From DEPARTMENT OF NEUROBIOLOGY, CARE SCIENCES AND SOCIETY
Karolinska Institutet, Stockholm, Sweden

THE ROLE OF SOCIO-EMOTIONAL FACTORS FOR COGNITIVE HEALTH IN LATER LIFE

Krister Håkansson

Karolinska Institutet

Stockholm 2016

Published by Karolinska Institutet.
Printed by E-Print AB 2016

ISBN 978-91-7676-449-7

THE ROLE OF SOCIO-EMOTIONAL FACTORS FOR COGNITIVE HEALTH IN LATER LIFE

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Krister Håkansson

Principal Supervisor:
Professor Miia Kivipelto
Karolinska Institutet
Department of NVS
Division of Neurogeriatrics and ARC

Co-supervisor(s):
Professor Abdul Mohammed
Linnaeus University
Department of Psychology

Professor Bengt Winblad
Karolinska Institutet
Department of NVS
Division of Neurogeriatrics

Opponent:
Associate Professor Francois Herrmann
University of Geneva
Department of Internal Medicine, rehabilitation and geriatrics

Examination Board:
Professor emeritus Bo Malmberg
Jönköping University
School of Health and Welfare
Division of Gerontology

Professor Katri Räikkonen-Talvitie
University of Helsinki
Institute of Behavioral Sciences

Associate Professor Michael Rönnlund
Umeå University
Department of Psychology

I dedicate this thesis to my parents because they have put up with me during all these years, and still do, and because they, after more than 60 years of marriage and through their life-style, exemplify a lot of what this thesis is about.

Live fish swim against the stream, while dead ones float with it.

Robert Daley, 1862

Whenever a theory appears to you as the only possible one, take this as a sign that you have neither understood the theory nor the problem which it was intended to solve.

Karl Popper, 1972

Old friends, old friends,
Sat on their park bench like bookends ...

Time it was, and what a time it was, it was
A time of innocence, A time of confidences
Long ago, it must be, I have a photograph
Preserve your memories; They're all that's left you

Paul Simon, 1968.

ABSTRACT

With increasing life expectancies in most parts of the world, the prevalence of dementia and other age-related chronic diseases is growing. Several factors affect future projections and are discussed in this thesis, including possible limits to a continued growth of life expectancy. A related question is to what extent healthy ageing per se affects cognitive functions in old persons. Previous studies have generally exaggerated ageing effects on cognition, by applying study designs that did not account for common confounders, such as birth cohort differences, and the effects of terminal decline and subclinical dementia. In contrast to healthy ageing, dementia neuropathologies dramatically reduce cognitive performance, and proposed mechanisms behind dementia are briefly discussed with focus on Alzheimer's disease (AD), on the role of genetic factors and on life course exposures. Three studies (study 1-3) investigated how cohabitant status and feelings of loneliness and hopelessness in midlife were associated with cognitive health in later life. Neurotrophic factors could potentially be involved in the biological mechanisms behind these and other associations between life-style factors and cognitive health. The fourth study aimed to explore how levels of brain-derived neurotrophic factor (BDNF), measured in serum, were affected by performing different activities; physical exercise, cognitive training, and mindfulness.

THE FOUR STUDIES

Study 1-3 were epidemiological association studies based on the Cardiovascular Risk Factor, Ageing and Dementia (CAIDE) Study, a population based cohort study on 1511 persons in eastern Finland, who at baseline were 50.4 years. Two re-examinations have been performed in the CAIDE Study, in 1998 when the participants were between 65 and 80 years, and between 2005-2008, averagely 25.3 years after the baseline examinations. The first two studies were based on the 1409 persons who fully participated in the first re-examination and the third study on the 1511 persons who participated in one or both re-examinations. In the first two studies logistic regression was the main statistical method with any cognitive impairment versus no cognitive impairment as outcome. In addition we performed analyses with mild cognitive impairment and Alzheimer's disease as separate outcomes. In Study 1 and 2 we also analysed how apolipoprotein epsilon 4 (ApoE4) status affected the associations with Alzheimer's disease. The statistical method in Study 3 was survival model analysis (Kaplan-Meyer and Cox regression) and the outcome variable was dementia, without subtyping. We compared the results from the analysis on the 1511 participants with the results when we used the total sample (by including register linked data on dementia diagnoses). We adjusted the associations for several potential confounding variables in all three studies.

In Study 4 we used 19 elderly healthy volunteers who were between 65 and 80 years (mean = 70.8 years). They performed three different activities during 35 minutes on separate occasions, i.e. a within-subject cross-over experimental design where we randomized the order of the three conditions between the participants. We sampled blood from a suitable

lower arm vein directly before and after each activity session and in addition at 20 and 60 minutes after the session had ended. After the serum had been analysed for BDNF levels, we used repeated measures ANOVA to calculate the differences in the effect of BDNF levels between the three conditions.

MAIN RESULTS

We found that living alone in midlife was associated with approximately a doubled risk of cognitive impairment during the re-examination. Among the non-cohabitants the risk increase was especially high for persons who were widowed in midlife and who had continued to live alone until the re-examination (odds ratio (OR) 7.67, 95% confidence interval (CI) 1.6 – 40.0). Feelings of loneliness were common both among cohabitants and non-cohabitants, but we found that such feelings were only associated with an increased dementia risk if these persons had also been living alone. Feelings of hopelessness in midlife, but not at follow-up, were associated with increased risk of cognitive impairment at the re-examination, especially of Alzheimer's disease (OR 2.90, CI 1.4 – 5.9). When we adjusted the association from midlife also for depression and hopelessness at the re-examination, this association was still statistically significant. Participants with a diagnosis of cognitive impairment had higher feelings of hopelessness at the re-examination, compared to the cognitively healthy group, but this difference between the groups existed already when they were in midlife. When we stratified the participants with reference to ApoE4 status, we found that participants who were also ApoE4 carriers had a dramatically increased risk of Alzheimer's disease compared to non-carriers without feelings of hopelessness, even after final adjustment for depression (OR 6.48, CI 2.4 –17.5). A similar stratification for ApoE4 status in Study 1 showed an even more dramatic increase in the association for persons who had lost their partner (widowed or divorced/separated) if they in addition were ApoE4 carriers.

In Study 4 we found that physical exercise, but not cognitive training or mindfulness, led to a statistically significant increase in BDNF levels of around 25%, compared to baseline. We also found that the individual differences in BDNF levels after the physical exercise correlated with working memory performance, measured on a separate occasion.

CONCLUSIONS

Social and emotional factors can have long-term consequences for cognitive health in later life. The long follow-up time in Study 1-3 suggests that the associations we found with dementia could reflect a causal, rather than a prodromal, relation. As other studies have found a range of adverse ill health consequences from both living alone and from depressive feelings, a possible mechanism behind the associations we found could be related to a systemic biological impact, and that the specific ill health outcome could be a result of individual vulnerability where genetic dispositions could play an important role. This conclusion seems consistent with the dramatic risk increases we found for AD when ApoE4

status was combined with the social factor of living alone and with the emotional dimension of hopelessness. At the micro level, as synaptic dysfunction and loss is characteristic of Alzheimer's disease, and as BDNF has a central role for synaptogenesis, impaired BDNF functionality could play a role in the development of Alzheimer's disease. More research is needed to further explore the role of BDNF in Alzheimer's disease and if the disease can be prevented, or the disease process halted, by activities that stimulate BDNF expression in the brain.

LIST OF SCIENTIFIC PAPERS

I. **Håkansson K**., Rovio S., Helkala E.-L., Vilska A-R, Winblad B, Soininen H., Nissinen A., Mohammed A H, Kivipelto (2009). Association between mid-life marital status and cognitive function in later life: population based cohort study. *British Medical Journal*, *339*, b2462. http://doi.org/10.1136/bmj.b2462 PMID: 19574312

II. **Håkansson K**, Soininen H, Winblad B, Kivipelto M (2015) Feelings of Hopelessness in Midlife and Cognitive Health in Later Life: A Prospective Population-Based Cohort Study. PLoS ONE 10(10): e0140261. doi: 10.1371/journal.pone.0140261 PMID: 26460971

III. **Håkansson K**, Feng L, Soininen H, Mohammed A H, Winblad B, Rusanen M, Laatikainen T, Ngandu T, Kivipelto M. Living and Feeling Alone in Midlife - Associations with Cognitive Health in later Life. Manuscript.

IV. **Håkansson K**, Ledreux A, Daffner KR, Terjestam Y, Bergman P, Carlsson R, Kivipelto M, Winblad B, Granholm A-C, Mohammed A. BDNF Responses in Healthy Older Persons to 35 minutes of Physical Exercise, Cognitive Training and Mindfulness: Associations with Working Memory Function. J Alzheimers Dis. 2017;55(2). In press.

CONTENTS

LIST OF ABBREVIATIONS

AD	Alzheimer's disease
ApoE4	Apolipoprotein E4 allele
BBB	Blood brain barrier
BDNF	Brain-derived neurotrophic factor
CAIDE	Cardiovascular Risk Factors, Aging and Dementia
CI	Confidence interval
CNS	Central nervous system
DMN	Default Mode Network
fMRI	Functional Magnetic Resonance Imaging
FTD	Frontotemporal dementia
HR	Hazard ratio
HR	Hazard ratio
MCI	Mild cognitive impairment
NFT	Neurofibrillary tangles
OR	Odds ratio
SAGE	Successful Ageing and Enrichment
VAD	Vascular dementia

1 PREFACE: ABOUT THE PERSONAL JOURNEY

A doctoral thesis is the outcome of an education, a manifestation of skills, and also, in the ideal case, a significant contribution to knowledge. On another level it also represents a personal journey. For me this journey has been an exciting adventure and I would like to start with this aspect of my thesis. This is the parallel personal and subjective version of this thesis, a part that is neither common, mandatory or needed in a doctoral thesis. It is written as a more accessible version especially for readers who are not scientists and for those who might have an interest in the more personal side of the research process. If you don´t belong to any of these categories, you can easily skip this part and go directly to Chapter 2.

In 1968 Simon & Garfunkel, one of my favourite groups at the time, released a new album called "Bookends". The lyrics were all about old people and their stories. When in high school we were allowed to freely chose a topic and conduct a study around that topic, my decision was greatly inspired by this album. I still remember many of the old persons I met, their life stories what they told me about getting old.

A few years later I worked at a hospital in Kristianstad for severely demented people. After more than 40 years I still have vivid memories of some of the very demented patients I met there. I imagine these experiences both have been important seeds to this thesis, and I also think that personal experiences of this kind has an important complementary role to what you learn from books and articles about ageing and dementia. These and similar experiences have kept reminding me that there are real people behind all the numbers and calculations you will encounter in other parts of this thesis.

In one way, to write a thesis about late-life cognitive health thus seemed like closing a circle by returning to experiences and questions I had asked myself when I was very young.

One day my colleague at the Linnaeus University, Abdul Mohammed, invited me to visit his department at Karolinska Institutet, where he also worked. At the visit to the department of Neurobiology, Care Sciences and Society I was fortunate to have a meeting scheduled with Bengt Winblad and Miia Kivipelto, who gave me access to the unique CAIDE[1] database from Finland, a population-based registry with baseline data from the seventies. For a person with a psychological background like myself, the large amount of unexplored information on social and psychological factors in this database was like a treasure. As I knew that other studies had found intellectual and social stimulation to be associated with better cognitive health, I made some initial calculations to compare people who had been married or living with someone in midlife with persons who had not, a variable that in medical research is usually considered as a possible confounder and something to adjust for while looking for other things. But like many others, I had from personal experience made the conclusion that living with someone is not only a social project, but also a cognitively challenging one. When

[1] CAIDE stands for Cardiovascular Risk Factors, Aging and Dementia

the number (the "odds ratio") appeared on my computer screen, indicating a seven-fold risk increase for widowed persons in this population, it was a flash bulb moment I will never forget. First I did not believe the numbers I got and thought I must have done a mistake in the calculation. After several double checks and recalculations I was finally convinced. I then subdivided the participants into carriers and non-carriers of the APOE4[2] allele, and the risk rocketed for those who both had been living alone after losing their partners in midlife and who in addition carried this allele. The odds ratios I had found for Alzheimer's disease, over 20 for the combined risk, were higher than I had seen in the literature for any other risk factor.

When I brought my results on the train to Stockholm I was quite nervous that I had "discovered" something others had already observed in this database before me. This was however not the case and everybody I talked to was happy and enthusiastic over the findings. I have often thought that this kind of reaction, the readiness to accept and reward new ideas, findings and accomplishments, could be one of the success factors for this institute. At Karolinska it has for me been easy to feel like a member in a soccer team and when you score, the team scores, and everybody is happy. (No, I am not naïve about competition and envy in the academic world at large, but this kind of attitude has been the rule rather than the exception in the groups I have worked with at Karolinska.)

To find an association of this kind is one thing. But trying to figure out what it means, and how to test such ideas, that is where the real fun starts. What was it about living with someone that was so favourable? If it was the cognitive stimulation in a close relation that mattered mostly, why were the singles so much better off than the divorced and especially the widowed? In contrast to the singles, at least they had had a portion of the possible health benefits from a cognitively challenging partner relation. Was it rather about the emotional consequences of losing a partner, especially if the partner dies from you, like feelings of hopelessness and loneliness? On the other hand, even if you live with someone, would not the quality of the relation matter more than living with someone per se? E.g. how many people feel even more alone from living with someone if the relation is not working? Trained in the experimental tradition, I also had permanent doubts about the validity of epidemiological association findings where a lot of possible confounders can not be fully controlled and where you can not even be sure about the direction of causality, if any, between the factor of interest and an "outcome". Especially in the case of dementia, with such a long subclinical disease phase, these doubts kept worrying me. These questions about the underlying mechanisms and the methodological concerns increased my curiosity and guided the studies that followed, most of them included in this thesis.

One of the issues that troubled me the most was the issue of reverse causation. I realized how lucky I was to work with a database with a follow-up time of over 20 years and with

---

[2] APOE4 is an allele that is known to increase the risk for Alzheimer's disease. Its prevalence varies largely across populations and around 20% carry this allele in Northern Europe [1].

participants only around 50 at baseline. Compared to most other studies of this kind, these features reduced the risk that even a subclinical dementia disease could have affected the variable of interest, the "predictor" variable, already at baseline. Another concern was the consequences of self-selected, rather than randomized, groups, for example persons who decide to marry versus the ones that don´t – or persons who embark on a long education versus people that don´t. How do I know that these differences are not also related to other factors, such as personality, intelligence or childhood experiences, factors that could not be included in the statistical adjustments, but could still be the ones that really mattered? In the first study I had been lucky also in that regard as the main difference was between two groups of persons who both initially married someone, and thus could be assumed similar also in such other respects.

It is probably true that both of these methodological concerns; the risk of reverse causation and the risk of uncontrolled confounders behind an association, have stamped the way I have approached the different research questions, and in the end even made me include a well-controlled experimental study to complement the epidemiological association study approach. Especially for depression, I quickly realized that reverse causation was a hot topic – are depressive feelings a true risk factor or is it an early consequence of even a sub-clinical dementia disease process? With the data on hopelessness in the CAIDE database, it was possible to show that persons with a dementia diagnosis in later life had higher levels of these feelings shortly prior to the diagnosis. But it was also possible to go back in time and show that the same difference existed decades before, already in midlife. An elegant and theoretically significant demonstration of depressive feelings as causal, rather than prodromal, I thought. Clearly, these results were in conflict with what many researchers thought to be the case – and perhaps less "spectacular", compared to the first study on marital status. For whatever reason, it proved difficult to get the article published in the more prestigious journals. I concluded that the quick and successful BMJ publication of the first study had spoiled me, and perhaps also given unrealistic expectations to others that my thesis would be a quick affair. Yes, it was very close a couple of times, but in the end I had to climb down the impact factor ladder a bit to finally get the second article published. At least it taught me a lot about publication policies and review processes in different journals. It made me realize that scientific journals and their editors are operating on a market and need articles that can give them public recognition and attention. That people who continue to live alone after a divorce or the death of a partner increase their dementia risk, is by these standards perhaps a more "spectacular" result than levels of hopelessness at different time points for persons that will, or will not, have a dementia diagnosis in later life. But theoretically, to understand disease mechanisms, was not the latter result of greater significance?

The idea to include an experimental study was welcomed by my supervisors and beneficial to develop my understanding of the relative merits of the two methodological approaches. Trained in the experimental tradition as a psychologist, I was happy to finally do a "clean" experimental study with perfect control of both reverse causation and confounding factors. But it soon became evident to me that each of the two approaches has to make different kinds

of sacrifices to address key issues in dementia research and that one of the methodological approaches is not inherently superior to the other, in spite of a present strong preference among journals for only one of these approaches. In this thesis I have devoted a special chapter to compare the pros and cons of the different methodological approaches I have used, and in what way one can complement the other.

Epidemiological studies can at best identify factors, many of which can be modified through life style changes, that are beneficial for healthy cognitive ageing or, stated the other way around, are somehow involved in the causal chain leading to a dementia diagnosis. Such knowledge is undoubtedly important for health professionals to design preventive measures for people at risk and give good health advice, for public health information and as a guide for anyone to modify your own life style in a more healthy direction.

The other benefit of such knowledge is to serve as a clue to find the underlying biological mechanisms of the disease. In that respect, to establish an association with e.g. physical activity, a certain diet or feelings of loneliness at one point in time, and a dementia diagnosis decades later, could be regarded as rather trivial in itself. The interesting question is why. Already when I had the first result I asked myself how losing a partner could trigger biological processes that could lead to a dementia disease decades later. In my mind, one key to answer this question is the multitude of other factors that show a similar association. Another observation is that these factors are usually not uniquely associated with one dementia disease, but to many, and in addition to other diseases as well. In what direction do these clues lead when we think of biological mechanisms behind e.g. Alzheimer's disease? I do realize that on one level every disease is very specific, but does it mean that non-specific disease factors are less important to consider? If we only focus on single diseases and single risk factors for that specific disease, could it sometimes make us blind to see what lies in front of us? One of many examples is the focus on beta amyloids in Alzheimer research. While epidemiological associations need to be related to the chain of events that later follow, would our understanding not in a corresponding way benefit from tracing the events that *preceded* the occurrence of this disease-related peptide? If the accumulation of amyloid plaques (or changes in the tau protein) is related to Alzheimer's disease, and if a much earlier emotional trauma also is, then somehow these more long term and short term antecedents of the disease need to be bridged. How does the chain of events look that can link them all together?

This means that, even if my background is in Psychology, I see the need to relate e.g. experiences and emotions with biology, which in turn means that I think that people with expertise in these different areas need to work and think together to solve this puzzle, a belief that is certainly not unique. Already in the first study, the results gave me a strong indication that social and emotional factors alone can´t give the whole story; when I combined the experience of living alone, especially after having lost a partner, with a genetic factor, this addition of the genetic component changed the picture dramatically. At the same time, if I only looked at the genetic risk, while controlling for other factors, the genetic risk increase per se was quite modest. My conclusion was that the event I had been looking at probably

constituted a general long-term health risk, but for those with the ApoE4 allele, Alzheimer's disease was a relatively more probable ill health outcome.

Related to the difference between establishing associations and identifying mechanisms is the distinction between postponing and preventing the disease. As long as the time point and the very fist stages of the disease are unknown, this distinction could be regarded as highly theoretical and abstract, perhaps of little use, even. Still I think it is a distinction that is crucial to make, but that few people seem very concerned about. As a result, it is common with sweeping and unfounded statements as to when the disease starts where risk factors are often confused with the disease itself. One example is if a difference in intelligence can be established already in childhood between persons with a higher or lower risk to be diagnosed with the disease when they get old. Does this mean that the disease started already in childhood? Of course not! It probably only means that once the disease has started, persons with a lower intelligence, because of lower cognitive reserve, will reach the level of cognitive functioning when the diagnosis can be made at an earlier point in time. Calculated on a group level, it will still seem as if a high intelligence "protects" against the disease, as fewer persons of a certain age will have a dementia diagnosis if they had a higher intelligence to start with. For the same reason, accumulating evidence now tells us that education does not prevent from dementia. Instead a higher education will mean that, due to better cognitive functioning, it will take longer time before a person with an underlying dementia disease will have reached the clinical criteria, in terms of cognitive functioning. At the same time, it is well possible that cognitive training can strengthen brain functioning in early phases of the disease to postpone the disease from a clinical point of view. In other words, education and cognitive training can probably postpone the disease in clinical terms, but probably not prevent or even delay the neuropathological initiation of the same disease process. While both of these aims are important, i.e. interventions to delay the clinical debut and measures to prevent the disease process to start, clearly the latter should be the holy grail of dementia research. The reason is simple: As far as we know, once a dementia disease like Alzheimer's disease, has started, it is an irreversible process that eventually, if the persons lives long enough, will literally ruin the persons life and brain, and also heavily affect the lives of persons around diseased person.

This debate refers to the basic question of what dementia is. Is it a clinical diagnosis of a disease or is it the neuropathological process that eventually will manifest in the clinical symptoms that fulfil criteria for such a diagnosis? A clinician with a more pragmatic attitude will probably vote for the first, while a neuroscience researcher or a pathologist will more probably lean towards the latter definition. At least it would make me happy to think that the emotional and social events I have studied have more to do with mechanisms that can trigger the disease process to start, rather than modifying the trajectory of a disease that is already in progress.

Many other thoughts and ideas have developed as I have been working with this project, but I hope this simple introduction can convey some of the fascination and curiosity that has

characterized the intellectual adventure and the personal journey behind. I also hope it can stimulate the appetite in readers without much experience of research to eventually make a similar journey.

2 INTRODUCTION

This thesis deals with possible long term effects on cognitive health from living alone, the trauma of losing a partner, feelings of hopelessness and loneliness, and in addition the possible implication of brain-derived neurotrophic factor (BDNF) in the biological mechanisms behind these associations and for cognitive health in general. As the original articles are included at the end of the thesis, the main purpose of the text that follows is to expand, complement and to put the included studies into a wider theoretical and methodological context, rather than repeating what was already written in the articles. Three of these studies describe and discuss long-term associations by using an epidemiological study approach, while the fourth experimental study has a more direct focus on biological mechanisms. One objective of this thesis is therefore also to bridge between these two methodological approaches to illustrate their complementary value. The focus in three of the articles is on risk factors for cognitive impairment, while the fourth has a focus on healthy cognition. In summary, this means that this thesis can be seen as a bold attempt to integrate the relevance of socio-emotional factors with biological mechanisms and also to apply the two main methodological approaches, epidemiological association studies and experimental intervention studies, in research on cognitive ageing.

After a short overview of demographic trends, their driving forces and implications for dementia prevalence, I will in the following discuss the concepts of ageing and cognitive ageing, the impact of ageing on cognition and possible mechanisms behind trajectories of cognitive ageing, dementia with a focus on Alzheimer's disease and possible underlying mechanisms, the relevance of life-course exposures for cognitive health and the possible role of neurotrophins, including brain-derived neurotrophic factor (BDNF), for healthy brain functioning. The pros and cons of the main methodological approaches in dementia research are also introduced before the aims and the different studies in this thesis are introduced.

3 COGNITION AND COGNITIVE HEALTH IN OLD AGE

3.1 DEMOGRAPHIC TRENDS AND THEIR IMPLICATIONS

As a result of increasing life expectancies, and in spite of a progressive decline in fertility rate, the global population is growing, and especially its proportion of old persons. The continued development of life expectancies is of critical importance for policy makers and politicians for several reasons: for adaptation of pension and taxation systems, for adequate allocation of health care resources, and for strategic research investments. If we can expect a continued increase in human life expectancies, it will become increasingly important to prevent, cure and delay age-related diseases, including dementia, both from a societal and economic perspective and from a welfare perspective. Two factors seem especially important to estimate what we can expect in this regard: the driving forces behind the on-going increase in global life expectancies and whether the human life span has a biological limit or not. In this chapter I therefore will shortly review hypotheses and empirical evidence related to these two issues.

During the last half century the fertility rate dropped from 5.2 to 2.7, but with a compensating parallel decrease in child and maternal mortality; between 1970 and 2010 child mortality dropped by around 50% [2] and maternal mortality has dropped annually by 1.5% since 1980 [3]. A related global trend is that more people reach a mature age and in addition have a higher remaining life expectancy thereafter [4]. The proportion of persons above 65 in the world was 6.9% in 2000 and is expected to grow to 16.3% in 2050, a proportion that has already been surpassed by countries such as Japan (24.8%), Italy (20.8%), Sweden (20,5%) (Fig 1), and Germany (20.9%) [5]. An even faster relative growth is taking place in the 85+ segment of the world population [6]. To exemplify, data from Human Mortality Data base [7] show that the 85+ proportion of the Swedish population was constantly below 0.5% during 200 years from 1750 until 1950, but has since then grown steadily from around 0.55% in the fifties to 2.65% at present, i.e. an approximate five-fold increase during the last sixty years (see Fig 1).

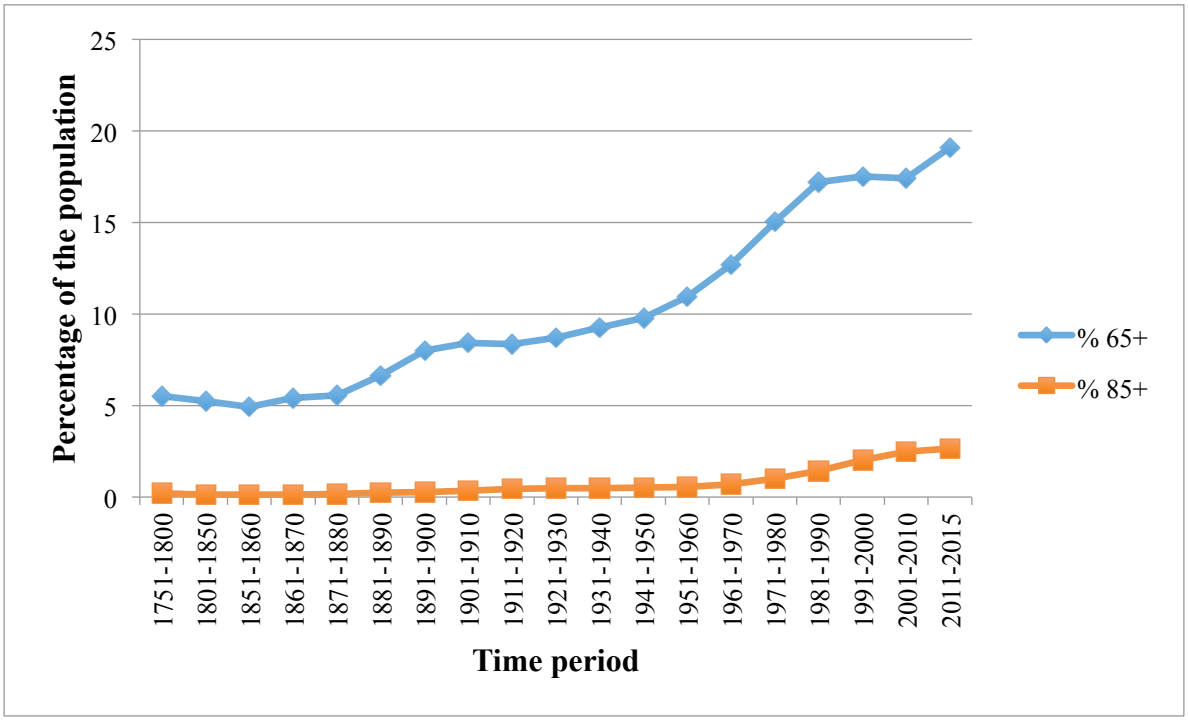

Figure 1. The proportions of persons above 65 and above 85 years in Sweden between 1751 and 2015.

