Innogenetics NV, Ghent, Belgium. ApoE levels in CSF were assayed by a slight modification of a commercial immunoassay method for ApoE in plasma BNProSpec Dade Behring system (Apolipoprotein E-kit insert – Dade Behring July 1998 edition). In the CSF apoE assay, undiluted CSF samples were analyzed, in contrast plasma assays where dilutions 1:20 were used. The method was linear up to 8 mg/L and the within run precision calculated to be 2.8% expressed as variation coefficient of a control pool

analysed 20 consecutive times. CSF levels of cholesterol, 24OHC and 27OHC were determined by isotope dilution-mass spectrometry (Leoni 2006).
Analysis of variance, analysis of covariance, and Student's *t*-test were used for group comparisons, while Pearson correlation coefficient was used for correlations. The level of significance was set to $p<0.05$.

3.2.2.2. Study V – plasma markers of peripheral and brain cholesterol metabolism

Study V is partly based on study IV, including 96 patients (33 with SCI, 36 with MCI and 27 with AD) who had available plasma and CSF samples and MRI images (all obtained during the diagnostic workup). Patients with psychiatric disorders (i.e. major depression, alcohol abuse) or other conditions (i.e. brain tumors, normal pressure hydrocephalus) were not considered for this study. Patients with SCI were used as control group in the analyses.
Plasma levels of 24OHC, 27OHC, lanosterol and lathosterol were assayed by isotope dilution-mass spectrometry (Leoni 2004). Total cholesterol, LDL-cholesterol and HDL cholesterol were measured by routine standard enzymatic assay (Modular Analytics; Roche Diagnostics, Mannheim, Germany).

MRI measurements (T1 weighted images, 1.5 T) were done using custom-made software BMAP/Volstat for volumetric brain analysis (developed in the imaging laboratory at Karolinska Institutet), running on a HERMES (Hermes Medical Solutions, Stockholm, Sweden) platform. BMAP performs brain extraction with inclusion of cortical CSF in the segmented volumes. The Volstat segmentation program is based on a fuzzy c means clustering (FCM) algorithm (Brandtberg 1999, Suckling 1999, Höppner 2000) enhanced by use of the distribution of signal intensity specific to each tissue type. In the Volstat FCM used for this study, the intra-cranial volume was segmented into 3 clusters (gray matter, white matter and CSF) assigning a partial cluster value (percentage) for all clusters in all voxels. Fuzzy volumes were used in the analyses: gray matter (GM), white matter (WM), and CSF volumes, total intracranial volume (TIV); GM fraction, WM fraction, CSF fraction and parenchymal fraction were also calculated.

Statistical analyses were performed with SPSS software for Windows, version 16.0 (SPSS Inc., Chicago, IL). $\chi 2$ tests and t-tests were used for comparisons between groups. As oxysterols are transported in the circulation by the same lipoproteins as cholesterol, and since

changes in the levels of these transport proteins may affect the results, we have calculated the ratios between the oxysterols and cholesterol. The relations between the 24OHC/cholesterol ratio (R_24OHC), 27OHC/cholesterol ratio (R_27OHC), cholesterol, LDL-C, HDL-C, lanosterol/cholesterol ratio (R_lanosterol), lathosterol/cholesterol ratio (R_lathosterol) and brain volumes were investigated in linear regression analyses. GM volume, WM volume, CSF volume, TIV, GM fraction, WM fraction, CSF fraction and parenchymal fraction were entered as dependent variables (they were normally distributed). Analyses were done for all patients and also stratified according to group (SCI, MCI and AD). All analyses were controlled for the presence of at least one APOEε4 allele and statin treatment (which are known to influence cholesterol homeostasis) and for socio-demographic variables that were significantly associated with brain volumes in this study population (age, sex).

4. RESULTS

4.1. Epidemiological studies

4.1.1. Characteristics of the populations

The general characteristics of the CAIDE and Kaiser Permanente populations are presented in Table 4.1. Follow-up time was about 21 years in CAIDE and 30 years in Kaiser Permanente. Sex distribution was similar for both populations. Race/ethnic group composition was different, with CAIDE being homogeneous and Kaiser Permanente more heterogeneous (26% subjects self-identified as non-white). As expected, midlife cholesterol levels were higher in the Finnish population. The APOE ε4 frequency in Finland is among the highest in the world (Ewbank 2004). Information on APOE genotype was not available from the Kaiser Permanente population.

Table 4.1. General characteristics of the CAIDE and Kaiser Permanente populations.

	CAIDE population (N=1449)	**Kaiser Permanente population (N=9844)**
***Age at baseline (years)**	50.4 (5.9)	42.4 (1.7)
***Age at late-life (years)**	71.3 (4.0)	79.1 (4.5)
Sex (♀)	62.1 %	63.9 %
Education:	*8.6 (3.4) years	45% college level and above
Race/ethnic group:		
Asian	-----	6.3 %
Black	-----	15.6 %
White	100%	73.9 %
Other	-----	4.2 %
***Midlife cholesterol (mmol/l)**	6.7 (1.2)	5.8 (1.1)
APOE ε4	35.4 %	-----
Dementia	61/1449 (4.2 %)	8.2% (Whitmer 2005)
Alzheimer dementia	48/1449 (3.3 %)	469/9944 (4.7 %)
Vascular dementia	8/1449 (0.6%)	127/9944 (1.3%)

Values are numbers and percentages (%) unless otherwise specified.

* Values are means (SD). Education levels are not directly comparable due to differences between the Finland and US educational systems.

Of the 1449 CAIDE participants, 61 were diagnosed with dementia (48 AD, 13 other dementias), 82 had MCI and the remaining 1306 persons formed the control group. The percentages of individuals with dementia and AD (Table 4.1) were in agreement with data from the Finnish population in general and for the given age range at the first CAIDE follow-up (65-80 years) (Koskinen 2006).
In the Kaiser Permanente population (9844 persons), 469 individuals had been diagnosed with AD and 127 with VaD (Table 4.1). The percentages of individuals with dementia, AD and VaD were as expected considering data from the US population and the age range of Kaiser Permanente members at the time of cognitive status ascertainment (61-88 years) (Plassman 2007). Also, the Kaiser Permanente population includes African Americans, a group known to have a higher prevalence and incidence of dementia.

Within each population, persons with dementia were significantly older compared to controls, had lower education levels and higher midlife cholesterol levels. A history of cardio/cerebrovascular conditions or events was more frequent among persons with dementia. In the CAIDE population, the percentage of APOE ε4 carriers was higher in the dementia or MCI groups in comparison with controls. Detailed descriptions of the subjects are provided in the Appendix (Study I, pages 752-754; Study II, page 1007; Study III, page 77).

For CAIDE, general information was available on non-participants. Age at midlife and sex distribution was similar for participants and non-participants; non-participants had fewer years of formal education, higher cholesterol levels, higher systolic and diastolic blood pressure, and higher BMI compared with participants. Data from patient records indicated that 10.2% of non-participants had been diagnosed with dementia (see Appendix, Study I, page 754).

4.1.2. Midlife serum total cholesterol and late-life cognition

The relation between midlife serum total cholesterol and late-life cognition (indicated either by dementia/AD/VaD diagnosis or by degree of cognitive impairment) is shown in Table 4.2. In both populations, higher cholesterol levels at midlife represented a risk factor for cognitive impairment or dementia (both AD and VaD) later in life.

Table 4.2. High midlife cholesterol as a risk factor for cognitive impairment/dementia.

Population	Outcome	Midlife cholesterol (mmol/l)	Risk
CAIDE OR (95%CI)	Higher degree of cognitive impairment (control-MCI-dementia)	<6.4	-reference-
		≥6.4	**1.8 (1.1-3.0)**
Kaiser Permanente HR (95%CI)	AD *	<5.2	-reference-
		5.2-6.2	1.2 (0.97-1.6)
		≥6.2	**1.6 (1.2-2.0)**
	AD **	<5.1	-reference-
		5.1-5.6	1.3 (0.96-1.6)
		5.7-6.4	**1.3 (1.0-1.7)**
		6.4-12.9	**1.6 (1.2-2.1)**
Kaiser Permanente HR (95%CI)	VaD *	<5.2	-reference-
		5.2-6.2	**1.5 (1.0-2.2)**
		≥6.2	1.3 (0.8-1.9)

* Cholesterol categorized according to ATP III guidelines; values are shown in mmol/l.
** Cholesterol categorized into quartiles.
SI conversion factor: To convert cholesterol from mmol/l to mg/dl, divide by 0.0259; to convert cholesterol from mg/dl to mmol/l, multiply by 0.0259.

