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LIFESTYLE RISK FACTORS FOR PARKINSON DISEASE AND AMYOTROPHIC LATERAL SCLEROSIS- AN EPIDEMIOLOGICAL PERSPECTIVE

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LIFESTYLE RISK FACTORS FOR PARKINSON DISEASE AND AMYOTROPHIC LATERAL SCLEROSIS- AN EPIDEMIOLOGICAL PERSPECTIVE

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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The thesis will be defended in public at Lecture hall David, Widerströmska huset, Tomtebodavägen 18A, 171 65 Solna, Friday October 28th 2022 at 9.00

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To my son Valentin

POPULAR SCIENCE SUMMARY OF THE THESIS

The prevalence of degenerative diseases of the brain and nerves are increasing globally, and no cure for neurodegeneration exists. Common characteristics for this group of disorders are slowly progressing decline of functions controlled by the nervous system such as sensory, motor, autonomous and cognitive functions. Parkinson disease (PD) is characterized by tremor, rigidity, and bradykinesia. Amyotrophic lateral sclerosis (ALS) on the other hand presents with progressive muscle weakness and muscle atrophy. Lifestyle habits may affect onset and progress of these diseases. Sitting time has increased, diets have changed dramatically over the last decades and obesity and depression are more frequent. This thesis investigates interconnected risk factors for PD, explores the link between desired Body Mass Index (BMI) and depression in obesity, and investigates the association between depression and ALS.

In **study I and II**, more than 40 000 Swedish participants completed a questionnaire about dietary habits, weight, height and how many hours/day they spent sitting. They were followed for more than ten years. Using Swedish patient registers, we linked everyone to a PD diagnosis and calculated the risk of PD depending on diet, BMI and sitting time. We found that a high intake of saturated fat increased the risk of PD. In **study III**, some 10 000 individuals in Stockholm completed a questionnaire about height, weight, desired weight, and symptoms of depression. We wanted to study how different levels of obesity was associated with the differences between ideal, desired and current BMI, and to what extent factors such as sex and depression were correlated to those BMI discrepancies. We found that among persons with obesity, women and depressed participants had a larger discrepancy between current and desired BMI, than men and non-depressed. In **study IV**, we investigated occurrence of depression and antidepressant use in every patient diagnosed with ALS in Sweden between 2005 and 2010. We found an increased risk of depression or antidepressant drug use both before and after ALS diagnosis.

The findings in this thesis highlight the importance of lifestyle habits as risk factors for neurodegenerative diseases and identify a previously unknown association between depression and subsequent ALS diagnosis, indicating common underlying mechanisms for depression and ALS. The thesis findings contribute to a deeper understanding of PD and ALS pathogenesis, paving the way for additional research to further increase our understanding of possible preventive measures for neurodegenerative diseases.

ABSTRACT

Following progressive aging of the population, the global prevalence of PD and ALS is expected to increase in the next decades. Primary prevention of the diseases is hampered by limited knowledge of preventable causes. Some suggestions have been made that an inactive lifestyle, obesity and dietary habits such as fat intake may have a role in the etiologies. Evidence is inconsistent regarding BMI and evidence is lacking regarding sitting time and different types of fat intake, and PD risk. The relationship between desired BMI and depression is not well explored. The prevalence of depression in ALS vary largely, and information about antidepression drug use in ALS patients is scarce. Little is known about depression prior to ALS. This thesis aims to investigate the association between sitting time, BMI and intake of dietary fat, and incident PD **(study I-II)**, to describe discrepancies between current, ideal and desired BMI and explore its relation to depression **(study III)**, and to investigate the association between depression and ALS, before and after ALS diagnosis (**study IV**).

Study I-II were prospective cohort studies based on the Swedish National March Cohort, following 42 000 individuals and measuring exposures from a baseline questionnaire and outcome of incident PD from the National Patient Register. The highest quartile of saturated fat intake was associated with a 41% increased risk of PD compared to the lowest quartile (HR Q4 vs. Q1: 1.41; 95% CI: 1.04–1.90). No association was found between sitting time, BMI and PD. **Study III** was a population based study in Stockholm. About 10 000 participants filled in a questionnaire, investing desired BMI. In persons with obesity, large discrepancies between desired BMI and BMI 25 kg/m^2 were predicted by male sex and high BMI, while large discrepancies between current and desired BMI were predicted by female sex, high BMI and depression. **Study IV** was a nested case control study and a matched cohort study based on a nationalwide samle of ALS patients. The risk of depression diagnosis or antidepression drug use was increased both the years before (multivariable adjusted OR 2.0, 95% CI: 1.7-2.2) and after (multivariable adjusted HR 13.5, 95% CI: 10.2-17.8) ALS diagnosis.

Further research efforts are encouraged to understand the pathogenesis of PD and ALS with focus on modifiable risk factors.

LIST OF SCIENTIFIC PAPERS

I. **Roos E**, Grotta A, Yang F, Bellocco R, Ye W, Adami HO, et al. Body mass index, sitting time, and risk of Parkinson disease. Neurology. 2018;90(16):e1413-e7

II. Hantikainen E*, **Roos E***, Bellocco R, D'Antonio A, Grotta A, Adami HO, et al. Dietary fat intake and risk of Parkinson disease: results from the Swedish National March Cohort. Eur J Epidemiol. 2022;37(6):603-13
**shared first authorship*

III. **Roos E,** Trolle Lagerros Y, Lönnroth K, Forsell Y. Factors associated with the discrepancy between current, desired, and ideal BMI in persons with obesity: results from a Swedish population-based study. *Manuscript*

IV. **Roos E**, Mariosa D, Ingre C, Lundholm C, Wirdefeldt K, Roos PM, et al. Depression in amyotrophic lateral sclerosis. Neurology. 2016;86(24):2271-7

Original research article not included in the thesis:

V. **Roos E**, Warmlander S, Meyer J, Sholts SB, Jarvet J, Graslund A, et al. Amyotrophic Lateral Sclerosis After Exposure to Manganese from Traditional Medicine Procedures in Kenya. Biol Trace Elem Res. 2021;199(10):3618-24

CONTENTS

LIST OF ABBREVIATIONS

ALS	Amyotrophic lateral sclerosis
AUDIT	Alcohol use disorders identification test
BMI	Body mass index
CI	Confidence interval
DALYs	Disability-adjusted life-years
DSM	Diagnostic and Statistical Manual of Mental Disorders
FTD	Frontotemporal dementia
HR	Hazard ratio
ICD	International classification of disease
MAOI	Monoaminne oxidase reuptake inhibitors
MDI	Major depression inventory
OR	Odds ratio
PD	Parkinson disease
PIN	Personal identification number
RCT	Randomized Clinical Trial
SD	Standard deviation
SSRI	Selective serotonine reuptake inhibitors
WHO	World Health Organization
YLD	Years lived with disability
YLL	Years of life lost

1 INTRODUCTION

1.1 NEURODEGENERATIVE DISEASES - A GLOBAL PUBLIC HEALTH CONCERN

The United Nation (UN) 2030 Agenda for Sustainable Development was adopted in 2015 by all UN Member States, who jointly pledged to reach the UN Sustainable Development Goal targets for 2030. Goal number 3 reads "to ensure healthy lives and promote well-being for all at all ages", and target 3.4 specifies the aim of the agenda to "reduce by one third premature mortality from non-communicable diseases through prevention and treatment and promote mental health and well-being" (1). Few years after this UN commitment to the goals, they reported that the goals would not be reached by 2030 given the current speed of progress, and that efforts to improve health and to reduce the burden of non-communicable diseases, including disorders of the nervous system, should be accelerated (2). Efforts to estimate and quantify the regional and international burden from disorders of the nervous system have been made in the Global Burden of Diseases, Injuries and Risk Factors Study (3), which reported that neurological disorders, including stroke (which alternatively may be classified as a cardio-vascular disorder) and dementias, are the second leading cause of death and the leading cause of disability-adjusted life-years (DALYs) worldwide. The neurodegenerative diseases Alzheimer´s disease and other dementias are among the top three contributors to neurological DALYs globally (3).

The two components contributing to DALYs are years lived with disability (YLD) due to ill health and years of life lost (YLL) due to early death. A long life expectancy associated with prolonged state of disability contributes to an increase in YLD and therefore to DALYs. Therefore, DALYs are better than mortality data as a measurement of the burden of neurodegenerative disorders, and are used throughout this thesis.

1.2 AN AGEING GLOBAL POPULATION

The majority of the world´s countries have an aging population with increasing life expectancy (Fig. 1) and a growing absolute and relative number of elderly (4). By 2050 the number of individuals aged 65 years or more is predicted to have doubled as compared to 2019, and one in six will be above 65 years (4). Professor Albert Hofman, editor of European Journal of Epidemiology summarized this phenomenon by saying "you live a week you gain a weekend". Moreover, the gender gap in terms of life expectancy is expected to slowly close

(4). Consequently, the incidence of neurodegenerative diseases associated with ageing will likely steeply increase. With an increasing older group in society, human and financial resources need to be allocated to prevention, health care and rehabilitation, with implications for health-care planning and prioritization.

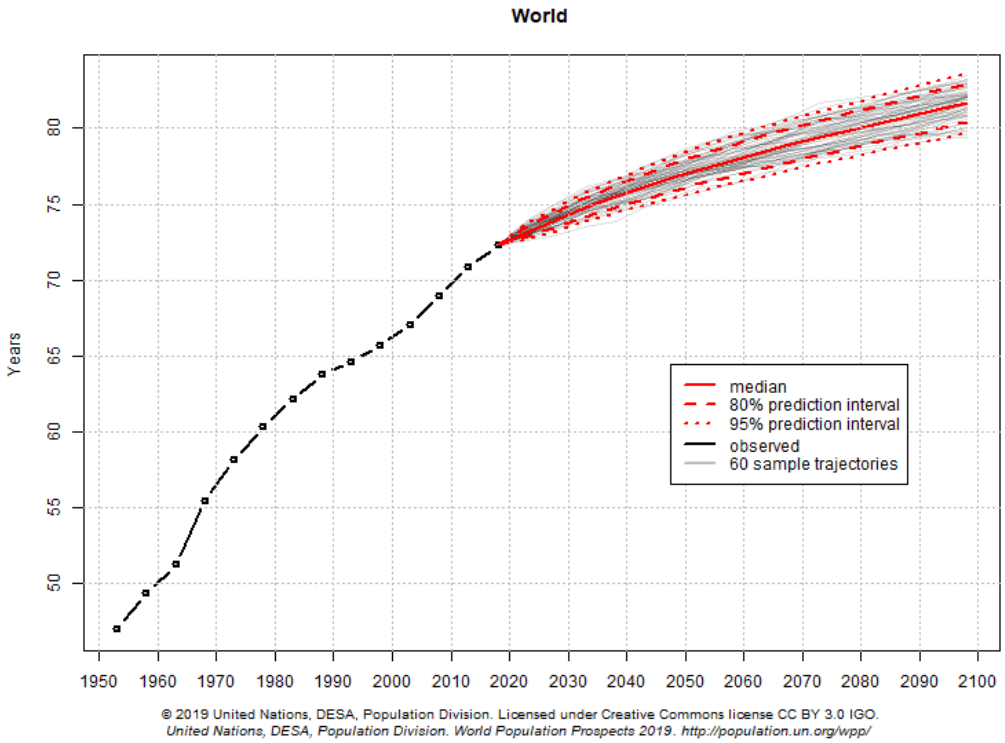

Figure 1. Life expectancy in years over time. A predictions of global ageing. The median life expectancy is anticipated to increase. *Reproduced with permission from United Nation World Population Prospects 2019.*

The severity of neurodegenerative diseases along with an increased prevalence and an ageing population call for identification of preventive measures. There is a growing body of literature that recognises the importance of lifestyle habits in the development of chronic diseases such as neurodegenerative diseases.

This doctoral thesis investigates some modifiable risk factors for the chronic neurodegenerative diseases Parkinson disease (PD) and amyotrophic lateral sclerosis (ALS), using Swedish cohorts and registers.

2 LITERATURE REVIEW

2.1 NEURODEGENERATIVE DISEASES

Neurodegenerative diseases are characterized by progressive nerve cell death in the central and/or the peripheral nervous system leading to problems with movement, sensation, balance, speech, memory and other cognitive functions. Neurodegenerative diseases include disease entities such as Alzheimer´s disease, PD, Multiple Sclerosis, Huntington´s disease, Lewy body disease, Friedreich ataxia, spinal muscular atrophy, and motor neuron diseases such as ALS.

Parkinson disease

Parkinson disease is the second most common neurodegenerative disease, next to Alzheimer´s disease. Its incidence ranges between 5 to 35 per 100,000 individuals and it is more common in men than in women (5). Some 90% of cases are sporadic in contrast to the 10% with a known family history defined as hereditary PD (6). Environmental, lifestyle and genetic factors contribute to the lifetime PD risk, particularly in genetically susceptible individuals. The neuropathology involves loss of dopaminergic neurons in the *substantia nigra* part of the brain, and aggregates of α-synuclein (6). Classical clinical characteristics of PD are muscle rigidity, bradykinesia and tremor. Parkinson disease also presents with several non-motor symptoms such as autonomic dysfunction, depression and cognitive decline (6). Despite progress in pharmacologic therapy, available treatment is merely symptomatic, aiming at substituting striatal dopamine to decrease motor symptoms. There is no cure for PD and progressive neurodegeneration eventually leads to severe disability, where resistance to treatment and dementia further diminishes the already poor prognosis.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis is a rare, invariably fatal, neurodegenerative disease affecting the anterior horn cells of the spinal cord and sometimes the frontal lobe of the brain. ALS incidence is about 2 per 100,000 individuals globally (7-9). The incidence of ALS is projected to increase by almost 70% until 2040 due to an increase in the older age group at risk of ALS (10). Like PD, an underlying genetic susceptibility to environmental and lifestyle factors seems to be responsible for most cases of ALS (11).