Values retrieved from Human Mortality Database [7]

This combined trend of decreasing birth rates and increasing longevity can be seen in all parts of the world, but has progressed farther in more developed regions. As a result, global life expectancy at birth has increased by over twenty years during the last six decades, from 46.8 years in 1950 to 70.5 years today - with a prognosis to approach 75 in 2050 [4], a life expectancy already surpassed in countries like Japan, Germany, Sweden, and Italy [5]. In Sweden, a country with the one of the best historic demographic registers in the world, the steady increase in life expectancy over three centuries, from below 40 in 1751 to over 80 years today, is illustrated in Figure 2.

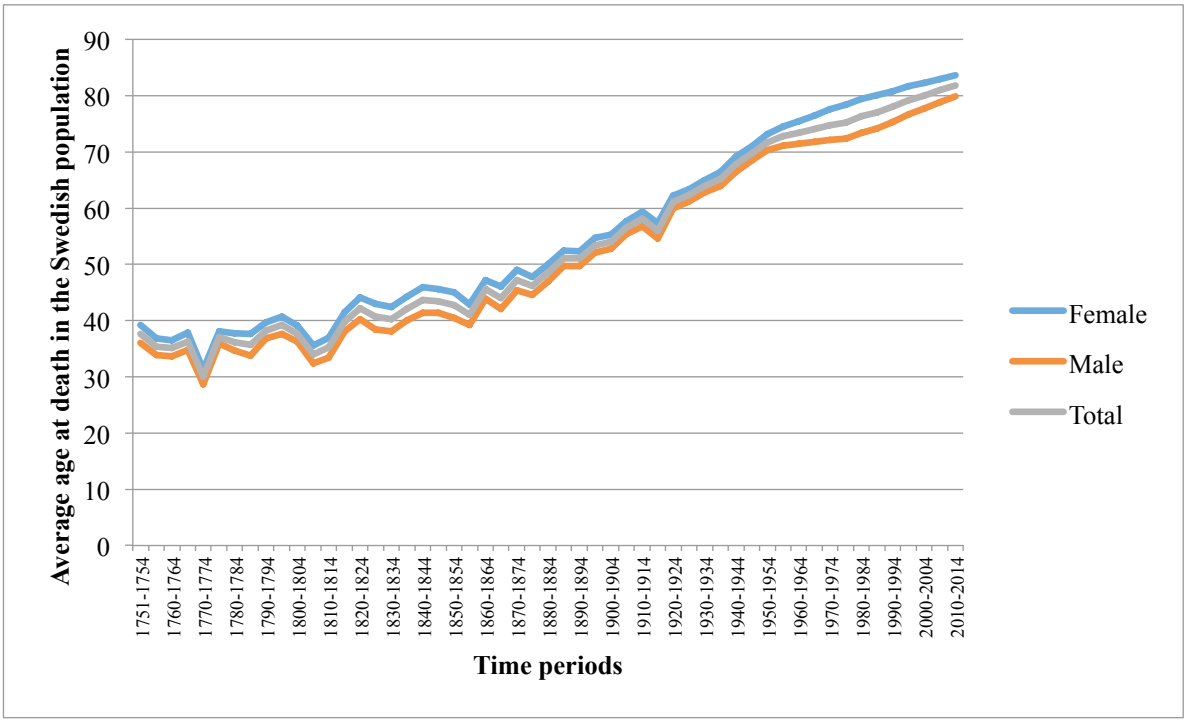

Figure 2. Development of Life expectancy in Sweden between 1751 and 2014

Values derived from Human Mortality Database [7]

3.1.1 Is there an absolute limit to the human life span?

It is easy to doubt that this trend can continue forever and that we at some point will have reached a limit of continued increases in life expectancy. Many attempts have been made to determine such a limit, starting with Dublin's in 1928 [8]. His calculations resulted in an estimation of 64.75 years [8], an estimation that was soon proven inadequately pessimistic. According to Oeppen and Vaupel [8] it has only taken five years on average from publication to break the fourteen suggested life expectancy limits that were presented between 1928 and 1990, including the 85 year limit presented in 1990 by Olshansky et al. [9]. If todays sceptics are equally wrong as those of the past, estimations have been made that a majority of the children born today in western Europe, the US, and Japan will experience their 100th birthday [6].

In contrast to life expectancies of populations, the maximum life spans of single individuals have increased to a much less extent. As exemplified by Swedish data, while life expectancy doubled during the last two centuries, there are repeated records from at least 1860 and onwards of many single individuals who lived more than 100 years [10]. Does this dramatic difference between the development of life expectancy at the population level and the very small increase in maximum life spans at the individual level indicate that there is an absolute limit to a human life span – and thus a limit to continued increases in human life expectancy? Some argue it does, and that this absolute limit is approximately 120 years [11,12].

A relevant distinction in this context is that between observed maximum life span and a theoretical "absolute" one [13]. When Fries wrote his classical paper in 1980, he stated, as

evidence for a fixed limit to the human life span that "…adequate data on the number of centenarians have been available in England since 1837; over this time, despite a great change in average life expectancy, there has been no detectable change in the number of people living longer than 100 years or in the maximum age of persons dying in a given year" (p 131) [14]. This argument does not apply today. The proportion of centenarians in the Swedish population is more than thirty-fold of the proportion that existed in the middle of the previous century. In line with Fries' argument, there were indeed repeated single instances of persons that reached the even higher age of 105 during the preceding centuries, but this number has increased dramatically during the last few decades (see Fig 3). If we choose the even more extreme age of 110 years, there were no records of such an old person in Sweden between 1751 and 1980, but since then several Swedish inhabitants have reached this age. Even if this proves that Fries was wrong about the premises of his argument, does it prove he was also wrong in his conclusion? Not necessarily. If we take the distinction between observed and absolute life span seriously, the problem with Fries' argument is instead its logic. An alternative interpretation is that the rapid growth of super-centenarians in Sweden (see Fig 3) could mean that observed maximum life spans is approaching the proposed absolute limit, possibly as a result of reducing "avoidable mortality" through more healthy life styles and improved health care [15].

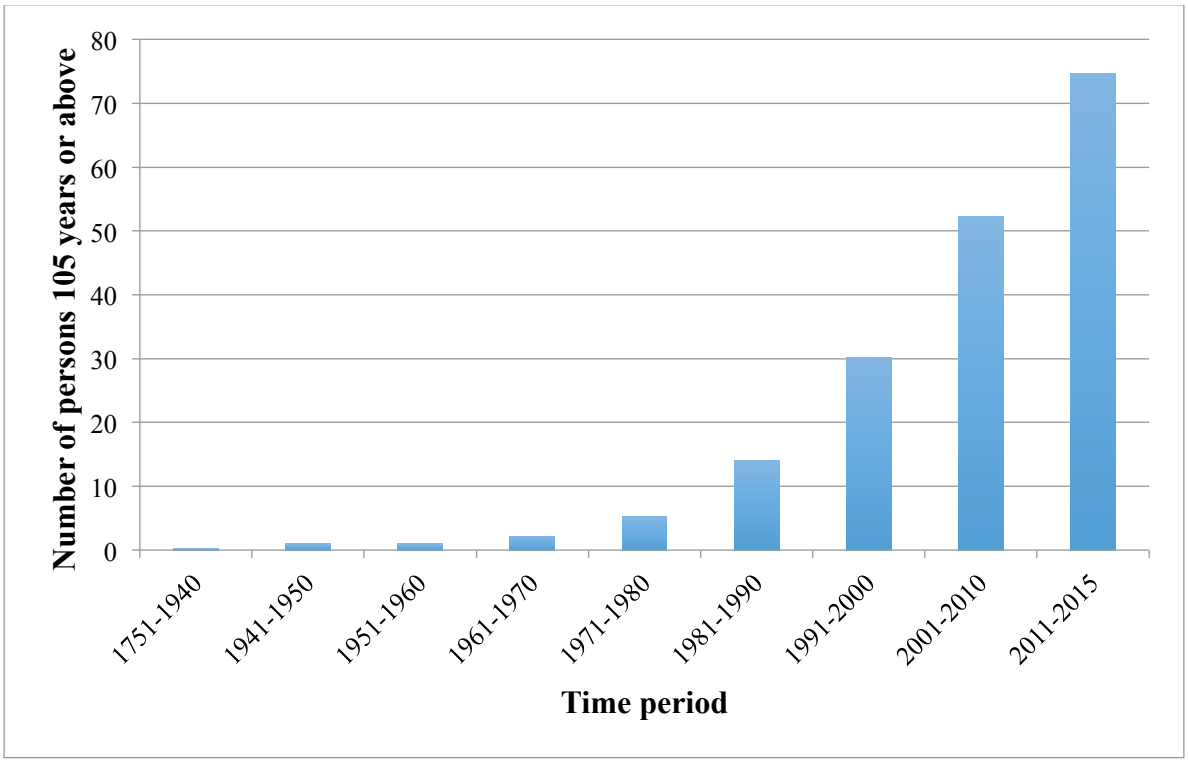

Figure 3. Number of persons 105 years or older in the Swedish population from 1751 to 2015.

Values derived from Human Mortality Database [7]

An expected consequence of the suggested 122 year limit to a human life span [11,12] should be a shift towards a more quadratic survival curve as more and more people enter into very

old age. If no compression of mortality can be demonstrated with prolonged life expectancies, this would seem to constitute a valid argument against the existence of an absolute life span limit for humans. However, in a recent and thorough analysis, Mathers et al [15] found no evidence for compression of mortality. There is even strong evidence to the contrary, a deceleration of mortality with very high age [16]. The meaning of this phenomenon is, perhaps surprisingly, that the risk of dying within the following year is higher for a single person of e.g. 85 years than for a person who has survived until e.g.105.

Related to compression of mortality is the "compression of morbidity" hypothesis {[14], based on a similar assumption of a biological human life span limit. According to this influential idea, improving life conditions will postpone the incidence of chronic diseases and hospitalisation until later in life. The evidence for compression of morbidity seemed convincing when first presented and may adequately have described the development during a large part of the previous century [14]. More recent evidence however suggests that people live longer today due to better survival in spite of chronic diseases [17] - or even in spite of *expanded*, rather than compressed, morbidity [18]. Thus, better health in old age does not seem as a valid explanation to account for increased life expectancies across the world. More importantly, for those who live until old age, modern medicine has become increasingly efficient in helping old persons survive many chronic conditions that previously were fatal.

In summary, life expectancies continue to increase across the world, and in Sweden, a country with especially good historic demographic records, this trend can be observed as accelerating up to now (Fig 2). It may seem natural to assume the existence of a biological and unsurpassable limit to a human life span, but some empirical data, especially the failure to empirically demonstrate compression of mortality, does not support such an assumption. As observed maximum life spans of single individuals have not increased over time at a rate near that of life expectancies in populations, the major driving force behind the on-going increase in life expectancy is most likely due to life style changes, such as the recent decrease in smoking in high-income countries [15], and progress in medical care, rather than a tendency for the oldest old to become even older before they die. On the other hand, if this scenario does not produce compression of mortality, which it should in the case of an absolute limit to human life span, it could either mean that we are not close to this limit yet – or that it does not exist.

For most of us, the main objective may however not be to live as long as possible, but to live well and to have our mental powers well preserved when we get old. What do we know about the prospects for this to happen?

3.2 AGEING AND COGNITION

The purpose of this chapter is to give a framework to the main research questions of this thesis and it is beyond the scope of this dissertation to cover all aspects and details of cognitive ageing, including distinctions between cognitive domains and their neural correlates. Instead the focus will be on general and methodological issues related to ageing and cognition.

3.2.1 Theories of Ageing and their Implications for Cognition

Many studies have described how trajectories of cognitive function decrease by age and given the impression that cognitive decline is a natural consequence of getting old. Although this to some extent is probably true, more recent evidence based on more adequate study designs illustrate that many of the previous claims have been overly categorical and pessimistic in this regard [19]. One of the key features in the association between aging and cognition is the amount of individual differences in both cognitive functioning at a certain age and the different trajectories of cognitive functioning with increasing age, including persons whose cognitive functioning seem more or less unaffected by advancing age [20].

3.2.1.1 Cognitive ageing as a function of a general ageing mechanism

One reason for cognition to actually suffer with ageing could potentially be related to any of the mechanisms of the ageing process itself. Over 300 theories of ageing have been proposed [21], but there is still no common agreement what constitutes a basic mechanism of ageing. In the following I will only touch upon a few of them, and try to relate them to one another.

A common view is based on the fact that as long as we live, cells divide and die, at different rates in different parts of the body. When a cell is about to copy itself, small errors may occur to make the copy less perfect than its mother cell, an imperfection that then will be reproduced in the coming generations of cells. One example of accumulated imperfections is the suggested progressive shortening of chromosome telomere length with each cell division [22]. Telomeres function as “caps” at the end of chromosomes to keep them intact and when telomeres become too short to perform this task, the functioning of the hosting cell will eventually become fatally disrupted. According to some evidence, telomere length is however not the only thing that matters for telomeres to perform their task; even very short telomeres can be efficient when they bind to a certain protein, the TRF2 [23], a protein that paradoxically has been reported to both contributes to telomere shortening and increasing telomere efficiency - but that also requires cell divisions to become expressed [23]. Although several, but not all[24], studies report a correlation between telomere length and biological age, several longitudinal studies have failed to establish telomere length as a predictor of morbidity and mortality [25] [26]. Although some studies indicate that telomere length has relevance for cognitive ageing[27], others failed to find support for an association [28]. For age-related neuropathologies, such as Alzheimer’s disease, the evidence for telomere shortening as mechanistically involved is also mixed [29].

As cell divisions occur at a certain rate through our lives, and if cell divisions imply accumulation of age-relevant disturbances in cell function, such a mechanism would mean that life is ticking towards a certain end when vital body functions can no longer be upheld. Such a "clock mechanism" seems highly consistent with the idea of a maximum human life span, as does the epigenetic clock theory of DNA methylations, suggested as a parallel clock to that of telomere shortening [30]. This theory has two aspects of relevance for ageing theory: that the rate of methylations is reduced with higher age and in addition that the basic individual rate of methylation predicts longevity and age-related diseases [31], including Alzheimer's disease [32]. Evidence from this research field indicates that centenarians typically have a lower methylation rate than expected from their chronological age, indicating a lower basic rate of methylation. One important difference between the telomere length and the methylation approaches is this emphasis in theories of methylation on individual differences. In common language, this difference means that clocks are ticking at different rates in different individuals, thereby introducing also a predicted difference in maximum individual life spans. The similarity in relation to ageing theory is on the other hand that they both assume that ageing is a pre-programmed process that imposes a definite life span limit.

Other theories state that ageing is the result of accumulated stochastic events, rather than a pre-programmed mechanism, such as DNA mutations from radiation or toxic exposures, events that could also lead to deficiencies in cell reproduction or cause other deviations from optimal functioning at the cell level. Related to this is the theory that repair mechanisms to resist accumulation of unfortunate events may also differ between individuals [33]. If these theories are correct, the length a human life span will to a greater extent be a consequence of luck in combination with accumulated exposures and efficiency of repair mechanisms, and thus partly a consequence of individual choices, rather than deterministically fixed by some kind of biological ageing program. This view would then be consistent with the view that a human life span does not have an absolute limit, common to all humans, and that longevity is better explained by a probabilistic function.

That different theories exist does not necessarily mean that they exclude each other; several of the theories I have shortly mentioned could have relevance and contribute in a complementary way. However, one problem for most of these ageing theories to also account for cognitive ageing is their reliance on cell divisions, a rare event in the organ that constitutes the biological basis for cognitive processes. According to present knowledge, only one part of the brain, the hippocampus, manifest cell division and neurogenesis. One reason for the brain being an exception in this regard could be evolutionary pressure to maintain brain cells and brain circuits over the life span due to their critical role for memory and identity, and thereby for survival[34]. This should mean that all ageing theories related to accumulated imperfections through cell divisions, whatever their causes and effects, are less relevant to explain brain ageing and cognitive ageing, in contrast to ageing processes in the rest of the body.

3.2.1.2 Cognitive ageing as a function brain ageing

Although cell division is such a rare phenomenon in the brain, there are other possibilities that ageing per se could account for cognitive decline. To exemplify, one theory suggests that brain cells could become less efficient with age through pre-programmed down regulation, leading to cognitive decline and eventually also neurodegeneration [35]. The reason for this, according to this theory, is related to the evolutionary advantage of saving energy. The brain is by far the body organ with the highest energy consumption in relation to its mass and economization is a pervasive principle in the brain at all levels, including re-uptake (recirculation) of transmittor substances at synapses and top-down processing in perception and neurological functioning [36]. According to this theory, with higher age the individual has acquired knowledge and experiences to a degree where further learning is becoming less and less critical for survival [35]. To make brain functioning less demanding, energy-consuming processes related to learning, such as synaptogenesis and formation of new neural networks, are therefore down regulated. Of possible relevance for evaluation for this hypothesis is the progressive reduction in metabolism with age, [37] which could mean that sustaining life with minimal energy expenditure may have a general evolutionary advantage. Evidence that DNA methylation decreases with age, signalling reduced epigenetic modifications in response to experience [36], also seems in line with this evolution-based theory. As always, evolutionary theories are difficult to test and it remains to be seen if a neuron "down regulation-by-age" program can be identified to account for cognitive decline in older age.

Another potential ageing mechanism that directly could affect the brain, also suggested by Harman [11], to account for an absolute limit in the human life span of 122 years, is the accumulation of free radicals with metabolism [11]. Especially the mitochondria, the power house of both nerve cells and other cells, should be especially susceptible to DNA and cell membrane damage caused by oxidative stress [38]. That metabolism itself could drive the ageing process also seems consistent with the anti-ageing effects of calorie restriction [39], and of anti-oxidants, both through an efficient endogenous antioxidant defence system [40], and probably also through intake of natural antioxidants in diet [41], but not through anti-oxidant supplements [42].

3.2.1.3 Cognitive ageing as a function of age-related deficiencies in brain-supporting systems

Another way to understand why ageing could affect cognition and the brain, in spite of neural cell divisions being non-typical, and as an alternative to theories specific to brain ageing, is to realise that the brain does not exist in isolation; brain processes are highly dependent on other organs and their functioning, organs that do not belong to the same exception as the brain in terms of cell division and accumulating cell copy imperfections with age. The cardiovascular system is perhaps the best example, of primary importance to provide oxygen, nutrition and vitamins to brain cells. With time there is also a tendency for blood vessels to become less efficient in their ability to nourish brain cells for other reasons than mechanisms related to

ageing per se, such as vascular damages from high blood pressure, diabetes, inappropriate diet, smoking and a host of other adverse life style factors. As the brain is so critically dependent on a continuous blood supply, both age-related and life-style related cardiovascular deficiencies have direct consequences for how well the brain can function and for survival of neurons [43]. An obvious case is stroke that could kill billions of neurons, but more and more attention has been given to "mini-infarcts", events in the brain that we normally do not even notice. A related event is a temporary reduction in blood supply, a transient ischemic attacks (TIA), usually caused by a clot that temporarily blocks the blood supply in a fine vessel, an event that is also associated with increased risk of stroke [44]. As a neuron in normal temperature can only survive around eight minutes without oxygen, such events present an obvious risk of neural cell death or at least impaired functioning due to lack of oxygen. When such events accumulate, the functioning of the brain as a whole will naturally suffer and could in the end affect cognitive functions to a degree that justifies a dementia diagnosis. As we shall see, a deficient cardiovascular system may not only affect the risk of vascular dementia, but, through mechanisms largely unknown, also the risk of dementia from Alzheimer's disease[43].

3.2.1.4 Individual differences in cognitive ageing as a function of accumulated exposures

As exposure effects accumulate, including mutations, errors in the cell copying machinery, and experiences with epigenetic consequences, the room for variation between individuals increases. We are all different in many regards; in personality, intelligence, body composition, physical constitution and much more. A universal feature seems to be that such individual differences get accentuated with age. Even homozygotic twins become progressively more different with advanced age, including in their DNA methylation marks, an observation commonly explained as "epigenetic drift", i.e. an effect of accumulating inter-individual differences in epigenetic influences on the genome [45].

3.2.2 Normal, Healthy and "Successful" Ageing – What is the Difference?

One question in relation to this variation is what constitutes "normal ageing" or "healthy ageing" - and the utility of these concepts. Obviously different old persons will manifest a continuum of better or worse functioning, including at the cognitive level, for already mentioned reasons. The discussion concerning normal or healthy ageing and cognition resembles a parallel discussion concerning the health concept itself. Is health synonymous with absence of diseases, as diagnosed through certain agreed-upon criteria, or is it something more? According to WHO, health should be defined as "a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity" [46]. A typical argument against applying this definition to ageing would be that it transforms healthy ageing to an extremely rare, almost non-existent entity. Already after 60 years of age, a majority of persons have at least one chronic disease, and the corresponding prevalence among octogenarians is over 80% [47].

Inherent in this statistical objection against the concept of healthy ageing as an absolute and ideal entity, is the preference for a more relative and "realistic" definition, i.e. that normal and healthy ageing is the level of functioning that is common or typical in a certain age group, sometimes called "normative ageing"[48]. The problem with such a definition is that if most persons at an advanced age have diseases, as we have seen that they indeed have [47], they would still be defined as belonging to the category of "healthy" or normal ageing. As disease by its definition is opposite to health, a valid argument could be that this represents a misuse of language. The relative definition has also been criticised for precisely its relative nature: whether someone is ageing in a normal/healthy fashion or not will depend on the level of health of everybody else in the same age category - and how the health of others may change.

One way to approach the concepts of normality and health in relation to ageing could be to distinguish between them, and at least avoid to define healthy ageing as inclusive of persons with diseases. But, to complicate matters even more, even the distinction between disease and non-disease is debatable and relative, as illustrated by the recent concept of sub-optimal health [49]. Persons with suboptimal health do not fulfil criteria for illness diagnoses, but still have impaired function due to suboptimal functioning on various levels. Exclusion of persons also with health impairments that do not qualify for illness diagnoses, would bring us back to a definition of healthy ageing analogous to the WHO health definition, i.e. ageing along with "complete physical, mental and social well-being". With this proposed distinction, normal ageing would, in contrast to the ideal of healthy ageing, or "successful ageing", be ageing that is typical for the birth cohort we belong to, apt to change with later cohorts. An obvious objective for research and the medical profession would then be to contribute to the transformation of normal ageing in the direction towards healthy ageing.

Although the concepts of healthy, successful, normal and normative ageing are extensively used and discussed, a possible alternative conclusion from the above could be that both these concepts are arbitrary and unnecessary and may be more about the human need to classify and simplify phenomena that naturally vary, than about their usefulness.

The complications to define healthy ageing are paralleled in the attempts to define healthy *cognitive* ageing[48]. If we apply the suggested definitions of healthy and normal ageing to cognitive functioning, healthy cognitive ageing would be the level of cognition that is optimal for a certain age cohort, whereas normal cognitive ageing would be a level of cognition that is most commonly found in the same age group. Besides the statistical argument, a problem with the concept healthy cognitive ageing is establishing the biological limits for optimal cognition for any single person. This can be exemplified by the concept of "cognitive decline", commonly used in ageing research. In both clinical and research settings, cognitive decline is often determined by measuring *the level* of cognitive performance. The most commonly used screening tool to detect cognitive decline is the Minimental Scale Examination (MMSE) [50] and a common threshold value is a performance score of 24 or below on that test. But, as critics have pointed out, the term decline denotes a change of performance, not a level of performance. Optimal functioning for a well-educated and

intelligent person would most probably correspond to a score of 30 on this test, while optimal functioning for another person could be considerably lower. If the first of these persons would perform at a level of 26, this could constitute a serious level of cognitive decline, while it might not for the second person. The fact that MMSE can be considered as a crude measure of cognitive performance does not change this argument. Even with the most sophisticated neuropsychological battery, we need at least two measure points in order to say anything about either cognitive decline or optimal cognitive performance for any person.

The fact that neurological conditions for cognitive performance naturally vary between individuals as a consequence of genetic factors, education and other types of environmental exposures through the life span, also means that there could be differences on group levels between different birth cohorts. Typical advantages of belonging to a later birth cohort include higher education, a more favourable socio-economic situation, and better nutrition over the life span, all of which contribute to improve cognitive performance. One manifestation of this is the "Flynn effect", meaning that persons at a certain age today typically have a higher intelligence than people of the same age had in the past [51]. The birth cohort effect constitutes one of the complications when ageing effects on cognition are determined by comparing the cognitive performance of persons of different ages. It is easy to forget that in a cross-sectional designs we are at the same time comparing groups of people who were born and who grew up under different social and material conditions.

3.2.3 What is the Isolated Effect of Ageing on Cognition?

The dramatic differences between estimations that consider, or do not consider, birth cohort effects are illustrated in figures 4 and 5, from Schaie (2005) [52]. The first figure gives the impression of a radical decrease in cognitive performance for four out of six different cognitive dimensions with increasing age. But when the estimation is based on intra-individual changes, performance does not change noticeable with age until after 60 years for any of the dimensions (Fig 5) [52].