In CAIDE, there was a 80% increased risk of higher degree of cognitive impairment for cholesterol levels above 6.4 mmol/l. High cholesterol levels as defined by the 2002 Adult Treatment Panel (ATP) III guidelines (≥240 mg/dl, or ≥6.2 mmol/l) represented a significant risk factor for AD as well, besides the well-known connection to cardiovascular disease risk. The results from the Kaiser Permanente population pointed to an even lower threshold, as additional analyses with cholesterol levels categorized into quartiles indicated that midlife cholesterol values above 220 mg/dl (5.7 mmol/l) increase the risk of developing AD three decades later. Testing of interaction terms by race/ethnic group and sex showed that effects were not statistically different for men and women or by race/ethnic group.

The association between midlife cholesterol and cognitive test performance in late-life in non-demented persons (CAIDE) is shown in Table 4.3. High midlife cholesterol (here >6.2 mmol/l) was associated with poorer performance on episodic memory and category fluency tests.

Table 4.3. (CAIDE) Midlife serum total cholesterol (TC) and cognitive test performance in late-life (non-demented persons)

COGNITIVE TESTS		MIDLIFE TC		
Domains	**Mean score (range)**	**Low (n=123)**	**Intermediate (n=365)**	**High (n=894)**
Global cognition	26 (16-30)	1.00	1.00	0.99
Episodic memory	5 (0.3-9.3)	0.97	1.00	**0.96 (p=0.02)**
Category fluency	19 (0-55)	0.94	1.00	**0.95 (p=0.02)**
Executive function	36 (-41 - 257)	0.98	1.00	0.99
Psychomotor speed	0.1 (-3 – 2.6)	1.04	1.00	0.99

Results are relative differences (with intermediate TC as a reference group); In cognitive tests, higher results mean better performance, except for executive function, where lower results indicate better performance. Model adjusted for age, sex, education, follow-up time, ApoE ε4 allele, BMI, systolic blood pressure and late-life history of myocardial infarction/stroke/diabetes mellitus, depressive symptoms and lipid-lowering medication.

4.1.3. Late-life serum total cholesterol and cognition

Late-life cholesterol levels, investigated in both CAIDE (Appendix, Study I, page 753; Study II, page 1008) and a subsample of 6600 Kaiser Permanente subjects (unpublished data), were not significantly different between the control and dementia groups and no significant association with late-life cognitive functioning was observed.

4.1.4. Cholesterol changes after midlife and cognition in late-life

In the CAIDE population, serum total cholesterol levels declined in most individuals during the 21 years of follow-up (Figure 4.1.; Appendix, Study I, page 753).

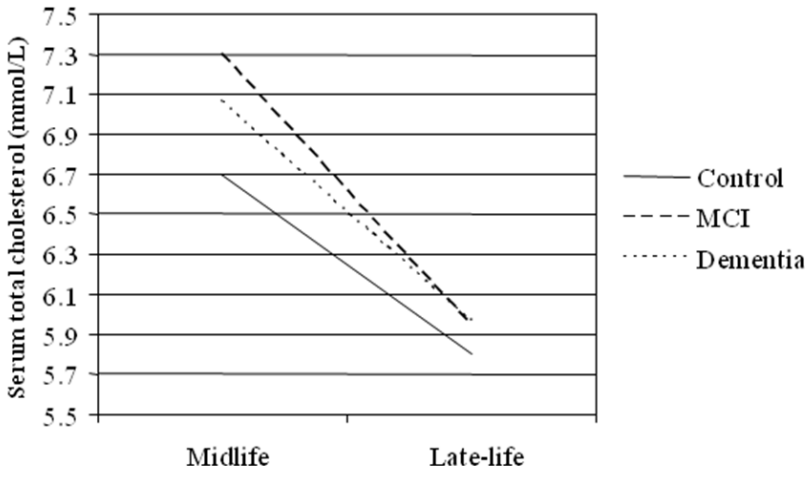

Figure 4.1. Cholesterol changes in the CAIDE population.

A moderate decrease in total cholesterol levels from midlife to late life (0.5-1 mmol/l) was significantly associated with increased risk of having a more impaired late-life cognitive status after adjusting for age, follow-up time, sex, years of formal education, midlife cholesterol, BMI changes, the presence of the APOE ε4 allele, history of myocardial infarction/stroke/diabetes, and lipid-lowering treatment (Figure 4.2).

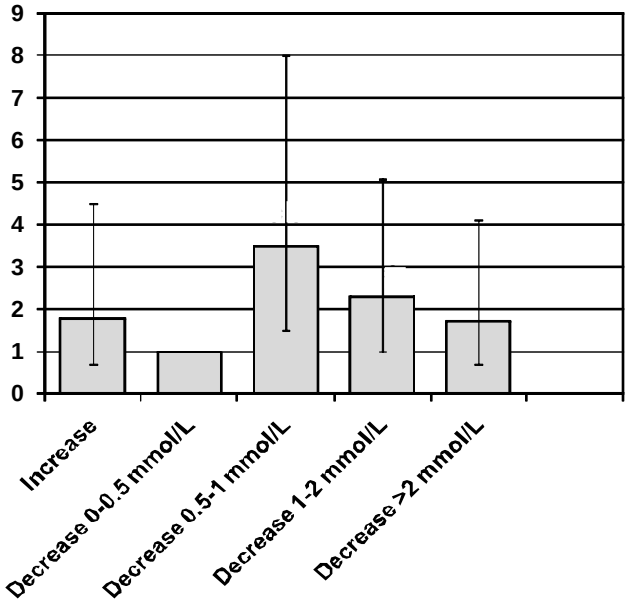

Figure 4.2. Cholesterol changes after midlife and OR (95% CI) for higher degree of cognitive impairment (control-MCI-dementia)

ORs for a worse cognitive outcome were more than three times higher for a 0.5-1 mmol/L decrease and more than two times higher for a 1-2 mmol/L decrease in total cholesterol levels. Using the classic approach (separating the three cognitive outcome categories and calculating ORs for MCI and dementia/AD unconnectedly) did not change the pattern of risk association with cholesterol changes. However, this approach led to somewhat wider CIs and less reliable estimates (Appendix, Study I, page 754).

There were no significant interactions between APOE genotype and cholesterol changes over time or between sex and cholesterol changes over time in relation to late-life cognitive impairment (results not shown). An analysis of APOE ε4 carriers and non-carriers separately showed the same pattern of association between cholesterol changes and late-life cognitive status, but for a moderate decrease in cholesterol (0.5 to 1 mmol/L), the OR (95% CI) for a worse cognitive outcome was 5.3 (1.4 to 20.7) for APOE ε4 carriers and 2.7 (0.9 to 7.7) for APOE ε4 non-carriers after adjusting for socio-demographic variables, midlife cholesterol, and history of myocardial infarction/stroke/diabetes. For men, the results were similar to those

for all subjects together, and the association between a moderate decrease in cholesterol (0.5-1 mmol/L) and the risk of a more impaired late-life cognitive status remained significant ($p<0.05$) after controlling for all above-mentioned variables. For women, both moderate and greater decreases in TC were associated with an approximately three times increased risk for worse late-life cognitive outcome ($p<0.1$).

A similar pattern of association between cholesterol changes after midlife and late-life dementia was also observed in a subsample of 6600 subjects from the Kaiser Permanente population (unpublished data).

The relation between changes in cholesterol after midlife and late-life performance in cognitive tests (non-demented persons, CAIDE) is shown in Table 4.4. A decrease in cholesterol levels was associated with poorer performance on episodic memory and psychomotor speed tests.

Table 4.4. Cholesterol changes after midlife and cognitive test performance in late-life (non-demented persons)

	Increase (n=325)	**No change /small decrease (n=234)**	**Decrease (n=823)**
Global cognition	0.98	1.00	0.99
Episodic memory	0.97	1.00	**0.95 (p=0.02)**
Category fluency	1.00	1.00	0.96
Executive function	1.00	1.00	1.00
Psychomotor speed	1.03	1.00	**0.94 (p=0.03)**

Results are relative differences (with no change/small decrease (0-0.5 mmol/L) as a reference group); In the cognitive tests, higher results mean better performance, except for executive function, where lower results indicate better performance. Model adjusted for age, sex, education, follow-up time, ApoE ε4 allele, midlife TC, changes in BMI, changes in systolic blood pressure and late-life history of myocardial infarction/stroke/diabetes mellitus, depressive symptoms and lipid-lowering medication.

No significant interactions were found between APOE genotype and cholesterol changes over time or between sex and cholesterol changes over time in relation to the degree of impairment in late-life cognitive functioning (control-MCI-dementia) or to performance in cognitive tests (non-demented persons) (Appendix, Study I, page 754).