Proposed pathophysiological mechanisms include protein aggregation, neuroinflammation and mitochondrial dysfunction leading to axonal degeneration (12). Amyotrophic lateral sclerosis is characterized by denervation of both upper and lower motor neurons, causing motor dysfunctions such as progressive muscle atrophy with weakness in limbs or difficulties to speak, swallow and breathe. Non-motor symptoms may include cognitive and/or behavioural impairments including problems with executive dysfunction, frontotemporal dementia and depressive symptoms (13, 14). There is no cure for ALS and most patients die within five years from diagnosis, often from respiratory failure (15).

Prevention of neurodegenerative diseases

Severe disability and high cost for society follows neurodegenerative diseases and no curative treatments exist. Several risk factors for these disorders are established, and age is the predominant one. Ageing is not modifiable; there is nothing we can do about the ageing itself, and more cases of neurodegenerative disease can be anticipated in the future. The scientific and clinical communities are trying to tackle these major disabling diseases with neurotherapeutic agents and support services. However, if significant modifiable risk factors can be identified and established, preventive measures may be implemented. The proverb “an ounce of prevention is worth a pound of cure” is certainly appropriate here. Indeed, cost-effectiveness studies have shown a favorable long-term return on investments in disease prevention and health promotion (16). The Global Burden of Disease Neurology collaborators recently called for action to encourage epidemiological studies for the development of new evidence for efficient prevention measures for neurodegenerative diseases (3). Sustainable and well-chosen policies are needed to promote healthy lifestyle habits throughout a long-life course. This doctoral thesis explores modifiable risk factors for PD and ALS.

2.2 RISK FACTORS FOR PARKINSON DISEASE AND AMYOTROPHIC LATERAL SCLEROSIS

For PD, some identified risk factors include pesticide exposure, intake of dairy products and a history of melanoma (17-19). In contrast, physical activity, and, surprisingly, smoking and caffeine seem to be protective (17, 19).

For ALS, established risk factors are age, male sex, family history and smoking. In addition, an increased risk of ALS has been observed after exposure to metals and organic chemicals as well as previous physical injury including head trauma (11, 20).

Non-modifiable risk factors – Age, sex and heredity

The peak age of onset for ALS is 65 years, and recent population-based studies have shown that ALS incidence indeed has a similar age pattern as Alzheimer´s disease and PD (7, 21). Male sex is an established risk factor for both ALS (7) and PD, and in most populations PD is twice as common in men than in women (6). Only a few percent of both PD and ALS have been explained by familial variants (12).

Modifiable risk factors

Environmental factors

Pesticides and metals

Pesticides and metals have been linked to both PD and ALS (17, 19). The neurotoxic metal manganese can cause parkinsonism (11). In addition to manganese, the neurotoxic metals lead and iron have been associated with ALS (11, 22). Metals may deposit into vulnerable regions of the central nervous system (18, 22).

Socioeconomy

High socioeconomic status is associated with a higher incidence of PD and elevated mortality from both PD and ALS (23, 24). Possible explanations that have been proposed are physically active demanding jobs, occupational exposures, the inverse association seen with smoking, or higher comorbidity (and thereby higher risk of death before developing PD or ALS) which all are more frequent in lower socioeconomic groups. Also, diagnosis bias may contribute to those with higher socioeconomic status receiving a diagnosis to a larger extent.

Depression

According to the 2017 Global Burden of Disease Study (25), more than 264 million people suffer from depression worldwide. Depression is one of the leading causes of disability and accounts for 564 DALYs per 100 000 individuals. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM), major depressive disorder is diagnosed in patients experiencing at least five depressive symptoms during a 2-week period, with at least one of those being depressed mood or loss of interest or pleasure (26). One of the symptoms is significant involuntary weight loss or weight gain or decrease or increase in appetite nearly every day. Further, these symptoms must cause the affected significant distress or impairment in social, occupational, or other important areas of functioning in order to be supportive for the diagnosis (26).

Depression as a risk factor for and consequence of neurodegeneration

It is now well established that many of the symptoms of depression occur in most neurodegenerative diseases, including PD and ALS. Depression and neurodegeneration seem to share similar neuronal dysfunctional pathways, including neuroinflammation, oxidative damage and a dysfunctional hypothalamic-pituitary-adrenal axis (27).

Depression and ALS

ALS patients show high levels of suicide ideation and among patients with different fatal disorders, ALS patients most frequently ask for physician-assisted suicide or euthanasia, in some studies up to one third of ALS patients (28). In Sweden where neither physician assisted suicide nor euthanasia is legalized, the risk of completed suicide was found to increase dramatically among ALS patients compared to the general ALS-free population, especially immediately after ALS diagnosis, although an elevated risk of suicide was also noticed years after ALS diagnosis (29). Although it is possible that increasing loss of independence might underlie the declining increment in suicide risk along with increasing time since diagnosis among ALS patients, the strong emotions evoked by receiving such a serious diagnosis as ALS may lead to an increased risk for suicide.

Suicide likely reflects however only the tip-of-the-iceberg of the psychological distress a patient of ALS may experience. It has been suggested (30, 31) that ALS patients in Sweden, Germany, the Netherlands and the USA, are more likely to develop depression compared to the rest of the population. The proportion of patients with ALS and concurrent depression is difficult to determine; the reported estimates of depression prevalence in ALS patients range from 0% (32) to 44% (33, 34). This wide variation might be a consequence of studies defining "depression" in different ways. The prevalence of depressive symptoms in 35 ALS patients in Sweden and their spouses was evaluated using a self-estimated depression scale (35); and the prevalence of depression was shown to be slightly higher among ALS patients and their spouses, compared to the general population. Other studies that have used structured interviews to diagnose depression according to the DSM IV criteria found a prevalence of depression of about 9-11% in ALS patients, compared to a 10% prevalence of depression in the general population (31). The global prevalence of depression in ALS patients has been estimated to 34%, according to the latest systematic review (36). Further studies are needed to assess if ALS patients have a higher prevalence of depression than the general population free of ALS.

An overlap between depression and ALS may be due to not only the fact that an ALS diagnosis provokes reactive depression. Although the general clinical impression does not support the hypothesis of a large over-representation of depression in pre-symptomatic ALS, given the fact that cognitive impairment is a part of the ALS symptomology, especially in patients with FTD (37, 38), it is possible that ALS patients may inherently have a higher than expected risk of depression (i.e., endogenous depression). The impairment of cognitive function in ALS, as in dementia (39), may include depressive symptoms. It remains to be determined if depression is also a potential prodromal symptom of ALS, and to our knowledge there are no previous studies investigating this. **Study IV** investigates if depression emerges before ALS diagnosis, if depression constitutes a risk factor for ALS, and investigates depression among those already diagnosed with ALS.

Lifestyle

Lifestyle is an umbrella term for how we live our life, in terms of activities and routines. Factors such as smoking, sleep, stress, diet, and physical activity comprise our lifestyles and affect health. A healthy living helps us to live longer. There seem to be an intergenerational transmission of health risks, including lifestyle habits (40), where children as young as infants are influenced by the habits of their parents. How well we practise and maintain healthy habits affects the onset and progress of many of the chronic diseases.

According to World Health Organization (WHO), most European countries report a high alcohol intake, low fruit and vegetable intake, inadequate physical activity, excessive fat intake and increasing obesity prevalence, which harm quality of life and shorten life expectancy (41).

Not only morbidity, but also the overall mortality is decreased when adopting a healthier lifestyle. A meta-analysis showed that mortality was reduced by 66% (95% CI 58%-73%) when adopting four of the healthy habits regular exercise, eating healthy (such as consuming five or more fruits and vegetables daily), drinking alcohol in moderation and not smoking (42). In one of the included studies, it was clear that every single one of those healthy habits matter. Matheson et al. followed almost 12,000 individuals for about 15 years, to investigate the effect of lifestyle on mortality. In their sample, adjusted by age, sex, education and marital status, they found that the overall mortality risk increased when removing one or more of the four healthy lifestyles (43). The mortality risk was more than three times increased in

individuals with no healthy habits, as compared to those with four healthy habits. The explicit benefits adopting a healthy lifestyle was shown for all BMI categories, with the greatest benefit in persons with obesity.

Overweight and obesity

BMI is widely used to quantify tissue mass in relation to weight and height. Body Mass Index categorize if a person is underweight (<18.5kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2) or obese. Obesity is further subdivided into class I (30.0-34.9 kg/m^2), class II (≥35.0-39.9 kg/m^2) and class III (≥40 kg/m^2) (44).

Obesity is increasing worldwide in both low, middle and high- income countries and is associated with significant morbidity and mortality (45-47). In Sweden, BMI is increasing among both men and women, especially in the young population (48). In a study of 447,925 adults; male sex, age, low education and rural residence were associated with a higher obesity prevalence (49). The obesity epidemic is complex with environmental, genetic, socioeconomic and behaviour factors intertwined (50). There is no doubt we live in an obesogenic infrastructure (51) and that rising obesity ratets are an increasing concern for public health.

Recent guidelines acknowledge the complexity of obesity as a chronic disease and suggest that the definition of obesity focus on how BMI impact physical and psychological health (52). Obesity is associated with unhealthy ageing and not only mortality, but also incidence and severity of several diseases such as cancer, type 2 diabetes, cardiovascular diseases and some infections, including COVID-19 (53-57). As an example of the magnitude of the severity of obesity, as much as 30% of COVID-19 hospitalizations during the ongoing coronavirus disease pandemic have been estimated to be attributed to obesity (58). Obesity seems to influence progression and prognosis of COVID-19 (56). The pathogentic mechanisms linking obesity to chronic diseases seem to be related to properties of the adipose tissue itself. The adipose tissue produces hormones, promoting low-grade chronic inflammation. (59, 60). The inflammatory cytokine TNF-alpha has been found in increased levels in plasma from persons with obesity (61). Also, brain dopamine receptor availability decreases with obesity, which may have implications for the risk of PD (62).

Further, persons with obesity often suffer from self-esteem issues, mental health problems and social- and work discrimination (63, 64). A reduction in weight by behavioural

interventions can reduce several of the health risks (65, 66). **Study I** investigates BMI and the risk of PD.

Obesity, desired- and ideal weight, and depression

According to de Wit et al., depression is associated with BMI outside the normal ranges, either low BMI or high BMI (67). This notion is strengthened by the fact that, within the diagnostic criteria for depression, the person could have both gained or lost weight, indicating that depression also induces weight changes. In addition to this, there may be other factors affecting both weight and depression, such as nutrition, alcohol intake and physical activity.

The normalization of individuals being overweight is increasing (68, 69). Physicians have difficulties identifying patients with obesity (70), and in a study by Kaplan et al. only 50% of individuals with obesity saw themselves as having obesity (71). Increased social acceptance of obesity is a double edge sword though. It might reduce the psychological effect of having obesity, which has been associated with depression (72, 73). But then again, acceptance of one's obesity may decrease the motivation for behavioural changes, and challenge preventive measures and effective obesity care (68). In terms of social acceptance of obesity and the perceived satisfaction of body appearance, sex, socioeconomy and ethnic factors may also play important roles (74, 75). The degree of rotundity which is desirable vary largely historically and culturally, probably due to food supply, the efforts required to obtain it and associated social implications.

The ideal weight is the one corresponding to what WHO define as normal BMI i.e. 18.5-24.9 kg/m^2. This is seen as a healthy weight. Discrepancy between desired and actual weight seem to play a role in health with associated comorbidities, notably psychologic and well-being, when the desired and actual weight do not align (76, 77). To date a few studies, with small sample sizes, have investigated the discrepancy between the desired weight, or BMI and current weight/BMI (78, 79). People who have the intention to lose weight have a lower all-cause mortality, even if they do not lose weight (80). Various psychologic factors may explain that association. They may show more acceptance of healthy lifestyle choices and therefore improved health, even if they do not lose weight. Alternatively, or possibly in combination, those without intention may lack motivation to maintain healthy behaviours. The lack of motivation itself may also be a consequence of environmental exposure or genetic predisposure.

Study III explores populations at risk of weight dissatisfaction i.e. those with the highest discrepancy between desired, current and ideal weight, and the link to depression.

Physical activity and sedentary behaviour

It is natural to sit down as it is a way for us to save energy. Sitting time, as a proxy for sedentary behaviour, can in turn be used as a proxy for lack of physical activity. Especially as we often sit in an inactive way, i.e. on a chair or sofa without activation of our legs and backs. Recent findings reveal that physical activity induces epigenetic changes in skeletal muscles in sedentary men (81), and that a sedentary behaviour increases the overall mortality (82). Inactivity, measured as prolonged sitting time, has been correlateted with thinness of the medial temporal lobe, the area of the brain involved in memory (83). Regular physical activity lowers the risk of PD (84), but the effect of BMI and sedentary behaviour on the PD incidence has not been extensively studied yet. Therefore, it is investigated in **study I**.