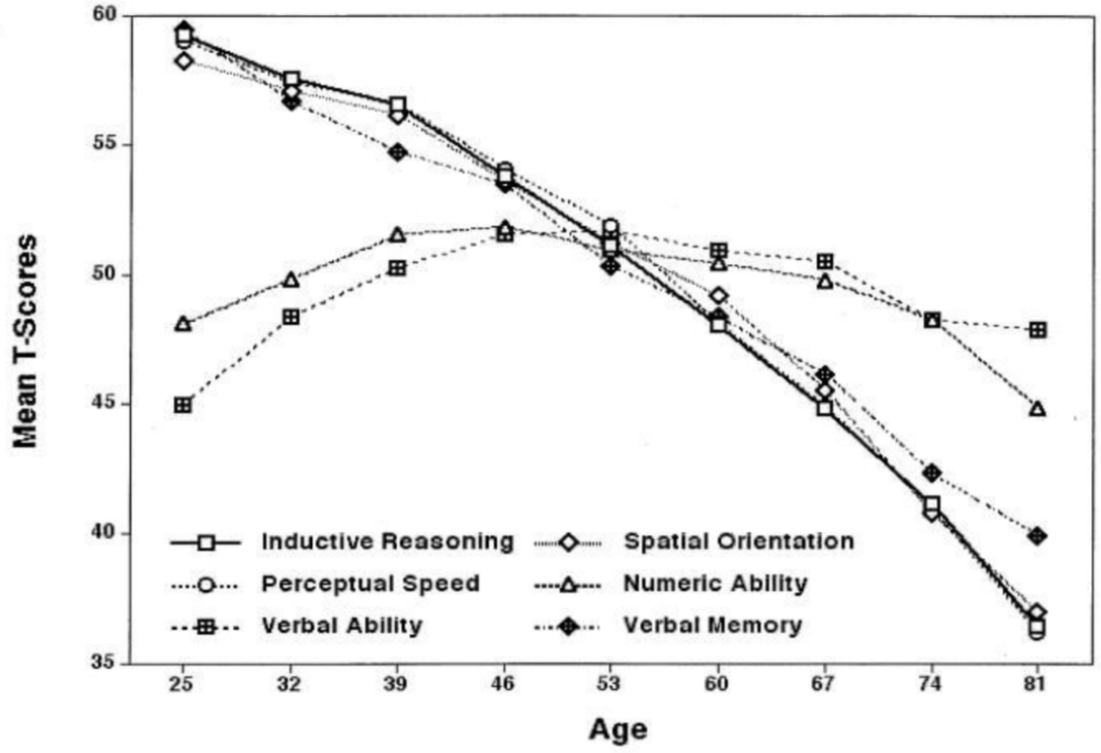

Fig 4. Estimated association between age and cognitive performance for six cognitive dimensions based on cross-sectional data. (From Schaie, 2005 [52])

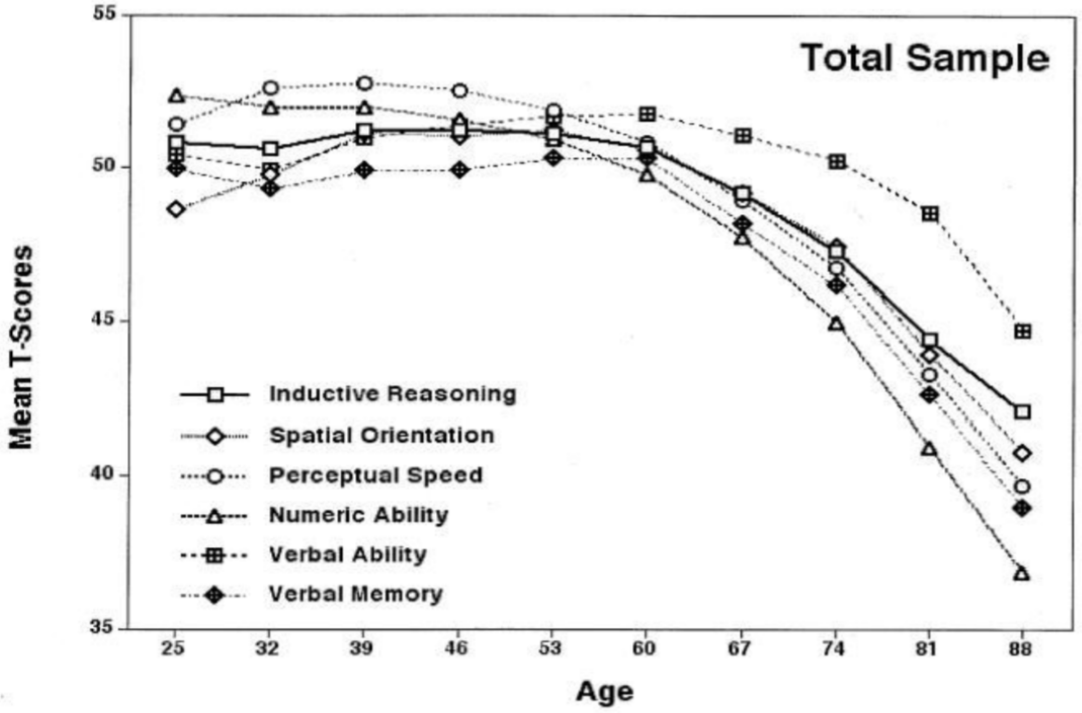

Fig 5. Estimated association between age and cognitive performance for six cognitive dimensions based on intra-individual changes with age. (From Schaie, 2005 [52])

To complicate things further, birth cohort effects have not only been demonstrated on *levels* of performance at different ages, but also on *cognitive trajectories* over age[53]. In other words, even if a certain birth cohort is followed longitudinally, the resulting trajectory of cognitive performance over age may differ compared to a cohort that was born earlier or later, and then followed and measured over the same age span. Such a birth cohort effect on cognitive trajectories is illustrated in Figure 6 for a younger and older cohort, but with overlapping age periods, from a study by Finkel et al[53]. Cognitive performance during the overlapping periods was always higher for the younger cohort, consistent with the Flynn effect (see above) [51]. But by only considering the younger cohort (the dashed curves), cognition appears stable or to slightly deteriorate with age, also during age spans when the older birth cohort increased in cognitive performance, creating a more curvilinear relationship (the solid line). The only exception for this difference in slopes was for processing speed, the only dimension where ageing seems to have a similar effect on the trajectories across these two birth cohorts.

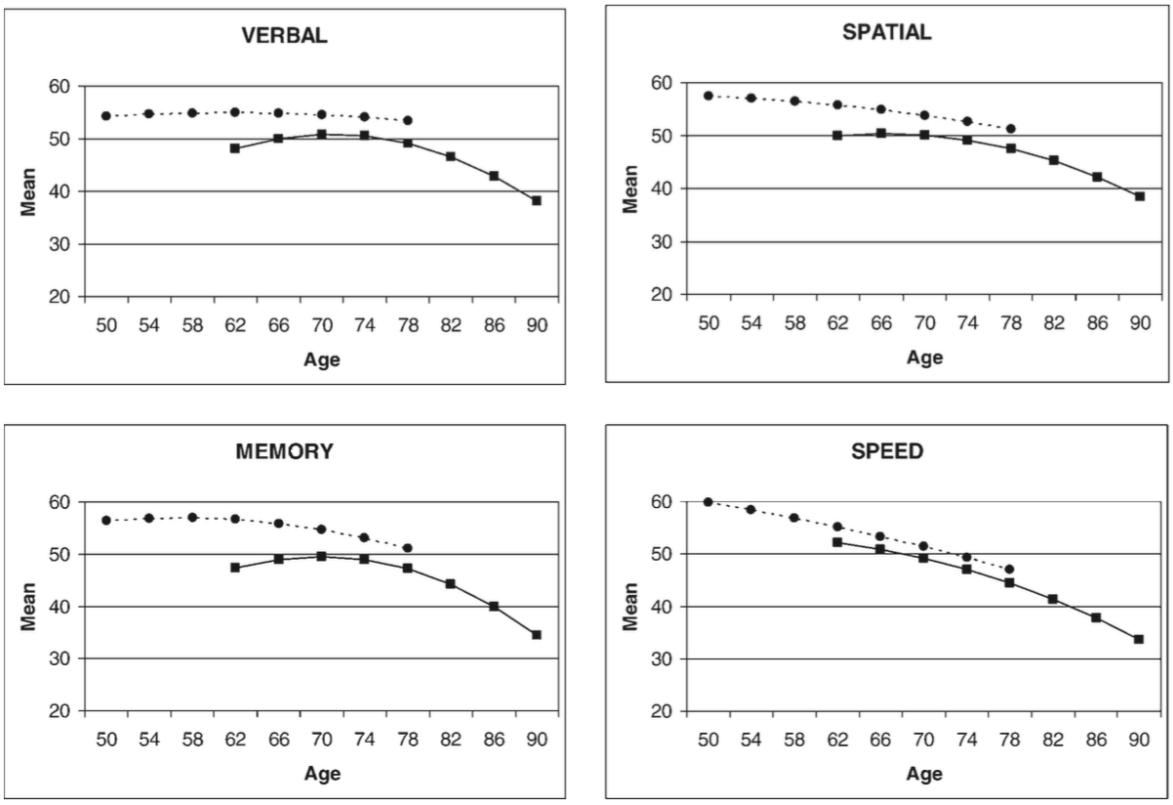

Fig 6. Illustration of birth cohort effects on trajectories of cognitive performance over age for two birth cohorts. From Finkel et al, 2007 [53].

A third source of contamination with age, even in longitudinal studies, is the effect of terminal decline. This concept implies that at a very late stage of life all functions in the body typically decline. If terminal decline will also affect cognitive performance, this effect will then be contaminated with the affect of age per se. If so, to what extent is the trajectory slope seen in old age, even in longitudinal studies, a reflection of terminal decline in the cognitive domain? Terminal decline effects in cognition do indeed seem to exist, and has been shown

to start at least three years before death[54]. According to one study this decrease in cognitive performance begins on average 7.7 years before death[55], and one study identified at terminal decline onset specifically for processing speed almost 15 years prior to death in dementia-free persons[56]. Others have shown that it is important to consider early and subclinical dementia, a common condition for old persons, when estimating terminal decline effects on cognition, and the effect of terminal decline is indeed smaller when persons with this condition are excluded[57]. An alternative study design to separate these effects from the effects of ageing itself is therefor to calculate age from death, rather than using the common measure of chronological age from birth. With such an approach it should be possible to adjust the cognitive trajectories in old age for the influence of terminal decline, that is not part of ageing per se, and in addition adjust for the previously discussed birth cohort effects, to better isolate the real effects of ageing on cognition.

While the scientific community of ageing research has clearly moved away from a purely cross-sectional approach to estimate ageing effects on cognition, it is till rare to find studies that also consider the fact that progressively more study participants are affected by terminal decline with increasing age, irrespectively of when they were born. I found no study that tried to map cognitive trajectories with increasing age that simultaneously adjusted for birth cohort effects, terminal decline effects, and effects of clinical and pre-clinical dementia. We saw previously that the effect of adjusting for birth cohort effects dramatically changed the relation between age and cognition up to at least 60 years. Additional research is needed to describe what happens to these trajectories after the age of 60 when adjustment for terminal decline and subclinical dementia are also added to the models.

Related to the unorthodox approach of calculating age from death, rather than from birth, is the recent distinction between chronological and biological age. In line with the above, two persons at a certain chronological age may show very different levels of functioning in terms of ageing. In common language, a person may “seem much younger (or older) than he or she is”, possibly due to differences in genetic factors that influence methylation rates and due to life-style differences that influence the rate of biological ageing. According to advocates of this distinction, biological age may be a more adequate description of a person’s real age, than what can be inferred from the year the same person was born.

3.2.4 Conclusions

In summary, there are many different theories of ageing and none of them enjoys unanimous support from the scientific community. The effect of ageing on cognition could therefore be a function of anything from a pre-programmed down-regulation of energy expenditure and/or brain plasticity with an evolutionary origin to an accumulation of unfortunate events in combination with less efficient repair mechanisms that causes deterioration of all body systems in old age, including the brain, and/or in systems that are vital to the brain. In order to measure ageing effects on cognition, it is important to separate ageing effects from the effects of other parameters that are associated with ageing, but is not ageing, such as differences between birth cohorts, effects of age-related diseases, and terminal decline. When

efforts have been made to control for any of these factors, the effect of ageing on cognition has been considerably reduced, and sometimes practically vanished. To evaluate the hypothesis that ageing in itself has an effect on cognition, also after control of age-related factors that is not ageing per se, the progressively larger variation in cognitive performance in older cohorts has to be accounted for, including the fact that some very old persons have a relatively preserved cognitive ability. One of the greatest challenges in estimating ageing effects on cognition is to control for under-lying neuropathological conditions that even its carrier may be unaware of. These conditions will be discussed in the next chapter.

3.3 COGNITIVE HEALTH IN OLDER AGE

The fact that we live longer naturally will have consequences for the prevalence of the most age-dependent diseases. For many of these diseases, like cancer and cardio-vascular diseases, we have witnessed impressive advances in medical technology and treatment that have made it possible to control these diseases, and, especially in the case of cancer, even cure many of them. For others, like Alzheimer's disease and several other dementias, no treatment has still been discovered to prevent, cure or efficiently halt their progression once a person has been afflicted[58]. As a consequence, with a growing proportion of old persons, especially the prevalence of dementia diseases has increased dramatically and continues to do so. Today around 50 million persons in the world are estimated to suffer from a dementia disease, with Alzheimer's disease as the most common type. By 2050 this number is projected to have surpassed 130 million, with dramatic consequences for the many afflicted individuals and their families, but also for societies in terms of drastically increasing care needs and costs[58]. While this increase in prevalence is driven by the fact that more and more people reach a higher age, there is however no indication that incidence, i.e. the risk for a certain individual at a certain age to have dementia, is increasing. On the contrary, some new evidence suggests that dementia incidence for different age cohorts of old people may actually be decreasing, at least in some parts of the world[58-60]. Although encouraging, this possible trend is however not even close to make up for the increased prevalence of dementia driven by the growing number of persons who live longer than before as increasing age is the primary driving force for dementia; for each five years after 70, the proportion of persons with dementia approximately doubles [61](Figure 7). The fact that age is so strongly associated with dementia incidence does however not mean that dementia is a feature of natural ageing, just as other age-related diseases like cancer or type 2 diabetes are not. Instead they are diseases that people in older age are more likely to get.

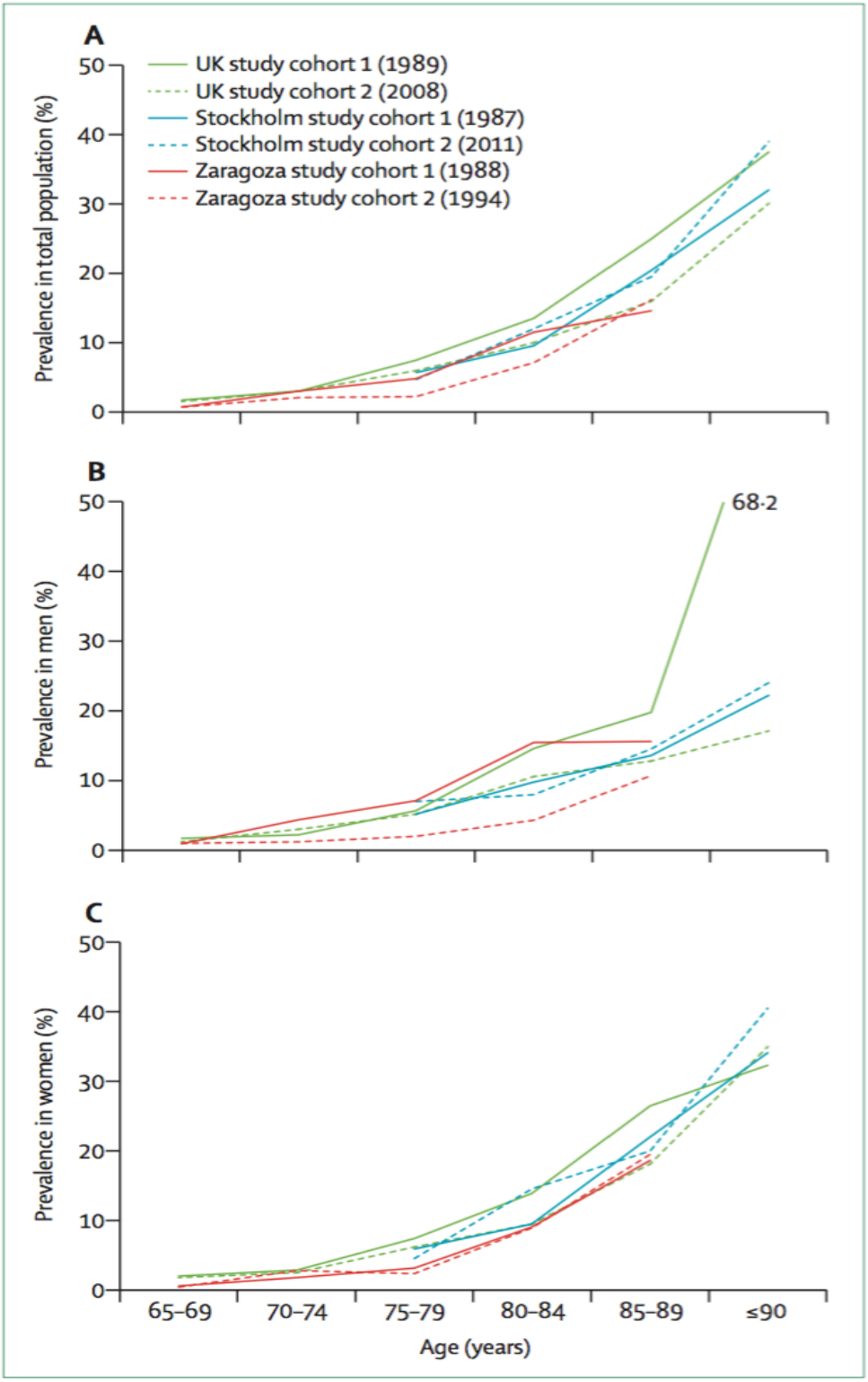

Fig 7. Increase of dementia prevalence with age in three different populations at different time points, with stratification for gender. (From Wu et al, 2015 [61])

3.3.1 Dementia

Dementia is an umbrella term for several neuropathological conditions with the common denominator of significantly impairing cognitive function. Depending on the type of

neuropathology, the types of cognitive domains mostly affected vary, as do the course of progression for each type of dementia. Without going into the details of different dementias and their different neurological consequences, the most common form of dementia, Alzheimer's disease (AD), usually affects memory functions initially, while frontotemporal dementia (FTD) usually has its early main effect on executive functions and regulation of emotions. Vascular dementia (VAD) is usually associated with more heterogenous symptoms that can vary due to the location and severity of the brain lesion. This exemplifies the differences in brain structures that are typically affected in the early stages of each of these common forms of dementias; typically the hippocampus area in AD, with consequences for working memory, the frontal regions in FTD with consequences for behaviour regulation, and failures of the vascular system to support various brain areas in VAD, with the consequence of affecting various brain structures with insufficient supply of oxygen and nourishment. But even more common than to have a single, specific type of dementia, is the case of mixed dementia. AD is the most common dementia disease and the most common scenario is to have AD mixed with some other dementia, often VAD especially among older persons [62] [63]. As AD is the most common type of dementia, either alone or in mixed dementia, and as two of the works in this dissertation has AD as a specific outcome, I will now give more specific attention to this particular type of dementia.

3.3.2 Alzheimer's Disease (AD)

The first record of AD was made by the German physician Alois Alzheimer in 1906 and the disease was then named after him. His patient, Auguste Deter, was diagnosed with this condition at only 51 years of age and died six years later. Through brain autopsy, Alzheimer and two Italian colleagues identified two types of abnormalities in her brain, amyloid plaques and neurofibrillary tangles (NFT), features that still today are used to describe the core characteristics of Alzheimer's disease. Today, an important distinction is usually made between the familial and late-onset types of AD. The rare familial type, with a prevalence of only around 1-2%, is a type of early-onset AD, often afflicting a person already between 40 and 50 years of age, or even earlier. As the inheritance is autosomal dominant, children to a parent with this mutation will have a 50% chance of inheriting the allele, that can either be a mutation in the amyloid precursor protein (APP), presenilin-1 (PS1), or presenilin-2 (PS2) genes. As far as is known, a person with the mutation will inevitably also get the disease if the person lives long enough. Besides the differences in terms of the genetic determination and the typical age of onset (averagely around 40 instead of around 75), the familial form of AD resembles late-onset sporadic AD in many ways. Some persons may also get the familial type of AD later in life, when they are over 65, which should make it virtually impossible to distinguish them from sporadic cases as the phenotypes are largely overlapping in older age [64]. As AD is very rare before 60 or even 65, with a prevalence of around only 35 persons per 100 000 in the age range 45-65 years [65], some researchers have suggested to simply use an age cut-off to distinguish the two types, usually at 65 or 60 years of age, and to use the terminology early versus late onset AD, without reference to the distinction in heritability. One argument that the two types of AD represent a continuum of the same disease is, besides

similarities in phenotypes [64], that the prevalence of AD in different populations is typically doubled for every progressive five-year age cohort already from 35 years of age [65], i.e. not only from 60 or 65 as is typically reported for dementia prevalence (e.g. see Fig 7). Some have also argued that the distinction between heritable and sporadic Alzheimer's disease is not valid, but that heritability is rather representing a continuum so that Alzheimer's disease at younger age to a larger extent is genetically determined and in later age relatively more by non-genetic factors. It is beyond this thesis to evaluate these claims, but it is important to note that most research on animal models with the aim of developing drugs against the common late-onset AD seem to be based on the assumption that familial AD is basically the same disease as late onset AD; the common method in this type of research is to study pharmaceutical effects on animals with genetic mutations of the same kind as found in familial AD. Evidence against such an assumption is that although the two phenotypes are similar they are not identical. Symptoms differ in some respects (e.g. in terms of motor functions), and biologically, familial AD is typically associated with higher amyloid burden and a more aggressive progression of the disease [64]. The difficulty in identifying genes of relevance for late-onset AD, except for the relatively modest impact of the ApoE4 allele (in comparison to an APP, PS-1 or PS-2 mutation) could also indicate a difference in disease mechanisms. Another indication in the same direction may be that drugs that have successfully relieved animals from their amyloid burdens, have not yet relieved humans with the common sporadic type of AD from their symptoms – or been able to halt the progression of the disease in them[58].

3.3.3 The role of ApoE4 in AD

Apolipoprotein E (ApoE) is a lipoprotein that can be produced in the liver and in astrocytes and has several biological roles, including cholesterol transport to the liver and, in the nervous system, also transport of fatty acids and phospholipids. It is coded by the same gene on chromosome 19, and exists in three isoforms called ApoE epsilon 2, 3 and 4. When the alleles combine it can thus theoretically result in six different combinations. The prevalence of the three isoforms vary somewhat across populations, but ApoE3 is the most common with a global prevalence of 78.3% and the prevalence of the rare ApoE2 isoform 6.4%[1], and in some populations completely absent[66]. The third isoform, ApoE4, is associated with a higher risk of Alzheimer's disease and also elevated risk of cardiovascular disease and a poorer prognosis after traumatic brain injury [67]. There is also evidence for ApoE4/4 implying a higher risk of Alzheimer's disease than the more common ApoE3/4 combination [66]. Conversely, ApoE2/3 implies a decreased risk of AD. As mentioned, the prevalence of the three isoforms varies somewhat across populations, and this is especially true for ApoE4 [68]. In the Nordic countries the prevalence of ApoE4 is typically around 25-30% [68], while the prevalence in the Caucasian population at large has been estimated to around 15% [69]. One meta-analysis estimated the average odds ratio for Alzheimer's disease to 2.7 (CI 2.2. – 3.2) for ApoE3/4 carriers versus non ApoE4 carriers (ApoE3/3 carriers) in the Caucasian population[69]. This means that many persons without this allele also get the disease. Several studies suggest that even if ApoE4 may be a risk factor in itself, the risk magnifies when

ApoE4 is combined with non-genetic risk factors such as physical inactivity, alcohol and smoking [70], living alone[71], and feelings of hopelessness[72]. The mechanisms behind the risk increase for Alzheimer's disease as a function of ApoE4 status is unknown, but several studies have reported that ApoE4 is also associated with AD biomarkers such as a higher amyloid burden and neurofibrillary tangles[66]. The possibility of an indirect link is indicated by the fact that ApoE4 is also associated with other risk factors for dementia such as depression [73] and cardiovascular disease [74]. While ApoE4 probably does not affect cognitive performance negatively in younger age, it has been reported to alter brain function in other ways, e.g. increased coactivation of brain areas in the default mode network (DMN), as measured through fMRI[67].

3.3.4 The amyloid β cascade hypothesis in AD

The probably most influential hypothesis concerning the biological mechanisms behind AD is the amyloid β cascade hypothesis, initially suggested by John Hardy and Gerald Higgins in a Science article in 1992 [75]. The core of this hypothesis is that accumulation of the amyloid β protein, leading to amyloid plaques in the brain, is the cause of Alzheimer's disease, and that other biological events associated with Alzheimer's disease that follow are a direct result of this amyloid accumulation. The theory has since been criticised and modified, also by John Hardy himself [76] [77], but is still the cornerstone in most biologically oriented research on AD. Fig 8 represents a common present view on the sequence of events in AD, following the proposed initial accumulation of amyloid β.[78].

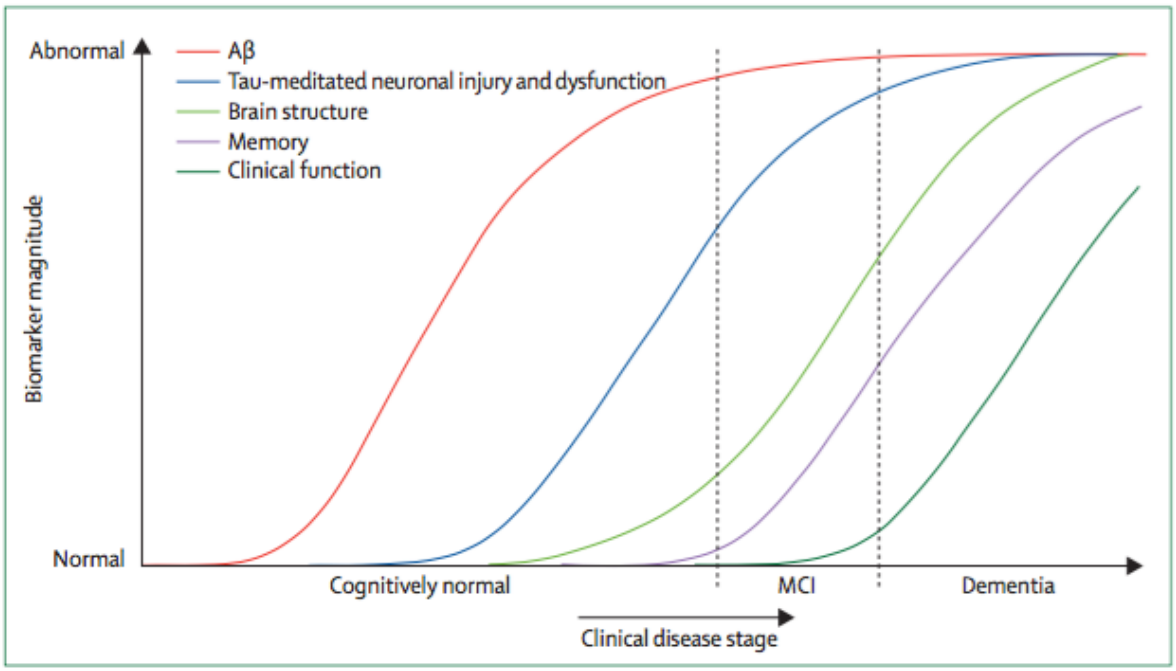

Fig 8. Sequence of events with measurable biomarkers in the development of AD.