4.1.5. Lipid-lowering drugs and late-life cognition in persons without dementia

The relation between lipid-lowering treatment and late-life cognitive performance in non-demented persons from the CAIDE population is shown in Table 4.5. Lipid-lowering treatment was related to better performance in episodic memory and psychomotor speed tests. Results were similar when only statins were considered (results not shown). Stratified analyses according to lipid-lowering treatment showed that cholesterol levels decline was related to poorer episodic memory and psychomotor speed in persons without treatment ($p = 0.021$), but not in treated subjects ($p = 0.266$).

Table 4.5. Lipid-lowering drugs and late-life cognitive test performance

	Treatment (n=192)	**No treatment (n=1190)**
Global cognition	1.00	1.00
Episodic memory	**1.05 (p=0.02)**	1.00
Category fluency	1.01	1.00
Executive function	1.00	1.00
Psychomotor speed	**1.08 (p=0.01)**	1.00

Results are relative differences between the treated and untreated group. In the cognitive tests, higher results mean better performance, except for executive function, where lower results indicate better performance. Model adjusted for age, sex, education, follow-up time, ApoE ε4 allele, midlife TC, changes in TC, changes in BMI, changes in systolic blood pressure and late-life history of myocardial infarction/stroke/diabetes mellitus and depressive symptoms.

4.2. Clinical studies

4.2.1. Population characteristics

Demographic data for patients referred to the Memory clinic at Karolinska University Hospital, Huddinge in 2003 and 2005, and patients in Study IV and V are presented in Table 4.6. Participants in Study V covered the whole spectrum of cognitive complaints (from subjective to mild objective impairment to Alzheimer dementia) and their characteristics were thus closer to those of the Memory clinic patients in general. Participants in Study IV were older, had fewer years of education and lower MMSE scores, since Study IV only included patients with MCI and AD dementia from the Memory clinic. Because Studies IV and V were focused on AD, they included a higher percentage of APOE ε4 carriers compared to the clinic in general.

Study IV also included a group of 43 controls (median age 50 years old; 28 females, and 15 males) from the Neurology clinic at the same hospital.

Table 4.6. Demographic data for patients referred to the Memory clinic at Karolinska University Hospital, Huddinge in 2003 and 2005, and patients in Study IV and V

	2003	2005	Study IV	Study V
N	293	435	37	96
Sex (♀)	55%	57%	47.4%	56.2%
Age (years)	62.3 (9.7)	63.0 (10.5)	65.7 (11.0)	62.1 (9.5)
MMSE	27.0 (3.1)	27.0 (3.0)	25.6 (4.5)	27.2 (3.1)
Education (years)	11.8 (4.0)	Not available	10.7 (3.1)	12.5 (3.7)
APOE ε4	44%	42%	46.7%	53.1%

Values are means (SD) unless percentage (%) is specified.

4.2.2. CSF markers of cholesterol metabolism – Study IV

Patients with AD were older than patients with MCI or controls. After controlling for age, levels of apoE, 24OHC and 27OHC were significantly higher in the AD and MCI groups compared to controls ($p<0.001$ for both), but no differences were observed for cholesterol. Age was not significantly correlated with any of these variables (Appendix, Study IV, page 80).

The correlations between apoE levels and other CSF markers are shown in Table 4.7.

Table 4.7. Correlations between apoE and other CSF markers

ApoE	Cholesterol	24OHC	27OHC	Qalb	CSFalbumin	Tau	P-Tau	Aβ42
Controls	**0.35 (0.04)**	0.1 (0.53)	0.08 (0.55)	0.2 (0.2)	0.09 (0.57)	---	---	---
MCI	0.42 (0.10)	**0.75 (<0.001)**	0.17 (0.48)	0.07 (0.6)	0.16 (0.50)	0.31 (0.23)	0.31 (0.22)	**0.54 (0.02)**
AD	0.41 (0.13)	**0.66 (0.004)**	0.30 (0.28)	0.16 (0.53)	0.17 (0.51)	**0.72 (0.001)**	**0.72 (0.002)**	-0.07 (0.80)

Results are Pearson correlation coefficients (p values).
--- Data not available.

ApoE was related to 24OHC in patients with MCI and AD, but not in controls. However, a significant correlation of apoE with cholesterol was only observed in controls. 27OHC, Qalb and CSFalbumin (indicators of blood-brain barrier integrity) were not related to apoE.
In patients with AD, apoE was correlated with both Tau and P-Tau levels (but not Aβ42), while in patients with MCI the relation of apoE with tau proteins was not significant.

4.2.3. Plasma markers of brain and peripheral cholesterol homeostasis – Study V

Patients presented to the Memory clinic quite early in the course of their condition (mean age about 62 years), when cognitive symptoms were still mild (mean MMSE score about 27). As expected, persons with AD were older, had lower education and lower MMSE scores compared to persons with MCI and SCI. The highest percentage of patients with at least one APOE ε4 allele was in the AD group and the lowest in the SCI group. There were no significant sex differences between the three groups. R_24OHC, R_27OHC, R_lanosterol and R_lathosterol were significantly lower in AD patients compared to MCI or SCI patients. The AD group tended to have lower total cholesterol, LDL-C and brain volumes, although the groups did not significantly differ with respect to total cholesterol, LDL-C, HDL-C and MRI measurements (Appendix, Study V, page 91).

24OHC – brain cholesterol homeostasis

The relationships between 24OHC and brain volumes are presented in Table 4.8. When the whole population was considered, R_24OHC was positively associated with GM fraction (i.e. higher GM fraction related to higher R_24OHC). In analyses stratified according to group, R_24OHC was positively associated to GM volume, GM and parenchymal fractions, and negatively associated with CSF volume and fraction, but only in persons with SCI.

Table 4.8. 24-hydroxycholesterol and brain volumes.

<table>
<tr><th></th><th>SCI</th><th>MCI</th><th>AD</th></tr>
<tr><td rowspan="2">R_24OHC</td><td colspan="3">GM fraction (β=0.3; p=0.03)</td></tr>
<tr><td>GM volume (β=0.5; p=0.03)
GM fraction (β=0.9; p=0.002)
Parenchymal fraction (β=0.7; p=0.009)
CSF volume (β= -0.7; p=0.007)
CSF fraction (β= -0.7; p=0.009)</td><td>NS</td><td>NS</td></tr>
</table>

Results are standardized β-coefficients (p-values) from linear regressions with brain volumes as dependent variables. All analyses are controlled for age, sex, the presence of at least one APOEε4 allele and statin treatment. Only statistically significant relations are shown. NS – not significant.

27OHC, total cholesterol, LDL-C, lanosterol, lathosterol–peripheral cholesterol homeostasis

Table 4.9 shows the patterns of relations between cholesterol precursors, total cholesterol, LDL-C, HDL, 27OHC and brain volumes in each group of patients. In the control group (but not MCI or AD) levels of cholesterol precursors were negatively related to brain volumes and positively related to CSF volumes (i.e. lower R_lanosterol associated with higher WM volume and fraction; lower R_lathosterol associated with higher parenchymal and WM fraction, and lower CSF volume and fraction). In patients with AD (but not MCI or SCI) total cholesterol and LDL levels were positively related to brain volumes and negatively related to CSF volumes (i.e. lower total cholesterol associated with lower parenchymal fraction, WM volume and fraction, and higher CSF volume and fraction; lower LDL-C associated with lower parenchymal fraction and higher CSF fraction). No significant relations were found between R_27OHC, HDL-C and MRI measurements.

Table 4.9. Patterns of relation between markers of extracerebral cholesterol homeostasis and brain volumes.

	SCI	MCI	AD
R_lanosterol	**WMV** (β= -0.4; p=0.024) **WM fraction** (β= -0.5; p=0.009)	NS	NS
R_lathosterol	**Parenchymal fraction** (β=-0.5; p=0.039) **WM fraction** (β= -0.4; p=0.036) **CSFV** (β=0.4; p=0.046) **CSF fraction** (β=0.5; p=0.039)	NS	NS
Total cholesterol	NS	NS	**Parenchymal fraction** (β=0.9;p<0.001) **WMV** (β=0.6; p=0.016) **WM fraction** (β=0.7; p=0.014) **CSFV** (β= -0.7; p=0.037) **CSF fraction** (β= -0.9;p<0.001)
LDL-C	NS	NS	**Parenchymal fraction** (β=0.9;p=0.028) **CSF fraction** (β= -0.9;p=0.028)
HDL-C	NS	NS	NS
27OHC	NS	NS	NS

Results are standardized β-coefficients and p-values from linear regressions with brain volumes (GM, WM, CSF volume, TIV, parenchymal fraction, GM, WM and CSF fractions) as dependent variables. All analyses are controlled for age, sex, the presence of at least one APOEε4 allele and statin treatment. Only statistically significant relations are shown. NS – not significant.