Dietary fat intake

Some dietary preferences, such as dairy products, have been associated with PD (17), as has a high energy intake (85). Pesticide contamination in milk or low levels of uric acid in high consumers of milk have been suggested to explain this link (86). Studies on dietary fat intake and PD have shown conflicting results. The latest meta-analysis showed an association between high total energy intake and PD incidence, but no statistically significant association with total or specific types of fat intakes (85). Polyunsaturated fatty acids have shown both anti-inflammatory, anti-apoptotic, anti-oxidant and neuroprotective properties, and monounsaturated fatty acids seem able to reduce oxidative stress (87). **Study II** investigates the relation between dietary fat intake and PD.

To summarize, life style and environmental factors seem to contribute to PD and ALS, yet the extent of these contributions remain to elucidate.

3 RESEARCH AIMS

Overarching aim of the doctoral thesis

The overarching aim of this doctoral thesis is to explore risk factors for the neurodegenerative disorders PD and ALS and to identify populations at risk to which neuroprotective measures can be applied.

Specific objectives

The specific objectives of the four studies included in this thesis are the following:

Study I: To investigate the association between BMI and sitting time, and PD

Study II: To investigate if dietary fat and intake of specific types of fat are associated with PD

Study III: To describe discrepancies between current, ideal and desired BMI in a population cohort in Sweden, and explore its relation to depression

Study IV: To investigate the association between depression and ALS, before and after ALS diagnosis

4 MATERIALS AND METHODS

4.1 MATERIALS

The National Registers of Sweden

Sweden has a long history of tracking its population. Already in the beginning of the 17th century, villagers were registered in the local Christian church books. The archbishop Olaus Martini urged his fellow priests in 1608 to write down births, baptisms, marriage, deaths and "opå alt annat, som wårdar någott" ("*everything else which cherish something*"). The post-war era in the early 1800th century was characterized by financial hardship, plague, and deaths. A royal decision was made to map the status of the population with the help of the church books, in order to plan the number of soldiers after a drop in the population number. Due to the establishment of nationwide population statistics, the Royal Swedish Academy of Sciences managed to calculate the size of the population for the first time in 1744; 2 097 000 inhabitants. This sparked an international interest in scientific statistical collaborations, but the statistics were not to be shared without caution outside of the State.

An attempt was also made to establish the birth rate. It was speculated that it may differ depending on regions: "ty den härrörer af folkets olika åldrar, hvar uppå de giftas och dö, hvilka åter kunna komma an på olika lefnads sätt och olika climater på åtskilliga orter" ("i*t comes from different ages, when they marry and die, which in turn could depend on the way of living and the difference in climate in different towns").* However, due to missing data on age in several church books, it was barely possible to proceed with such accurate calculations. This was the beginning of demographic calculations on population based data in Sweden. Despite typical epidemiological challenges such as missing data, it had a crucial sociopolitical role and made visible concerns with deaths and emigration at this time. Preventive measures could be initiated such as establishments of more hospitals and training of medical staff (88).

Total Population Register and Personal Identity Number

Today Statics Sweden keeps the register of the total population (Registret över Totalbefolkningen) and every individual recorded in this register has been assigned a Personal Identity Number (PIN) by the National Tax Board (89). The system with the PIN was established in 1947. It works as a unique identifier and is now an invaluable tool when

linking medical registers. With the collaboration between Statistics Sweden and the Swedish National Board of Health and Welfare, using the PIN, information can be drawn from different registers and the same individual can be linked in between the registers allowing for unique research in register-based medical research (90, 91). For each of my studies, data from multiple registers were added to one single data file, which was then used for analyses. We used several registers, each with a specific purpose. For example, the migration register and the register of population changes were used to censor participants lost to follow-up due to migration in the cohort studies. The Swedish Population and Housing Census contains demographic information such as employment and household income, and was used for demographic background variables. The Swedish Education Register was established in 1985 by Statistics Sweden and includes all Swedish residents at the age of 16-74 years and is updated annually. We used it to assess education as a covariate. The next chapter will describe the main registers and cohorts used.

The National Patient Register

The Swedish healthcare system is divided into regions that are financially and administratively independent. The vast majority of patients visit a public healthcare centre in the region of residency. Hospital visits that encounter a hospital admission and a hospital-based outpatient specialist care are registered in the National Patient Register, using the PIN.

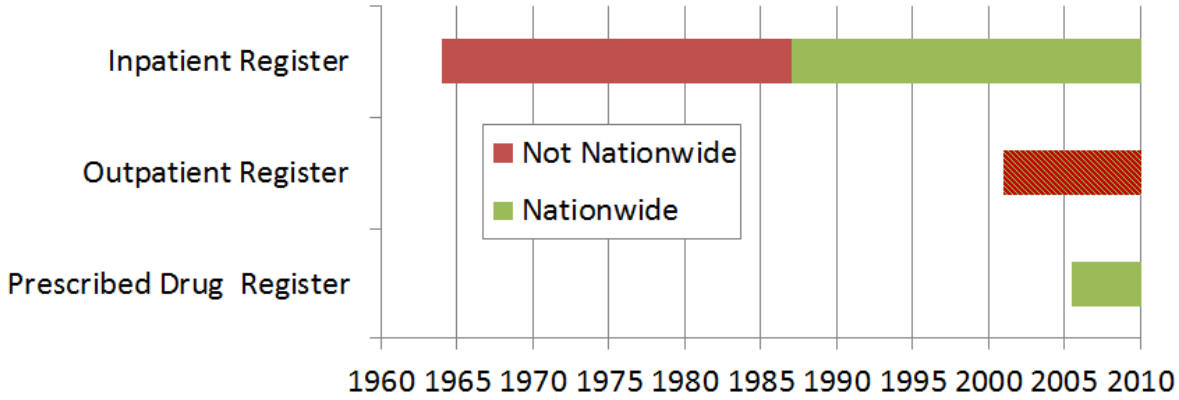

Figure 2. Coverage of three Swedish National Registers. The outpatient register is nationwide and cover hospital-based specialist visits only, and no primary care. *Figure by Daniela Mariosa.*

Medical diagnoses are coded according to the Swedish Revisions of the International Classification of Diseases (ICD). ICD-9 codes were used from 1987 to 1996, ICD-10 since

1997 and in a couple of years the new ICD-11 will be implemented. The codes are collected by the Swedish National Board of Health and Welfare and linked to each individual via the PIN. The National Patient Register has two parts: The Swedish Inpatient Register, which has full national coverage since 1987, and the Swedish Outpatient Register. The Outpatient Register was initiated in 2001 and covers specialist care non-admitted outpatient visits. Primary care is not yet registered in the National Patient Register (Fig. 2) (92).

The Cause of Death register

The Swedish National Board of Health and Welfare also compiles a register over causes of deaths, established in 1952. It includes variables such as underlying cause of death based on ICD-codes, secondary causes of death, place of death and death abroad or in Sweden, along with personal data (93).

The Swedish Prescribed Drug Register

The Swedish Prescribed Drug Register was established in 2005 with a purpose to improve pharmaceutical patient safety. Drugs that are prescribed by a physician and dispensed at any Swedish pharmacy are recorded in the register along with information about the prescriber, cost, brand name, substance, dosage, drug classification code according to the Anatomical Therapeutic Chemical classification system, and about the dispensed amount. Non-dispensed drugs, drugs used during inpatient care, and drugs that can be purchased without prescription are not included in the register. Additional useful information from this register is date of prescription, which gives an idea of time of diagnosis or significant symptoms. The date of dispense could be used as a proxy for time of start of medication use (94).

Swedish National March Cohort

The Swedish National March Cohort was initiated 1997 to create a large prospective cohort to investigate associations between lifestyle factors and chronic disease. A national fundraising event was organized by the Swedish Cancer Society in 3 600 cities in Sweden 10-14 September 1997. It included scientific programs on television, galas, and local activities like the possibility to look at cancer cells in a microscope, meet a researcher and the possibility to donate 50 SEK and to take a walk, the “National March”. During the fund raising events, people were also given the possibility to “donate time”. They were asked to donate one hour for research, filling in a 36-page questionnaire covering lifestyle and medical history. The questionnaires were returned, no postage required, in special mailboxes at the local supermarkets. Next, milk trucks transported the mailboxes to Statistics Sweden for

scanning. 43 880 questionnaires were returned. After excluding incomplete PINs, the final cohort consisted of 43 865 women and men (95).

The cohort has been linked to a number of National Registers such as the Total Population Register, National Patient Register (92), Cause of Death Register, Cancer Register, Prescribed Drug Register and Register of Population Changes, the latter providing information about emigration which is of value when following up the cohort. The cohort has so far been followed up through record-linkage from 1997 to 2016.

PART study

The PART study (Acronym for *Psykisk hälsa, Arbete, RelaTioner* - Mental Health, Work and Relations) is a longitudinal population-based study aiming at identifying risk- and protective factors for mental health (96). At baseline (1998) Swedish citizens aged 20-64 residing in the Stockholm county were randomly selected. Only Swedish citizens were chosen as the questionnaire was in Swedish. The Stockholm county population fulfilling the inclusion criteria at the time was 858 000. Between 1998-2000 five random samples were drawn for the first wave of PART. From the Total Population Register, 19 742 persons from Stockholm were invited to fill in a self-administered questionnaire. The questionnaire was answered by 53% (n=10 443). The questionnaire included demographics, financial status, social network, somatic illnesses, stressful life events, a scale on psychological well-being and screening instruments for alcohol use, drug use and psychiatric diseases. Some of those who screened positive for psychiatric diseases, including depression, were invited to a diagnostic interview to validate the screening instruments. The cohort has been linked to several Swedish registers including the National Patient Register.

4.2 METHODS

Study design

The evidence based medicine pyramid of evidence (97) helps researchers identify the most appropriate study design to answer a research question at hand. Systematic reviews and meta-analyses of, preferably double-blinded, randomized clinical trials (RCT´s) are considered to generate the highest quality of evidence synthesis. Hence, RCT is the optimal design to obtain empirical evidence underpinning evidence synthesis. However, ethical, practical, and financial considerations often constrain the use of RCTs. Trials that expose individuals to

potential risk factors are generally considered unethical. When investigating risk factors for disease rather that treatments, well-designed cohort and case-control studies, two types of observational studies, produce high quality evidence without risks for the participants. For all study designs, the size of the study and quality of the design are important factors in the evidence quality evaluation.

In this thesis work, I have used three types of observational study designs: Cohort study (study I, II and IV), nested case-control study (study IV) and cross-sectional study (study III). An overview of the studies is presented in Figure 3.

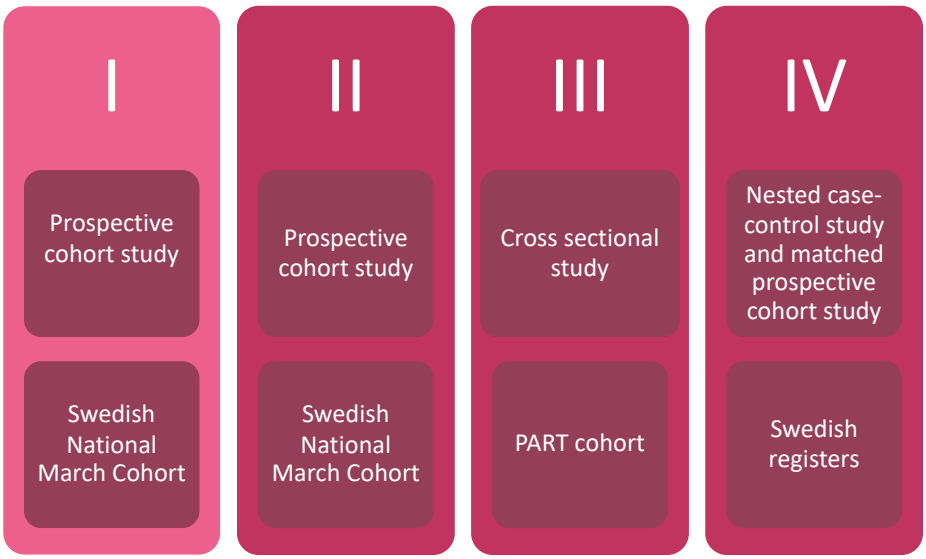

Figure 3. Overview of the four studies included in this thesis: study designs and data sources.

Cohort studies

In general, a cohort is set up to study a specific group of people over time, prospectively or retrospectively. Comparisons can be made between cohort members who have been exposed to a variable, and those who have not been exposed. Cohort studies are advantageous when studying potentially harmful factors, as participants are not deliberately exposed. The exposure has to be present before the outcome. The incidence of an outcome, and the effect of the exposure in relation to the effect of no exposure on the probability of developing the outcome, i.e., the relative risk, can be calculated.

Nested case-control studies

Case-control studies investigate causes of disease, especially rare ones, by including people affected by an outcome and suitable controls free of the outcome. The exposure prior to the outcome is often ascertained retrospectively and compared in both groups to determine if the exposure is a predictor of the outcome. In a nested case-control study, cases and controls are chosen from a defined parent cohort, in which exposure data was already collected prior to the outcome. All cases occurring in the cohort are included. Controls, who at the diagnosis date of the index case themselves are free from the diagnosis, are then randomly selected from the cohort and matched with the cases. The nested design can reduce recall bias, and a cohort design as basis can ensure higher coverage of outcomes measured. The prevalence of an outcome, and the odds ratio (OR), an estimation of the relative risk, of the outcome can be calculated.