MCI stands for Mild Cognitive Impairment. From Jack et, 2010 [78]

This proposed sequence of events is characterized by hyperphosphorylation in the tau protein in the second phase. This protein has a central role in microtubuli for intracellular transport and for the architectural integrity of the neuron. As the disease develops, progressively larger areas of the brain are affected through both synaptic loss and cell death, leading to measurable atrophy in the brain, resulting in enlarged ventricles that can typically be observed in brain images also in relatively early stages of AD development. As these changes develop, they will eventually lead to impaired memory and deterioration of other cognitive functions, and eventually clinical symptoms to enable diagnosis [78]. AD is a progressive and lethal disease with an average survival time after diagnosis of 3-4 years [79].

The emphasis on amyloid β as the primary causal agent in AD has been questioned, as some observations seem at least party inconsistent with such a role. For example, there is no association between amyloid burden and cognitive decline in AD [80], amyloid plaques are a universal phenomenon in persons with AD, but is also common in old persons without any symptoms of AD or other cognitive decline[81], amyloid β has recently been reported to have antimicrobial properties and be part of the innate immune system [82], and, as mentioned previously, pharmaceutical interventions targeting amyloid β, while relatively successful in mouse models, have failed to improve symptoms in humans and in some trials even worsened the symptoms [83] [81] [84]. One possibility for the pervasiveness of the amyloid cascade hypothesis is that amyloid β accumulation seems to be a universal phenomenon in persons who will develop AD. But, to apply simple logics, an association between these two events is no proof that there is also causation involved, and even if it is, that it has to be in the assumed direction. If the association indeed reflects causation, could it be possible that the direction of causality is opposite to that proposed by the amyloid β cascade hypothesis? Some critics of the amyloid β cascade hypothesis have suggested precisely this alternative possibility[85], and even John Hardy, the originator of the amyloid β cascade hypothesis[75], has mentioned this as "a worrying possibility"[76].

3.3.5 Strategies to discover new mechanisms

Alzheimer's disease, like any other disease, can be described and explained at different levels, and the previous discussion about the role of amyloid β should illustrate this important difference between description and explanation. It seems unclear whether amyloid β accumulation causes the disease, is a characteristic of the disease, or perhaps even a protective reaction to the disease. In contrast, tau phosphorylation and the related formation of neurofibrillary tangles (NFT), and loss of synapses are most certainly integral characteristics of the disease itself, directly related to its consequences for cognition, behaviour and the clinical picture[78]. In the case of amyloid β, growing doubts concerning its role, while recognizing the fact that amyloid β accumulation is always found in early stages of AD, has instigated proposals to look at the wider, but still largely unknown, role of this peptide in biological systems in order to find new mechanistic clues[76]. After all, there is no reason to believe that evolution equipped us with this peptide in order to cause Alzheimer's disease.

To go from characteristic to explanation, i.e. to look "upstream" from any significant characteristic of the disease, is to look for precursors, for what may be behind these characteristics of the disease. Synaptic loss has been proposed by some as the most characteristic and universal feature of AD[86], and possibly also of other dementias[87]. An "upstream strategy" in the search for causal mechanisms based on this characteristic, could then be to look at neurotrophic factors, including brain-derived neurotrophic factor (BDNF), as they are of critical importance for neurogenesis, synaptogenesis and neuroplasticity[88,89] [90] [91-94]. In other words, if Alzheimer's disease is caused by dysfunction related to nerve growth, synaptogenesis and neurogenesis, will we find new clues to understand and cure Alzheimer's disease if we look for factors that regulate BDNF functionality? That neurotrophic factors are hampered in AD is not only indirectly indicated by synaptic loss in AD, but also by an inverse association between BDNF levels and severity of neuropathology [95].

The strategy to look for "what is behind" biological phenomena can easily be criticised for being reductionist; there is always something behind everything – and the search never ends. Instead of arriving at an explanation, the risk is that we find yet another level of descriptive characteristics. A complementary strategy to understand the mechanisms of Alzheimer's disease and other dementias is to look for associations at a very different level, at the level of behaviours, life styles and psychosocial factors. One of the main points of this thesis is that understanding of Alzheimer's disease and other dementias cannot come only from descriptions of biological events at an atomistic level. On the other hand, an association between a certain behaviour or emotional condition during the life course, and incidence of dementia decades later, does not add much to the understanding if we cannot even imagine a link between such an association and the biological events that lead to the disease. They should all be regarded as pieces of the same puzzle.

3.3.6 Life-course Exposures and the Brain Reserve Hypothesis

Classical experiments by Mark Rosenzweig, Marian Diamond and colleagues have demonstrated that an enriched environment can increase cortical thickness and total brain weight in rodents[96]. Enriched animals also showed a richer neuronal dendritic network and improved learning abilities compared to less stimulated individuals[97]. More recently it has also been shown that an enriched environment can increase brain levels of neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) [98]. Some experimental studies also suggest that social stimulation may have similar effects as enriched environments and physical exercise[99,100].

A large number of epidemiological studies on humans have shown that especially education[101] [102], but probably also other forms of intellectual stimulation, is associated with lower dementia risk[103]. A common explanation for this effect is the brain reserve hypothesis[104]. The core of the brain reserve hypothesis is that a brain with rich neural networks, at least partly as a result of prior stimulation, has better ability to resist and compensate against an underlying neurodegenerative disease process[105]. Empirical data

support a number of factors that seem to help building such a brain reserve, including support for education, a range of other intellectual activities, physical exercise - and social factors[106]. Related to the brain reserve hypothesis is also the finding of a probable link between a higher cognitive capacity / intelligence early in life and a lower risk of dementia in old age[107].

The brain reserve hypothesis may not seem very original in the sense that it adds little to, and builds closely on, previously well-established characteristics of the nervous system, such as its plasticity; it has long been known that the brain is capable of repairing itself and of reallocating neural processes after injury to regions adjacent to the injured one and that this ability is more pronounced in younger individuals. As mentioned above, the effects of enriched environments on neural networks, dendritic spines and synapses, which are all related to the brains capacity to be modified by experience, are also well known.

Another weakness of this hypothesis is that it seems mainly relevant for explaining individual differences in the latency between the point of disease initiation and diagnosis. There is no evidence that this hypothesis has anything to say about factors that may prevent the disease process from being initiated in the first place. This point is illustrated by recent findings that higher education is often associated with higher cognitive performance, but not with less cognitive decline in later life[108,109]. This should mean that persons with high education typically have a longer period of cognitive decline before their cognition has dropped to a level where criteria are met for a dementia diagnosis and when it does, that the disease at the neuropathological level has reached a more advanced stage[110]. This may also be the reason why a highly educated person typically has a faster progression of the disease after the time of an AD diagnosis[108]. In other words, education does not protect against dementia, but it typically delays its diagnosis.

On the other hand, any knowledge that is relevant for postponing cognitive decline to a debilitating level are welcome, both in terms of adding more years with a higher quality of life for the affected individual and in terms of attenuating rampant societal costs for dementia care in advanced stages of the disease. In addition to this, there is epidemiological evidence that the same type of activities that may build brain reserve capacity in a life-span perspective, possibly can have such an influence also in later life[111], perhaps even if exercised during early stages of cognitive impairment. Several clinical trials are on-going to test this hypothesis in elderly people, some of them in early stages of cognitive impairment and the results are so far promising[112].

As dementia is a progressive disease with a confirmed preclinical phase of up to twelve years[113], this means that a decline in cognitive ability may have lasted varying lengths of time for a certain individual at the time of diagnosis, depending on at least three factors: a / baseline level of cognitive functioning at the point when the subclinical disease process started, b / the rate of cognitive decline from this point and onwards and c / neuronal and other resources available to the individual in order to compensate for the progressive degenerative process. All of these factors should be related to the amount of brain reserve the

individual had accumulated at the time when the neurodegenerative process started - and possibly also on brain reserve-relevant activities after that point.

3.3.7 A Multitude of Life-style Associations not unique for Dementia: What does it mean?

It is common in medical research to look for causes and mechanisms that are specific for different diseases. In research on Alzheimer's disease the focus on the role of beta-amyloids and tau phosphorylation exemplify such an approach. Although it seems obvious that specific mechanisms such as these, unique for any disease, need to operate on a micro level for a specific disease to develop, some argue that non-specific disease mechanisms, as well as protective mechanisms against the same diseases, may be important to recognize. What is meant by a "non-specific disease mechanism"? It is related to a factor that increases the risk of disease in a general way, and that is not specifically tied to any specific disease. Ageing itself could in this perspective be considered as a factor that increases the risk for disease in a non-specific way; with increasing age the biological system gets more fragile and will become less able to withstand health threats of many kinds, from a virus infection to cancer. Sleep is intimately related to immune function, and poor sleep during an extended period can also be considered as a non-specific health threat, possibly due to its impact on immunological efficiency, with many possible specific disease outcomes as possible [114].

A related assumption is that psychological and biological phenomena are closely linked and that their interrelatedness is a key to understand mechanisms behind health and disease. This idea can be exemplified by the biological consequences of mental states, such as stress, grief or depression, which are reflected in bodily processes that could have health consequences, rather than being isolated from these processes.

These two ideas of a general, or non-specific, health mechanism and the interrelatedness of psychological and biological phenomena are corner stones in the psychoneuroimmunological approach [115]. It also rests on the assumption that efficient communicative pathways exist between the immune system, the nervous system and the endocrine system, an assumption that seems empirically supported [116]. The generality of this approach, relates to the protective role of a well-functioning immune system against practically any kind of heath threat, and conversely that immunological dysfunction may have a wide range of disease consequences.

Although the term was coined as late as 1983, the basic assumptions behind psychoneuroimmunology are not new; to "rest and recover" is probably still a very common medical advice when a person feels sick, with or without other medical interventions. The notion that immune-disruptive exposure to e.g. stress, bad sleep or negative emotions constitute a general health risk is both part of ancient and common wisdom. More recently, empirical studies have confirmed that one and the same psychological stressor, with a high likelihood of immunological relevance, can indeed lead to a range of different diseases [117]. But can it also lead to Alzheimer's disease and other dementias?

Before addressing this question directly, we will consider the diversity of factors that have been suggested as protective against cognitive impairment. Among these are physical exercise [118], a generally active life style [119], social interactions [120], and a "Mediterranean" diet (a large proportion of vegetables, fish and fruit) [119]. Among suggested risk factors we find loneliness [121,122], depression [123], hopelessness [72,124], stress [125,126], obesity [127,128], midlife hypertension [129], and frailty [130]. It should be evident that few, if any, of these factors have a specific relevance to explain cognitive impairment, but equally well apply to many other health conditions. Is this an indication that they operate through a common factor? If so, is the immune system a possible candidate?

Before addressing this question, it should be noted that the hypothesis of a non-specific health mechanism does not preclude the possibility that a factor could work through such a mechanism, thereby contributing to disease proneness in a general way, but in addition have a more specific risk impact. To exemplify, smoking undoubtedly has a specific link to impaired lung function and increased risk of lung cancer. But smoking may in addition present a burden on the immune system, thereby resulting in elevated risk for a range of other diseases. According to some studies, dementia is one of them[131]. Having a chronic disease may in a similar way present an added burden on the immune system, and thereby in a non-specific manner also increase the risk of other diseases to develop. To exemplify, multimorbidity is more common in persons with diabetes, and while some of the links between diabetes and other diseases may be more direct, leading to different pattern of comorbidity compared to comorbidity in persons with other chronic diseases [132], persons with a chronic disease like diabetes have been found more vulnerable to develop multimorbidity when they are also exposed to other risk factors [133]. From a psychoneuroimmunological perspective, this may be due to the pre-existing immunological burden from the original chronic disease.

On logical grounds it could be argued that whatever specific events that at a micro level constitute a certain disease, there has to be a chain of events leading from a risk-related factor and, at the end of the line, the specific events and processes that constitute and characterize the disease, events that in the case of Alzheimer's disease are exemplified by tau phosphorylation and synaptic loss. If we move upstream from tau phosphorylation, beta-amyloids seem to have an important role, although not yet fully understood[134]. Which are the chances that immunological mechanisms reach into these processes and affect their initiation and outcome? Without a credible bridge from behaviour and emotions to immunological consequences, and then to immunology-related consequences of relevance for disease-specific events and processes, the psychoneuroimmunological theoretical framework may not appear as a convincing one.

For at least some of the suspected emotional risk factors for Alzheimer's disease and dementia, such evidence now seems to exist; in the case of depression, it is known to increase pro-inflammatory reactions in the brain, including increased levels of cytokines[135]. These reactions in turn have been proposed as implicated in the development of Alzheimer's disease[136,137]. One experimental study showed that increased levels of glucocorticoids,

which in humans are related to depressive feelings, anxiety and stress, led to increased beta-amyloid and tau pathology, with increasing APP levels and beta-APP cleaving enzyme, in an animal AD model[138]. This should mean that also Alzheimer's disease could be one of many possible disease outcomes from a psychoneuroimmunological perspective. A schematic summary of this link between depression, immunology and AD is found in figure 9, adapted from Irwin et al, 2007[139]

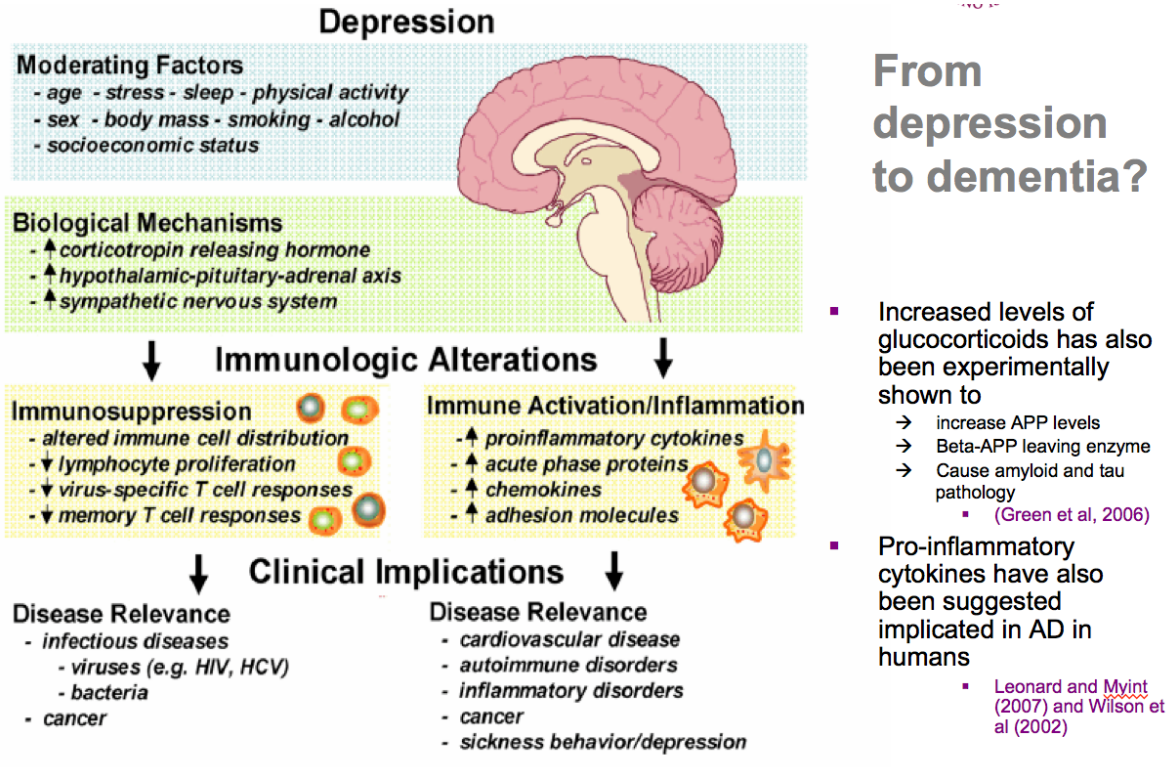

Fig 9. A schematic illustration of brain-immune interactions in depression and how it relates to dementia.

Adapted from Irwin, 2007[139]

3.4 THEORETICAL AND METHODOLOGICAL CONSIDERATIONS

The long pre-clinical period during which symptoms develop gradually and subtly, presents a major methodological challenge to identify life style and environmental factors that can increase the risk of the disease. The imprecise nature of the pre-clinical period can also lead to dramatic over estimation of its length without a proper distinction between antecedents/predictors of the disease – and the disease itself.

3.4.1 The Difference between AD Diagnosis and AD Neuropathology

An important distinction in this regard is between carrying the disease (at the neuropathological level) and being clinically diagnosed with the same disease, a distinction we previously discussed in relation to the question of high education being protective against

dementia or not (see section 3.3.5). Measurable cognitive decline has been demonstrated to start up to twelve years before clinical diagnostic criteria are fulfilled[113], which means that any longitudinal study with a follow-up time from baseline of less than twelve years runs the risk that any association between a factor at baseline and the subsequent diagnosis of a disease can reflect influences of subclinical phases of the same disease already at baseline, i.e. reflect reverse causation. To exemplify, if social interactions at baseline is found to predict dementia a few years later, this association could mean that that an underlying disease had already started to affect the person's social behaviour at the start of the study. To avoid the risk of reverse causation in dementia research, long follow-up times are needed and preferably also baseline measurements when the participants are relatively young. It is uncommon that both these methodological requirements are fulfilled, and the CAIDE Study, on which three of the studies in this thesis are based, is one of very few examples.

3.4.2 The difference between Precedents and Characteristics

Another important distinction in understanding Alzheimer's disease is that between precedents and characteristics of the disease. To exemplify, if accelerated cognitive decline can be demonstrated ten years before diagnosis, compared to persons of the same age who will not become diagnosed, it should mean that the observed cognitive decline that preceded the diagnosis is not only a precedent, but in addition an early manifestation of the disease at the neuropathological level. But if persons with a lower IQ already before 20 years of age are more likely to become diagnosed with AD when they get old, as has been found in the famous Nun studies by Snowdon et al.[107], that does not mean that their AD started already in childhood. First of all, AD is a progressive and lethal disease, meaning that lower intelligence in itself cannot be a manifestation of the disease, unless a progressive decline in intelligence and/or cognitive ability can be demonstrated. A more likely explanation is that people with lower IQ can be diagnosed earlier, which, as in the case with low education/a lower brain reserve, easily can be confused with a higher risk of actually getting the disease. Another possibility is that people with lower IQ are more vulnerable to develop AD. In neither of these cases does it mean that this exemplified antecedent is a symptom of the disease itself. Difficulties in understanding this distinction may lead to exaggerated and unwarranted claims concerning the typical age when AD starts to develop on the neuropathological level.

3.4.3 Main Methodological Approaches in Dementia Research: Pros and Cons

Main methodological approaches in dementia research include experiments on AD animal models, intervention studies on humans and epidemiological association studies on humans. Unfortunately none of these approaches alone offers a royal road for dementia research, as we shall see.

3.4.3.1 Animal Studies: Pros and Cons

The main advantage with animal studies, although some would strongly disagree, is that ethical limitations are less of a problem. Animals can be genetically modified, exposed to high levels of different exposures in a systematic and controlled fashion and they can be killed ("sacrificed") at a time when it is most convenient for immediate identification of structural and biochemical effects. In the case of drug trials, this means that at the time when the drugs are tried in humans, successful outcomes in preceding phases on animals, reduces the risk of casualties, suffering and other adverse effects when the same drug is tried in humans. In the case of non-drug animal experiments, the effects of various environmental exposures can be invaluable to discover important brain mechanisms or establish health effects from a specific exposure. One example of the first is the research that dramatically illustrated the effect of environmental stimulation on neuronal density and cortical thickness[96]. Animal experiments confirmed smoking as causative for lung cancer, a demonstration with enormous impact on public health.

The main problem with animal studies is that animals are animals – not humans. If we are interested to see if depressive feelings, cultural activities, singing in a choir or mindfulness meditation are beneficial for cognitive health, animal studies have little to contribute. For other factors, like physical exercise or intellectual stimulation, animal experimentation may however seem a less remote possibility. In the case of AD research, mice do not naturally get a disease that resembles AD, and, as mentioned above, genetically manipulated mouse models can yet only be made to carry some biological characteristic of the disease, like heavily elevated levels of amyloids, but in other respects share little resemblance with the full phenotype of human AD. In spite of huge advantages in terms of experimental control and the kinds of analyses that can be made, animal experimentation, especially on mice, should be either non-feasible or have little generalizability to directly test hypotheses on either causal links to AD in humans, or cures against the disease. Development of new and more adequate AD mouse models could of course make such a conclusion less valid in the future than it probably is today.

Even if there are doubts concerning the potential of animal experimentation to directly test hypotheses concerning causes and cures for AD in humans, for reasons mentioned, animal experiments can still be of value to test mechanisms that are related to AD in some way and to translate such findings into studies on humans. In other words, animal experiments can be valuable by generating mechanistic hypotheses of relevance that can then be tested on humans. Paper 4 in this dissertation is an example of such a translational approach. This paper builds on the hypothesis that neurotrophic factor dysfunction could be relevant for AD. Animal experiments have not only shown that environmental enrichment can produce synaptogenesis and improved learning ability in mice, but also indicated that BDNF availability in the brain is a prerequisite for this to occur[140-142]. Intuitively, AD in humans appears to represent the antipode of increased inter-neural connectivity, synaptogenesis and neurogenesis, plastic processes where BDNF seems to play central role - in combination with

different types of beneficial environmental exposures. With this in mind, the idea with Study 4 was to see how different types of activity could affect BDNF levels in humans. This example brings us to consider pros and cons of experimental/intervention studies in humans.

3.4.3.2 Human Intervention Studies: Pros and cons

The general advantage of an experiment is that participant can be randomly assigned to different conditions, thereby evading the main problem of epidemiological association studies; that of self-selected groups and confounding influences from variables that differ between the groups, in addition to the predictor variable of interest. A classical parallel groups design with random assignment of conditions dispenses with the need to adjust for potential confounders; in a well-performed experiment with parallel groups, only one factor differs between the groups, the factor of interest. If there is a difference in outcome, it can be safely attributed to the causal influence of this independent variable.

Besides the parallel groups design, or between-groups design, many other experimental (and quasi-experimental) designs exist [143]. While it is beyond the scope of this thesis to discuss all of them, mention should be made of a second major experimental design that, when feasible, is superior to a parallel groups design. This is the within-subjects, or crossover, design. In this design the different conditions are not assigned to different groups, but each individual is exposed to each condition and intra-individual differences in effects between the different conditions are compared. The advantage of this design refers to the fact that although individuals in a parallel groups design are randomized to the different conditions, they are each different from each other in many ways, although these differences are not systematically distributed between groups. This creates variability in effects within each parallel group that easily can mask the systematic difference between groups that is attributable to the difference between conditions. When each individual is compared to him/herself across conditions, the inter-individual "noise" in a parallel groups design is eliminated and the true effect of the intervention, if there is one, can emerge as a stronger, and potentially detectable, signal. In statistical terms, fewer participants are needed to attain sufficient power, compared to a parallel groups design. The main threat in this design is from sequence effects, but this is easily dealt with by systematically varying the order of conditions between individuals to attain a fully balanced design. When feasible, which is not always the case, a within-subjects/cross-over design is therefore always preferable to a parallel groups design. Paper 4 illustrates the application of a within subjects/cross-over design in an intervention study on healthy elderly humans.

If the problem with animal experiments is that animals are animals and not humans, in a way the problem with a human intervention study is that humans are – humans. Humans are social animals that are sensitive to even the subtlest signal of a desired performance from experimenters. The experimenter effect, i.e. the effect of expectations that are more or less subtly communicated, has proven powerful[144]. The social nature of humans can also make us wanting to live up to expectation of others and in addition wanting to present ourselves in a favourable way to others, called the social desirability effect [145]. In other words, an

elegant experimental design is no guarantee that the results are trustworthy if the experiment was not carried out in a proper way in other respects to circumvent these and other threats, especially when the participants were humans.

At the end of the previous section, we discussed how paper 4, an intervention study on human subjects, was inspired by results from animal experiments. In the translation of this into an experiment on humans, several drawbacks, in relation to an animal experiment, should be obvious. For ethical reasons, we did not want to measure BDNF levels in the brains of our participants, not even in their cerebrospinal fluids, which, from a purely scientific point of view, would have been the most relevant. The compromise was to measure BDNF in peripheral blood serum. This compromise made the interpretation of the results a lot more complicated and speculative than they otherwise would have been.

No intervention study on humans can directly test if a certain exposure of interest will increase the risk of Alzheimer's disease. This would not have been a problem with mice, not even if we would find mice that can develop AD of the human type. Instead, and for ethical reasons, we focused in that study on finding positive brain health effects, or rather, probable correlates of such effects. Human intervention studies are in practice limited to studying factors that can prevent dementia, rather than identifying factors that could cause the disease. From a clinical perspective, this is not a problem of course, but for more basic research questions concerning possible triggering mechanisms behind Alzheimer's disease, human intervention studies are out of question. This is in contrast to both animal studies, and as we shall see, epidemiological association studies.

Another problem with intervention studies is that many of the most important factors that have emerged from epidemiological association studies are not possible to assign to different persons in an experimental setting. For example, traumatic experiences, stress, depressive feelings – or any feelings, positive or negative, for that matter, cannot be "assigned" to persons because they belong to a specific group in an experiment. The potentially "positive" factors we finally chose in Study 4 were mindfulness, which could be seen as the opposite of stress, physical exercise and cognitive training. These choices were guided by ethical considerations and evidence from both animal experiments and epidemiological association studies.

This brings us to another ethical dilemma in intervention studies to prevent dementia: what do we do with the control group? A control group should be a group that is not subjected to the factor of interest, e.g. a combination of stimulating activities. But if the researchers fear that they thereby deprive the unfortunate individuals that ended up in the control group of something that could prevent or delay dementia, does that not indirectly mean that they contribute to dementia developing earlier in them? On the other hand, if the control group is active in a way that does not make them disadvantaged in relation to the group that received the intervention, we are deliberately ruining the chances of testing the hypothesis the study was designed for. The solution is often an active control group that participates in something of probable benefit, but hopefully with less benefit than the factor of interest. The FINGER

Study[146] exemplifies this dilemma by offering the control group regular health advice and monitoring their cardiovascular health. After two years of intervention, both groups had improved their cognitive performance, although the "real" intervention group even more so than the active control[112]. The question then arises: what was the real effect of the intervention? Was the improvement in the control group, in spite of getting two years older, a retest effect, which is the purpose of a control group to control for? Or was it related to the activities, including social interactions that the control group performed in parallel to the intervention in the other group? Or to life-style changes inspired by the health advice? To balance ethical and methodological considerations in designing what a control group should do in an intervention study, often carried out over several years, represents a delicate dilemma for the researchers.

A final problem with intervention studies on human subjects in dementia research refers to the previously discussed time difference between the point of diagnosis and the point of disease initiation (see section 3.4.1). The golden grail for researchers on Alzheimer's disease is to find the answer to the question "What causes Alzheimer's disease?" But with a preclinical period of at least 10-15 years, and due to ethical limitations previously discussed, human intervention studies have to limit themselves to the more trivial question: "How can we delay the progression of an Alzheimer's disease that has already started?" To perform a proper experiment to answer the "golden grail question", we would have to expose persons, probably already in midlife, to something we think will prevent them from having Alzheimer's disease when they get old – and then wait 20 years to find out if we were right. To do that, in the first place we would need a hypothesis that, on the basis of existing knowledge, seems to have a very high probability of being correct – and to have a lot of confidence in that hypothesis to motivate us to even think about such a venture. We would also need long term funding and a lot of patience. Another option is to rely on nature's experiments and on choices that people have made by their own will. That option is to conduct an epidemiological association study.