5. DISCUSSION

The present project confirms the existence of a relation between cholesterol and AD as well as VaD. While the significance of high total cholesterol at midlife as a risk factor for late-life cognitive impairment, dementia, AD, VaD or poorer performance in cognitive tests is quite straightforward, the meanings of cholesterol levels at older ages become more difficult to disentangle, as cholesterol itself may be influenced by the very disease(s) it has contributed to. In addition, the results of the project indicate a role for the 24OHC - apoE system in the regulation and control of neurodegeneration, as well as for plasma 24OHC levels as a marker of the number of metabolically active neurons in gray matter.

5.1. Midlife serum total cholesterol and late-life cognitive status

5.1.1. Cholesterol and dementia/cognitive impairment

In the present set of studies, high midlife serum total cholesterol consistently emerged as a risk factor for late-life dementia/cognitive impairment in two different populations and with different approaches.

These results confirm previous reports from the CAIDE and Kaiser Permanente populations (Kivipelto 2001a, b; Whitmer 2005). Preceding analyses of the CAIDE data considered AD (Kivipelo 2001a) or MCI (Kivipelto 2001b) as outcomes separately, categorized as present/absent. In the current project (Study I) a more dimensional approach was taken and the cognitive continuum behind the categories of ´control´, ´MCI´ and ´dementia´ was acknowledged by defining the outcome (in ordinal regression analyses) as degree of cognitive impairment (MCI as higher degree of cognitive impairment compared to control, and dementia as higher degree of cognitive impairment compared to MCI). Moreover, in Study II the focus was switched from the clinically extreme category of dementia to performance in cognitive tests in persons without dementia. High midlife cholesterol (>6.4 mmol/l) remained a risk factor for cognitive impairment/poorer performance in cognitive tests even when changes in cholesterol levels after midlife were taken into account.

In the Kaiser Pemanente population, elevated midlife cholesterol was previously shown to be a risk factor for dementia, irrespective of type (Whitmer 2005). In Study III, the follow-up information is updated to 2007 and the focus is specifically on AD and VaD as ascertained

from electronic medical records (ICD 9 CM codes 331.0 for AD and 290.4 for VaD). It is thus a typical categorical approach, with AD and VaD as present/absent and mutually exclusive. The finding that elevated midlife cholesterol was a risk factor for both AD and VaD becomes particularly interesting in this context. As expected, high cholesterol levels as defined by the 2002 Adult Treatment Panel (ATP) III guidelines (≥240 mg/dl, or ≥6.2 mmol/l) represented a significant risk factor for AD, besides the well-known connection to cardiovascular disease risk. Study III points to an even lower threshold, as additional analyses with cholesterol levels categorized into quartiles indicated that midlife cholesterol values above 220 mg/dl (5.7 mmol/l) increase the risk of developing AD three decades later. Therefore, even moderately elevated cholesterol in midlife was associated with an increased risk of AD.

There are few population-based long-term follow-up studies with midlife as starting point that have investigated cholesterol and late-life cognition (Table 1.2). Data from the Finnish Cohort of the Seven Countries Study also indicated a role for high midlife total cholesterol as a risk factor for AD (Notkola 1998). In the Honolulu-Asia Aging Study (HAAS), clustering of cardiovascular metabolic risk factors (including total cholesterol) at midlife increased the risk of dementia in general (not AD), but no significant relation was found between cholesterol and dementia of any type (Kalmijn 2000). Also, no significant association between cholesterol and dementia was found in the Israeli Ischemic Heart Disease Study (IIHDS) (Beeri 2004). The differences in findings between studies could be due to differences in population characteristics, since HAAS included Japanese-American men, and IIHDS included Israeli men from several geographic areas of origin (Europe, Middle East, Northern Africa, and Israeli born), while the Finnish CAIDE population and the multiethnic Kaiser Permanente US population included both women and men. Moreover, 7453 of the 10059 subjects of the original IIHDS cohort died before the follow-up; the deceased were reported to have significantly higher levels of several cardiovascular risk factors (Beeri 2004), which may have resulted in an underestimation of the cholesterol-dementia relation. Since dementia cases were identified by telephone screening (Modified Telephone Interview for Cognitive Status, TICS-m) followed by a face to face interview with all subjects with a TICS-m score of 27/50 or lower, cases of incipient dementia may have remained undiagnosed, again underestimating the role of cholesterol as a risk factor for dementia. When interpreting results from different populations, the socioeconomic context may also be important. In the IIHDS population in the beginning of the 1960s, higher cholesterol levels were related to higher socioeconomic status (as a composite measure of education and occupation) (Goldbourt 2007), perhaps reflecting

(at least partly) better chances to live in a context of austerity (post-World War II food rationing ended in 1959 in Israel). In the CAIDE population in the 1970s and the beginning of 1980s (food rationing in Finland ended in mid-1950s), higher cholesterol levels were related to lower education (linear regression analysis controlled for age and sex; standardized β-coefficient -0.159, $p<0.001$), partly reflecting a less healthy lifestyle. Such differences between populations and their environments in long-term epidemiological studies need to be further investigated.

Although data on midlife cholesterol was available in the Framingham study, the methodology used to analyse it was different compared to the other long-term epidemiological studies with midlife as baseline. Cumulative cholesterol values, averaged over long periods of time during and after midlife (16-30 years) were used in the Framingham analyses, which may be the main reason why no relation with dementia was found (Tan 2003).

5.1.2. Cholesterol and VaD

Most available studies have so far focused on dementia/AD, leaving out VaD or mixed (AD and VaD) dementias. In the Kaiser Permanente population, borderline cholesterol levels as defined by the 2002 Adult Treatment Panel (ATP) III guidelines (200-239 mg/dl, or 5.2-6.2 mmol/l) significantly increased the risk of developing VaD three decades later. High cholesterol levels (≥240 mg/dl or ≥6.2 mmol/l) tended to increase VaD risk as well, but the association did not reach statistical significance. One reason for this could be the small sample size of VaD (127 persons in total, 39 with high cholesterol).

HAAS reported no significant association of cholesterol with either VaD or AD with cerebrovascular disease, although there was a risk increase with clustering of cardiovascular metabolic risk factors (including total cholesterol) at midlife (Kalmijn 2000). Differences in populations may partly explain the different results. However, the most important issue is that ´VaD´ represents a markedly heterogeneous group of disorders (Hachinski 2006), and VaD subtypes are very difficult to account for in epidemiological studies. According to current protocols, dementia must be diagnosed before diagnosing VaD, but dementia criteria are heavily biased towards AD, and AD must exclude vascular pathology. VaD is thus by default less prevalent than AD. Moreover, diagnostic criteria such as ICD-9-CM (currently used in USA) are mainly focused on multi-infarct dementia, leaving out other VaD subtypes.

Despite its strong link with coronary artery disease, elevated serum cholesterol has a less straightforward relation with stroke, since stroke subtypes are not accounted for in many studies (Romero 2007). Moreover, persons with stroke do not always get dementia, and VaD may also be the result of small and clinically silent infarcts (which seem to be even more common than clinically manifest strokes) (Hachinski 2008). Risk factor profiles may be slightly different for different types of lesions contributing to VaD: atherosclerosis in larger brain vessels is thought to be related primarily to blood pressure and secondarily to blood lipids; atherosclerosis in smaller vessels is thought to be related to blood pressure alone and not affected by blood lipids (He 2007). Since The Kaiser Permanente cohort included fewer VaD cases, and since current diagnostic criteria are not able to differentiate between VaD subtypes, more refined conclusions on cholesterol and VaD are difficult to formulate.

5.2. Late-life total cholesterol and cognitive status

Late-life total cholesterol was not associated with dementia/cognitive impairment or with performance in cognitive tests in the CAIDE population (Study I, II). No significant differences in total cholesterol levels were observed between the control, MCI and dementia groups at follow-up (Study I). Similarly, the SCI, MCI and AD groups in the clinical sample (Study V) were not significantly different with respect to total cholesterol values in plasma. These results are in line with reports from several other studies (Table 1.3). Interestingly, shorter follow-up studies with older populations at baseline show either no association between cholesterol and cognitive impairment/dementia, or opposite results compared to long-term follow-up studies with midlife as baseline (higher cholesterol as protective against cognitive impairment/dementia).