Cross-sectional studies

Data from a cohort or a group is examined at a certain point in time and provides prevalence of exposures and outcomes. Since both exposure and outcome is measured at the same point in time an association cannot distinguish between cause and effect. This design is useful when generating a hypothesis from the findings, to create a research question for a more in-depth research study, or to determine that a certain variable is not frequent in a certain condition, leading to the conclusion that further cohort studies are not be needed. High response rates are advantageous, to not miss differences in variables between responders and non-responders (98, 99). Self- administered questionnaires may result in low response rates. Interviews are more likely to yield higher response rate than questionnaires, but are more time-consuming and expensive, and may limit the sample size.

Study I and II

The research question in the first study was: Is there any association between BMI, sitting time and PD?

The research question of the second study was: Is there any association between dietary fat intake and PD?

Study population

Study I and II are cohort studies using data from the Swedish National March Cohort and the Swedish national registers. In total, 41 638 (study I) and 41 597 (study II) participants were

included in the study after exclusion of those who before start of follow up died (n=8), emigrated (n =41), had a PD diagnosis (n = 10), were below 18 years old (n= 1 732), lacked a valid PIN (n=11) or reported values of total caloric intakes which seemed unrealistic (n=459).

Study design

A prospective cohort study design was chosen. Participants completed a printed self-administered baseline questionnaire in September 1997. Follow-up started 1 October 1997 and ended at index date which was defined as date of PD diagnosis, date of death, emigration, or end of follow-up 31 December 2010 (study I) or 2016 (study II), whichever occurred first.

Exposures

BMI was calculated by dividing the self-reported weight by the self-reported squared height. BMI was classified into normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obesity (≥30 kg/m^2) according to World Health Organization criteria (100). We also assessed the waist circumference, divided in low (<94 cm for men and <80 cm for women), medium (94-102 cm for men and 80-88 cm for women) and high (>102 cm for men and > 88 cm for women).

Sitting time as a proxy for sedentary lifestyle, and physical activity, as a potential confounding factor in study I-II, were assessed using a questionnaire specially validated for this study (101). Examples of activities performed sitting down were office work, bathing, and knitting (Fig. 4). The cut-off for sitting time was set to six hours of sitting/day.

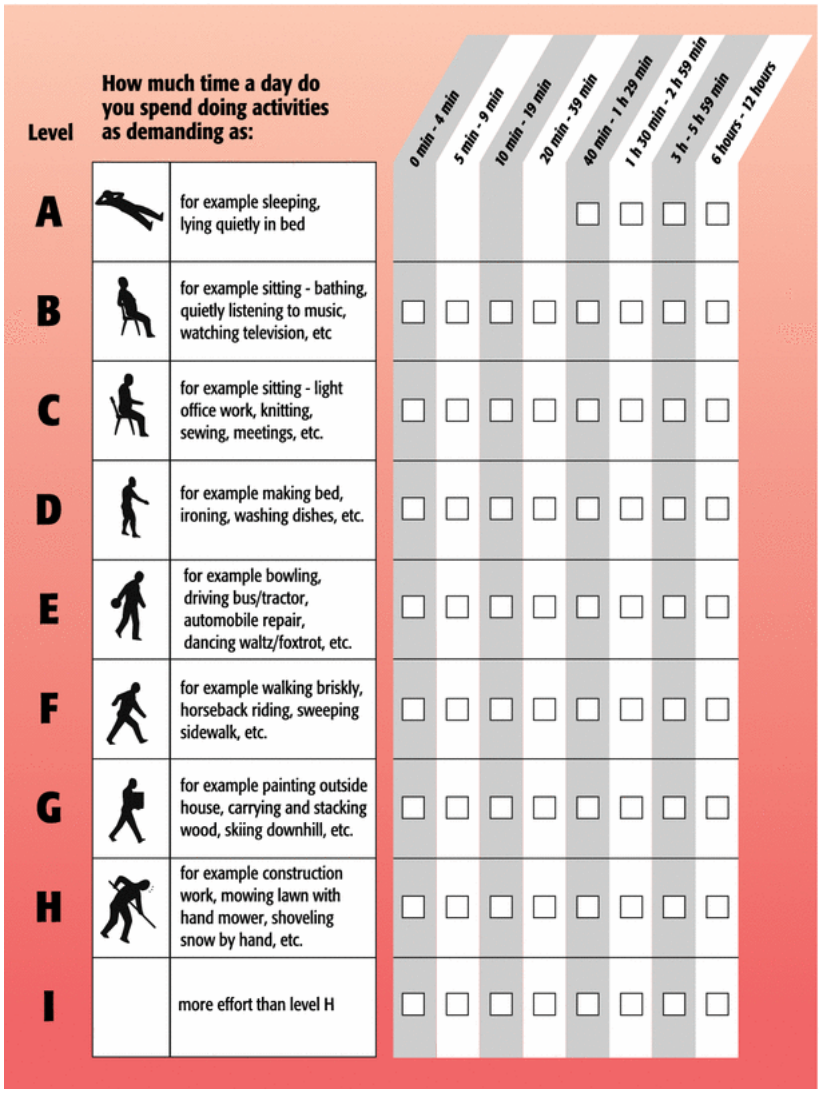

Figure 4. The sitting time questionnaire in the Swedish National March Cohort questionnaire.

An 85-item validated semi-quantitative food frequency questionnaire (FFQ) was included in the baseline questionnaire of the Swedish National March Cohort. By reporting what type of food items and how often they were consumed (Fig. 5), intake of both energy (kcal/day) and dietary fat (g/day) could be assessed. This was calculated by linking the Swedish National March Cohort to the Swedish Food Composition Database from the National Food Agency of Sweden. This database holds information on specific food items and their total energy content and the three macronutrients fat, carbohydrates, and proteins, which enables a translation from intake of a specific food item to intake of gram fat and kcal.

Hur mycket dricker/äter du av följande:
Kryssa för ett alternativ per rad. (7+ betyder 7 gånger eller mer)

	Glas per dag (1 glas=2 dl)							
	0	1	2	3	4	5	6	7+
Lätt/minimjölk	☐	☐	☐	☐	☐	☐	☐	☐
Mellanmjölk	☐	☐	☐	☐	☐	☐	☐	☐
Standardmjölk	☐	☐	☐	☐	☐	☐	☐	☐
Lättfil/lättyoghurt	☐	☐	☐	☐	☐	☐	☐	☐
Filmjölk/yoghurt	☐	☐	☐	☐	☐	☐	☐	☐
Saft/läsk	☐	☐	☐	☐	☐	☐	☐	☐
Lättöl	☐	☐	☐	☐	☐	☐	☐	☐

Vilka typer av matfett brukar du använda?
Kryssa för ett eller flera alternativ för smörgås, sedan för matlagning

	Smörgås	Matlagning
Smör	☐	☐
Bregott	☐	☐
Smörgåsmargarin (t. ex. Flora)	☐	☐
Lättmargarin (t. ex. Lätta)	☐	☐
Hushållsmargarin (t. ex. Milda)	☐	☐
Olivolja	☐	☐
Rapsolja		☐
Matolja (t. ex. majs-, solrosolja)		☐
Flytande margarin		☐
Jag använder inget matfett	☐	☐

Figure 5. Questions about fat intake in the Swedish National March Cohort questionnaire. *Image reproduced with permission from the illustrator Lukas Produktion.*

Outcome

The outcome was incident cases of PD, defined by first-ever specialist outpatient contact or hospital discharge with a primary or secondary diagnosis of PD, identified by ICD codes and PIN from the National Patient Register.

Statistical analysis

First, the baseline characteristics of the cohort were stratified by BMI, waist circumference and sitting time in study I, and by quartiles of total fat intake in study II. Then, age-standardized PD incidence rates were calculated.

Cox proportional hazards regression model

Age was chosen as the underlying time scale and therefore inherently adjusted for in a Cox proportional hazards regression model, which was fitted to estimate hazard ratios and the corresponding 95% confidence intervals (CIs) of PD at certain amounts of sitting time, waist circumference levels, various BMI levels, and quartiles of fat intakes, initially adjusted for sex and later for additional potential confounders. The following potential confounders were identified based on subject knowledge and assessed from the baseline questionnaire: Sex, smoking status, alcohol consumption, coffee consumption, education level, physical activity in household activities and commuting, waist circumference, BMI, and sitting time.

We conducted a linear trend test to assess any potential linear relationship between exposures and outcome. In this test, we handled the exposures as continuous variables by using the median values from the BMI groups, waist circumference, sitting time, quartiles of total fat and specific types of fat.

Isocaloric substitution model

In study II, we performed an isocaloric substitution. The aim of this substitution model calculation was to understand if an association between high fat intake and PD could actually be explained by a high calory intake, rather than the fat itself. We chose to substitute 10% of the energy (kcal) deriving from one energy source (fat) with 10% energy from other sources (carbohydrates, proteins, or alcohol). We also tested to substitute different type of fats, such as saturated fat, monounsaturated and polyunsaturated fat, with each other. In the model, the substituted energy source is excluded, while total energy intake and the remaining energy sources are added as confounders, resulting in an effect of the energy source replacement on the outcome PD, while keeping the energy intake constant.

Sensitivity analyses

Finally, we did three types of sensitivity analyses, to determine how different values of the exposures affected the outcome under the given set of assumptions. First, we tested for reverse causation bias. A certain diet may be due to PD itself. In order to test this, the first

two, three or five years of follow-up were excluded to remove any PD cases occurring during this time. Secondly, PD cases from the Cause of Death Register were added. Thirdly, the robustness, the strength of the statistical model, was assessed by multiple imputation based on the assumption that data was missing at random.

Study III

The research question of the third study was: Are there any discrepancies (differences) between current, ideal, and desired BMI, and what is their relation to depression?

Study population and study design

Study III is a cross sectional study using data from the PART cohort. In total, 10 443 adults from Stockholm were included. A self-administered questionnaire provided information about height, weight, desired weight, demographic variables, alcohol use and symptoms of depression. The patient register was used to assess background variables such as previous psychiatric disorder.

Exposure

The exposures included socio-demographic variables such as age, sex, educational level, and ethnicity. In addition, BMI, hazardous alcohol use and presence of major depression were considered as exposures. The Alcohol Use Disorders Identification Test was included in the questionnaire and used to define hazardous alcohol use. Major depression diagnosis was determined using the Major Depression Inventory (MDI), according to the DSM-IV criteria. The MDI responses from the questionnaires were scored by summary index, which has shown high validity (102).

Outcome

From the questionnaire data, BMI and desired BMI were calculated. Absolute and relative (percentage) difference between desired BMI and the upper limit of ideal BMI (25 kg/m^2) were calculated to determine the discrepancy between the desired BMI of participants with obesity and the ideal BMI of 25 kg/m^2. Similarly, absolute and relative differences between current BMI and desired BMI were calculated to determine the discrepancy between current and desired BMI in participants with obesity.

Statistical analysis

Descriptive statistics was calculated for the entire study population, and stratified by BMI groups including different levels of obesity. Differences, first in desired BMI, and then in the discrepancies (desired vs. BMI 25 kg/m^2, and current vs. desired BMI, respectively) between sub-groups (based on BMI, sex, ethnicity, educational status, hazardous alcohol use and presence or not of depression) were determined using two-sided Student's t-test. Using univariate and multivariate linear regression, factors predicting a higher or lower discrepancy (desired vs. BMI 25 kg/m^2, and current vs. desired BMI, respectively) among persons with obesity could be identified.

Study IV

The research question of study IV was: Is there any association between depression and ALS?

Study population

The study population consisted of all Swedish residents born in Sweden enlisted in the 1990 Swedish Population and Housing Census. After exclusion of those with previous ALS diagnosis, death, migration, or without information on region of residence 6,337,988 individuals remained for a population-based nested case control study.

Study design

Using the PIN, the study population was cross-linked to the Swedish Patient, Cause of Death, and Migration Registers. All individuals in the study population were first followed from July 1st, 2005 until a first diagnosis of ALS, death, emigration out of Sweden, or December 31, 2010, whichever came first.

The first part of the study was a nested case-control study (Fig. 6), designed to evaluate the association of depression and the use of antidepressant drugs with ALS risk. The second part of the study was a matched cohort study. The ALS cases free of previous exposure to depression or antidepressant drug use, were followed over time to estimate the association with ALS diagnosis and the subsequent risk of depression and use of antidepressant drugs.

Ascertainment of ALS diagnosis

An ALS diagnosis was identified and defined if an inpatient or outpatient hospital visit record with ALS either as the main or a secondary discharge diagnosis, classified by ICD code, was

found in the National Patient Register. The first date of hospital contact for ALS was used as the proxy for date of ALS diagnosis.

Ascertainment of depression and anti-depressant drug use

Depression diagnosis (clinical depression) and antidepressants drug use were used as proxies for depression in the study. Clinical diagnosis of depression was extracted from the National Patient Register from January 1st, 2001 to the index date using ICD codes for both cases and controls. Information on prescription of antidepressants was retrieved from the Prescribed Drug Register. Specific antidepressants analysed were: Non-selective monoamine reuptake inhibitors, selective serotonin reuptake inhibitors, monoamine oxidase A inhibitors, and other. Regular use of antidepressants among ALS patients was estimated by identifying how many times the drug was dispensed. One dispense may be occasional while two may indicate regular use of the drugs. Only individuals with two or more dispenses of antidepressants were defined as regular users of antidepressants and included as antidepressant drug users in the study.