3.4.3.3 Epidemiological Association Studies: Pros and Cons

An epidemiological study typically establishes associations between a behaviour or a condition, e.g., smoking or feeling depressed, at some point in life with a subsequent outcome, e.g. a dementia diagnosis, rate of cognitive decline or structural characteristics found through brain imaging. But an association in itself is not synonymous with causation, only a necessary requirement. Another requirement for an association to be causal is related to time; that the proposed causal factor existed before the outcome that it allegedly caused. Although these conditions may seem trivial, many epidemiological studies claim causality from associations without having fulfilled them. Referring to the above-mentioned "holy grail question" in dementia, epidemiological association studies with less than 15 years of follow-up cannot possibly ascertain that observations at baseline are causally related to the initiation of an Alzheimer's disease process if the subclinical progress can be assumed to be at least more than 15 years, which most estimation say it is [147]. In addition, the risk of reverse

causation is obvious (see chapter 1 and 3.4.1). In the ideal case, the time of baseline has to be at or before the time window when a factor potentially could have the effect to initiate the disease process. We also have to consider that, as far as known, AD is a progressive disease once it has been initiated. No known studies have reported evidence that the disease can be stopped at earlier phases of this process, although many hope that earlier detection could break the trend of all failed attempts we have witnessed so far. If the disease process is indeed irreversible once it has started, what is the point of relating a candidate risk factor in parallel to the disease, only a few years prior to the stage when the same disease can be diagnosed?

On the other hand, if there is an association and in addition the time window requirement is met, the epidemiological association study have some unique advantages over animal studies and intervention studies, in spite of its relative logical weakness. First of all, the target outcome, e.g. AD, can actually be included as outcome without ethical problems, and events or behaviours that are impossible to use as independent variables in an intervention study can be related to as they with this study design are part of natural exposures or individual choices. In a time when the pendulum has swing so heavily in the direction of intervention studies, these advantages are important to keep in mind.

In addition, it is often possible to circumvent the requirement of very long prospective designs if it is possible to reliably establish events and circumstances retrospectively. The problem with retrospective data is often that these measures are unreliable, e.g. when they are established through interviews, especially if the interviewed person is old with some degree of cognitive impairment. Even in perfectly healthy persons, memory research has demonstrated that memories are often false and constructed and/or modified unconsciously in retrospect [148]. A better possibility is probably to use archival data, or reliable registers. The mentioned studies by Snowdon[107], where letters written early in life were found and analysed according to their semantic complexity, is a well-known example of this strategy. The CAIDE Study, on which three of the scientific papers in this thesis is based (1-3), also represents a creative way to build on data that was originally collected for another purpose[149], and to relate the detailed information from those measurements to data from clinical re-examinations up to more than 30 years later.

The major drawback with all epidemiological association studies is identical to the strongest feature in experimental designs: that categories of participants have placed themselves in those categories, rather than through a procedure of randomization by the researcher. To deal with potential confounding variables is therefore a major challenge in these types of studies. A common procedure to do that is to identify variables that were measured and that show an association to either or both the predictor and the outcome, with addition of variables selected on the basis of theoretical relevance, and then to statistically adjust for the influence from these variables in order to isolate the unique association with the factor of interest. It is difficult to avoid a certain degree of subjectivity in this process and the pressure to generate statistically significant and publishable results in the academic world does not help to make this procedure more objective and reliable. Another problem is the variables that were never

measured and that potentially could have altered the associations. One example of adjustments that are rare in dementia research is personality. At the same time it is likely that personality factors affect both the choices and the life styles people have and in addition may be associated with health. In the end, it is impossible to adjust for "everything", both for statistical and practical reasons. The relative advantage of an experiment in this regard is that the randomization process ideally accomplishes just that.

When a sample is invited to participate in a study of this kind, it is a universal feature that some invited persons do not accept the invitation or that they later drop out. If these persons were a random sample from the total sample, this would not present a problem, but they typically are not. To estimate the effect of participation bias it is common to describe the characteristics of this non-participant subsample with the true participants. The results from Paper 3 illustrates that this may be insufficient. In that study we did not only have baseline data from both participants and non-participants, but through linked registers we also knew what the outcome (dementia incidence) was in the group that did not participate in the follow-up. When we compared incidences between participants and non-participants we found a dramatic difference, indicating that participation bias may be a much larger problem than commonly assumed, even when the participation rate is as high as in the CAIDE Study (around 75% during the re-examinations).

3.4.3.4 Concluding remarks

From the above it should be obvious that dementia research cannot rely solely on any of these approaches, but need to combine them. This has been a fruitful strategy in other cases where the outcome of interest was ethically impossible to include as outcome in an experiment on humans. Perhaps the most famous example is the case of smoking and lung cancer. The conclusion of a causal relationship was arrived at by combining findings from epidemiological studies that found a higher incidence among smokers than non-smokers with findings in animal experiments where animals were exposed to cigarette smoke with lung cancer as a frequent result. Public health recommendations have led to decreased smoking in many countries, and as a result the prevalence of lung cancer has gone down dramatically – besides resulting in other kind of health benefits[15].

Intervention studies are often regarded as the golden standard in dementia research, but it seems that a comparative evaluation of pros and cons should justify an important role for high-quality epidemiological association studies also in the future.

4 AIMS

4.1 GENERAL AIMS

The general aim of this thesis was to investigate the long-term impact of social and emotional factors for cognitive health in later life. The purpose was to accomplish this by applying and combining both epidemiological and experimental methods, and by building on different types of data and methods of data analysis. A further aim was to investigate biological mechanisms of possible relevance for associations between life-style factors and brain health in old age.

4.2 SPECIFIC AIMS OF THE DIFFERENT STUDIES

The first paper aimed to investigate the association between cohabitant status in midlife and cognitive health in later life and how ApoE4 status modified the association with Alzheimer's disease.

The second paper aimed to evaluate one of the hypotheses emanating from the first study; whether the increased dementia risk detected especially for widowed could reflect that feelings of hopelessness is a risk factor for cognitive impairment in later life.

The third paper, also building on the results from the first study, aimed to investigate the separate and combined effects of living and feeling alone in midlife on the estimated risk of a subsequent dementia diagnosis, and in addition to evaluate how non-participation bias influences the estimation of these effects.

The fourth study aimed to investigate effects of different activities on levels of brain-derived neurotrophic factor (BDNF) in healthy elderly persons.

5 PARTICIPANTS AND METHODS

5.1 STUDIES 1-3

The first three studies were based on the Cardiovascular, Aging and Dementia (CAIDE) Study, carried out in Eastern Finland. CAIDE is a large, population-based study focusing on vascular, life-style and psychosocial risk factors for cognitive functioning and dementia in later life. With baseline examinations carried out already in midlife and a long follow-up period, these data offer a unique opportunity to study the relevance of these factors for cognitive health in a life-course perspective.

The participants in the CAIDE Study were the survivors in 1997 of four independent populations samples, originally investigated between 1972 and 1987 in Eastern Finland as part of the North Karelia Project and the WHO MONICA project. The original purpose of these investigations was to investigate cardiovascular health and life style factors that could potentially explain an exceptionally high incidence of cardiovascular diseases and mortality in Finland, the highest in the world for young men at the time [149]. Participation rate was high, ranging from 83% to 93% [150]. The protocols for these baseline survey methods at baseline were standardized to comply with international recommendations and followed the WHO MONICA protocol, or were similar to these [151].

The Cardiovascular, Aging and Dementia (CAIDE) Study was later initiated to use data from these investigations in order to study longitudinal associations between health/life style factors in midlife and cognitive health in later life. Thus, in 1997 a random sample of 2000 survivors between 64 and 79 years were invited for a first re-examination that took place during the following year, and then to a second re-examination that took place between 2005 and 2008. In addition to the same measures that had been applied at baseline, when the participants were in their midlife years, the re-examinations included screening for cognitive impairment and careful clinical diagnosing for possible cases of mild cognitive impairment (MCI) or any specific type of dementia. (For further details on diagnostic procedures, see Paper 1.)

Of the 2000 persons who were invited for the re-examinations in 1998 and 2005-2008, a total of 1551 persons accepted and fully participated in one or both of these re-examinations. In addition to re-examination data on these participants, we linked register data on dementia diagnoses and mortality from various register sources for all the 2000 persons who were initially selected to form the CAIDE Study population. Figure 10 schematically summarizes the design, including both re-examinations, and with cognitive health, MCI and dementia as outcomes. Table 1 is a summary of demographic data, stratified for re-examination participation and with outcomes (with or without a dementia diagnosis) and mortality as found through linked registers (from paper 3 in this thesis). Studies 1-3 are part of the CAIDE Study; The CAIDE Study was approved by the ethical committees at Kuopio University Hospital in Finland (D nr. 24/97 and 124/2004) and Karolinska Institutet (D nr. 04-103).

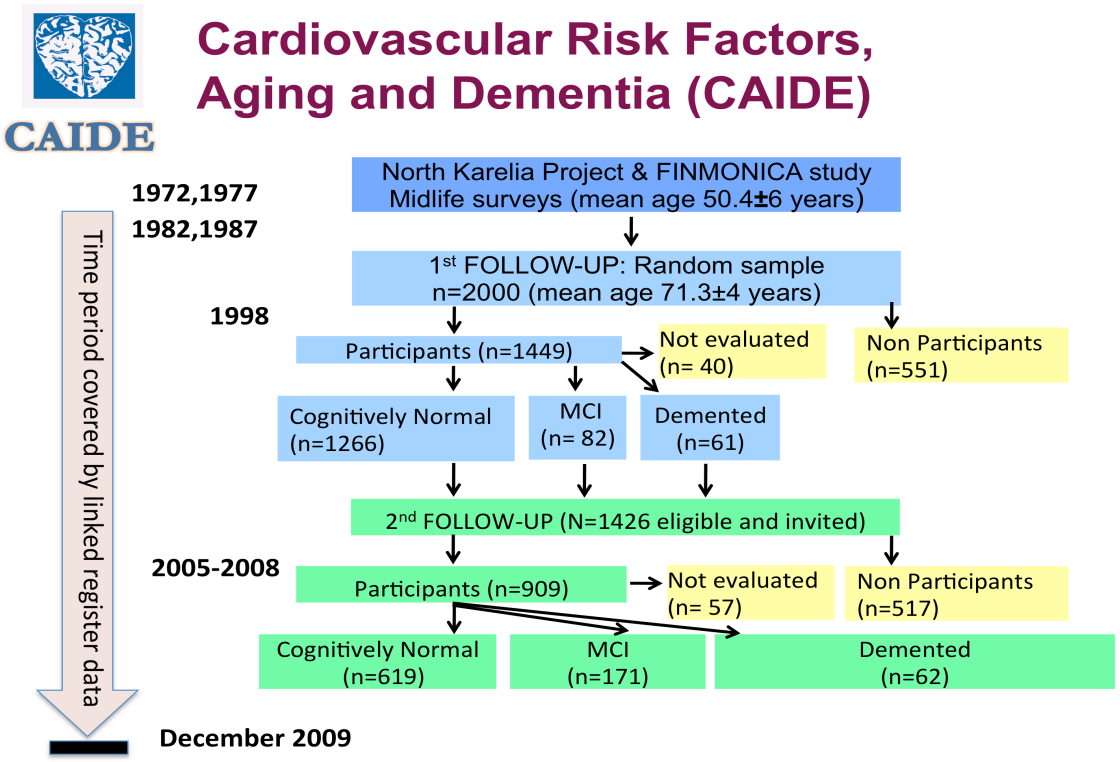

Fig 10. Flow chart of the CAIDE study covering the time period from 1972 until December 2009.

Baseline variables (N = 2000)	Full CAIDE sample (N = 2000)	Stratified by re-examination participation		
		Participants (N = 1511)	Non-participants (N = 489)	p-value
Age at baseline	50.6 (6.00)	50.3 (6.00)	51.5 (6.00)	<0.0005
Gender (% female)	62.50%	62.30%	63.00%	0.828
Feeling lonely (N = 2000)	22.40%	21.20%	26.30%	0.022
Cohabitant status (% Non-cohabitants)	21.90%	20.20%	27.30%	0.001
Education in years	8.31 (3.35)	8.59 (3.40)	7.43 (3.04)	<0.0005
Systolic blood pressure	146 (20.6)	144 (20.0)	151 (21.4)	<0.0005
Diastolic blood pressure	89.9 (11.1)	89.3 (11.0)	92.0 (11.2)	<0.0005
KTL cholesterol	6.80 (1.24)	6.75 (1.22)	6.96 (1.31)	0.002
BMI	26.8 (3.96)	26.6 (3.78)	27.4 (4.44)	<0.0005
Persons with feelings of hopelessness (%)	46.2%	42.7%	57.7%	<0.0005
Variables related to outcome				
Age in 1998 (N = 2000)	71.6 (4.07)	71.2 (3.97)	72.8 (4.17)	<0.0005
Persons with a hospital dementia diagnosis (N = 327)	16.40%	14.20%	23.30%	<0.0005
Age at time of diagnosis	79.4 (5.39)	80.5 (5.02)	77.5 (5.57)	<0.0005
Died before end of study (N = 738)	36.90%	28.40%	64.10%	<0.0005
Age at time of death	79.6 (5.38)	80.4 (5.46)	78.5 (5.09)	<0.0005

Table 1. Characteristics of the CAIDE population, with stratification by participation in re-examinations. (From paper 3)

For continuous variables, values are means (SD). P-values are from one-way ANOVA analysis for continuous variables and from χ^2 analyses for categorical variables.

In Study 1 and 2, we used mainly logistic regression to calculate the associations between the social and emotional predictor variables and the cognitive health outcome variables, including AD and MCI as specific outcomes.

In Study 1, the main predictor variable was marital/cohabitant status in midlife. Participants were classified as cohabitants (living with a partners independent of formal marital status), single (never-married/cohabitant), divorced or widowed. In some calculations we also used marital status at follow-up for categorization of transitions in cohabitant status between midlife and follow-up, e.g. to identify participants who had been cohabitants at both occasions. We adjusted for several relevant health and life-style variables, including age, gender, education, smoking, physical activity, depressive feelings (feelings of hopelessness and loneliness), blood pressure, and cholesterol, all measured in midlife, and in addition for ApoE4 status, measured at follow-up in 1998. The main statistical method was logistic regression.

In study 2, we used feelings of hopelessness at midlife as predictor, as measured through responses to the following statements: "I feel that it is impossible to reach the goals I would like to strive for" and "The future seems to me to be hopeless, and I can't believe that things are changing for the better". A five-point Likert scale was used, originally coded as 0 = absolutely agree; 1 = somewhat agree; 2 = cannot say; 3 = somewhat disagree; or 4 = absolutely disagree. In the data analysis we reversed the scores in order for higher scores to reflect a higher degree of hopelessness and summed them into a scale ranging from 0 - 8. In the first analysis this scale was entered as a five-level ordinal scale after collapsing categories with fewer than 10% of the observations (scores 0-1 and 5-8). To facilitate comparison with other studies, additional analyses were based on a median split dichotomization of the original hopelessness scale. The statistical analysis was of the same type as in Study 1.

In Study 3, with access to a continuous data of time points for dementia diagnoses and dates of death, we used survival models to analyse the associations based on outcome data from linked registers. In this study we included participants also from the second follow-up in the CAIDE population, in addition to verified dementia diagnoses from registers, both on participants (N=1511) and non-participants (N=489) in the two re-examinations (see Table 1). As we only had access to dementia as diagnosis, without subtypes, from the registers, we used any type of dementia as outcome in this study in order to compare and combine information from registers and re-examinations. We first performed a log rank Meyer-Kaplan survival analysis and censored all who died without a prior dementia diagnosis or who were alive at the end of Study. In the Cox regression survival analysis we also adjusted for several potential confounders with an association to both living and feeling alone and to dementia incidence, including age at baseline, gender, education, occupation, cholesterol and feelings of hopelessness. These analyses were performed by either using dementia diagnoses from the re-examinations

For a more complete description of methods and participants, see the corresponding papers 1-3.

5.2 STUDY 4

This study was part of the Successful Ageing and Enrichment (SAGE) study, led by the Linnaeus University and Harvard Medical School, and with several collaborating institutions. Study 4 related to the aim of addressing biological mechanisms of possible relevance for associations of the type demonstrated in the previous three studies, and to the aim of complementing an epidemiological association study design approach with an experimental approach. In this study the participants were nineteen healthy volunteers aged averagely 70.8 years, ranging from 66 to 78 years, eleven women and eight men, from a region in the Växjö city area, south Sweden. For further demographical information, see Table 2 (from paper 4).

Variables	**Mean value (±SEM) or % (N=19)**
Gender (% female)	57.9
Age (years)	70.8 ± 0.8
Formal education (years)	8.02 ± 1.24
Continued adult education (total time in years)	2.63 ± 0.23
MMSE score (range 0-30)	28.9 ± 0.2
Cognitive performance in CogMed, average ratio of correct answers	0.72 ± 0.01
Physical activities in daily life. Index score (range 0-40)	19.1 ± 1.2
Systolic blood pressure (mm Hg, average of three measures)	145 ± 2.8
Diastolic blood pressure (mm Hg, average of three measures)	84 ± 1.4
Resting pulse (average of three measures)	75.8 ± 0.8
Self-rated health (1-4, where 4 is best health)	2.53 ± 0.77
Health problems, average index score (0-1 on 35 symptoms)	8.05 ± 1.22

Table 2 Characteristics of participants in study 4.

Numbers are mean values with SEM for continuous variables and % participants for the nominal variable (gender).

We used an experimental within-subjects design to measure effects on BDNF levels in serum from three different activities that the participated performed during 35 minutes. The three activities were a/ aerobic physical exercise of moderate intensity, b/ a cognitive training session, and c/ a session of mindfulness. A specific sequence of conditions was randomly assigned to each participant to obtain a fully balanced design. The time interval between sessions was at least one week for each participant, and we kept the time of day as similar as possible within each person. In addition, all sessions were performed between 09 and 11 am for all participants. (For a schematic overview of this design, see Fig 11.)

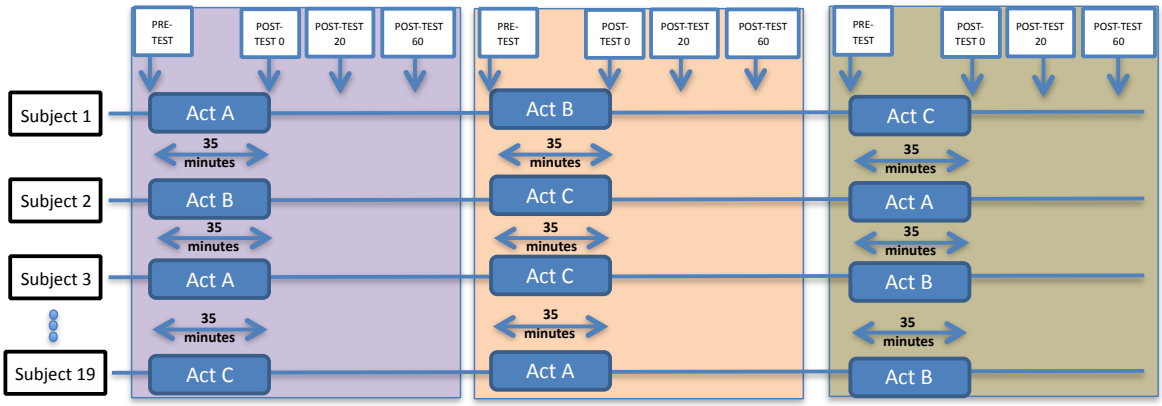

Fig 11. Schematic illustration of the within-subjects experimental design in study 4.

We collected 8 ml blood samples from a lower arm vein directly before each session started and at three time points (0, 20 and 60 minutes) after the conclusion of each session. The blood samples were kept at room temperature for 30 min to allow for clotting, and were then centrifuged at 2000 *g* for 10 min at 4°C to separate serum. The different samples were marked and frozen at -80 degrees C until analysis. We used an ELISA type of analysis to estimate levels of BDNF in the different serum samples after diluting them according to instructions from the manufacturer of the ELISA kit we used (Human BDNF Quantikine ELISA, DBD00, R&D Systems, Minneapolis, MN).

To take statistical advantage of the within-subjects cross-over design, we used repeated measures ANOVA to estimate the difference in effects on BDNF levels from each activity within each subject.

For a more complete description of the Methods we used in this study, see paper 4.

This study was approved by the regional ethical review board in Linköping (decision dnr 2013/154-31).

6 SUMMARY OF RESULTS

In this summary some of the most salient results from each study have been selected and illustrated. For a detailed presentation of all results, see the corresponding papers that are included in this thesis.

6.1 STUDY 1

We found 285 individuals among the CAIDE participants who at midlife had been living without a partner. Compared to the 1147 cohabitants, they had approximately a two-fold risk of cognitive impairment (MCI or dementia) at follow-up, after full adjustment for potential confounders (OR 2.09, CI 1.3 – 3.4). For those who had lived alone both at midlife and in later-life, the corresponding odds ratio was 2.89 (CI 1.7 – 5.0). The associations were similar in relation to each cognitive health outcome (any cognitive impairment, and separately to MCI and AD), as illustrated in Fig 12.

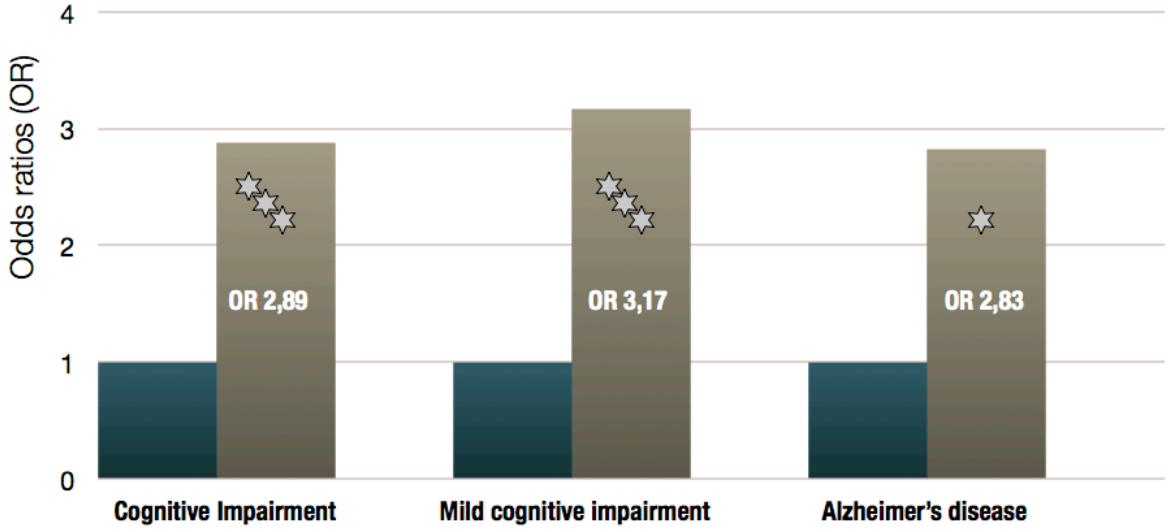

Fig 12. Associations with any cognitive impairment, mild cognitive impairment (MCI) and AD for non-cohabitants (brown bars) both at baseline and follow-up in relation to cohabitants (blue bars).

Stars indicate level of statistical significance (≤ 0.05, ** ≤ 0.001, *** ≤ 0.0005)*

We also found that among non-cohabitants in midlife, the risk for widowed persons was higher than for any other category (single or divorced), compared to cohabitants. This difference was accentuated when we also stratified for ApoE4 carrier status, and especially when the outcome was Alzheimer's disease, as compared to mild cognitive impairment (MCI). Fig 13 illustrates these associations when the risk for ex-cohabitants (widowed and

divorced) was compared to those who were cohabitants both in midlife and in later life, including stratification for ApoE4 status.

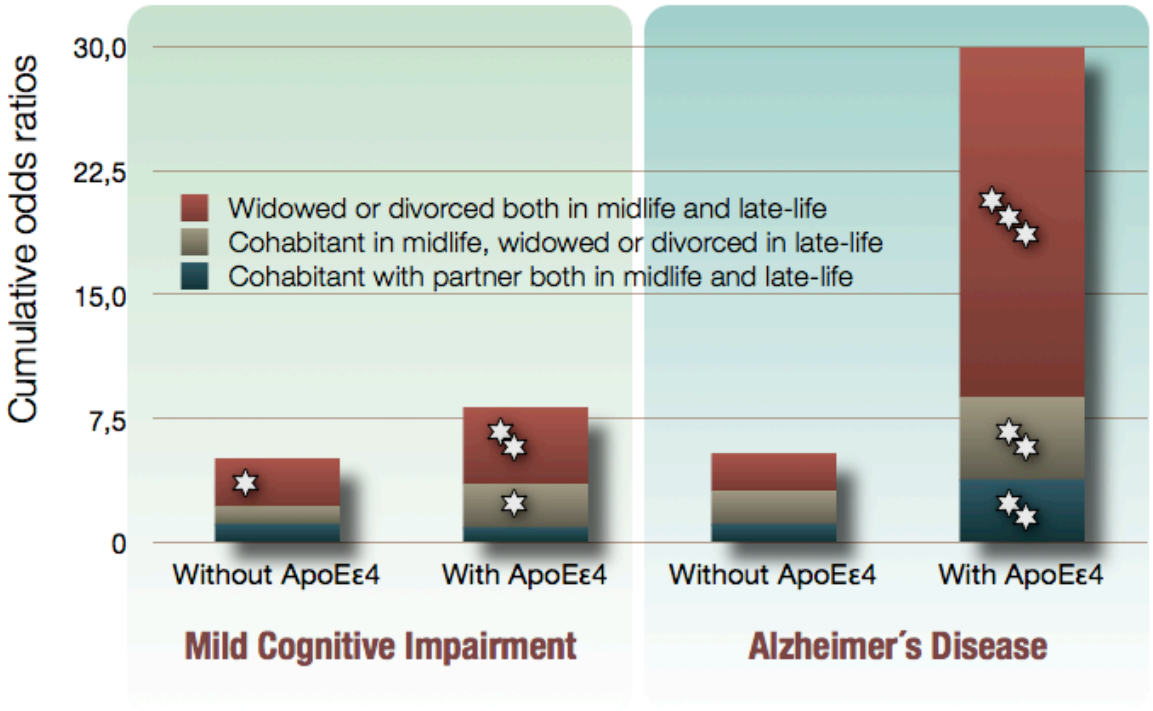

Fig 13. Associations with mild cognitive impairment (MCI) and AD for three groups with respect to cohabitant status at midlife and in later life. stratified for ApoE4 status.