Chronic diseases with a long preclinical phase pose inherent difficulties in the identification of their risk factors. Since disease onset cannot be pinpointed, chances are that true risk relationships (factors increasing the probability of getting the disease) and reverse causality (the effects of the disease itself on various factors) get confused. At late-life, any apparently normal population will be a mixture of individuals without the disease(s) and individuals who have the disease(s), although clinically silent and undiagnosed. Midlife is thus more suitable as a start point when looking for risk factors, since AD or cerebrovascular pathology are less likely to already be present.

5.3. Serum cholesterol and cognition: bidirectional association

5.3.1. Epidemiological data

In the CAIDE population, total cholesterol levels decreased in most individuals during the 21 years of follow-up. A moderate decrease in cholesterol from midlife to late-life was significantly associated with the risk of having a more impaired late-life cognitive status, even after adjusting for a wide range of confounders (Study I). Likewise, in persons without dementia a decrease in cholesterol was related to poorer late-life performance on episodic memory and psychomotor speed tests (Study II).

Lower cholesterol levels have been observed in association with clinical and subclinical diseases (Jacobs 1992). Dementia/AD resembles many other chronic diseases, in which several factors, such as BMI or blood pressure, decrease before the clinical signs become visible (Skoog 1996). Serum cholesterol may also decrease as a result of the pathologic processes leading to dementia (Kalmijn 2000, Mielke 2005). Interestingly, data from the HAAS study indicated that a decline in serum total cholesterol after midlife may be associated with early stages in the development of dementia (Stewart 2007). Whereas dementia-related decline for BMI or blood pressure becomes detectable about 3–6 years before the clinical expression of the disease, cholesterol starts declining much earlier, with little subsequent acceleration prior to dementia onset (Stewart 2007). The results of the present project support this hypothesis. After controlling for several possible confounders, the decrease in cholesterol was related both to poorer late-life cognition in non-demented persons (some of whom may develop dementia later on), and to higher degree of cognitive impairment assessed as control-MCI-dementia.

These results provide a possible explanation for the apparent contradictions between long-term follow-up studies with midlife as baseline and shorter-term follow-up studies in older populations. The pattern of cholesterol change may be more relevant for late-life cognition than cholesterol levels at given time points after midlife. Averaging multiple cholesterol measurements over a long period of time may mask this pattern and show decreasing cholesterol as ‘low cholesterol’ related to poorer cognition (Elias 2005). Also, as the significance of cholesterol may change over time (from risk factor to risk marker), grouping

individuals with a very wide age range (from young to very old adults) may make it more difficult to show the cholesterol–cognition association (Teunissen 2003).

Several other factors may have contributed to the decline in cholesterol between 1970s and 1990s in the CAIDE population. Greater decreases in cholesterol levels (>2 mmol/l) were more likely due to other causes (e.g., cardiovascular-related conditions or other diseases) because the relationship with late-life cognition was not significant in any of the models. Also, because subjects whose cholesterol apparently increased over time had the lowest TC levels at midlife and subjects whose cholesterol decreased more than 2 mmol/l had the highest TC levels at midlife, the cholesterol changes in these two groups may reflect the statistical phenomenon of regression toward the mean. From baseline to the first follow-up, cholesterol increased in 325 persons, but it is unclear whether the values increased at first and then decreased, although they remained higher compared to baseline. Adding a third time point (the second CAIDE follow-up, now completed) will refine the analyses. Cholesterol-lowering strategies (including statins since the mid-1990s) were directed especially toward persons with the highest cholesterol levels. However, all subjects (not just those with high cholesterol) were followed over time in the CAIDE study, so regression toward the mean cannot entirely explain the results.

The decrease in cholesterol after midlife may be partly explained by physiologic ageing (Abbott 1997, Ferrara 1997). Unintentional or voluntary changes in lifestyle-related factors (such as diet, physical activity, or smoking) may have also contributed to this pattern. Research on serum lipids in the cardiovascular diseases field has led to interventional programs meant to lower cholesterol levels, and the North Karelia Project has caused a significant decrease of the average cardiovascular risk level (including serum cholesterol concentrations) between 1970s and 1990s in the Finnish population (Vartiainen 2000). Similar patterns of cholesterol level changes have also been reported in other populations (Kuulasmaa 2000, Lanti 2005).

Variations in cholesterol measurement methods are unlikely to have influenced cholesterol modifications over time in the CAIDE study because all measurements were performed in the same central laboratory and the appropriate correction for the difference between the baseline and re-examination biochemical methods was made.

5.3.2. Clinical data

The existence of a bidirectional relation between cholesterol and AD is also supported by clinical data (Study V). The patterns observed for lanosterol, lathosterol, and total cholesterol in relation to brain volumes in patients with AD compared to MCI and SCI are consistent with a central nervous system - induced depressing effect of neurodegeneration on extracerebral cholesterol homeostasis.

Plasma R_lanosterol and R_lathosterol levels (indicating a lower rate of endogenous cholesterol synthesis) were lower in persons with AD compared to persons with MCI or SCI. Also, in patients with AD (but not MCI or SCI) lower plasma total cholesterol and LDL-C were related to lower brain volumes/higher CSF volumes. In contrast, in the SCI group lower levels of the cholesterol precursors lanosterol and lathosterol were related to higher brain volumes/lower CSF volumes. Lower lanosterol and lathosterol levels have been previously associated with better cognitive performance in a study of a cognitively normal ageing population (Teunissen 2003). To which extent the subjects with MCI in our study represent pre-clinical stages of AD is not known, but our results suggest that patients with AD may have a disturbance in cholesterol homeostasis, perhaps with decreased cholesterol synthesis.

The present and the previous (Leoni 2004) findings of lower R_27OHC in the circulation of AD patients are also in line with this contention since substrate availability is an important factor in the production of this oxysterol (Björkhem 2006). The nature of the CNS-related effect can only be speculated on at the moment and the possibility cannot be excluded that it is, at least partly, a consequence of the changes in lifestyle-related factors (such as diet and nutritional status) which can accompany dementia onset. Also, the results of Study V need to be validated with larger samples and longitudinal designs.

5.3.3. Possible mechanisms of association

The exact mechanisms behind the association of cholesterol with cognitive impairment/ dementia are still unclear, but several possible pathways have been proposed. High cholesterol levels can lead to cardiovascular pathologies which increase the risk of cognitive impairment (such as atherosclerosis, coronary artery disease, myocardial infarction, heart failure, atrial fibrillation) (Jefferson and Benjamin 2009, van Vliet 2009). Cerebral hypoperfusion is one possible pathophysiological mechanism. For example, impaired cardiac function in heart failure may lead to insufficient blood flow to the brain; patients with

dementia have been shown to have a lower cerebral blood flow. Cerebrovascular disease could also explain the association, since atherosclerosis, cerebral infarctions, or white matter lesions have been associated with an increased dementia risk (van Vliet 2009). Interestingly, in the present project high midlife cholesterol remained a risk factor for cognitive impairment/dementia even after controlling for several vascular factors and cardio/cerebrovascular conditions.

In addition, vascular pathology has been associated with executive dysfunction (Price 2006). In the CAIDE population, total cholesterol was indeed related to category fluency and psychomotor speed, tests partly relying on executive functions. However, cholesterol was also related to episodic memory after taking into account vascular factors and cardio/cerebrovascular conditions. The mechanisms behind the cholesterol - cognition association are thus partly independent of the vascular pathway.

Vascular risk factors may directly induce AD neuropathology, or lead to arteriosclerosis, impaired brain blood flow and metabolism, and neuronal dysfunction. Vascular risk factors may also induce small and large vessel cerebrovascular disease and infarcts. The accumulation of various lesions can lead to an increased risk of development of clinically manifest dementia, be it AD or VaD or a combination of both.

5.4. Lipid-lowering treatment and cholesterol decline after midlife

Lipid-lowering treatment (especially statins) was related to better performance in episodic memory and psychomotor speed tests in persons without dementia from the CAIDE population. Moreover, the decline in cholesterol levels was significantly related to poorer performance in cognitive tests only in persons without treatment (not in treated subjects).

That statins may be beneficial for cognition in non-demented elderly is supported by several studies (Table 1.5). One possible mechanism is a direct consequence of serum lipid level reduction. However, after adjusting for both midlife cholesterol and changes in cholesterol, the results were still significant. Statins may act through changes in both serum and brain cholesterol. As an intact blood-brain barrier prevents cholesterol influx from the circulation into the brain, it is not yet known to what extent the effect of statins on serum cholesterol influences brain cholesterol. Statins were introduced in Finland in the mid-1990s, and not many persons used them in 1998, making it difficult to analyze statins according to their ability to cross the blood-brain barrier and directly influence brain cholesterol. Lowering only serum cholesterol or both serum and brain cholesterol may not have the same impact on

cognition. Besides their lipid-lowering effect, statins have many other actions which may be important for brain health (Alegret and Silvestre 2006).