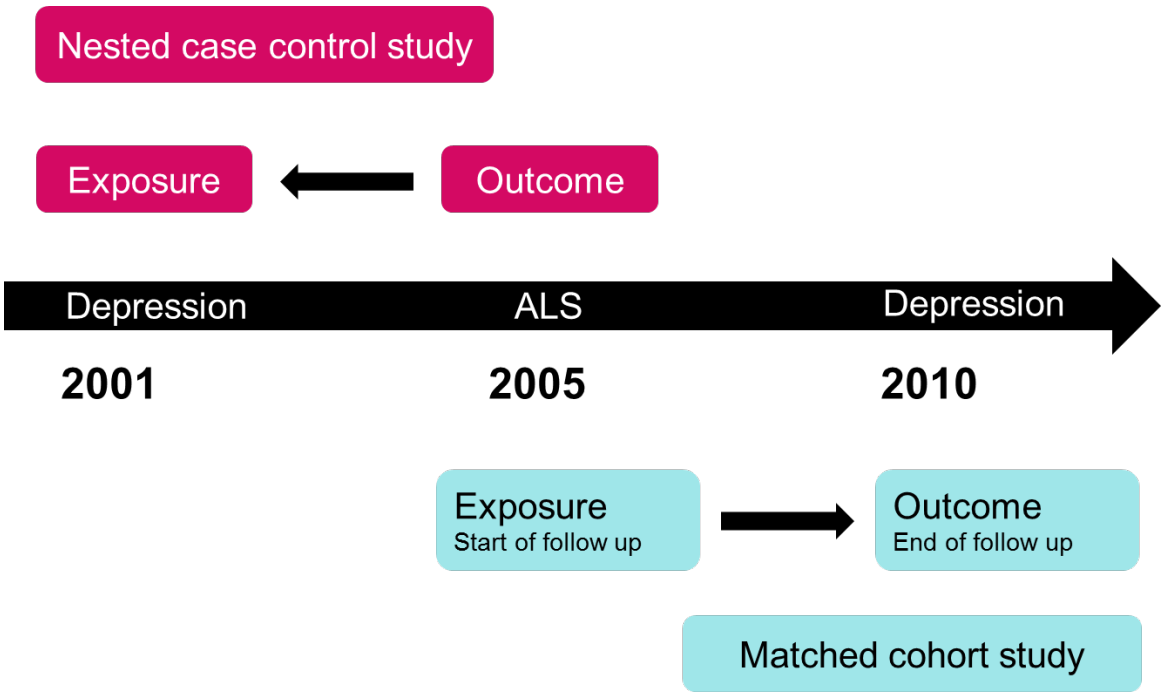

Figure. 6. Overview of the study design in study IV.

Nested case-control study – depression and the subsequent risk of ALS

A nested case-control study was conducted by identifying within the above defined study base the ALS cases newly diagnosed between July 2005 and December 2010. The nested

case-control study was performed to compare the frequency of the exposure depression in ALS patients, with the frequency of the exposure to depression among ALS-free individuals.

Controls were selected with the method of incidence density sampling. Five controls that were free of ALS at the time of diagnosis for every index ALS case were randomly selected from the study base and matched to the index case based on year of birth, sex, and area of residence. The index date for cases was defined as the date of ALS diagnosis, and for controls the index date was the date of diagnosis of their matched index cases. In total, 1752 cases and 8760 controls were enrolled in this nested case-control study. Through the PIN, the cases and controls were cross-linked to the National Patient Register for ascertainment of hospital records for depression diagnosis and the Prescribed Drug Register for ascertainment of antidepressants use.

Matched cohort study – ALS diagnosis and subsequent risk of depression

A matched cohort study was then performed to compare the risk of depression among ALS patients after their diagnosis, to the risk of depression among ALS-free individuals. In this analysis, ALS diagnosis was considered the exposure, and depression the outcome.

For this analysis, all cases and controls enrolled in the above nested case-control study were followed from the index date (ALS diagnosis) to a first clinical diagnosis of depression, first prescription of antidepressants use, death, or December 31, 2010, whichever came first. The follow-up was again performed through cross-linking the cases and controls to the National Patient Register, Prescribed Drug Register, and Cause of Death Register. Cases and controls of the original nested case-control study who had experienced depression before the index date were excluded from the analysis. Follow-up of the controls was also censored if they developed ALS during the follow-up period.

Statistical analysis

Descriptive statistics were produced to describe the prevalence of depression and use of antidepressants among the ALS cases and their matched controls in both the nested case-control study and the matched cohort study.

Depression and subsequent ALS risk

In the nested case-control study, conditional logistic regression models were used to estimate the ORs with 95% CIs as an estimate of the association between depression and the subsequent risk of ALS. To test if ALS patients had an increasing prevalence of depression

as they approached their date of diagnosis, the association between depression and ALS were separately analysed for different time windows; >3 years, 3rd year, 2nd year, 1st year before the index date.

ALS and the subsequent risk of depression

In the matched cohort study, conditional Cox regression models were used to estimate the hazard ratios (HR) with 95% CIs as an estimate of the association between ALS and subsequent risk of depression. Risk estimates were initially adjusted for the matching variables only, given the matched nature of the study design, and secondly also for education level and socioeconomic status in both the nested case-control study and in the matched cohort study.

4.3 ETHICAL CONSIDERATIONS

This thesis is based on epidemiological data extracted from self-reported questionnaires (study I-III) and Swedish nationwide patient registers (study I, II, IV) including the National Patient Register, Cause of Death Register and Prescribed Drug Register. No animal experiments were conducted, no biological tissues or fluids collected and no patient-physician relationships established. However the four ethical principles for research involving humans need consideration in this context: autonomy, beneficence, non-maleficence, and justice.

Autonomy

All studies in this thesis comply with the Declaration of Helsinki. For the studies using self-reported questionnaires from the Swedish National March (study I-II) and PART (study III) cohorts, all participants were informed about aims of study, of the linkage to registers, and informed consent was given prior to participation. Personal Identification Numbers were provided from the participants, acting as unique personal identifiers allowing linkage to registers. A potential threat to autonomy may be that participants signed the consent form at baseline, and the follow up of the cohort stretches over several years, and several studies and register linkages may have been added. However, the consent forms stated the long-term goal and participants were informed, in written form, about their right to opt out from the cohort at any point and thereby not participating in any future register linkages. Questionnaires were sent by surface mail and no remuneration was given. Those who found the questions too

intrusive did probably not participate. Statistical results are presented at group level and not individual levels, and therefore pose no threat to the integrity of the individual participant.

Beneficence

Using high quality register-based data is cost- and time efficient. The aims of the studies include exploring risk factors for neurodegenerative diseases. Possible benefits of the studies include a chance to better target risk factors with preventive measures. This benefits the study participants, their families and friends and populations at a national and international level. We believe our research leads to humanitarian and financial benefits in the society in the long run.

Non-maleficence

When participants fill in questionnaires about lifestyle, physical and psychological health they most probably reflect on their replies and consequences of them. Uncomfortable emotions may arise when realizing that one has obesity, depression, consume high amounts of alcohol, or have an unhealthy diet, for example. However, this may also spark motivation for lifestyle changes.

To ensure the validity of observational studies, no interventions should be done. This collides with beneficence, the will of doing good for study participants when answers of concern are observed. In study III, the limit of intervention has been carefully selected to signs of psychological illness only. If this was obvious from the questionnaires, these participants were selected for in depth-interviews and if deemed appropriate, were offered help from psychiatric services. Those with signs of being acute severely unwell, such as suicidal, were called immediately. However, if a participant was found to have obesity or a diet of concern, there was no intervention. One may question what responsibility we have to act on such information. In the PART cohort (study III), a separate information letter was attached to the questionnaire, including contact details to health care staff should questions or concerns arise.

Another aspect of non-maleficence is the access to a large amount of data linked to each individual. It is of greatest importance that the information is not misused. All data was anonymized so no individuals could be identified during data analysis. To maintain the integrity of the data, only researchers directly involved had access to the data. Data from the Swedish National March cohort and the PART cohort were stored in de-identified databases

and no personal information was accessible to the researchers. The data was stored in accordance with the European GDPR.

Justice

The questionnaires were in Swedish only, and we could therefore offer participation only to those who knew Swedish. Due to limited financial resources we could unfortunately not translate the questionnaires.

All studies were approved by the regional ethics review board, Karolinska Institutet, Stockholm, Sweden, with the following reference numbers:

Study I-II: DNR 1997-205 and DNR 2017/796-31

Study III: DNR 1996/260

Study IV: DNR 2012/1814-31/4

5 RESULTS

Sedentary behavior, BMI, diet, and risk of Parkinson disease (Study I-II)

Among the 41 638 (study I) and 41 597 (study II) participants from the Swedish National March Cohort, 36% were men and the mean age at enrolment was 52 ± 16 years. Few participants were underweight (≤18.5 kg/m^2, n=579) and were therefore included in the group "normalweight". Participants with obesity were less educated than normalweight and overweight participants. Those with a prolonged sitting time were more educated, smoked more and drank more alcohol, than those sitting a shorter time per day. Participants with higher total fat intake, were more likely to have obesity, be smokers and be less educated. During the 13 years (study I) and 18 years (study II) of follow up, 286 and 465 incident cases of PD were detected.

Sitting time, waist circumference and BMI as risk factors for Parkinson disease

We did not find an association between prolonged sitting time, waist circumference or BMI and the risk of developing PD (Table 1). The multivariable HR and corresponding 95% CIs of the association between 6 hours or more of sitting per day of as compared to less than 6 hours, and risk of PD was 1.06 (95% CI 0.76–1.47). For the association between BMI ≥30 kg/m^2 vs. BMI <25 kg/m^2 and risk of PD, the HR was 1.13 (95% CI 0.60–2.12). Age-and sex adjusted analyses showed similar results for sitting time and BMI. The linear trend assessment in study I did not show any significant evidence for a trend. The *p for trend* was 0.727 for sitting time and 0.849 for BMI groups, indicating that there were deviations from a linear association between prolonged sitting time or a high BMI and PD.

When investigating the specific risk of developing PD from a high saturated fat intake, HR Q4 vs. Q1 was 1.41; 95% CI 1.08-1.85; *p for trend: 0.01* in the age-and sex adjusted model. An increased risk remained significant in all quartiles, a risk which remained, and increased further in the multivariable adjusted model. HR Q4 vs. Q1 was again 1.41; 95%. CI 1.04-1.90, HR Q3 vs. Q1: 1.45 (1.07-1.95) and Q2 vs. Q1: 1.38 (1.02-1.86), *p for trend*: 0.03.

For total fat, the HR in Q4 vs. Q1 was 1.29. (95% CI 1.00-1.68) in the age- and sex adjusted model. There was no significant association for Q2 or Q3 compared to Q1, *p for trend*: *0.05*. In the multivariable adjusted model, the associations did not remain.

The link between saturated fat intake and PD was further strengthened when investigating continuous saturated fat intake rather than intake by quartiles. The risk of PD was increased

by 10% per 1 SD increment of saturated fat intake (multivariable adjusted HR: 1.10; 95% CI 1.00–1.22). Again in line with these results, spline analyses did not reveal any deviation from a linear association between saturated fat intake and PD, indicating a linear dose-response relationship between saturated fat intake and risk of PD (Fig.7).

Table 1. Age standardized incidence rates and hazard ratios (HRs) with 95% confidence intervals (CIs) of Parkinson disease for BMI, waist circumference and sitting time.

	Parkinson disease				P for trend
	n	IR	HR	95% CI	
Multivariable adjusted*					
Sitting time (hours/day)					
<6	145	7.12	1.00	Ref	
≥6	53	11.51	1.06	0.76-1.47	
					0.727
Body Mass Index (kg/m^2)					
<25	108	7.79	1.00	Ref	
25-30	74	6.75	0.96	0.68-1.38	
≥30	16	6.86	1.13	0.60-2.12	
					0.849
Waist circumference					
Low[a]	88	7.53	1.00	Ref	
Medium[b]	59	7.49	0.92	0.64-1.31	
High[c]	51	7.16	1.06	0.67-1.66	
					0.851

a ≤94 cm for men and <80 cm for women | b= 94-102 cm for men and 80-88 cm for women | c ≥102 cm for men and > 88 cm for women

**Adjusted for: Age (model time scale), sex, education, smoking, alcohol consumption, Body mass Index, waist circumference, sitting time and household and commuting physical activity.*

n = Number of Parkinson disease cases after excluding cases with missing values on at least one of the covariates included in the model.