Fully adjusted odds ratios in relation to cohabitants without ApoE4 (reference group). Stars indicate level of statistical significance (≤ 0.05, ** ≤ 0.001, *** ≤ 0.0005)*

The separate odds ratio for those who were ex-cohabitants both in midlife and at follow-up and in addition carried the ApoE4 allele was a high 25.55 (5.7 - 114.5, P=<0.0005), in relation to the reference group (cohabitant non-ApoE4-carriers at both time points).

6.2 STUDY 2

We found higher levels of hopelessness *in midlife*, but not at follow-up, to be associated with cognitive impairment at follow-up; the adjusted odds ratio for each step of the five-level hopelessness scale was 1.30 (95% confidence interval 1.11-1.51, P = 0.001) for any cognitive impairment and 1.37 (95% 1.05-1.78, P = 0.020) specifically for Alzheimer's disease. These associations remained significant also after the final adjustments for depressive feelings and for hopelessness at follow-up.

Also when measured at follow-up, feelings of hopelessness were significantly more pronounced among participants who were to be diagnosed as cognitively impaired, but this association was largely eradicated when adjustments were made, except for Alzheimer's disease as separate outcome (OR 1.38, 95% confidence interval 1.00 – 1.90, P = 0.051). This association also became statistically non-significant after final adjustment for depressive feeling at the re-examination.

We found no significant differences in scores of hopelessness between baseline and follow-up *within* any of the outcome groups, including within the cognitively healthy reference group. In other words, persons who in later life would be found with cognitive impairment, had higher levels of hopelessness, but this difference in hopelessness feelings between the groups existed already at midlife. The pattern of these results is illustrated in Fig. 14.

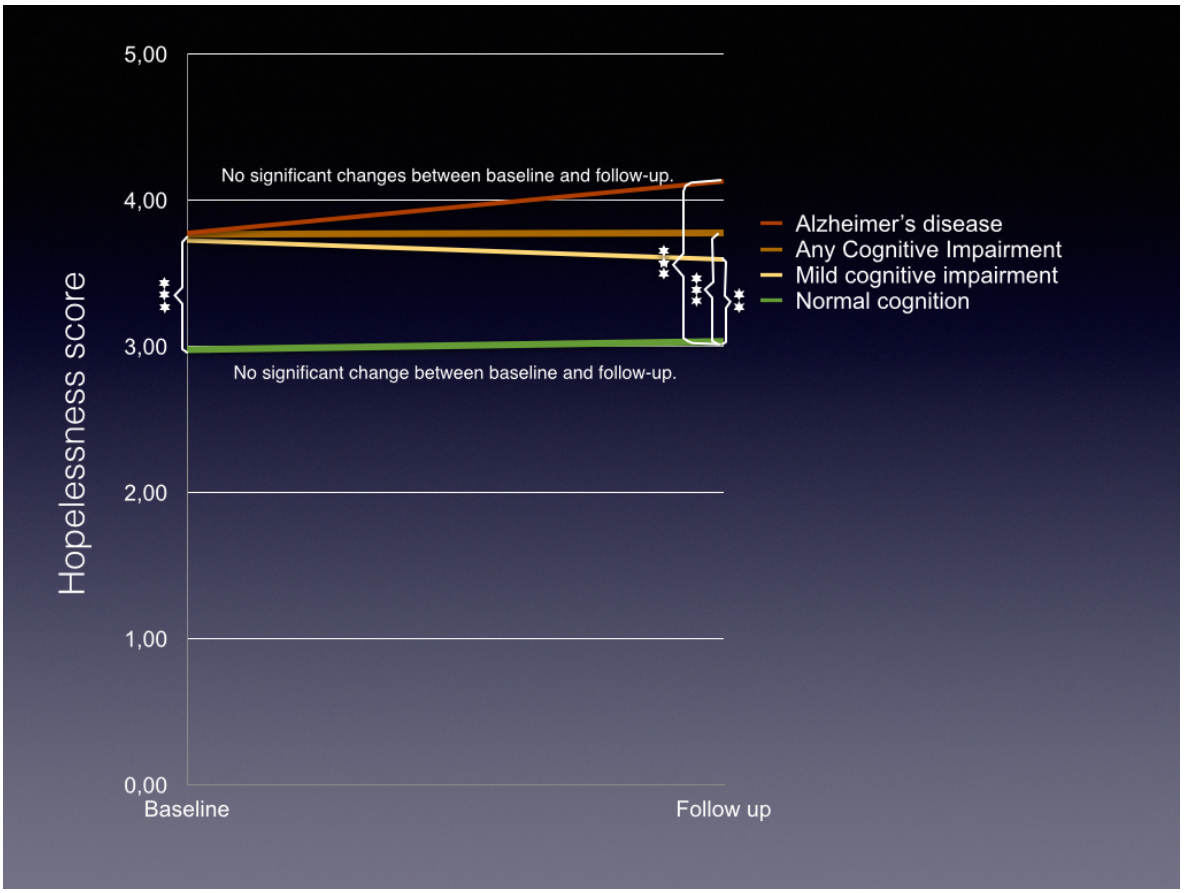

Fig 14. Levels of hopelessness at baseline and follow-up, stratified by cognitive health status at follow-up.

When we stratified the participants into ApoE4 carriers and non-carriers, the association between midlife levels of hopelessness and cognitive impairment in later life was more pronounced among ApoE4 carriers, especially when Alzheimer's disease was the outcome. With non-carriers with low levels of hopelessness at midlife as reference, this subgroup had an adjusted odds ratio of 8.08 (3.1-21.1). After final adjustment also for depressive feelings during the follow-up, the corresponding odds ratio was reduced to 6.48 (95% confidence interval 2.4-17.5, P = < 0.0005).

6.3 STUDY 3

We found that persons who lived alone and in addition felt lonely in midlife had a higher risk of a dementia diagnosis decades later compared to cohabitants, both to cohabitants who felt alone and especially to those who did not. Another consistent result in this population was the absence of a risk increase for persons who lived with a partner, but who felt lonely. These results were consistent whether the calculations were based on registered dementia diagnoses or if also data from the clinical diagnoses at the re-examinations were included in the analysis, and they were largely independent of potential confounding variables.

The log rank Kaplan-Meyer analysis, based on incidence of verified dementia diagnoses in registers, showed a statistically significant overall difference in the survival distributions between the four groups, $\chi^2(3) = 18.50$, $p < 0.0005$ (Mantel-Cox log rank analysis). Pairwise comparisons (Mantel-Cox) showed that the risk of a dementia diagnosis after the baseline measurements was significantly shorter for persons who both lived alone and felt lonely, both in relation to cohabitants who did or did not feel lonely ($\chi^2 = 6.86$, $p = 0.009$, and $\chi^2 = 11.71$, $p = 0.001$, respectively), but not in relation to non-cohabitants without feelings of loneliness. The non-cohabitants without feelings of loneliness differed in relation to the two cohabitant groups (with and without feelings of loneliness), but to a weaker extent ($\chi^2 = 9.54$, $p = 0.002$ and $\chi^2 = 3.81$, $p = 0.051$, respectively). Cohabitants who felt alone did not significantly differ from cohabitants without such feelings. The pattern of these results is illustrated in Fig. 15.

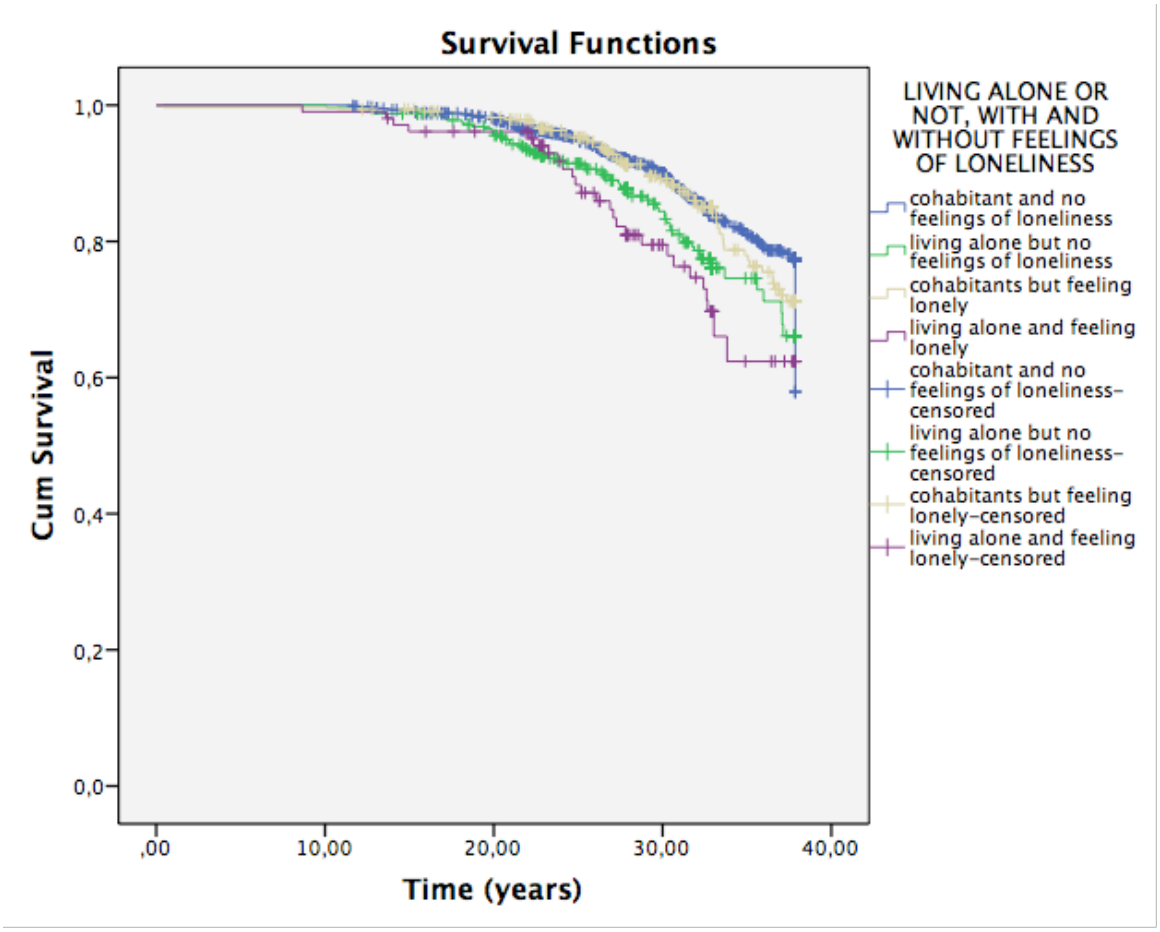

Fig 15. Survival functions for incidence of dementia among persons who lived and/or felt alone.

From Kaplan-Meyer log rank analysis based on register data.

Cox regression analyses with adjustments did not change this pattern and the hazard ratio (HR) for a dementia diagnosis for the group who in midlife had both lived alone and felt lonely was statistically significant in all models, also after adjustment for feelings o hopelessness at baseline (1.67, CI 1.06 – 2.61, P = 0.025 after full adjustments).

When corresponding analyses were performed exclusively on the 1511 participants who participated in any or both re-examinations, the pattern of results was maintained, but statistical significance was lost. When re-examination data and registry data were combined, the results were almost identical to the calculation based only on registry data (HR 1.65, 95% CI 1.08 – 2.53, P = 0.022). When the calculations were stratified for gender, based on the combined information from both registers and re-examinations, the pattern was reproduced separately within each gender.

6.4 STUDY 4

In this study we found a significant immediate increase in serum BDNF levels in healthy older individuals after a 35-minute physical exercise session, but not in the same individuals when they participated in either cognitive training or mindfulness practice for the same duration of time. The mean post-intervention BDNF level was 22.5 ± 0.99 ng/mL after the physical exercise session, compared to 19.2 ± 1.17 ng/mL at baseline ($p = 0.004$, paired t-test, Cohen's $d = 0.75$). Figure 16 depicts these changes in BDNF levels from baseline in relation to each of the three post-intervention time points (at 0, 20 and 60 minutes), and in addition to the average of these three post-intervention measures.

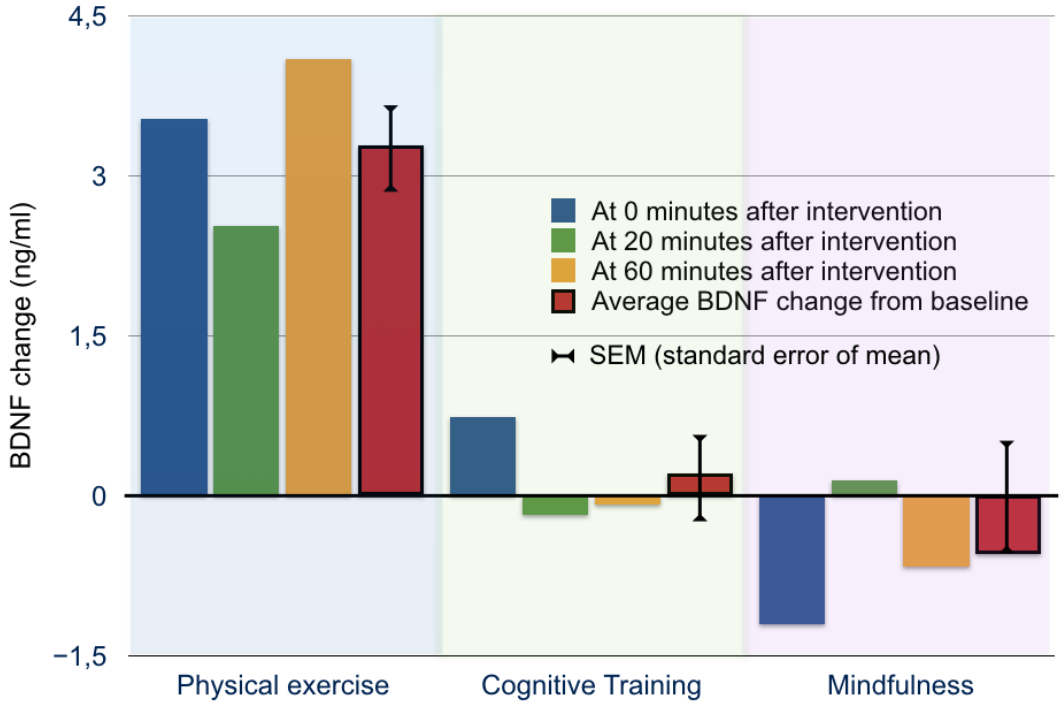

Fig 16. BDNF level changes from baseline after physical exercise, cognitive training and mindfulness practice.

Bars represent the difference in ng/ml serum between baseline and each post-intervention time point, in addition to average change.

We also found considerable individual variation in BDNF responses across the nineteen individuals. When we in post-hoc analyses explored possible sources behind this variation we

found a negative correlation with BDNF level at baseline and the degree to which BDNF levels increased during the intervention (r_{xy} -0.49, p = 0.03), meaning that participants with higher baseline levels of BDNF tended to have a smaller BDNF response. We also found a significant correlation between working memory performance and post-intervention BDNF values ($r_s = 0.50$, p = 0.03) after physical exercise, but not to basal levels or to degree of BDNF change in our participants (Table 3). When we, according to the hypothesis of a ceiling effect for the BDNF response, used tertiles to stratify participants according to their baseline BDNF values, we found a significant correlation with working memory performance also for BDNF change within the subgroup with the lowest baseline values ($r_{sy} = 0.89$, p = 0.02) – but still only in the physical exercise condition (Table 3).

Intervention		**Physical exercise**			**Cognitive training**			**Mindfulness**		
BDNF outcomes		Baseline level	Response	Post-interv level	Baseline level	Response	Post-interv level	Baseline level	Response	Post-interv level
All participants (N=19)		0.25 p = 0.31	0.22 p = 0.38	**0.50** p = **0.03**	0.08 p = 0.74	0.09 p = 0.72	0.03 p = 0.90	-0.12 p = 0.63	-0-13 p = 0.60	-0.09 p = 0.72
Stratified by baseline BDNF	Participants with lower levels (N=6)	-0.60 p = 0.21	**0.89** p = **0.02**	0.71 p = 0.11	0.07 p = 0.88	0.36 p = 0.43	0.32 p = 0.48	-0.03 p = 0.96	-0.20 p = 0.70	-0.37 p = 0.47
	Participants with higher levels (N=6)	0.71 p = 0.11	0.31 p = 0.54	0.77 p = 0.07	0.26 p = 0.63	-.66 p = 0.16	-0.43 p = 0.40	0.26 p = 0.62	-0.37 p = 0.47	0.14 p = 0.79

Table 3. Correlations between working memory performance and BDNF levels at baseline, BDNF level increases and BDNF post-intervention levels for the different interventions, stratified according to baseline BDNF levels.

Numbers are coefficients from Spearman correlations with exact p-values beneath.

The stratification of participants into lower versus higher baseline levels of BDNF was according to first and third tertiles.

It should be pointed out that these correlations were based on only nineteen subjects, and even fewer after the stratification shown in the lower part of Table 3. Although the participants were the same across conditions, this means that the statistical strength of the cross over design does not fully apply to these calculations, in contrast to the main calculations. The pattern of results could therefore be of greater interest than any single association, i.e. that strong associations with working memory performance were all found in the single condition where we also showed a main effect from the intervention.

7 DISCUSSION

As the results from each of the four studies are separately discussed in paper 1-4, the main purpose of this chapter is to integrate the results from these studies and discuss their possible implications for cognitive health and possible underlying mechanisms.

7.1 SOCIO-EMOTIONAL FACTORS

7.1.1 Cohabitants and non-cohabitants

Study 2-4 emanated from the results of the first study and the questions they raised. In that study we showed that living without a partner at midlife was associated with approximately a doubled risk in later life of being diagnosed with mild cognitive impairment or dementia, averagely twenty years later. When we compared persons who had been living alone both at midlife and in later life with cohabitants at both time points, the associations were even stronger. The credibility of these results was strengthened by the similar associations we found for mild cognitive impairment and Alzheimer's disease as independent outcomes.

Another important feature in these results were the differences *within* the non-cohabitant group, with a marked risk increase for widowed persons as compared to singles, defined as persons who had never been married or lived with a partner. Previous studies that found a risk increase from living alone[152,153]had interpreted the association as a possible effect of less cognitive stimulation from living alone, i.e. an interpretation related to the brain reserve hypothesis[104]. We could not exclude this as a possible explanation, especially for the risk increase for the non-cohabitant group as a whole. But the largest contribution to the risk increase among non-cohabitants did not come from the singles, but from the widowed, i.e. from participants who in most cases had been cohabitants for a substantial portion of their lives. At least in a life-course perspective, this did not seem to support the brain reserve hypothesis as a full explanation for the cohabitant advantage. Could the emotional consequences of losing a partner be an alternative or additional explanation to account for the increased risk we saw especially for the widowed group? Other studies have shown that negative feelings, e.g. global depressive feelings, feelings of hopelessness and feelings of loneliness had been found to increase the risk of mortality [154-156], morbidity [154,157-159] and also specifically cognitive impairment in later life [121,160-170]. Thus, did the risk increase we saw reflect the emotional consequences of being widowed already in midlife?

7.1.2 Feelings of hopelessness

When we consulted the CAIDE database we found that feelings of hopelessness had been measured at both midlife and follow-up by two questions. In addition, the same two questions had been used in at least three other studies that found an increased risk of mortality and carotid atherosclerosis [171], and they had also been found as a better predictor in these regards than global depression [172]. Others have suggested that hopelessness may be a central dimension in depression of equal or greater relevance than global depression for health and mortality [157] [173], including risk of suicide [174].

When we compared levels of hopelessness between widowed and non-widowed, we found that such feelings were indeed more common in the widowed group (unpublished results), but we also saw that such feelings were not uncommon among other participants, independent of cohabitant status. This observation was also in line with an epidemiological study that later reported a relatively high prevalence of hopelessness feelings in Eastern Finland from where our population had been sampled [175]. When we calculated the associations between feelings of hopelessness at baseline and cognitive health at follow-up, it confirmed our hypothesis that hopelessness increased the risk of dementia independently of being widowed or not.

As hopelessness was measured in exactly the same way both at baseline and during follow-up it also gave us a unique opportunity to investigate whether such depressive feelings reflect a prodromal or a causal relation with cognitive impairment. Many studies report that depression is a common dementia comorbidity [176,177], and that the two conditions in addition may be mechanistically linked[178]. But very few studies have actually followed individuals over a sufficiently long period of time to substantiate the claim that depression is a prodromal symptom of dementia. One of the few research groups that studied development of depression during the prodromal phase, did not find depressive feeling to increase at all [170], and in another population "not noticeable" so [179]. Compared to our study, we had a considerably longer follow-up time (averagely 21 years, compared to 11 and 8-9 years in these other studies). The long pre-clinical phase before a dementia diagnosis would in that sense put us in an even better position to address the causality versus prodromal hypotheses.

In our study we found that persons with a dementia diagnosis had higher levels of hopelessness feelings at the time they were diagnosed, compared to persons who were cognitively healthy at follow-up, consistent with depression as a prodromal symptom. But when we traced the same persons back to when they were only in midlife, we found the same difference already then, and with no noticeable increases between the two time points. (From Figure 14, an increase can be seen only among persons who later would develop Alzheimer's disease, but also this difference was not close to being statistically significant.) In summary, these findings should add to the credibility that depressive feelings are somehow implicated in the causal mechanisms, rather than only a prodromal symptom, in the development of both mild cognitive impairment and Alzheimer's disease.

Compared to the studies by Wilson et al[170] [179], we had not screened the participants for dementia or measured cognitive performance at baseline. With participants only in midlife and a mean survival time of averagely only 4-5 years[79], we could at least exclude the possibility that any of them would have had early clinical dementia averagely twenty years prior to follow-up. But we could not with certainty exclude the possibility that some of them had been in an early pre-clinical phase already at baseline, especially for those with a shorter follow-up time than average. In that case, the association we found could still reflect reverse causation, at least to some degree. We tested this possibility by dividing the persons in a younger (35-50 years) and an older (50+) subgroup from baseline. When we calculated the

associations separately for these groups, we found no support for this possibility. On the contrary, the association was somewhat stronger for those who were only between 35-50 years at baseline.

7.1.3 Feelings of loneliness and cohabitant status

The fact that feelings of hopelessness were common also among non-widowed participants and the fact that we had adjusted the original associations between cohabitant status and dementia also for feelings of hopelessness, suggest the involvement of other factors behind this association. In addition we did also see a risk increase for other non-cohabitant subgroups. If emotions can have a long-term impact on cognitive health, feelings of loneliness seemed a natural candidate behind a risk increase for persons who live alone. It seemed also possible that such feelings could have been more pronounced after losing a partner than for persons who were singles, which could contribute to the elevated risk for this non-cohabitant subgroup. Similar to feelings of hopelessness, feelings of loneliness had in other studies been found to increase the risk of mortality[180], morbidity[181] and dementia[121]. In line with previous studies [182] we noticed that feelings of loneliness were not exclusively found in persons who lived alone. From a statistical point of view this was also a necessary condition in order to separate their long-term associations, if any, with cognitive health in later life. In an attempt to achieve this aim, we collapsed the three non-cohabitant categories (divorced, widowed and singles) in order to use the same dichotomous cohabitant – non-cohabitant variable we had used in the first study. Participants were also categorized into two groups of relatively higher or lower levels of loneliness feelings, as described in the Methods section. By combining these two dichotomous variables we could categorize the participants into four groups (cohabitants and non-cohabitants with high or low levels of loneliness) and relate to the dichotomous outcome of either having a subsequent dementia diagnosis or not. The results indicated a more complicated association to the risk of a dementia diagnosis than we had expected. While the combined status of living alone and feeling alone had a consistent and robust association with the risk of a subsequent dementia diagnosis, indicating an additive relationship between the objective and subjective aspects of alone-ness, there was no indication that feeling lonely increased dementia – if the participant lived had lived with a partner at baseline. Expressed in other terms; living with a partner seemed to protect against the otherwise adverse health effects of feeling alone. Although statistical significance was only consistently found for non-cohabitants who in addition felt lonely in relation to the reference group, the same pattern re-emerged whether we based the analyses on register data, on clinical evaluations during the re-examinations, or when we combined the two sources of information. The unadjusted log rank Kaplan-Meyer analysis and the Cox regression also gave a very similar picture, and the pattern was not dramatically affected by adjustments for different potential confounders. The same pattern was also repeated when we made separate analyses on men and women.

Some of these results may seem counter-intuitive. Why was there an increased dementia risk from living alone if these persons did not also feel lonely? Although many studies report that

living alone, social isolation and feelings of loneliness all contribute to increased mortality [180], few studies have attempted to directly investigate how the two are related. One study investigated mortality risk over seven years with a similar aim as ours to investigate the relation between social isolation, measured through contact with family and friends and social participation, and feelings of loneliness [156]. Both variables predicted mortality separately, but the association with social isolation was more robust and remained also after adjustment for loneliness, while loneliness did not when social isolation and other factors were was adjusted for. Despite the differences between the studies, in follow-up time, the predictor (social isolation versus living alone) and the outcomes (dementia versus mortality), being alone emerged as the strongest predictor in both studies. This is in contrast to a study by Wilson et al [121], who found that feelings of loneliness increased the risk of Alzheimer's disease, also after adjustment for social isolation.

The focus in our study was on living alone, rather than social network size or number of friends. In a recent meta-analysis on social relationships and dementia risk, Kuiper et al [120] concluded that the element of social interaction, rather than network size, was the most relevant aspect in social relationships and the relation with cognitive health. One meta-analysis that compared associations with mortality from living alone and the size of social networks/social isolation as separate dimensions, also found that living alone had a somewhat stronger association [180].

7.2 THE RELEVANCE OF GENERAL HEALTH MECHANISM FOR AD

In a wider context, it may seem surprising, or even suspicious, that not only living alone, the loss of a partner, and feelings of hopelessness and loneliness, i.e. factors in focus of this thesis, but a host of other factors, including depression, stress, high blood pressure in midlife, high cholesterol, and low level of physical activity, have all been reported to increase the risk of Alzheimer's disease and other types of dementia. As mentioned in the chapter 3.3.7, not only are many of these factors associated with Alzheimer's disease and other types of dementia, but to many other diseases as well. This seems to indicate the existence of a common mechanism for several diseases that could also be relevant for the risk of Alzheimer's disease and other types of dementia. In that chapter I proposed that the immune system could be involved in such a mechanism; when weakened or dysfunctional, whether due to stress, depression, an already existing disease, long-term sleep deprivation, toxic exposures, frailty due to high age, or any other reason, the risk should increase for various ill health conditions to develop, due to immunological dysfunction and inefficiency [183]. The specific disease that will result in such a scenario would then, according to this hypothesis, be determined by specific vulnerabilities, where genetic factors could have an important role. A proper analogy to illustrate this hypothesis of general disease proneness leading to a specific disease, as determined by specific vulnerabilities, could be that of a chain, with some links stronger, others weaker. When under pressure, the chain will naturally break where the weakest link is. For a person with a genetic disposition to develop AD, that disease could be the more probable specific disease outcome. We found indirect support for this hypothesis

when we stratified persons according to ApoE4 status and whether they had been exposed to either the loss of a partner (Study 1) or feelings of hopelessness (Study 2). In both studies, and especially in Study 1, the ApoE4/risk factor combination lead to a dramatic risk increase for specifically AD, rather than mild cognitive impairment. A similar magnification effect of carrying the ApoE4 allele together with different non-genetic risk factors has also been reported by others[70]. One way to test this hypothesis further could be to investigate if also genetic vulnerabilities for other diseases magnify the risk of the associated disease, when such vulnerabilities are combined with factors that seem to magnify the risk of AD in ApoE4 carriers, especially factors that are known to impair immunological efficiency. Depressive feelings, traumatic experiences, extensive sleep problems, and chronic stress are factors with well-known immunological effects[114,116,117,139] that could be of special interest to investigate further with such an aim.