5.5. LDL- and HDL-cholesterol

In the present project, information on LDL and HDL was available only in the clinic-based population (late-life LDL and HDL, Study V). Since laboratories gradually began to add LDL and HDL to their test menu only during the 1970s (Delahunty 1998), these assessments were less accessible during the baseline examinations in epidemiological studies.

As expected, late-life LDL and HDL levels were not significantly different between patients with SCI, MCI or AD. A recent meta-analysis has also reported no significant associations of HDL or LDL with dementia, AD or VaD (Anstey 2008). However, some studies have found higher LDL to increase the risk of VaD or dementia with stroke (Moroney 1999, Reitz 2004), and to relate to WML (Amarenco 2006).

In Study V, lower LDL levels were related to lower parenchymal fraction and higher CSF fraction in patients with AD, but not with SCI or MCI, supporting the hypothesis of a disease-related effect on peripheral cholesterol metabolism (see also 5.3.2).

No significant relationship was found between plasma HDL levels and brain volumes, although low HDL levels have been associated with lower hippocampal volumes in some (Wolf 2004) but not all (den Heijer 2005) studies.

5.6. The role of the APOE ε4 allele

In the CAIDE population, high midlife cholesterol and the APOE ε4 allele were independent risk factors for cognitive impairment or poorer performance on cognitive tests. Also, the association between cholesterol changes after midlife and late-life cognition remained significant even after controlling for the presence of at least one APOE ε4 allele. A tendency to interaction between APOE genotype and cholesterol changes over time (and also between sex and cholesterol changes over time) in relation to the degree of impairment in late-life cognition was observed in Study I, but because of the limited sample sizes in the stratified analyses, it is not possible to draw firm conclusions about potential differences between APOE ε4 carriers and non-carriers (or between men and women).

5.7. Apolipoprotein E in the CSF

In Study IV, CSF levels of apoE were significantly increased in AD and MCI patients. Since apoE was not correlated with albumin or Qalb (markers of blood brain barrier functionality), this increase cannot be explained by a dysfunction of the blood–brain barrier. Also, there was no correlation between apoE and 27OHC (most 27OHC in CSF originates from extracerebral sources (Leoni 2005)). Previous measurements of apoE in CSF from patients with neurodegeneration have given contradictory results and increased (Lindh 1997, Fukuyama 2000, Moreira 2005), decreased (Blennow 1994, Pirttilä 1994, Landen 1996, Hesse 2000) or unchanged levels have been reported (Lefranc 1996, Hahne 1997). These different results may be due to methodological differences as well as to the characteristics of the chosen control population.

Apart from its role as cholesterol transporter in the circulation and within the brain, apoE is involved in a constant transport of excess cholesterol from the brain into the circulation via the CSF (Pitas 1987). There is also a flux of 24OHC from the brain into the CSF (Leoni 2005). Since apoE is the dominating lipoprotein in CSF, it may play an important role in this efflux. The highly significant correlation between levels of apoE and 24OHC found in Study IV in patients with AD and MCI is in line with in vitro data indicating that 24OHC has a direct effect on apoE secretion from astrocyte cells (Pfrieger 2003). A similar strong correlation was not observed in healthy control subjects. A possible explanation could be that the coupling between 24OHC and apoE secretion is most important under conditions with increased levels of 24OHC in CSF. Levels of 24OHC in CSF increase under conditions of neurodegeneration (Leoni 2005, Björkhem 2006), presumably reflecting populations of dying neuronal cells (and consequently increased levels of free cholesterol). Under these specific conditions the 24OHC-mediated increase in secretion of apoE may be more important than under normal conditions. Although apoE is a cholesterol-transporting lipoprotein, no significant correlation was found between apoE and cholesterol in CSF from patients with AD or MCI. However, a low but significant such correlation was observed in control subjects.

ApoE levels were also significantly related to T-Tau and P-Tau in patients with AD, and with Aβ42 in patients with MCI. These results are consistent with a direct role of the 24OHC-apoE system in the regulation and control of neurodegeneration. The details of the mechanisms

involved (as well as the diagnostic potential of apoE levels in CSF) need to be further investigated.

5.8. 24-hydroxycholesterol

Brain cholesterol levels may be altered in neurodegenerative diseases, but changes in brain cholesterol content during neurodegeneration cannot be directly analysed. Since 24OHC in the circulation originates from the brain, 24OHC measurements can provide a window into this otherwise analytically inaccessible compartment.

In the present project, CSF levels of 24OHC were significantly higher in patients with AD and MCI than in controls (Study IV), while plasma 24OHC was lowest in the AD group, highest in the SCI group with intermediate levels in the MCI group (Study V). These findings are in agreement with previous studies (Björkhem 2006). It is known that less than 1% of the total excretion of 24OHC occurs via CSF. This minor fraction appears to reflect neuronal damage and rate of neuronal loss, rather than the total number of metabolically active neuronal cells (which is reflected in plasma 24OHC levels). The changes in CSF are thus more marked than those in the circulation (Björkhem 2006).

5.8.1. 24-hydroxycholesterol in CSF

The correlation between 24OHC and apoE in CSF of patients with AD and MCI (but not controls) (Study IV) suggests that the 24OHC-mediated secretion of apoE creates an overcapacity for transport of steroids from CNS under conditions of neurodegeneration but not under normal conditions. Under in vitro conditions, cholesterol increases APP cleavage via the amyloidogenic pathway, whereas 24OHC has been reported to have an opposite effect (Brown 2004). If the neurodegenerative process results in increased levels of free cholesterol, 24OHC may have a role both as a suppressor of amyloidogenesis and as a stimulator of apoE-mediated removal of cholesterol.

The transcription of the CYP46A1 gene, coding for cholesterol 24S-hydroxylase, has been found to be resistant to most regulatory factors tested, and availability of substrate cholesterol may be the most important factor under normal conditions (Björkhem 2006). Oxidative stress is one of the few factors capable to up-regulate the gene at a transcriptional level. Since oxidative stress occurs in CNS of patients with AD (Moreira 2005), it is possible that this may result in increased formation of 24OHC and increased flux of apoE from the astrocytes.

5.8.2. 24-hydroxycholesterol in plasma

In the whole population, R_24OHC was positively related to GM fraction. Interestingly, when each group was considered separately, the association of R_24OHC with GM volume, GM fraction and parenchymal fraction was significant in control subjects only. The positive association between R_24OHC and GM volume, GM fraction and parenchymal fraction in the SCI group adds further evidence that plasma 24OHC reflects the number of metabolically active neurons in the brain. A recent study has also linked plasma 24OHC to hippocampal size in middle-aged normal individuals (Koschack 2007) and another to the dimension of the striatum nucleus and its degree of atrophy (Leoni 2008). In case of neurodegeneration, such as in AD, the observed reduced levels of 24OHC depend upon a reduced activity of the cholesterol 24-hydroxylase, the neuronal enzyme responsible for 24OHC synthesis. The expression of this enzyme is rather stable in neurons, and the reduced number of active neuronal cell present in the gray matter is then mirrored by a parallel reduction of plasma 24OHC.

A possible explanation for the lack of significant associations with brain volumes in the MCI or AD groups could be the abnormal expression of the CYP46 enzyme in glial cells that was shown in the brain of patients with AD (Bogdanovic 2001, Brown 2004), which occurs as a compensatory mechanism in neuronal degeneration. This may represent a limitation for the use of 24OHC as a biomarker in patients with AD. The situation may be different in other types of neurodegenerative diseases, however, and at present it is unknow if there is an abnormal expression of CYP46 also in other diseases.

5.9. Methodological issues

The main strength of this project lies in its integrative approach. Although each study design, method and population has its own inherent problems and limitations, combining them can only reinforce the results.

5.9.1. Epidemiological approach

Studies I and II have a prospective population-based design (Finnish population), while study III has a retrospective, register-based design (Kaiser Permanente of Northern California, a non-profit, group-practice health integrated delivery system, with members representative of

the sociodemographics of the local population). The project combines thus data from a more homogeneous population (CAIDE) with data from a heterogeneous US population.