IR = Incidence Rate. Presented per 10,000 person-years

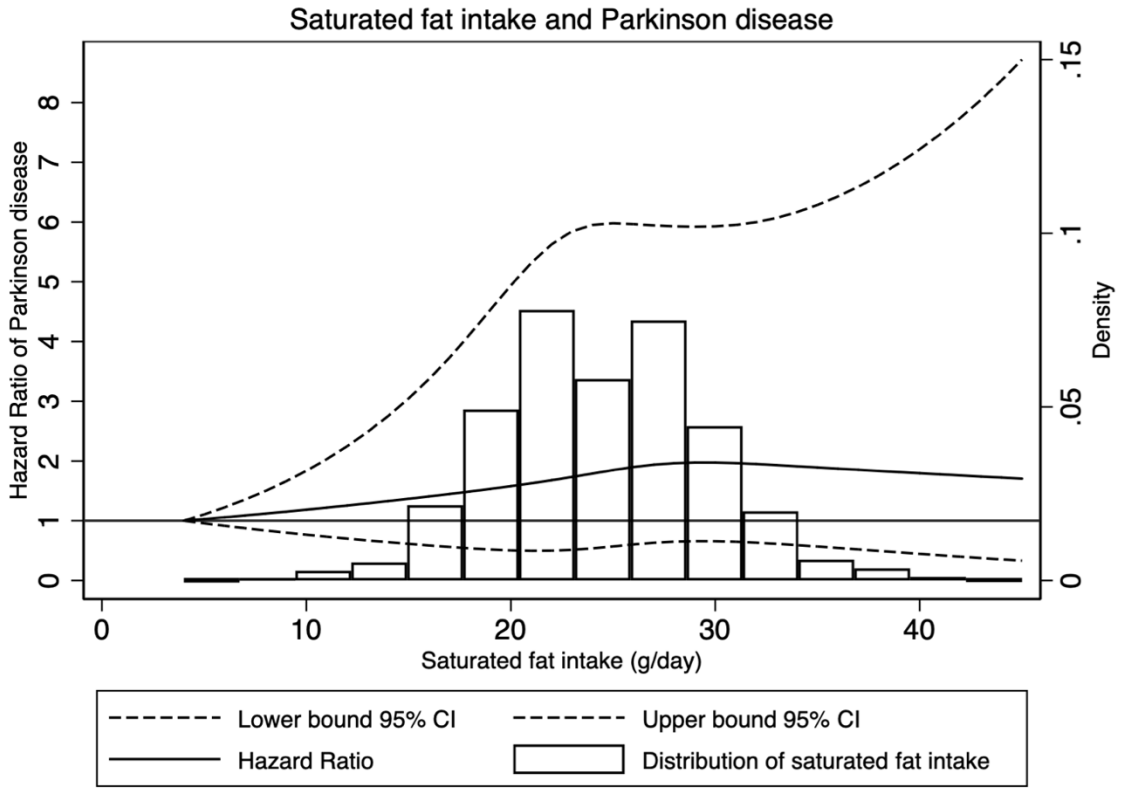

Figure 7. Multivariable-adjusted restricted cubic spline curve for the association between dietary intake from saturated fat, and the risk of Parkinson disease. Adjustments were made for age (underlying time scale), sex, intake of coffee, dietary intake of Vitamin E, BMI, education, smoking status, and physical activity, including household and commuting activity.

In the isocaloric substitution models a few findings, however not significant, indicated the potential importance of saturated fat intake on PD risk (Table 2). When replacing 10% of monounsaturated fat with saturated fat, the risk of PD increased by 81% (HR 1.81, 95% CI 0.56-5.83). Similarly, when replacing 10% of polyunsaturated fat with saturated fat, the risk of PD increased by 76% (HR 1.76, 95% CI 0.65-4.76). When examining the opposite situation, replacing 10% saturated fat with mono- or polyunsaturated fats, the PD risk decreased (HR 0.37, 95% CI 0.06-2.32 and HR 0.44, 95% CI 0.05-4.15). Replacing fat with other macronutrients did not have any effect.

Table 2. Multivariable-adjusted hazard ratios with 95% confidence intervals for Parkinson disease risk from isocaloric substitution models, when replacing 10% of the total energy from total fat with the same amount of energy from other macronutrients or when replacing 10% of the total energy from saturated or unsaturated fats with the same amount of energy from other types of fats.

Substitution of:	**HR**	**95% CI**
10% of the total energy from fat with:		
Proteins	0.93	0.50 - 1.75
Carbohydrates	0.85	0.66 - 1.11
Alcohol	0.87	0.67 - 1.13
Substitution of:	**HR**	**95% CI**
10% of the total energy from saturated fat with:		
Monounsaturated fat	0.37	0.06 - 2.32
Polyunsaturated fat	0.44	0.05 - 4.15
10% of the total energy from monounsaturated fat with:		
Saturated fat	1.81	0.56 - 5.83
Polyunsaturated fat	0.61	0.04 - 8.44
10% of the total energy from polyunsaturated fat with:		
Saturated fat	1.76	0.65 - 4.76
Monounsaturated fat	0.0	0.07 - 2.41

HR = Hazard Ratio; CI = Confidence Interval.
Analyses performed with Cox Proportional Hazards regression model, with attained age as timescale, adjusting for sex (male/female), coffee intake (0, 1-2, 3-4, ≥5 cups/day), physical activity (≤2, 3-4, 5-6, >6 hours/week), smoking (never, former, current), intake of vitamin-E (mg/day) and total energy intake (kcal).

Most of the sensitivity analyses in our studies on BMI, sitting time or dietary fat intake and risk of PD did not significantly affect our findings. However, when excluding the first five years of follow up, the multivariable adjusted HR´s remained significant for saturated fat intake Q4 vs. Q1: HR 1.39 (95% CI 1.01–1.92) with a non-significant *p for trend (= 0.07).* When excluding the three first years of follow up, the association between saturated fat intake and PD turned non-significant.

Desired BMI in persons with obesity and the presence of depression (Study III)

Out of 10441 participants, 212 (2%) were underweight, 6359 (62%) normal weight, and 2963 (29%) overweight. There were 808 (8%) persons with obesity, divided in three groups: 618 (6%) persons with BMI 30-35 kg/m^2, 122 (1%) persons with BMI 35-40 kg/m^2, and 68 (1%)

persons with BMI ≥40 kg/m^2. The mean desired BMI was 27 kg/m^2 in persons with obesity. Persons with less severe obesity BMI <40 kg/m^2 desired a considerably lower BMI than those with more severe obesity BMI≥ 40 kg/m^2 (26 vs. 36 kg/m^2, *p<0.001*). Also, women desired a slightly lower BMI than men (26 vs. 28 kg/m^2, *p<0.001*).

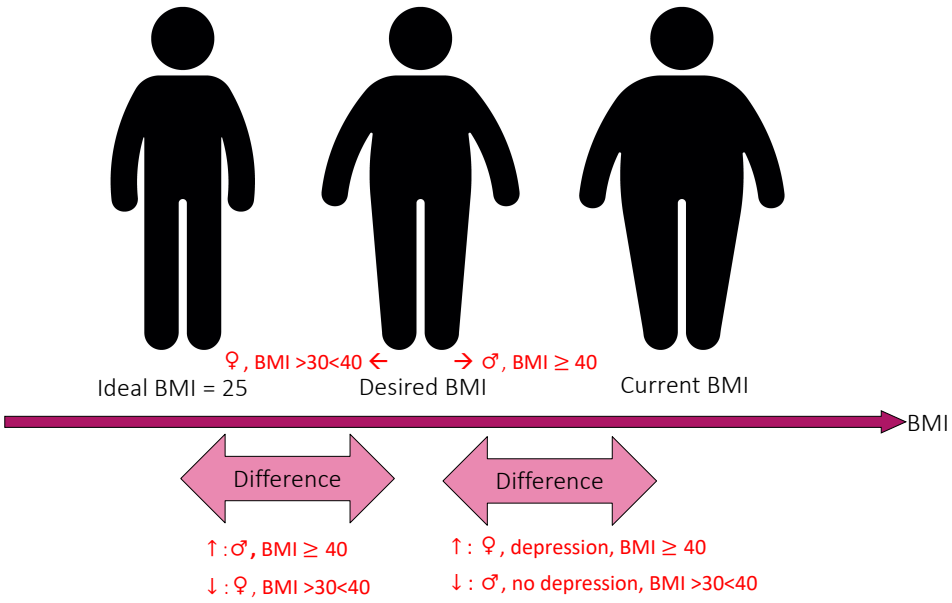

Figure 8. Variables indicating larger or smaller discrepancies (differences) between current and desired BMI, and between desired BMI and BMI 25 kg/m^2. The unit for BMI is kg/m^2. Arrows above the line indicate variables that determine a larger or smaller desired BMI. Arrows below the line indicate variables which contribute to an increase or decrease in discrepancy.

In persons with obesity, the discrepancy between current and desired BMI was larger in women, those with higher BMI, and those with depression. The discrepancy between desired BMI and BMI 25 kg/m^2 was larger in men and in those with higher BMI (Fig. 8).

The results from the regression model were in line with our previous findings on discrepancies. Being female, having a high BMI and being depressed were predictors for a larger discrepancy between current and desired BMI. Being a man and having a high BMI were predictors of a larger discrepancy between desired BMI and BMI 25 kg/m^2. In addition, depression seemed to be a predictor of a smaller discrepancy between desired BMI and BMI 25 kg/m^2 (Table 3).

Table 3. Predictors of discrepancy between desired BMI and BMI kg/m^2, and between current and desired BMI, in persons with obesity.

Participants with obesity i.e. BMI >30 kg/m^2	**Multivariable analysis***	
Difference desired and BMI 25 kg/m^2 (%)	**Coefficient**	**95% CI**
Age (years)	-0.0	-0.1 to 0.0
Sex, male	7.2	5.1 to 9.3
BMI (kg/m^2)	2.6	2.4 to 2.7
Born abroad	1.5	-1.5 to 4.4
University degree	0.0	-2.7 to 2.7
Hazardous alcohol use	-0.1	-2.8 to 2.6
Major depression diagnosis	-5.1	-8.7 to -1.5
Difference current and desired BMI (%)		
Age (years)	-0.0	-0.1 to 0.1
Sex, male	-7.6	-10.3 to -5.0
Born abroad	-2.8	-6.4 to 0.9
University degree	-0.0	-3.3 to 3.3
Hazardous alcohol use	-0.2	-3.6 to 3.1
Major depression diagnosis	6.9	2.5 to 11.4

**adjusted for age, sex, BMI, born abroad, university degree, hazardous alcohol use and major depression diagnosis.*

Depression in ALS (Study IV)

In total 1 752 ALS patients and 8 760 controls were studied. The frequencies of matching variables were, by the nature of the study design, equal among the cases and controls. 56.6% were men, 30% were above 75 years old and 42.8% were white collar workers. The number of cases and controls with depression up to 10 years before and 5 years after index date are presented in Table 4.

Table 4. Depression diagnosis, antidepressant drug use and "any depression" (depression diagnosis or antidepressant drug use) among ALS cases and matched controls.

Years before index date		**ALS cases** **n (%)**			**Controls** **n (%)**	
	Depression diagnosis	*Anti-depressants*	*Any depression*	*Depression diagnosis*	*Anti-depressants*	*Any depression*
10	1 (0.1)	.	1 (0.1)	1 (<0.1)	.	1 (<0.1)
9	1 (0.1)	.	1 (0.1)	6 (0.1)	.	6 (0.1)
8	2 (0.1)	.	2 (0.1)	13 (0.1)	.	13 (0.1)
7	3 (0.2)	1 (0.1)	3 (0.2)	16 (0.2)	4 (<0.1)	20 (0.2)
6	5 (0.3)	20 (1.1)	23 (0.3)	26 (0.3)	114 (1.3)	27 (1.4)
5	4 (0.2)	40 (2.3)	40 (2.3)	22 (0.3)	150 (1.7)	161 (1.8)
4	6 (0.3)	52 (3.0)	53 (3.0)	40 (0.5)	215 (2.5)	221 (2.5)
3	13 (0.7)	46 (2.6)	51 (2.9)	28 (0.3)	219 (2.5)	215 (2.5)
2	13 (0.7)	77 (4.4)	75 (4.3)	37 (0.4)	246 (2.8)	239 (2.7)
1	29 (1.7)	163 (9.3)	162 (9.2)	41 (0.5)	224 (2.6)	216 (2.5)
Years after index date						
1	34 (1.9)	176 (15.7)	184 (16.6)	28 (0.3)	93 (1.5)	93 (1.5)
2	3 (0.5)	30 (7.4)	29 (7.2)	28 (0.4)	50 (1.1)	54 (1.2)
3	1 (0.3)	8 (4.2)	9 (4.7)	13 (0.3)	31 (1.0)	32 (1.1)
4	0 (0.0)	3 (4.3)	3 (4.4)	7 (0.2)	14 (0.8)	15 (0.9)
5	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.2)	2 (0.4)	2 (0.4)

ALS patients are at higher risk of *any depression*, defined as either depression diagnosis or antidepressant drug use, both prior to and after ALS diagnosis.

Depression before ALS

Before the index date, 77 of 1752 patients (4.4%) and 2.6% of controls were diagnosed with depression. 355 of 1473 of ALS patients (24.1%) and 14.2% of controls used antidepressant drugs. Depression diagnosis or antidepressant drug use doubled the risk of subsequent ALS (OR 2.0, 95% CI 1.7-2.2). In those above 65 years of age, the risk was increased 2.4-fold (OR 2.4, 95% CI 1.9-3.1).