Although the immune system has developed through evolution over millions of years to the present level of perfection, it is common in AD research to focus on the other side of the coin, if immunological implications in AD are at all considered, namely on the effect of inflammation as a possible causal mechanism in AD – or even the idea that AD could be an auto-immune disease [184]. Inflammation is the common process through which the immune system attacks antigens and accomplishes repair after injury, in other words, accomplishes the task it is designed for. The idea that inflammation could have a causal role for the development of AD has probably been nourished by the fact that amyloid β accumulation has been observed as a precursor to clinical AD, and that inflammation and amyloid load have been found to coexist[185]. New findings, indicating that amyloid β could have antimicrobial properties, and could be even part of the innate immune system [82], could, in addition to other sources of doubt concerning the role of amyloid β[83] [81] [84], lead to a drastic re-interpretation of why inflammation and accumulation of beta-amyloids co-exist; that they both represent an immunological attempt to counteract continued development of an Alzheimer's disease process, probably in early phases of the disease. This interpretation is also consistent with the observation that many persons with large amounts of amyloid plaques have no other signs of AD or other cognitive impairments[81]. With an alternative interpretation, amyloid plaques in cognitively healthy persons may be remnants from previous successful attempts to stop the disease from developing further, as has also been suggested by others[186,187]. Taken together, these findings could justify a shift of focus into regarding the normal role of the immune system; to fix health problems, not to create them, as an alternative way to relate immunological function and Alzheimer's disease. From such an alternative perspective, development of clinical AD could reflect immunological inefficiency to accomplish this task, either due to factors that have weakened the immune system or in situations when the genetic odds are too overwhelming. The factors studied in this dissertation are closely related to factors that according to previous studies can cause immunological imbalances and inefficiency, such as grief, depression and chronic stress [116]. Such an effect on immunological functioning may be the reason why social and

emotional factors can affect the risk of Alzheimer's disease, especially when a presence of AoE4 makes the person more vulnerable to develop this specific disease.

7.3 BDNF: PART OF A SPECIFIC MECHANISM BEHIND ALZHEIMER'S DISEASE?

Even if such a broad general heath mechanism has relevance also for Alzheimer's disease on a macro level, it still contributes little to understand the specific mechanisms behind the initiation and development of the disease at the micro level. One of the core symptoms in AD, and possibly also in other neuropathological diseases [87], is synaptic dysfunction and loss [188]. Some studies have reported that toxic amyloid β oligomers may be responsible[189], but as neurotrophic factors are critically involved in synaptic repair and synaptogenesis[97], and as decreased BDNF expression has been found in brains of persons with AD[95], BDNF functionality could also be of interest as a possible key to better understand this disease. BDNF is of central importance for brain plasticity, where BDNF plays a key role, but plasticity is severely reduced or eliminated with Alzheimer's disease. Based on this, Lu et al[188] have even suggested that BDNF-based therapies against AD and other neuropathological conditions should be developed.

The fourth study is based on the assumption that BDNF functionality is relevant for cognitive health and dementia. It aimed to investigate the immediate effects on BDNF levels from different types of activities in healthy elderly persons. In spite of the theoretical relevance of BDNF for cognitive health, and the urgent priority to counteract cognitive decline in old age in an ageing world population, we were surprised that no one seemed to have performed a study of this kind before. We found that 35 minutes of physical activity increased BDNF levels on average 25%, but found no such effects from the other activities, cognitive training or mindfulness. In previous studies it had been assumed that increased BDNF levels in serum after physical exercise reflects increased BDNF levels in the brain. The pattern of our results led us to conclude that the immediate BDNF increases in serum from this exercise instead had a peripheral, not a CNS origin. In spite of its name (brain-derived neurotrophic factor), BDNF can be produced in a range of cells outside the nervous system, including in immune cells, in the kidney, in neuromuscular joints, and in salivary glands[190-192]. In addition, some studies have reported that brain BDNF does not pass the blood-brain-barrier (BBB) [193], that BDNF increases in the brain are not reflected in blood[194], or at least that the efflux from the brain into the blood stream is relatively slow[195]. It also seemed counter-intuitive, considering the role of BDNF for healthy brain functioning, that physical activity would stimulate the brain to produce BDNF to a higher extent than a cognitively demanding task would.

Another finding was that the variation we saw between individuals in the BDNF responses they had, and especially the levels after physical exercise, correlated with individual differences in working memory performance. As a high level of cognitive function should require good BDNF functionality in the brain, these two findings in combination could mean that BDNF functionality in one part of the body is a marker for the ability to produce BDNF

also in other parts, e.g. in the brain. This speculation, based on a correlation only among 18 individuals, of a systemic component needs to be further investigated. If the speculation is correct, and if the finding of a relatively slow BDNF efflux from the brain is correct[195], it should mean that an intervention over weeks and months could show that BDNF effects from cognitive training are also reflected in elevated BDNF levels in serum. As BDNF is central for brain plasticity, and if synaptic dysfunction and loss is an early event in AD, a dramatic consequence of this proposed hypothesis is that BDNF responsivity in serum after physical exercise could be marker of brain health, and possibly also an early marker of AD. The hypothesis could also be tested by investigating if persons who gain relatively more, in terms of cognitive performance improvements, also show a parallel response in terms of BDNF levels during the intervention period.

In conclusion, this study raised more questions than it answered and some of the results were unexpected. It scratched the surface of a possibly relevant micro level mechanism behind AD development and generated many ideas for future studies.

7.4 CONCLUSIONS

To live with someone may be beneficial for cognitive health in a long-tem perspective and feelings of loneliness can dramatically increase the risk of dementia if persons who feel lonely also live alone. To live alone after having lost a partner was associated with a unique risk increase, as compared to having always lived without a partner. Hopelessness, a central emotional dimension in depression, was also associated with a higher dementia risk. The fact that not only social and emotional factors can increase the risk of dementia, but a host of other factors, and that many of these are also related to other bad health outcomes, could indicate a general systemic effect that results in specific diseases as a result of specific vulnerabilities where genetic dispositions could play a role. The dramatic risk increase we found when ApoE4 status was related to social and emotional factors in two of the studies seems to support the relevance of such a general health mechanism also for Alzheimer's disease. On the micro level, BDNF functionality seems as a promising field to generate and test new hypothesis concerning early dementia development, early detection and potentially also to discover new therapeutic interventions.

Medical research usually has a focus on disease and disease-related factors. If loneliness, living alone and depressive feelings imply increased dementia risk, the other side of the coin should mean that close relations and interactions, and positive feelings promote cognitive health.

7.5 FUTURE DIRECTIONS

One of the factors I have only touched upon in this thesis, is sleep. Sleep is of critical importance for immunological efficiency, and both sleep deprivation and poor sleep quality are associated with increased risk of mortality and a range of health disorders [114]. If the common and non-specific health mechanism proposed here, with the immune system as a suggested key agent, is indeed relevant also for the development of Alzheimer's disease and

other dementias, sleep quality should show a long-term association with cognitive health in later life. Although many cross-sectional studies exist that describe sleep disorders in persons who have dementia, no well-designed prospective studies with sufficient follow-up time were found. Such studies could be of value to understand and prevent dementia, and also contribute to evaluate the role of immune function for dementia development.

Participant bias may be seriously underestimated as a source of error in prospective population studies. The common procedure to describe baseline differences, if such data are at all available, between participants and non-participants, is, based on the results in Study 3, clearly insufficient to account for this. Linkage of registers with careful verification of diagnoses, or other outcome variables of interest, would probably dramatically increase the validity of results from prospective cohort studies.

Inconsistent evidence and therapeutic failures could mean that amyloid-based theories on the causal mechanisms behind AD need to be revised and/or that other mechanisms may be more relevant. The role of neurotrophic factors for synaptic dysfunction and loss of synapses in Alzheimer's disease should be explored further with the aim to find new mechanisms of relevance for understanding and possibly also develop therapeutic strategies against AD and other dementias.

8 ACKNOWLEDGEMENTS

Someone told me it is not uncommon for recipients of a thesis to first go this chapter to find out if they are included. This is scary to think about, because there are so many I am grateful to, and it will be impossible not to forget to mention someone who should have been mentioned. Still I want to start to express my gratitude to some persons who will probably never read this thesis. First I think of all the participants in the different studies, in the CAIDE Study and in the SAGE Study in Växjö. Without your efforts and sacrifices, this thesis would not have been possible – and not a lot of other studies either.

I have had some great teachers that I think have indirectly, but strongly, contributed to this thesis. I am especially thinking of Alf Kristiansson, my teacher in elementary school who gave me confidence to continue with higher education, the late professor Gudmund Smith who encouraged me to dare to write in a personal way and to avoid scientific jargon, and to Jarl Risberg who was both my boss and my teacher and who introduced me to brain research in his rCBF (regional cerebral blood flow) lab in Lund.

Among probable readers, and among persons who have had a more direct influence on me and this thesis, I first want to thank my supervisors, and most of all my main supervisor Miia Kivipelto, and for many things: for always being so positive, encouraging and supportive, for having given me so much freedom to develop and test ideas – just what a curious person like me needs to be highly motivated – and for her ability to tell me I am wrong, when I am, in such a nice way that even a stubborn person like myself can accept it; Abdul Mohammed for inviting me to NVS the first time, for his sense of linguistic elegance that have helped me develop my writing, and for all the long and enlightening discussions we have had both about academic matters and other things; Bengt Winblad for having believed in me from the beginning and supported me all the way, for personifying a balance between warmth and ambition that is constructive for people around him, and for the childish, spontaneous and overwhelming happiness he still feels and expresses with every new publication. I also want to thank my mentor and friend Mats Wahlgren for good advice and support when I have needed and for enlightening discussions about the nature of science and about rules and conventions in the academic world.

Then we have all the others, and here is the point where I will miss to mention people who should be mentioned. Many persons have helped me develop my thinking through eye-opening academic conversations, even if some of them may not know it, and persons who definitely belongs to this category are Åke Wallin, Kirk Daffner, Pär Lundqvist, Laura Fratiglioni, Tiia Ngandu, Suvi Rovio, Ove Almqvist, Johan Fastbom, Babak Hoosmand, Mirza Baig, Carl-Axel Hageskog, Anna Herbert, Chengxuan Qiu, Marti Parker, Nancy Pedersen, Anna Dahl Aslan, Lucas Morin, Alina Solomon, Alina Codita, Giola Santoni, Hui-Xin Wang, Shireen Sindi, Weili Xu, Giulia Grande, Mårten Lagergren, Pär Schön, Lars Sonde, Lars Andersson and of course Pingo (Ingemar Kårehult).

I also want to thank members and friends in the Nordic Brain Network (NBN), including our members on the Finnish side at University of Eastern Finland in Kuopio and National Institute of Public Health in Helsinki. It has been a privilege to have access to such a fantastic database as CAIDE and the support from the Finnish side, including the discussions we have had and the information I have received from Eeva-Liisa Helkala, Aulikki Nissinen, Alina Solomon, Jenny Kulmala, Hilkka Soininen and Minna Rusanen, has been invaluable for the studies I have worked on. I feel especially indebted to Eeva-Liisa Helkala who was the psychologist who ensured that so many variables of psychological relevance were measured in the CAIDE Study already at baseline.

It has also been a privilege to belong to the Medical Sector group at ARC, and to ARC at large. I can hardly think of a better and friendlier environment to develop ideas, where you can get so much stimulation and inspiration by just talking to other persons in your room, across the corridor or at seminars, and through the integration with Demenscentrum and Äldrecentrum, also get insights from people who work with elderly people in a more direct way than researchers often do. And finally, whatever people say these days, Karolinska is a great place to be, with an enormous range of courses, seminars and other opportunities to learn from some of the best experts in the world. I have always felt privileged to work in one of the best ageing research groups in the world and to be a PhD student at one of the best medical universities in the world.

During my years at ARC I have also continued to work at the Linnaeus University in parallel, and I want to thank that university for accepting this arrangement. I have always felt encouraged to succeed with my PhD studies, by the Head of Psychology department, Judit Lindqvist, by my colleagues, by Inger Törnqvist, the former director of the HR department, by rector Stephen Hwang and by many others.

Finally, but not least, I am grateful to those who have sponsored my studies financially. Without the generous grant from the Kamprad Family Foundation I and my colleagues in Växjö would not have been able to realize the BDNF study on healthy elderly persons, and I am both grateful to the foundation and to Lena Fritzén and Anna Carlström at the foundation who have always been so encouraging and enthusiastic about this project. Many have contributed to the financing of the CAIDE Study and some of them are Academy of Finland, Special Government Funding (EVO, Finland), Kuopio University Hospital and the Carita Foundation. I am also grateful for the grants I have received from Demensfonden and Stohne Foundation that have helped to pay for expenses in relation to my studies.

At the very end I want to thank all those I forgot to mention, but should have been mentioned.

9 REFERENCES

1 Eisenberg DTA, Kuzawa CW, Hayes MG. Worldwide allele frequencies of the human apolipoprotein E gene: climate, local adaptations, and evolutionary history. *Am J Phys Anthropol* 2010;**143**:100–11. doi:10.1002/ajpa.21298

2 Rajaratnam JK, Marcus JR, Flaxman AD, *et al.* Neonatal, postneonatal, childhood, and under-5 mortality for 187 countries, 1970–2010: a systematic analysis of progress towards Millennium Development Goal 4. *The Lancet* 2009;**375**:1988–2008. doi:10.1016/S0140-6736(10)60703-9

3 Hogan MC, Foreman KJ, Naghavi M, *et al.* Maternal mortality for 181 countries, 1980-2008: a systematic analysis of progress towards Millennium Development Goal 5. *Lancet* 2010;**375**:1609–23. doi:10.1016/S0140-6736(10)60518-1

4 Culpepper D. World Population Ageing. New York: : United Nations 2016. http://www.un.org/en/development/desa/population/publications/pdf/ageing/WPA2015_Report.pdf

5 Central Intelligence Agency. *The CIA World Factbook 2014*. Skyhorse Publishing, Inc. 2013. https://www.cia.gov/library/publications/the-world-factbook/

6 Christensen K, Doblhammer G, Rau R, *et al.* Ageing populations: the challenges ahead. *The Lancet* 2009;**374**:1196–208. doi:10.1016/S0140-6736(09)61460-4

7 Max Planck Institute for Demographic Research Germany, University of California, Berkeley USA, editors. Human Mortality Database. mortality.org.

8 Oeppen J, Vaupel JW. Demography. Broken limits to life expectancy. *Science* 2002;**296**:1029–31. doi:10.1126/science.1069675

9 Olshansky SJ, Carnes BA, Cassel C. In search of Methuselah: estimating the upper limits to human longevity. *Science* 1990;**250**:634–40.

10 Wilmoth JR. Demography of longevity: past, present, and future trends. *Exp Gerontol* 2000;**35**:1111–29.

11 Harman D. Aging: Overview. *Ann N Y Acad Sci* 2001;**928**:1–21. doi:10.1111/j.1749-6632.2001.tb05631.x

12 Ruiz-Torres A, Beier W. On maximum human life span: interdisciplinary approach about its limits. *Adv Gerontol* 2005;**16**:14–20.

13 Carey JR. Life span: a conceptual overview. *Population and Development Review* 2003.

14 Fries JF. Aging, natural death, and the compression of morbidity. *N Engl J Med* 1980;**303**:130–5. doi:10.1056/NEJM198007173030304

15 Mathers CD, Stevens GA, Boerma T, *et al.* Causes of international increases in older age life expectancy. *Lancet* 2015;**385**:540–8. doi:10.1016/S0140-6736(14)60569-9

16 Lynch SM, Brown JS. Reconsidering mortality compression and deceleration: an alternative model of mortality rates. *Demography* 2001;**38**:79–95. doi:10.1353/dem.2001.0007

17 Crimmins EM, Beltrán-Sánchez H. Mortality and morbidity trends: is there compression of morbidity? *Journals of Gerontology Series B-Psychological Sciences and Social Sciences* 2010;**66**:75–86. doi:10.1093/geronb/gbq088

18 Walter S, Beltrán-Sánchez H, Regidor E, *et al.* No evidence of morbidity compression in Spain: a time series study based on national hospitalization records. *Int J Public Health* 2016;:1–10. doi:10.1007/s00038-016-0829-5

19 Nyberg L, Lövdén M, Riklund K, *et al.* Memory aging and brain maintenance. *Trends Cogn Sci* 2012;**16**:292–305. doi:10.1016/j.tics.2012.04.005

20 Han L, Gill TM, Jones BL, *et al.* Cognitive Aging Trajectories and Burdens of Disability, Hospitalization and Nursing Home Admission Among Community-living Older Persons. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 2016;**71**:766–71. doi:10.1093/gerona/glv159

21 Viña J, Borrás C, Miquel J. Theories of ageing. *IUBMB Life* 2007;**59**:249–54. doi:10.1080/15216540601178067

22 Honig LS, Kang MS, Cheng R, *et al.* Heritability of telomere length in a study of long-lived families. *Neurobiol Aging* 2015;**36**:2785–90. doi:10.1016/j.neurobiolaging.2015.06.017

23 Karlseder J, Smogorzewska A, de Lange T. Senescence Induced by Altered Telomere State, Not Telomere Loss. *Science* 2002;**295**:2446–9. doi:10.1126/science.1069523

24 Harris SE, Martin-Ruiz C, Zglinicki von T, *et al.* Telomere length and aging biomarkers in 70-year-olds: the Lothian Birth Cohort 1936. *Neurobiol Aging* 2012;**33**:1486.e3–1486.e8. doi:10.1016/j.neurobiolaging.2010.11.013

25 Martin Ruiz CM, Gussekloo J, Heemst D, *et al.* Telomere length in white blood cells is not associated with morbidity or mortality in the oldest old: a population‐based study. *Aging Cell* 2005;**4**:287–90. doi:10.1111/j.1474-9726.2005.00171.x

26 Bischoff C, Petersen HC, Graakjaer J, *et al.* No Association Between Telomere Length and Survival Among the Elderly and Oldest Old. *Epidemiology* 2006;**17**:190–4. doi:10.1097/01.ede.0000199436.55248.10

27 Yaffe K, Lindquist K, Kluse M, *et al.* Telomere length and cognitive function in community-dwelling elders: Findings from the Health ABC Study. *Neurobiol Aging* 2011;**32**:2055–60.

28 Harris SE, Deary IJ, MacIntyre A, *et al.* The association between telomere length, physical health, cognitive ageing, and mortality in non-demented older people. *Neuroscience Letters* 2006;**406**:260–4. doi:10.1016/j.neulet.2006.07.055

29 Boccardi V, Pelini L, Ercolani S, *et al.* From cellular senescence to Alzheimer's disease: The role of telomere shortening. *Ageing Research Reviews* 2015;**22**:1–8. doi:10.1016/j.arr.2015.04.003

30 Marioni RE, Harris SE, Shah S, *et al.* The epigenetic clock and telomere length are independently associated with chronological age and mortality. *International Journal of Epidemiology* 2016;**45**:424–32. doi:10.1093/ije/dyw041

31 Hannum G, Guinney J, Zhao L, *et al.* Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell* 2013;**49**:359–67. doi:10.1016/j.molcel.2012.10.016

32 Siegmund KD, Connor CM, Campan M, *et al.* DNA Methylation in the Human Cerebral Cortex Is Dynamically Regulated throughout the Life Span and Involves Differentiated Neurons. *PLoS ONE* 2007;**2**:e895. doi:10.1371/journal.pone.0000895

33 Kirkwood TBL. Understanding the Odd Science of Aging. *Cell* 2005;**120**:437–47. doi:10.1016/j.cell.2005.01.027

34 Damasio AR. *Self Comes to Mind: Constructing the Conscious Brain*. Pantheon Books 2010.

35 Reser JE. Alzheimer's disease and natural cognitive aging may represent adaptive metabolism reduction programs. *Behav Brain Funct* 2009;**5**:13. doi:10.1186/1744-9081-5-13

36 Gilbert CD, Sigman M. Brain States: Top-Down Influences in Sensory Processing. *Neuron* 2007;**54**:677–96. doi:10.1016/j.neuron.2007.05.019

37 Roberts SB, Rosenberg I. Nutrition and Aging: Changes in the Regulation of Energy Metabolism With Aging. *Physiological Reviews* 2006;**86**:651–67. doi:10.1152/physrev.00019.2005

38 Richter C. Oxidative damage to mitochondrial DNA and its relationship to ageing. *Int J Biochem Cell Biol* 1995;**27**:647–53.

39 Fontana L, Weiss EP, Villareal DT, *et al.* Long-term effects of calorie or protein restriction on serum IGF-1 and IGFBP-3 concentration in humans. *Aging Cell* 2008;**7**:681–7. doi:10.1111/j.1474-9726.2008.00417.x

40 Kachiwala SJ, Harris SE, Wright AF, *et al.* Genetic influences on oxidative stress and their association with normal cognitive ageing. *Neurosci Lett* 2005;**386**:116–20. doi:10.1016/j.neulet.2005.05.067

41 Peng C, Wang X, Chen J, *et al.* Biology of ageing and role of dietary antioxidants. *Biomed Res Int* 2014;**2014**:831841–13. doi:10.1155/2014/831841

42 Bjelakovic G, Nikolova D, Gluud LL, *et al.* Mortality in Randomized Trials of Antioxidant Supplements for Primary and Secondary Prevention: Systematic Review and Meta-analysis. *JAMA* 2007;**297**:842–57. doi:10.1001/jama.297.8.842

43 Qiu C, Fratiglioni L. A major role for cardiovascular burden in age-related cognitive decline. *Nat Rev Cardiol* 2015;**12**:267–77. doi:10.1038/nrcardio.2014.223

44 Rothwell PM, Johnston SC. Transient ischemic attacks: stratifying risk. *Stroke* 2006;**37**:320–2. doi:10.1161/01.STR.0000200555.89117.d2

45 Fraga MF, Ballestar E, Paz MF, *et al.* Epigenetic differences arise during the lifetime

of monozygotic twins. *Proc Natl Acad Sci USA* 2005;**102**:10604–9. doi:10.1073/pnas.0500398102

46 World Health Organization. World report on Ageing And Health. 2015. http://apps.who.int/iris/bitstream/10665/186463/1/9789240694811_eng.pdf

47 Santoni G, Angleman S, Welmer A-K, *et al.* Age-related variation in health status after age 60. *PLoS ONE* 2015;**10**:e0120077–10. doi:10.1371/journal.pone.0120077

48 Daffner KR. Promoting successful cognitive aging: a comprehensive review. *J Alzheimers Dis* 2009;**19**:1101–22. doi:10.3233/JAD-2010-1306

49 Yan Y-X, Liu Y-Q, Li M, *et al.* Development and evaluation of a questionnaire for measuring suboptimal health status in urban Chinese. *J Epidemiol* 2009;**19**:333–41. doi:10.2188/jea.JE20080086

50 Folstein M, Folstein S, McHugh P. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;**12**:189–98.

51 Flynn JR. Massive IQ gains in 14 nations: What IQ tests really measure. *Psychol Bull* 1987;**101**:171–91. doi:10.1037/0033-2909.101.2.171

52 Schaie KW. What Can We Learn From Longitudinal Studies of Adult Development? *Res Hum Dev* 2005;**2**:133–58. doi:10.1207/s15427617rhd0203_4

53 Finkel D, Reynolds CA, McArdle JJ, *et al.* Cohort differences in trajectories of cognitive aging. *Journals of Gerontology Series B-Psychological Sciences and Social Sciences* 2007;**62**:P286–94.

54 Wilson RS, Beck TL, Bienias JL, *et al.* Terminal cognitive decline: accelerated loss of cognition in the last years of life. *Psychosomatic Medicine* 2007;**69**:131–7. doi:10.1097/PSY.0b013e31803130ae

55 Muniz-Terrera G, van den Hout A, Piccinin AM. Investigating terminal decline: Results from a UK population-based study of aging. *Psychology and …* 2013.

56 Thorvaldsson V, Hofer S, Berg S, *et al.* Onset of terminal decline in cognitive abilities in individuals without dementia. *Neurology* 2008;**71**:882–7.

57 Laukka EJ, MacDonald SWS, Bäckman L. Terminal-Decline Effects for Select Cognitive Tasks after Controlling for Preclinical Dementia. *The American Journal of Geriatric Psychiatry* 2008;**16**:355–65. doi:10.1097/01.JGP.0000300630.24668.64

58 Winblad B, Amouyel P, Andrieu S, *et al.* Defeating Alzheimer's disease and other dementias: a priority for European science and society. *The Lancet Neurology* 2016;**15**:455–532. doi:10.1016/S1474-4422(16)00062-4

59 Qiu C, Strauss von E, Bäckman L, *et al.* Twenty-year changes in dementia occurrence suggest decreasing incidence in central Stockholm, Sweden. *Neurology* 2013;**80**:1888–94. doi:10.1212/WNL.0b013e318292a2f9

60 Satizabal CL, Beiser AS, Chouraki V, *et al.* Incidence of Dementia over Three Decades in the Framingham Heart Study. *N Engl J Med* 2016;**374**:523–32.

doi:10.1056/NEJMoa1504327

61 Wu Y-T, Fratiglioni L, Matthews FE, *et al.* Dementia in western Europe: epidemiological evidence and implications for policy making. *Lancet Neurol* Published Online First: 20 August 2015. doi:10.1016/S1474-4422(15)00092-7

62 Wang BW, Lu E, Mackenzie IRA, *et al.* Multiple pathologies are common in Alzheimer patients in clinical trials. *Can J Neurol Sci* 2012;**39**:592–9.

63 Schneider JA, Arvanitakis Z, Bang W, *et al.* Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology* 2007;**69**:2197–204. doi:10.1212/01.wnl.0000271090.28148.24

64 Day GS, Musiek ES, Roe CM, *et al.* Phenotypic Similarities Between Late-Onset Autosomal Dominant and Sporadic Alzheimer Disease: A Single-Family Case-Control Study. *JAMA Neurol* 2016;**73**:1125–32. doi:10.1001/jamaneurol.2016.1236

65 Harvey RJ, Skelton-Robinson M, Rossor MN. The prevalence and causes of dementia in people under the age of 65 years. *Journal of Neurology Neurosurgery and Psychiatry* 2003;**74**:1206–9.