5.9.1.1. CAIDE

CAIDE is based on population-based random samples of individuals that were investigated twice during the study. Participation rates were high, ranging from 83 to 93% at baseline and 72% at follow-up. The prospective population-based design and relatively high participation rates increase the reliability of the findings. The follow-up time was long, on average 21 years, which created the opportunity to investigate risk factors that were present already at midlife, and to study the true risk relationship, as well as the hypothesis of reverse causality. No information was available on the participants´ cognitive status at baseline. However, at the time of the midlife examination, subjects were 39 to 64 years old, and it is unlikely that they had dementia at that time. If there were persons showing early signs of dementia already at the baseline examination, they would have probably not survived and participated in the follow-up examination.
Prospective studies are known to be time-consuming and expensive to conduct, although they allow the investigators better control over exposure and outcome information, which is collected directly from the participants and according to protocols formulated specifically for the study. CAIDE offers therefore the advantage of data on APOE genotype, as well as on the continuum of cognitive functioning (results of detailed cognitive tests). Not only dementia or AD is investigated, but also MCI.

CAIDE included 1449 participants, a sufficiently large population to investigate the relations between cholesterol and cognition/dementia. However, in some of the stratified analyses (i.e. APOE ε4 carriers vs non-carriers) the sample sizes did not allow any definite conclusions about potential gene-environment interactions. Also, the number of persons with dementia types other than AD was too small to consider such dementia types (i.e. VaD) as outcomes in the analyses.

Only participants with MMSE $\leq$ 24 in the screening phase entered the clinical phase at the follow-up examination. It would have been ideal if all persons had participated in both screening and clinical phases, but the three-phase protocol was probably high enough in sensitivity and specificity to detect AD. Nearly half of all participants with MMSE below the

cut-off point in the screening phase were considered as cognitively normal in the clinical phase, suggesting that the cut-off score was sufficiently sensitive for detecting AD in this population. Using a higher cut-off would have resulted in the inclusion of a very large proportion of the population in the clinical phase, as the median MMSE was 26. While MMSE may have been sensitive enough to capture manifest AD, the use of MMSE may have resulted in underdiagnosis of dementia types in which memory deficits are not the main initial manifestation of disease. Persons diagnosed with dementia in the clinical phase underwent brain imaging in the differential diagnostic phase. Autopsy data were not available to confirm the clinical diagnoses, but a previous neuropathological study conducted in the clinic in Kuopio has shown that the accuracy of clinical AD diagnosis is good (96% for probable AD and 86% for possible AD) (Kosunen 1996).

The different criteria for VaD (including NINDS-AIREN) seem to have low sensitivity, but higher specificity (Knopman 2001). It is known that current VaD diagnostic criteria select etiologically and clinically heterogeneous groups (Erkinjuntti 2000). The Hachinski Ischemic Score (HIS) has been reported to have both high sensitivity and specificity (Moroney 1997). While lacking neuroimaging criteria, HIS has been suggested as more suitable for identifying the majority of dementia patients with at least some cerebrovascular pathology. HIS was used in the CAIDE study to aid in VaD identification, but VaD was diagnosed using NINDS-AIREN criteria. Thus, the real prevalence of VaD in the CAIDE population was probably higher than the detected prevalence.

The identification of MCI was essentially based on clinical judgement: the persons did not fulfill the criteria for dementia, but had some subjective and objective cognitive impairment. Some cases of MCI and dementia may have been lost due to the MMSE cut-off score. The CAIDE study was designed to detect dementia, and it is unclear how sensitive and specific the screening procedure was in identifying those persons with MCI. However, the prevalence of MCI in CAIDE was similar to that of another population-based study conducted in the same region with a population of corresponding age, suggesting that the detection bias was minimal (Hänninen 2002).

Cholesterol levels were measured as non-fasting values. Non-fasting measurements are often used in epidemiological studies, mainly for practical reasons and also because non-fasting values are not significantly different from fasting levels for an individual (Bachorik 1991).

Thus, any variation in cholesterol levels caused by these conditions of measurement would be equally distributed between all subjects and would not be expected to change the results. In addition, a person usually spends most of the time in a non-fasting state.

Information about vascular events was obtained from self-administered questionnaires, which may introduce some reporting bias. However, a proxy informant (spouse, other relative) can increase information reliability, since significant medical events such as myocardial infarctions or strokes are likely to be remembered.

Non-participation may have influenced the results. No information was available on baseline non-participants with regard to risk factors or cognitive status. However, non-participation rates at baseline were low, and the main reasons were: address information not up to date, temporarily away from home, or unable to participate. Very few individuals refused to participate (Puska 1995). The role of non-participation at follow-up could be evaluated to some extent. High cholesterol levels at midlife are associated with increased cardiovascular risk and mortality, as well as with increased risk of MCI and dementia. Non-participants at follow-up had higher cholesterol values at midlife, a higher prevalence of dementia and worse health status later in life, so their cholesterol levels would be expected to markedly decrease from midlife to late life. Thus, the results would rather represent an underestimation of the association between cholesterol and the risk of worse cognitive outcomes. The same is true for selective survival, if we assume that among the dead there were more persons with high midlife cholesterol levels who would have been more likely to have dementia/cognitive impairment and a worse health status later in life.

5.9.1.2. Kaiser Permanente

The main advantage of retrospective cohort studies is the quicker and less expensive access to large amounts of information on various risk factors and outcomes. Study III is so far the largest longitudinal study to investigate the link between midlife serum total cholesterol and dementia. The number of persons with dementia allowed the consideration of both AD and VaD as outcomes. The study is based on the Kaiser Permanente electronic database, and includes information from comprehensive health examinations at midlife, a long follow-up period, and a multiethnic representative sample including both men and women with equal access to medical care. Also, because cholesterol was measured in people aged 40 to 45 years,

it is highly unlikely that subclinical dementia was present at baseline; thus the temporality of the associations is clear.

Several potential confounders were controlled for in the analyses. However, information on APOE genotypes and on lipid-lowering treatments was not available for this study.

Since outcomes were obtained electronically from chart diagnoses, AD and VaD definitions are based on ICD 9 CM codes (as the ICD 9 CM system is currently in use in the US for registering morbidity data). It is thus possible that a portion of the population may have had undiagnosed dementia. It is also likely that some AD or VaD cases were missed in participants who died prior to 1994, the onset of the ascertainment. However, this would tend to bias the results toward an underestimation of the effect of midlife cholesterol on AD or VaD. In addition, having to rely entirely on ICD 9 CM codes has restricted the choice of outcomes to manifest dementias, to the detriment of milder forms of cognitive impairment.

Neuropathological data regarding the diagnoses of AD and VaD in the Kaiser Permanente cohort was not available. Diagnostic criteria used in current clinical practice are known to have a bias towards AD due to the emphasis on memory impairment in dementia diagnosis. As a result, some VaD cases may have been labelled as AD. Also, current diagnostic criteria define AD and VaD as entirely separated from one another, at the cost of mixed dementia aetiologies. Although the cohort in Study III does not include persons with mixed dementia as recognized by ICD 9 CM, the concomitant presence of neurodegenerative and vascular pathologies in a portion of the sample cannot be excluded. Moreover, the term ´VaD´ covers many types of dementia, but it was not possible to do more detailed analyses within the VaD group, partly because of the diagnostic criteria (which focus mainly on multi-infarct dementia) and partly because of the VaD sample size.

Due to the study design, AD or VaD status could only be assessed in persons who were still Kaiser Permanente members at the time of the ascertainment. However, post hoc analyses revealed no significant differences in any of the midlife cardiovascular risk factors by health plan membership status in 1994.

5.9.2. Clinic-based approach

Studies IV and V are case-control studies with cross-sectional analyses. The clinic-based approach offers the advantage of very detailed evaluations of participants, and the Memory clinic at Karolinska University Hospital, Huddinge, Sweden was chosen for its standardized comprehensive examination protocol which routinely includes neuropsychological testing,

brain imaging, and plasma and CSF measurements (with CSF Aβ42, T-tau and P-tau levels used in establishing dementia aetiology). The populations in studies IV and V are thus highly selected compared to studies I-III, since they specifically include individuals referred to a hospital-based setting due to memory-related concerns. This is an important advantage for studying AD, but it makes studying VaD difficult, as most VaD cases are evaluated in traditional Neurology clinics and only a small number can be found in memory clinics.

The selection of an appropriate control group is perhaps the most difficult and critical issue in a case-control study. Two different control groups were used in the clinical studies: patients referred to the Neurology clinic (Study IV) and patients with SCI from the Memory clinic at at Karolinska University Hospital (Study V). Patients from the same hospital as the controls, and who have been admitted for conditions other than the disease being studied, are often used as control groups, since they are likely to have been subjected to the same selection factors that influenced the cases to come to a particular hospital. The chief disadvantage of hospital-based controls is that they have by definition health-related problems and may therefore be different from healthy individuals. However, the comprehensive examination protocols used at Karolinska University Hospital excluded the presence of AD (as defined by current criteria) in these patients.