To test if ALS patients had an increasing prevalence of depression as they approached their date of diagnosis, the association between depression and ALS were separately analyzed for different time windows, i.e., >3 years, 3rd year, 2nd year and 1st year before the index date. One year before diagnosis, ALS patients showed about four times higher risk of depression diagnosis than controls (OR 3.6; 95% CI 2.2-5.8). Similar associations were also noted for 1-2 years (OR 1.8; 95% CI 0.9-3.3) and 2-3 years (OR 2.4; 95% CI 1.2-4.8) before the index

date. The same pattern was seen for use of antidepressants and for *any depression*. Slightly higher ORs were seen for antidepressant use than for depression diagnosis (Table 5).

Table 5. Depression and ALS risk.

	Depression diagnosis		Antidepressant use		Any depression	
years prior to ALS	OR (95%CI)	OR (95%CI) adjusted	OR (95%CI)	OR (95%CI) adjusted	OR (95%CI)	OR (95%CI) adjusted
< 1	3.6 (2.2-5.8)	3.5 (2.1-5.6)	4.2 (3.4-5.2)	4.3 (3.5-5.3)	4.4 (3.5-5.4)	4.4 (3.6-5.5)
1-2	1.8 (0.9-3.3)	1.8 (1.0-3.5)	1.8 (1.3-2.3)	1.8 (1.4-2.3)	1.8 (1.4-2.3)	1.8 (1.4-2.4)
2-3	2.4 (1.2-4.8)	2.5 (1.3-4.9)	1.2 (0.8-1.6)	1.2 (0.8-1.6)	1.3 (1.0-1.8)	1.3 (1.0-1.8)
>3	0.9 (0.6-1.4)	0.9 (0.6-1.5)	1.3 (1.0-1.6)	1.3 (1.1-1.7)	1.2 (1.0-1.5)	1.3 (1.0-1.6)

The effect sizes were similar when adjusting for the matching variables age, sex, and region of residence or additionally adjusting for socioeconomic status and educational level.

Depression after ALS

After the index date, 38 of 1675 ALS patients (2.3%) and 0.9% of controls were diagnosed with depression. 217 of 1118 ALS patients (19.4%) and 3.0 % of controls used antidepressant drugs.

Table 6. ALS and subsequent risk of depression.

	Depression diagnosis		Antidepressant use		Any depression	
years post ALS	HR (95%CI)	HR (95%CI) adjusted	HR (95%CI)	HR (95%CI) adjusted	HR (95%CI)	HR (95%CI) adjusted
Total	4.1 (2.6-6.5)	4.6 (2.9-7.3)	12.8 (9.7-16.9)	13.2 (10.0-17.5)	13.1 (10.0-17.2)	13.5 (10.2-17.8)
1	6.7 (3.9-11.5)	7.9 (4.4-14.3)	15.3 (11.0-21.2)	16.1 (11.5-22.6)	16.5 (11.8-23.0)	17.4 (12.3-24.5)
2	1.6 (0.4-5.8)	2.3 (0.6-9.1)	9.5 (4.9-18.3)	10.2 (5.1-20.1)	9.2 (4.7-17.7)	9.7 (4.9-19.0)

Risk of *any depression* was significantly increased after ALS diagnosis, especially for antidepressant use (Table 6). The risk of depression (HR 6.7; 95% CI 3.9-11.5) and antidepressant use (HR 15.3; 95% CI 11.0-21.2) was significantly elevated within the first year after ALS diagnosis. The increased risk tapered after the first year after ALS diagnosis (Table 6).

The strength of the associations between ALS and depression was stronger after adjustments for educational level and socioeconomic status. The adjusted HR for any depression within the first year since ALS diagnosis was 17.4 (95% CI 12.3-24.5).

When the results from the nested case-control study and the matched cohort study were taken together, it became clear that ALS patients tended to have a higher risk of both depression diagnosis and use of antidepressant drugs both before and after the time of ALS diagnosis (Fig. 9).

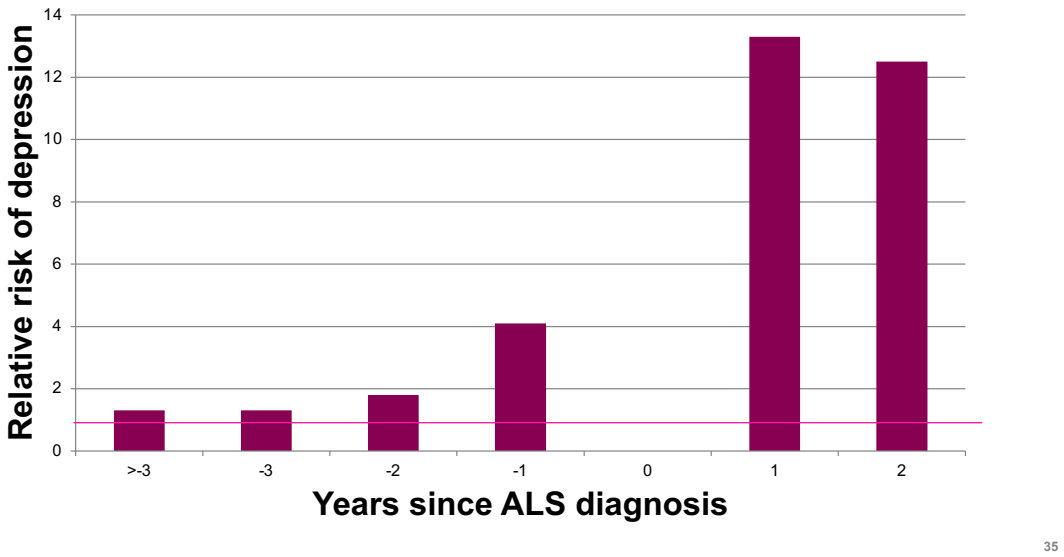

Figure 9. Estimations of relative risks (presented as ORs or HRs) of *any depression* (depression or antidepressant drug use) before and after index date, defined as date of ALS diagnosis for cases.

Antidepressant medication

Specific types of antidepressants used were also analyzed. It was found that several types of non-selective monoamine oxidase inhibitors (MAOI) and selective serotonin reuptake inhibitors (SSRI) and one type of selective MAOI were used among ALS cases. Two drugs, tryptophan and nortriptyline were only used by the controls.

To estimate if there was a difference in the types of antidepressants used before and after diagnosis among ALS cases, the distribution of different antidepressants used before and after diagnosis were investigated (Figures 10 and 11).

No remarkable differences in the class of antidepressants used before and after ALS diagnosis were detected. SSRI´s were most commonly used.

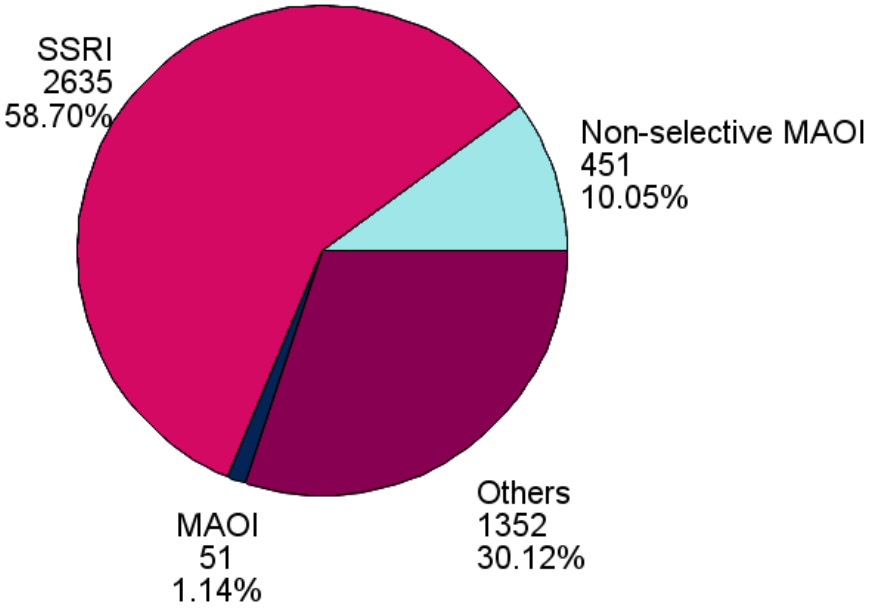

Figure 10. Classes of antidepressant drugs used before ALS diagnosis.

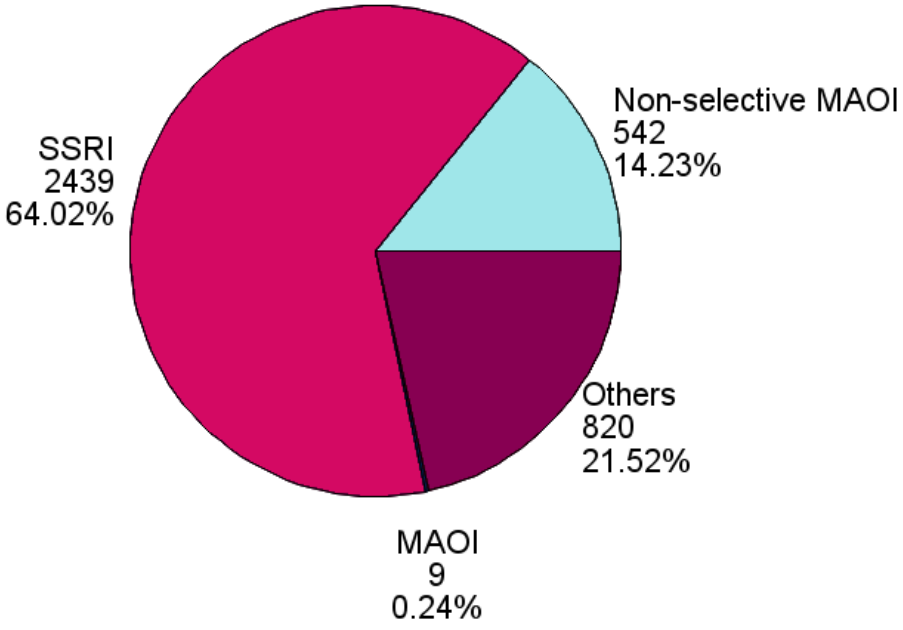

Figure 11. Classes of antidepressant drugs used after ALS diagnosis.

6 DISCUSSION

6.1 INTERPRETATIONS OF MAIN FINDINGS

Saturated fat intake increases the risk of PD, while BMI and sitting time do not

Our data indicate that a high saturated fat intake increase the risk of incident PD. We found this association in a cohort study (study I) which measured exposure prior to a known outcome. We found the association both in univariaible and multivariable adjusted analyses, when assessing the exposure variables as categorical and continuous, and we found a significant linear trend. Contrary to our hypothesis, prolonged sitting time and high BMI were however not risk factors for PD (study II).

These studies were based on a large sample size with a long follow-up. The latest meta-analysis revealed nine studies about dietary fat intake and PD (85). The observation that saturated fat intake increases PD risk has previously been discussed. We found two case-control studies (103, 104) and one cohort study (105) in line with our results on saturated fat. One of the larger studies, Nurses' Health Study and the Health Professionals Follow-up Study, found an effect on PD from saturated fat when performing an isocaloric substitution with polyunsaturated fat (106).We found similar results, although non-significant.

Similar to previous studies (106, 107), we found no association between total fat intake and PD, despite high energy intake previously being suggested to be a risk factor for PD (85). When we adjusted for high energy intake we still did not find any significant effect. This highlights the likely importance of saturated fat itself as a risk factor for PD.

The relationship between sitting time and PD was studied here for the first time. To our knowledge, no similar studies have been performed since. The absence of any association between BMI and PD has been reported in a previous study (108).

The possible molecular mechanisms contributing to an increased PD risk from consumption of saturated fat are unknown. The saturated fat intake and PD risk may be explained by increased chronic inflammation and oxidative stress. In mice given a high-fat diet, impaired dopaminergic neuronal function has been noted (109). A possible link between saturated fat and exposure to environmental neurotoxic agents with lipophilic properties has been

suggested (110). Effects on neuronal inflammation and oxidative stress by intake of saturated fat are also possible (111).

Desired BMI vary with BMI, sex and depression

In study III we found that in persons with obesity, men desired a slightly higher BMI than women. A larger discrepancy between desired BMI and BMI 25 kg/m^2 was predicted by male sex and BMI >40 kg/m^2, while a larger discrepancy between current and desired BMI was predicted by female sex, BMI >40 kg/m^2 and depression. Previous studies have also reported that persons with obesity desire a higher BMI than normalweight persons, and women desire a lower BMI than men (112, 113).

Persons with obesity and depression had a large discrepancy between current and desired BMI. The direction of this association remains to be clarified; in persons with obesity, does depression lead to BMI discrepancy or does BMI discrepancy lead to depression? A possible explanation might be that some persons would like to, but do not manage to, lose weight and this inability could lead to depression. Alternatively, the discrepancy indicates a general dissatisfaction often seen in already depressed persons.

That men with obesity and persons with pronounced obesity (BMI >40 kg/m^2) tend to desire a BMI significantly higher than WHO´s recommended BMI may have several explainations: Firstly, they may simply have a more realistic weight goal. Secondly, it may indicate that they have reconciled with having obesity and thirdly they believe that their current BMI is closer to BMI 25 kg/m^2 than it is, and that their pronounced obesity does not pose a significant health risks. Which of these explanations that fit our results best remains to be explored.