66 Huang Y. Mechanisms linking apolipoprotein E isoforms with cardiovascular and neurological diseases. *Current Opinion in Lipidology* 2010;**21**:337–45. doi:10.1097/MOL.0b013e32833af368

67 Filippini N, MacIntosh BJ, Hough MG, *et al.* Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. *Proc Natl Acad Sci USA* 2009;**106**:7209–14. doi:10.1073/pnas.0811879106

68 Ewbank DC. The APOE gene and differences in life expectancy in Europe. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences* 2004;**59**:16–20.

69 Farrer LA, Cupples LA, Haines JL, *et al.* Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA* 1997;**278**:1349–56. doi:10.1001/jama.1997.03550160069041

70 Kivipelto M, Rovio S, Ngandu T, *et al.* Apolipoprotein E epsilon4 magnifies lifestyle risks for dementia: a population-based study. *J Cell Mol Med* 2008;**12**:2762–71. doi:10.1111/j.1582-4934.2008.00296.x

71 Hakansson K, Rovio S, Helkala E-L, *et al.* Association between mid-life marital status and cognitive function in later life: population based cohort study. *BMJ* 2009;**339**:b2462–2. doi:10.1136/bmj.b2462

72 Håkansson K, Soininen H, Winblad B, *et al.* Correction: Feelings of Hopelessness in Midlife and Cognitive Health in Later Life: A Prospective Population-Based Cohort Study. *PLoS ONE* 2015;**10**:e0142465. doi:10.1371/journal.pone.0142465

73 Forsell Y, Corder E, Basun H, *et al.* Depression and dementia in relation to apolipoprotein E polymorphism in a population sample age 75. *Biological Psychiatry* 1997;**42**:898–903.

74 McCarron MO, Delong D, Alberts MJ. APOE genotype as a risk factor for ischemic

cerebrovascular disease: a meta-analysis. *Neurology* 1999;**53**:1308–11. doi:10.1212/WNL.53.6.1308

75 Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 1992;**256**:184–5.

76 Hardy J. The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. *J Neurochem* 2009;**110**:1129–34. doi:10.1111/j.1471-4159.2009.06181.x

77 Hardy J, Bogdanovic N, Winblad B, *et al.* Pathways to Alzheimer's disease. *Journal of Internal Medicine* 2014;**275**:296–303. doi:10.1111/joim.12192

78 Jack CR, Knopman DS, Jagust WJ, *et al.* Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* 2010;**9**:119–28. doi:10.1016/S1474-4422(09)70299-6

79 Wolfson C, Wolfson D, Asgharian M, *et al.* A reevaluation of the duration of survival after the onset of dementia. *New England Journal of Medicine* 2001;**344**:1111–6.

80 Arriagada PV, Growdon JH, Hedley-Whyte ET, *et al.* Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* 1992;**42**:631–9.

81 Herrup K. The case for rejecting the amyloid cascade hypothesis. *Nat Neurosci* 2015;**18**:794–9. doi:10.1038/nn.4017

82 Soscia SJ, Kirby JE, Washicosky KJ, *et al.* The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. *PLoS ONE* 2009;**5**:e9505–5. doi:10.1371/journal.pone.0009505

83 Pimplikar SW. Reassessing the amyloid cascade hypothesis of Alzheimer's disease. *Int J Biochem Cell Biol* 2009;**41**:1261–8. doi:10.1016/j.biocel.2008.12.015

84 Karran E, Mercken M, Strooper BD. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nat Rev Drug Discov* 2011;**10**:698–712. doi:10.1038/nrd3505

85 Lee HG, Castellani RJ, Zhu X, *et al.* Amyloid-β in Alzheimer's disease: the horse or the cart? Pathogenic or protective? - Lee - 2005 - International Journal of Experimental Pathology - Wiley Online Library. *International journal of ...* 2005.

86 Hamos JE, DeGennaro LJ, Drachman DA. Synaptic loss in Alzheimer's disease and other dementias. *Neurology* 1989;**39**:355–61.

87 Scheff SW, Neltner JH, Nelson PT. Is synaptic loss a unique hallmark of Alzheimer's disease? *Biochem Pharmacol* 2014;**88**:517–28. doi:10.1016/j.bcp.2013.12.028

88 Lu B, Chow A. Neurotrophins and hippocampal synaptic transmission and plasticity. *J Neurosci Res* 1999;**58**:76–87.

89 Agranoff BW, Cotman CW, Uhler MD. *Synaptic Plasticity as a Model for Learning and Memory Research*. Philadelphia: : Lippincott-Raven 1999.

90 Zagrebelsky M, Korte M. Form follows function: BDNF and its involvement in sculpting the function and structure of synapses. *Neuropharmacology* 2014;**76 Pt C**:628–38. doi:10.1016/j.neuropharm.2013.05.029

91 Waterhouse EG, An JJ, Orefice LL, *et al.* BDNF promotes differentiation and maturation of adult-born neurons through GABAergic transmission. *J Neurosci* 2012;**32**:14318–30. doi:10.1523/JNEUROSCI.0709-12.2012

92 Lee J, Duan W, Mattson MP. Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. *J Neurochem* 2002;**82**:1367–75. doi:10.1046/j.1471-4159.2002.01085.x

93 Scharfman H, Goodman J, Macleod A, *et al.* Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. *Exp Neurol* 2005;**192**:348–56. doi:10.1016/j.expneurol.2004.11.016

94 Taliaz D, Stall N, Dar DE, *et al.* Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. *Mol Psychiatry* 2009;**15**:80–92. doi:10.1038/mp.2009.67

95 Buchman AS, Yu L, Boyle PA, *et al.* Higher brain BDNF gene expression is associated with slower cognitive decline in older adults. *Neurology* 2016;**86**:735–41. doi:10.1212/WNL.0000000000002387

96 Diamond MC, Rosenzweig MR, Bennett EL, *et al.* Effects of environmental enrichment and impoverishment on rat cerebral cortex. *J Neurobiol* 1972;**3**:47–64. doi:10.1002/neu.480030105

97 Mohammed A, Zhu S, Darmopil S, *et al.* Environmental enrichment and the brain. *Prog Brain Res* 2002;**138**:109–33. doi:10.1016/S0079-6123(02)38074-9

98 Pham TM, Winblad B, Granholm A-C, *et al.* Environmental influences on brain neurotrophins in rats. *Pharmacol Biochem Behav* 2002;**73**:167–75.

99 Cirulli F, Berry A, Bonsignore LT, *et al.* Early life influences on emotional reactivity: evidence that social enrichment has greater effects than handling on anxiety-like behaviors, neuroendocrine responses to stress and central BDNF levels. *Neurosci Biobehav Rev* 2010;**34**:808–20. doi:10.1016/j.neubiorev.2010.02.008

100 Branchi I, D'Andrea I, Sietzema J, *et al.* Early social enrichment augments adult hippocampal BDNF levels and survival of BrdU-positive cells while increasing anxiety- and 'depression'-like behavior. *J Neurosci Res* 2006;**83**:965–73. doi:10.1002/jnr.20789

101 Katzman R. Education and the prevalence of dementia and Alzheimer's disease. *Neurology* 1993;**43**:13–20.

102 Ngandu T, Strauss von E, Helkala E-L, *et al.* Education and dementia: what lies behind the association? *Neurology* 2007;**69**:1442–50. doi:10.1212/01.wnl.0000277456.29440.16

103 Marx J. Preventing Alzheimer's: A Lifelong Commitment? *Science* 2005;**309**:864–6.

104 Valenzuela M, Sachdev P. Brain reserve and dementia: a systematic review. *Psychol Med* 2006;**36**:441–54.

105 Schofield P. Alzheimer's disease and brain reserve. *Australasian Journal on Ageing* 1999;**18**:10–4.

106 Fratiglioni L, Paillard-Borg S, Winblad B. An active and socially integrated lifestyle in late life might protect against dementia. *Lancet Neurol* 2004;**3**:343–53.

107 Snowdon D, Greiner L, Markesbery W. Linguistic ability in early life and the neuropathology of Alzheimer's disease and cerebrovascular disease. Findings from the Nun Study. *Ann N Y Acad Sci* 2000;**903**:34–8.

108 Cadar D, Stephan BCM, Jagger C, *et al.* The role of cognitive reserve on terminal decline: a cross-cohort analysis from two European studies: OCTO-Twin, Sweden, and Newcastle 85+, UK. *Int J Geriatr Psychiatry* 2016;**31**:601–10. doi:10.1002/gps.4366

109 Lenehan ME, Summers MJ, Saunders NL, *et al.* Relationship between education and age-related cognitive decline: a review of recent research. *Psychogeriatrics* 2014;**15**:154–62. doi:10.1111/psyg.12083

110 Scarmeas N, Stern Y. Cognitive reserve: implications for diagnosis and prevention of Alzheimer's disease. *Curr Neurol Neurosci Rep* 2004;**4**:374–80.

111 Fratiglioni L, Wang H. Brain reserve hypothesis in dementia. *J Alzheimers Dis* 2007;**12**:11–22.

112 Ngandu T, Lehtisalo J, Solomon A, *et al.* A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER):a randomised controlled trial. *The Lancet* 2015;:1–9. doi:10.1016/S0140-6736(15)60461-5

113 Amieva H, Le Goff M, Millet X, *et al.* Prodromal Alzheimer's disease: successive emergence of the clinical symptoms. *Annals of Neurology* 2008;**64**:492–8. doi:10.1002/ana.21509

114 Irwin MR. Why sleep is important for health: a psychoneuroimmunology perspective. *Annu Rev Psychol* 2015;**66**:143–72. doi:10.1146/annurev-psych-010213-115205

115 Ader R. Developmental psychoneuroimmunology. *Dev Psychobiol* 1983;**16**:251–67. doi:10.1002/dev.420160402

116 Irwin MR. Human psychoneuroimmunology: 20 Years of discovery. *Brain Behav Immun* 2008;**22**:129–39. doi:10.1016/j.bbi.2007.07.013

117 Kiecolt-Glaser J, McGuire L, Robles T, *et al.* Emotions, morbidity, and mortality: New perspectives from psychoneuroimmunology. *Annu Rev Psychol* 2002;**53**:83–107.

118 Rovio S, Kåreholt I, Helkala E-L, *et al.* Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease. *The Lancet Neurology* 2005;**4**:705–11. doi:10.1016/S1474-4422(05)70198-8

119 Qiu C, De Ronchi D, Fratiglioni L. The epidemiology of the dementias: an update. *Current Opinion in Psychiatry* 2007;**20**:380–5.

120 Kuiper JS, Zuidersma M, Oude Voshaar RC, *et al.* Social relationships and risk of dementia: A systematic review and meta-analysis of longitudinal cohort studies. *Ageing Research Reviews* 2015;**22**:39–57. doi:10.1016/j.arr.2015.04.006

121 Wilson RS, Krueger KR, Arnold SE, *et al.* Loneliness and risk of Alzheimer disease. *Archives of General Psychiatry* 2007;**64**:234–40. doi:10.1001/archpsyc.64.2.234

122 Cacioppo JT, Hawkey LC. Perceived social isolation and cognition. *Trends Cogn Sci* 2009;**13**:447–54. doi:10.1016/j.tics.2009.06.005

123 Ownby R, Crocco E, Acevedo A, *et al.* Depression and risk for Alzheimer disease - Systematic review, meta-analysis, and metaregression analysis. *Archives of General Psychiatry* 2006;**63**:530–8.

124 Håkansson K, Soininen H, Winblad B, *et al.* Feelings of Hopelessness in Midlife and Cognitive Health in Later Life: A Prospective Population-Based Cohort Study. *PLoS ONE* 2015;**10**:e0140261. doi:10.1371/journal.pone.0140261

125 Johansson L, Guo X, Waern M, *et al.* Midlife psychological stress and risk of dementia: a 35-year longitudinal population study. *Brain* Published Online First: 20 May 2010. doi:10.1093/brain/awq116

126 Sindi S, Hagman G, Håkansson K, *et al.* Midlife Work-Related Stress Increases Dementia Risk in Later Life: The CAIDE 30-Year Study. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences* 2016;:gbw043. doi:10.1093/geronb/gbw043

127 Kivipelto M, Kivipelto M, Ngandu T, *et al.* Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Arch Neurol* 2005;**62**:1556–60. doi:10.1001/archneur.62.10.1556

128 Hassing LB, Dahl AK, Thorvaldsson V, *et al.* Overweight in midlife and risk of dementia: a 40-year follow-up study. *Int J Obesity* 2009;**33**:893–8. doi:10.1038/ijo.2009.104

129 Kivipelto M, Helkala E-L, Laakso MP, *et al.* Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. *BMJ* 2001;**322**:1447–51.

130 Buchman A, Boyle P, Wilson R, *et al.* Frailty is associated with incident Alzheimer's disease and cognitive decline in the elderly. *Psychosomatic Medicine* 2007;**69**:483–9.

131 Reitz C, Heijer den T, van Duijn C, *et al.* Relation between smoking and risk of dementia and Alzheimer disease - The Rotterdam Study. *Neurology* 2007;**69**:998–1005.

132 Alonso-Morán E, Orueta JF, Esteban JIF, *et al.* Multimorbidity in people with type 2 diabetes in the Basque Country (Spain): Prevalence, comorbidity clusters and comparison with other chronic patients. *Eur J Intern Med* 2015;**26**:197–202. doi:10.1016/j.ejim.2015.02.005

133 Lynch CP, Gebregziabher M, Axon RN, *et al.* Geographic and racial/ethnic variations in patterns of multimorbidity burden in patients with type 2 diabetes. *J Gen Intern Med* 2015;**30**:25–32. doi:10.1007/s11606-014-2990-y

134 Mangialasche F, Solomon A, Winblad B, *et al.* Alzheimer's disease: clinical trials and drug development. *Lancet Neurol* 2010;**9**:702–16.

135 Irwin M. Psychoneuroimmunology of depression: Clinical implications. *Brain Behav Immun* 2002;**16**:1–16. doi:10.1006/brbi.2001.0654

136 Leonard BE. Inflammation, depression and dementia: Are they connected? *Neurochem Res* 2007;**32**:1749–56. doi:10.1007/s11064-007-9385-y

137 Wilson C, Finch C, Cohen H. Cytokines and cognition - The case for a head-to-toe inflammatory paradigm. *Journal of the American Geriatrics Society* 2002;**50**:2041–56.

138 Green KN, Billings LM, Roozendaal B, *et al.* Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. *J Neurosci* 2006;**26**:9047–56. doi:10.1523/JNEUROSCI.2797-06.2006

139 Irwin MR, Miller AH. Depressive disorders and immunity: 20 years of progress and discovery. *Brain Behav Immun* 2007;**21**:374–83. doi:10.1016/j.bbi.2007.01.010

140 Rossi C, Angelucci A, Costantin L, *et al.* Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. *Eur J Neurosci* 2006;**24**:1850–6. doi:10.1111/j.1460-9568.2006.05059.x

141 Griesbach GS, Hovda DA, Gomez-Pinilla F. Exercise-induced improvement in cognitive performance after traumatic brain injury in rats is dependent on BDNF activation. *Brain Research* 2009;**1288**:105–15. doi:10.1016/j.brainres.2009.06.045

142 Mu JS, Li WP, Yao ZB, *et al.* Deprivation of endogenous brain-derived neurotrophic factor results in impairment of spatial learning and memory in adult rats. *Brain Res* 1999;**835**:259–65.

143 Thompson CB, Panacek EA. Research study designs: experimental and quasi-experimental. *Air Med J* 2006;**25**:242–6. doi:10.1016/j.amj.2006.09.001

144 ROSENTHAL R. THE EFFECT OF THE EXPERIMENTER ON THE RESULTS OF PSYCHOLOGICAL RESEARCH. *Prog Exp Pers Res* 1964;**72**:79–114.

145 Nederhof AJ. Methods of coping with social desirability bias: A review. *European Journal of Social Psychology* 1985;**15**:263–80. doi:10.1002/ejsp.2420150303

146 Kivipelto M, Solomon A, Ahtiluoto S, *et al.* The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER): Study design and progress. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2013;:–. doi:10.1016/j.jalz.2012.09.012

147 Dubois B, Hampel H, Feldman HH, *et al.* Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimer's & dementia : the journal of the Alzheimer's Association* 2016;**12**:292–323.

doi:10.1016/j.jalz.2016.02.002

148 Loftus EF. Make-believe memories. *Am Psychol* 2003;**58**:867–73. doi:10.1037/0003-066X.58.11.867

149 Vartiainen E, Jousilahti P, Alfthan G, *et al.* Cardiovascular risk factor changes in Finland, 1972-1997. *International Journal of Epidemiology* 2000;**29**:49–56.

150 Puska P. Communication with the population: the North Karelia Project experience. *J Hum Hypertens* 1995;**9**:63–6.

151 Vartiainen E, Puska P, Pekkanen J, *et al.* Changes in risk factors explain changes in mortality from ischaemic heart disease in Finland. *BMJ* 1994;**309**:23–7.

152 Helmer C, Damon D, Letenneur L, *et al.* Marital status and risk of Alzheimer's disease: a French population-based cohort study. *Neurology* 1999;**53**:1953–8.

153 van Gelder B, Tijhuis M, Kalmijn S, *et al.* Marital status and living situation during a 5-year period are associated with a subsequent 10-year cognitive decline in older men: The FINE study. *Journals of Gerontology Series B-Psychological Sciences and Social Sciences* 2006;**61**:P213–9.

154 Stamatakis KA, Lynch J, Everson SA, *et al.* Self-esteem and mortality: prospective evidence from a population-based study. *Annals of Epidemiology* 2004;**14**:58–65. doi:10.1016/S1047-2797(03)00078-4

155 Tilvis RS, Laitala V, Routasalo PE. Suffering from loneliness indicates significant mortality risk of older people. *Journal of Aging ...* 2011.

156 Steptoe A, Shankar A, Demakakos P, *et al.* Social isolation, loneliness, and all-cause mortality in older men and women. *Proc Natl Acad Sci USA* 2013;**110**:5797–801. doi:10.1073/pnas.1219686110

157 Anda R, Williamson D, Jones D, *et al.* Depressed Affect, Hopelessness, and the Risk of Ischemic-Heart-Disease in a Cohort of United-States Adults. *Epidemiology* 1993;**4**:285–93.

158 Everson S, Goldberg D, Kaplan G, *et al.* Hopelessness and risk of mortality and incidence of myocardial infarction and cancer. *Psychosomatic Medicine* 1996;**58**:113–21.

159 Cacioppo J, Hawkley L, Crawford L, *et al.* Loneliness and health: Potential mechanisms. *Psychosomatic Medicine* 2002;**64**:407–17.

160 Devanand DP, Sano M, Tang M-X, *et al.* Depressed Mood and the Incidence of Alzheimer's Disease in the Elderly Living in the Community. *Archives of General Psychiatry* 1996;**53**:175–82. doi:10.1001/archpsyc.1996.01830020093011

161 Luchsinger J, Honig L, Tang M, *et al.* Depressive symptoms, vascular risk factors, and Alzheimer's disease. *Int J Geriatr Psychiatry* 2008;**23**:922–8. doi:10.1002/gps.2006

162 Andersen K, Lolk A, Kragh-Sorensen P, *et al.* Depression and the risk of Alzheimer disease. *Epidemiology* 2005;**16**:233–8. doi:10.1097/01.ede.0000152116.32580.24

163 Modrego PJ, Ferrández J. Depression in Patients With Mild Cognitive Impairment Increases the Risk of Developing Dementia of Alzheimer Type: A Prospective Cohort Study. *Arch Neurol* 2004;**61**:1290–3. doi:10.1001/archneur.61.8.1290

164 Wilson R, Barnes L, de Leon C, *et al.* Depressive symptoms, cognitive decline, and risk of AD in older persons. *Neurology* 2002;**59**:364–70. doi:http://dx.doi.org/10.1212/WNL.59.3.364

165 Palmer K, Berger A, Monastero R, *et al.* Predictors of progression from mild cognitive impairment to Alzheimer disease. *Neurology* 2007;**68**:1596–602. doi:http://dx.doi.org/10.1212/01.wnl.0000260968.92345.3f

166 Saczynski JS, Beiser A, Seshadri S, *et al.* Depressive symptoms and risk of dementia: The Framingham Heart Study. *Neurology* 2010;**75**:35–41. doi:10.1212/WNL.0b013e3181e62138

167 Dal Forno G, Palermo MT, Donohue JE, *et al.* Depressive symptoms, sex, and risk for Alzheimer's disease. *Annals of Neurology* 2005;**57**:381–7. doi:10.1002/ana.20405

168 Geerlings MI, Heijer den T, Koudstaal PJ, *et al.* History of depression, depressive symptoms, and medial temporal lobe atrophy and the risk of Alzheimer disease. *Neurology* 2008;**70**:1258–64. doi:10.1212/01.wnl.0000308937.30473.d1

169 Deckers K, van Boxtel MPJ, Schiepers OJG, *et al.* Target risk factors for dementia prevention: a systematic review and Delphi consensus study on the evidence from observational studies. *Int J Geriatr Psychiatry* 2015;**30**:234–46. doi:10.1002/gps.4245

170 Wilson RS, Arnold SE, Beck TL, *et al.* Change in depressive symptoms during the prodromal phase of Alzheimer disease. *Archives of General Psychiatry* 2008;**65**:439–45. doi:10.1001/archpsyc.65.4.439

171 Everson SA, Kaplan GA, Goldberg DE, *et al.* Hopelessness and 4-year progression of carotid atherosclerosis. The Kuopio Ischemic Heart Disease Risk Factor Study. *Arterioscler Thromb Vasc Biol* 1997;**17**:1490–5.

172 Whipple MOM, Lewis TTT, Sutton-Tyrrell KK, *et al.* Hopelessness, depressive symptoms, and carotid atherosclerosis in women: the Study of Women's Health Across the Nation (SWAN) heart study. *Audio, Transactions of the IRE Professional Group on* 2009;**40**:3166–72. doi:10.1161/STROKEAHA.109.554519

173 BECK AT, Wenzel A, Riskind JH, *et al.* Specificity of Hopelessness about Resolving Life Problems: Another Test of the Cognitive Model of Depression. *Cognitive Therapy and Research* 2006;**30**:773–81. doi:10.1007/s10608-006-9081-2

174 Beck AT, Steer RA, Kovacs M, *et al.* Hopelessness and eventual suicide: a 10-year prospective study of patients hospitalized with suicidal ideation. *American Journal of Psychiatry* 1985;**142**:559–63.

175 Haatainen K, Tanskanen A, Kylmä J, *et al.* Factors associated with hopelessness: a population study. *Int J Soc Psychiatry* 2004;**50**:142–52.

176 Potter G, Steffens D. Contribution of depression to cognitive impairment and dementia in older adults. *Neurologist* 2007;**13**:105–17.

177 Novais F, Starkstein S. Phenomenology of Depression in Alzheimer's Disease. *J Alzheimers Dis* 2015;**47**:845–55. doi:10.3233/JAD-148004

178 Byers AL, Yaffe K. Depression and risk of developing dementia. *Nat Rev Neurol* 2011;**7**:323–31. doi:10.1038/nrneurol.2011.60

179 Wilson RS, Hoganson GM, Rajan KB, *et al.* Temporal course of depressive symptoms during the development of Alzheimer disease. *Neurology* 2010;**75**:21–6. doi:10.1212/WNL.0b013e3181e620c5

180 Holt-Lunstad J, Smith TB, Baker M, *et al.* Loneliness and social isolation as risk factors for mortality: a meta-analytic review. *Perspect Psychol Sci* 2015;**10**:227–37. doi:10.1177/1745691614568352

181 Ong AD, Uchino BN, Wethington E. Loneliness and Health in Older Adults: A Mini-Review and Synthesis. *Gerontology* 2015;**62**:443–9. doi:10.1159/000441651

182 Coyle CE, Dugan E. Social Isolation, Loneliness and Health Among Older Adults. *J Aging Health* 2012;:–. doi:10.1177/0898264312460275

183 Kiecolt-Glaser J, McGuire L, Robles T, *et al.* Psychoneuroimmunology and psychosomatic medicine: Back to the future. *Psychosomatic Medicine* 2002;**64**:15–28.

184 Carter CJ. Alzheimer's disease: a pathogenetic autoimmune disorder caused by herpes simplex in a gene-dependent manner. *Int J Alzheimers Dis* 2010;**2010**:140539–17. doi:10.4061/2010/140539

185 Philippens IH, Ormel PR, Baarends G, *et al.* Acceleration of Amyloidosis by Inflammation in the Amyloid-Beta Marmoset Monkey Model of Alzheimer's Disease. *J Alzheimers Dis* 2016;:1–13. doi:10.3233/JAD-160673

186 LEE H-G, CASADESUS G, ZHU X, *et al.* Challenging the Amyloid Cascade Hypothesis: Senile Plaques and Amyloid-β as Protective Adaptations to Alzheimer Disease. *Annals of the New York Academy of Sciences* 2004;**1019**:1–4. doi:10.1196/annals.1297.001

187 Castellani RJ, Lee H, Siedlak SL, *et al.* Reexamining Alzheimer‘s Disease: Evidence for a Protective Role for Amyloid-β Protein Precursor and Amyloid-β - Journal of Alzheimer’s Disease - Volume 18, Number 2 / 2009 - IOS Press. *Journal of Alzheimer's …* 2009.

188 Lu B, Nagappan G, Guan X, *et al.* BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. *Nat Rev Neurosci* 2013;**14**:401–16. doi:10.1038/nrn3505

189 Pozueta J, Lefort R, Shelanski ML. REVIEW SYNAPTIC CHANGES IN ALZHEIMER’S DISEASE AND ITS MODELS. *Neuroscience* 2013;**251**:51–65. doi:10.1016/j.neuroscience.2012.05.050

190 Phillips C, Baktir MA, Srivatsan M, *et al.* Neuroprotective effects of physical activity on the brain: a closer look at trophic factor signaling. *Front Cell Neurosci* 2014;**8**:170. doi:10.3389/fncel.2014.00170

191 Brunelli A, Dimauro I, Sgrò P, *et al.* Acute exercise modulates BDNF and pro-BDNF protein content in immune cells. *Med Sci Sports Exerc* 2012;**44**:1871–80. doi:10.1249/MSS.0b013e31825ab69b

192 Smith PA. BDNF: no gain without pain? *Neuroscience* 2014;**283**:107–23. doi:10.1016/j.neuroscience.2014.05.044

193 Pardridge WM. Blood-brain barrier delivery. *Drug Discov Today* 2007;**12**:54–61. doi:10.1016/j.drudis.2006.10.013

194 Lanz TA, Bove SE, Pilsmaker CD, *et al.* Robust changes in expression of brain-derived neurotrophic factor (BDNF) mRNA and protein across the brain do not translate to detectable changes in BDNF levels in CSF or plasma. *Biomarkers* 2012;**17**:524–31. doi:10.3109/1354750X.2012.694476

195 Pan W, Banks WA, Fasold MB, *et al.* Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology* 1998;**37**:1553–61.