Although in the smaller, pilot study on CSF markers of cholesterol metabolism the Neurology clinic served as source of controls (Study IV), persons with SCI from the Memory clinic were chosen as control group for the larger study on plasma markers of cholesterol homeostasis (Study V). If a clinic is known to be a referral centre for the treatment of a particular condition (i.e. AD), it becomes a good source of cases but these patients may not be comparable with individuals from other clinics in the same hospital. However, all exclusion criteria used in the identification of cases were also applied to controls in both clinical studies.

6. SUMMARY AND CONCLUSIONS

Physicians tend to confine Alzheimer and cerebrovascular disease to geriatric age borders and address symptoms as they occur. The results of this project point out the importance of addressing risk factors as early as midlife, before the underlying disease(s) or the symptoms appear. High midlife serum total cholesterol increases the risk of both AD and VaD, a finding that adds to the existing body of evidence on a degree of overlap between the two dementia types in terms of risk factors, symptoms and neuropathology.

Cholesterol–dementia and cholesterol–health relationships are controversial, and the benefit/risk ratio for cholesterol-lowering drugs in old age is still discussed. The present studies show that high serum total cholesterol at midlife is a risk factor for subsequent development of cognitive impairment or dementia, but serum cholesterol levels may later reflect ongoing pathologic processes in the brain and may represent a risk marker for late-life cognitive impairment or dementia. The significance of circulating cholesterol likely differs between younger and older adults mostly in those with normal or low cholesterol, not in those with high cholesterol. Elderly with low cholesterol may have had lifelong low levels, or may have low cholesterol secondary to different diseases (for example AD). High cholesterol carries risk even in old age, and results from clinical trials in vascular diseases support the benefit of lipid-lowering treatment in elderly patients. Lipid risk profiles seem to change with time, and the interpretation of low cholesterol levels in old age must be done with respect to the patients' health and cognitive status. In the CAIDE study, statin treatment seemed to be beneficial for cognitive functioning even after taking into account the decrease in serum cholesterol after midlife.

The relation of lipids with vascular pathology and the relation of protein aggregation with neurodegeneration have long been regarded as separate matters of investigation. The results of this project emphasize the involvement of brain cholesterol metabolism in AD pathophysiology, and suggest a potential role (and also limitations) for cholesterol-related markers in identifying the disease in a clinical setting. However, much research is still needed before the level of knowledge on brain cholesterol homeostasis can reach the level of knowledge on circulating cholesterol metabolism.

7. FUTURE DIRECTIONS

Current evidence supports the potential of cholesterol-modifying strategies for dementia. However, more data still needs to be gathered before this potential can be used in clinical practice. Additional research on brain cholesterol metabolism and on its interactions with circulating cholesterol is particularly important. A key point suggested by the RCTs with statins in patients with AD is the optimal timing of treatment in order to achieve benefit. It seems that mild to moderate AD is too late, a stage too advanced in the AD pathogenic cascade. Early diagnosis of the disease, preferably before dementia becomes manifest, may lead to a different response to treatment. Unfortunately, current diagnostic criteria are based on a dichotomy between ´dementia´ and ´non-dementia´, without taking into consideration that cognitive impairment occurs across a continuum, not over a threshold. Revised research criteria for AD have been recently proposed, in an attempt to move away from the current two-step approach (first identification of dementia, then identification of dementia type) towards diagnosis of the underlying disease before dementia onset. Such criteria need to be tested and adjusted for use in day to day clinical practice.

RCTs with statins in AD treatment had very strict enrollment criteria, including only patients with no other indication for statin treatment, and leaving out persons with AD and increased cardiovascular risk. The latter group of patients may respond better to statin therapy. It is also difficult to estimate treatment effects in AD patients who also have significant concurrent cardio/cerebrovascular disease, since such persons are usually excluded from RCTs. This exclusion of an important group of patients is the result of the dichotomy between the categories of ´neurodegenerative´ and ´vascular´ in dementia diagnosis. `Pure´ AD and ´pure´ VaD should be considered the opposite ends of a dementia aetiology continuum, where most cases are ´in between´ and have combinations of AD type and vascular changes in different degrees. More attention has to be directed towards similarities and interactions between various pathophysiological processes, instead of attempting to create disease classification systems where the main purpose is only to accurately pigeonhole patients.

Cholesterol-modifying strategies may be most effective when used to prevent dementia/AD. However, medication need not be the first choice of strategy in all cases (especially if there are no other indications for statin treatment). In addition, since AD is a multifactorial disease and cholesterol is only one piece of the puzzle, integrative strategies targeting

hypercholesterolemia together with other vascular and lifestyle-related risk factors (such as hypertension, diabetes, obesity, or physical inactivity) may be more prone to succeed. Gene-environment interactions may make genetically susceptible persons more likely to benefit from such interventions, and this is an important area of further research. Tools for predicting the risk of dementia, which can be useful for targeting preventive measures to those most at risk, have already been developed (i.e. CAIDE Dementia Risk Score).

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APPENDIX:
ORIGINAL PUBLICATIONS

I

Serum cholesterol changes after midlife and late-life cognition.
Twenty-one-year follow-up study

A. Solomon, I. Kåreholt, T. Ngandu, B. Winblad, A. Nissinen, J. Tuomilehto, H. Soininen, M. Kivipelto**.**

Neurology 2007;68;751-756

II

Serum total cholesterol, statins and cognition in non-demented elderly

A. Solomon, I. Kåreholt, T. Ngandu, B. Wolozin, S.W.S. MacDonald,
B. Winblad, A. Nissinen, J. Tuomilehto, H. Soininen, M. Kivipelto

Neurobiology of Aging 30 (2009) 1006–1009

III

Midlife Serum Cholesterol and Increased Risk of Alzheimer's and Vascular Dementia Three Decades Later

A. Solomon, M. Kivipelto, B. Wolozin, J. Zhou, R.A. Whitmer

Dement Geriatr Cogn Disord 2009;28:75–80

IV

Levels of ApoE in cerebrospinal fluid are correlated with Tau and 24*S*-hydroxycholesterol in patients with cognitive disorders

M. Shafaati, A. Solomon, M. Kivipelto, I. Björkhem, V. Leoni

Neuroscience Letters 425 (2007) 78–82

V

Plasma levels of 24S-hydroxycholesterol reflect brain volumes in patients without objective cognitive impairment but not in those with Alzheimer's disease

A. Solomon, V. Leoni, M. Kivipelto, A. Besga, A-R. Öksengård, P. Julin, L. Svensson, L-O. Wahlund, N. Andreasen, B. Winblad, H. Soininen, I. Björkhem

Neuroscience Letters 462 (2009) 89–93

PUBLICATIONS
SERIES OF REPORTS, DEPARTMENT OF NEUROLOGY

1. **Juhani Partanen (1978):** Time-locked phenomena of human motor unit potentials. An electromyographic study of satellites and doubles.
2. **Eeva Leino (1981):** Clinical and biochemical studies on progressive myoclonus epilepsy.
3. **Hilkka Soininen (1981):** Senile dementia. A clinical, neurochemical and etiological study.
4. **Rolf Danner (1982):** Adverse effects of anticonvulsive treatment on peripheral nerve conduction and posterior dominant EEG rhythm.
5. **Markku Saksa (1982):** The autonomic nervous system in experimental allergic neuritis. A functional, morphological and biochemical study.
6. **Juhani Sivenius (1982):** Studies on the rehabilitation, epidemiology and clinical features of stroke in East Central Finland.
7. **Asla Pitkänen (1987):** Somatostatin in epilepsy. An experimental and clinical study.
8. **Esa Mervaala (1987):** Evoked potential in human epilepsy. A neurophysiological study.
9. **Kari Reinikainen (1988):** Neurotransmitters in Alzheimer's disease.
10. **Tapani Keränen (1988):** Epilepsy in adults. An epidemiologic study in Eastern Finland.
11. **Jukka Jolkkonen (1988):** Vasopressin in the central nervous system. A study based on cerebrospinal fluid measurements.
12. **Jouni Sirviö (1989):** The cholinergic system in ageing and dementia. With special reference to acetylcholinesterase.
13. **Hannu Koponen (1989):** Delirium in the elderly. A clinical, neurochemical, neuropsychological and neuroradiological study.
14. **Asla Pitkänen (1989):** Somatostatin in experimental and human epilepsy.
15. **Eeva-Liisa Helkala (1990):** Memory in patients with Alzheimer's disease and demented patients with Parkinson's disease.
16. **-**
17. **Paavo Riekkinen Jr (1990):** Animal models of age-related degeneration of subcortical regulatory systems. With special reference to cholinergic, noradrenergic and serotonergic systems.
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