Study III is a hypothesis-generating cross-sectional study providing information about BMI discrepancies. The study is population based with a quite large sample size, which in particular is an advantage when studying stratified levels of obesity, still allowing enough power in each obesity group. Depression is often diagnosed in primary care, but primary care visits are unfortunately not included in the National Patient Register. Therefore, there may be a potential underestimation of participants with a previous depression diagnosis, included in the study. The data was collected 25 years ago. The way we look at obesity may have changed over time. The obesity prevalence was lower in the past. Today, there seem to be an increased acceptance of obesity in society perhaps along with an increased social pressure

about appearance. Had our study been performed today, the desired BMI would perhaps have been closer to BMI 25 kg/m^2 and thereby discrepancies between desired BMI and BMI 25 kg//m^2 would have been smaller, while discrepancies between current and desired BMI would have been larger. The associations seen with sex, high BMI and depression may however not have been affected.

Persons with depression are at increased risk of subsequent ALS, and ALS patients are at increased risk of subsequent depression

Depression or antidepressant use risk was not only elevated directly post ALS-diagnosis, but 16 times increased within two years from the ALS diagnosis. Interestingly, ALS patients were also more likely to be depressed prior to ALS diagnosis.

This study is the largest of its kind and is based on high-quality data. The results are in accordance with earlier studies which show a higher risk of developing depression after receiving an ALS diagnosis (31). Previous studies, including all studies reviewed in (31), used Standard Clinical Interview according to DSM-IV or questionnaires for depression ascertainment. The number of patients in previous studies ranged from 27 to 131 and the prevalence of depression varied between 0 and 44%. In a study (114) including 127 ALS patients that completed an ALS depression inventory and provided information on antidepressant drug use, 38% used antidepressant drugs, most commonly SSRIs , in accordance with our results. In a US study the prevalence of severe depression was only 6%, but selection bias cannot be ruled out as non-depressed ALS patients may have been more likely to participate in the study. The prevalence of mild depression was 29%. In our study we did not separate mild and severe depression.

Depression before ALS

ALS patients were more often diagnosed with depression before ALS diagnosis. The reason for this is unclear. The ALS diagnostic process may continue for several months or years and the patient during that time experience several ALS symptoms such as muscle weakness and reduced performance in daily life. The patient might suspect ALS without having the disease confirmed and may experience loneliness and helplessness along with progress of deterioration leading to depression. In the case of bulbar ALS problems to talk and swallow further taxes the resources of the patient, maybe contributing to depression. Speed of ALS progression varies individually which might also affect anxiety level differently. Consequently, the patient may develop depression before diagnosis due to anxiety and lack

of support during the diagnostic process and disease progression, or depression as a reaction on the diagnosis itself.

Another interpretation of this elevated depression risk before ALS diagnosis is that depression is a comorbidity biologically linked to ALS. Historically the general impression among clinicians has been that motor symptoms are the first symptoms of ALS (personal communication). Our study shows that ALS patients may be depressed before ALS diagnosis; for some individuals even 5 years before diagnosis. Some but not all ALS patients are cognitively affected, maybe related to similar cellular changes as the ones that appear in the ALS disease. Depression may be perceived as a prodromal symptom to ALS itself and may be perceived as a proxy for cognitive impairment before ALS diagnosis. Our results suggest a common underlying factor for both depression and ALS among those patients who developed depression before ALS. A proportion (45%) of ALS patients show cognitive impairment or even frontotemporal lobe dementia (FTD) (15%) (39), where endogenous depression is a part of the FTD symptom spectrum. Depression as an early manifestation of frontal lobe degeneration in a proportion of ALS patients may possibly be used as an upper motor neuron sign heralding a more widespread cortical affection. It is of interest for future studies to know if the patients developing depression before ALS diagnosis are the same individuals who subsequently develop FTD. Those data were not extracted in the present study.

ALS patients are not routinely examined by a neuropsychologist, in contrast to patients with other neurodegenerative disorders such as Alzheimer´s disease or PD, where depression is an established part of the disease symptom flora (115). Depression may be considered a prodromal symptom of PD (116). In the final stages of PD, cognition is dramatically impaired and up to 16% of PD patients with REM sleep behavior disorders demonstrate depression. However, PD patients are on average older than ALS patients and the elderly in general have a higher incidence of depression.

Because of the lack of diagnostic routines for depression in the ALS diagnostic workup, depression may be both over- and underreported. The former due to the possibility that depression may mimic structural nerve impairment in ALS, resulting in higher than real depression prevalence before ALS diagnosis; and the latter since depression was missed in the ALS diagnostic procedure.

Depression after ALS

An almost eight times higher risk of depression was found one year after ALS diagnosis. This is not surprising as a crisis reaction can be anticipated after the diagnosis of such a devastating disease. This process is normal and patients pass through it with varying ease. It may seem normal to be sad and anxious after the diagnosis of a disease. On the other hand, endogenous depression might be overseen because of this. The way the diagnosis of ALS is delivered may also affect the patient´s reaction and consequently potential depression. In patients receiving other diagnoses such as breast cancer diagnosis an increased risk for depression (rate ratio 1.7, 95% CI 1.4-2.1) an increased risk for use of antidepressants (rate ratio 3.1, 95% CI 3.0-3.2) has also been found (117).

The distinction between diagnosed clinical depression, used for the calculations in this study, and a wish to die, often expressed by patients with incurable disease, must be emphasized. Thoughts about death, an elevated interest in end-of-life issues and speculations about means to end life may be perceived as rational and reasonable given the progressive nature and lame prognosis of ALS, however not enough to motivate a diagnosis of depression. In a multicenter study only 37% of those ALS patients who expressed a wish to die were actually clinically depressed (118).

Use of antidepressants in ALS patients

Citalopram, a SSRI drug, is the most widely and frequently used antidepressant in Sweden according to clinicians, verified by our results. We suspected that specific drugs used before ALS diagnosis might indicate endogenous depression and that specific other drugs used after ALS diagnosis would indicate reactive depression, and that it would be possible to observe a shift in drug use for those who develop depression after ALS diagnosis. However, we could not identify any changes in type of drugs used before and after ALS diagnosis and the same drugs seem to be used for all types of depression regardless of relation to ALS diagnosis time.

Antidepressants are sometimes prescribed for reasons other than depression, especially among ALS patients, and may not be an ideal proxy for depression. For example, the anticholinergic side effects of antidepressants are commonly used to treat pseudobulbar symptoms, such as excessive salivation, in ALS (114). Very common side effects of one of the most common SSRI, citalopram, are dry mouth, fatigue, drowsiness and somnolence.

SSRIs are often given to ALS patients to treat anxiety, dysphoria and emotional outbursts in frontal lobe affected patients.

It is interesting for clinicians to know to what extent the patient actually takes the drug prescribed. In some cases we noted a delay of up to one year between prescription date and dispensation date. In our study we only used cases who collected the drug at the pharmacy twice or more as such a dispensation pattern indicates regular use of the drug. By the other token it is never possible to know for sure if the patient avoids taking the drug which would lead to a non-differential misclassification. Compliance may be hampered by depressive behaviour,or by the simple fact that ALS patients experience problems due to their motor symptoms (such as difficulties in opening the pill box due to muscle weakness). It is possible that they do not consider themselves to be depressed, or they doubt that the drug will help them and therefore avoid medication.

6.2 METHODOLOGICAL CONSIDERATIONS

The epidemiological investigations in this thesis aim at providing accurate estimations of disease occurrence and associations between exposures and outcomes of interest. We have made efforts to minimise errors and draw appropriate conclusions, while considering methodological aspects summarised here. Errors in epidemiological studies can be random or systematic, and both can lead to study results differing from the true value.

Validity

With high internal as well as external validity it is possible to draw inferences from the study findings, for the study population and for the population which study participants are intended to represent - the source population and ultimately the target population. For example, in the PART cohort (study III) the source population is adults from Stockholm and the target population is all adults.

Random error

Due to chance, even a randomly selected study population´s values may not represent the true population values and thereby cause imprecise measures of occurrence and association. How random errors affect the precision of the estimates is reflected by a p-value or confidence interval. The precision increases with samples size. In all studies, attempts were

made to have a sufficiently large sample in order to reduce random errors related to sampling. For example, in study IV, a case-control study, five controls per case were randomly selected to improve precision.

Systematic error

Bias occurs when a systematic error alters the estimates. It could be an error within the study design which contributes to a wrong conclusion about the true association between exposure and outcome. Systematic errors in this thesis may be selection bias, misclassification (measurement or information) bias and confounding (119).

Selection bias

Selection bias occurs when the study participants in a non-random way do not represent those eligible to participate (119). This can occur when the study population sampling frame does not include all in the source or target population, when some invited people are more likely to participate than others, or when there is differential loss of follow-up. Mainly in study I-III, selection bias was a concern. The Swedish National March Cohort (study I-II) was based on a cancer fundraising event. Participants with a close relative affected by cancer or other diseases may have been more likely to participate, which may have introduced selection bias since they could be genetically or environmentally more sensitive to disease. They may also be more health-conscious, causing a healthy volunteer bias. Compared to the Swedish population, participants tended to smoke less (9.6% vs. 19.2% of the adult population), be slightly less educated (only compulsory schooling in 39% vs. 25%) and have a slightly higher BMI (43% vs.40% with BMI >25 kg/m^2). With smoking as a generally accepted undesirable behavior, the low reported numbers could be explained by an underestimation, i.e. a potential systematic measurement error. However, the probability of self-selection in the Swedish National March study is not likely associated with the outcome of Parkinson disease, due to the prospective nature of the study design (120). In study III, compared to the general Swedish population, participants more often had a Nordic origin, higher education and income, were married and had no previous psychiatric diagnosis.

Misclassification

Information bias, measurement bias or misclassification, may occur when we classify participants incorrectly (119). The self-reported data from questionnaires (study I-III) is prone to information bias, such as inaccurate or incomplete data about BMI, sitting time or diet. Body mass index is often underestimated when self-reported, especially among those

with higher BMI (121, 122) and this was likely the case in study I and III. Due to the prospective design, any misclassification related to BMI as exposure was likely non-differential, and recall bias was likely minimal in the cohort studies (study I, II and IV). When studying neurodegenerative diseases such as PD, a prospective design is a good choice to avoid recall bias due to memory deficits or recall bias influenced by disease status.

Confounding

A confounding factor is a covariate with an association to both the exposure and outcome, and consequently affects the observed effect (119). Confounding is a threat to the validity of a study. In all our studies, we performed multivariable regression to adjust for potential confounders, carefully selected based on known confounders from the literature. We also stratified the exposures to keep selected potential confounders constant in each stratum.

7 CONCLUSIONS

STUDY I: No association was found between prolonged sitting time, overweight or obesity, and Parkinson disease.

STUDY II: High intake of saturated fat was associated with an increased risk of Parkinson disease.

STUDY III: Persons with obesity desired a BMI higher than the ideal BMI recommended by WHO. High BMI and male sex were predictors of a large discrepancy between desired BMI and BMI 25 kg/m^2. High BMI, female sex and depression were predictors of a large discrepancy between current and desired BMI.

STUDY IV: Depression was associated with ALS, both before and after ALS diagnosis.

8 POINTS OF PERSPECTIVE

Prolonged sitting time is not associated with increased PD risk, as shown in **STUDY I.** Focus should be directed to other potential risk factors for PD. To what extent sitting time affects other neurodegenerative diseases remains to elucidate, and this parameter may be included in future studies on disease risk.

Dietary recommendations in Sweden (123) favor a level of fat intake above the level found in **STUDY II.** Additional large-scale studies are needed to corroborate these findings. However, dietary interventions may in the future prove useful in the treatment of PD. These findings may also be relevant for other neurodegenerative diseases such as Alzheimer´s disease (124), and for cardiovascular diseases such as myocardial infarction and stroke. Death occurs earlier from stroke than from PD, and the benefits of limiting saturated fat intake may have an impact on both disease groups.

STUDY III was a cross-sectional study generating hypotheses on the effects of BMI discrepancies. To chart the direction of possible associations between BMI discrepancies and depression we suggest longitudinal studies for the future, including further analyses of available data from the three waves in the PART cohort. In addition, we recommend qualitative studies to better understand the underlying mechanisms for discrepancies between desired and ideal BMI, and between current and desired BMI. Depending on the underlying causes, health care providers could then take necessary measures: Pay attention to certain groups with large discrepancies between desired and ideal BMI, including persons with depression or with a high BMI; prevent misleading misperceptions about one´s BMI; motivate a positive change or provide support to those with depression.

Depression may be a prodromal symptom of ALS, as indicated in **STUDY IV.** This finding, described here for the first time, is a useful tool in future ALS diagnostic workups. Further studies could investigate if individuals with depression prior to ALS diagnosis are the same persons who develop FTD. Attention could also be drawn towards high usage of antidepressant medication as an indicator of possible ALS, but this potential relationship needs further exploration. Additional studies are needed to establish if depression can be considered a risk factor for ALS. Extended studies are also needed to explore potential common denominators for both depression and ALS as these disorders may share mechanisms of pathogenesis.

Lifestyle behaviors studied in this thesis should be considered as modifiable in contrast to current treatments both for PD and ALS which are symptomatic treatments, with no cure in sight for neither disease. Global dietary patterns affect obesity and exposure to environmental toxins follow eating habits. Future research efforts in the direction of understanding the pathogenesis of PD and ALS should focus on modifiable risk factors.

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