The timing of environmental risk factors and prodromal signs of multiple sclerosis

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To my dear friend and example Christa Schad

Scientific environment

The studies included in this thesis were conducted at The Norwegian Multiple Sclerosis Competence Center at the Department of Neurology, Haukeland University Hospital, as well as at the Department of Global Public Health and Primary Care and the Department of Clinical Medicine, University of Bergen, Bergen, Norway.

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Abbreviations

BMI	Body mass index
CI	Confidence interval
CIS	Clinically isolated syndrome
CNS	Central nervous system
DAG	Directed acyclic graph
DMT	Disease-modifying treatment
EAE	Experimental autoimmune encephalomyelitis
EBV	Epstein-Barr virus
EBNA	Epstein-Barr virus nuclear antigen
EDSS	Extended disability status scale
EnvIMS(-Q)	Environmental Risk Factors in Multiple Sclerosis (questionnaire)
Gd	Gadolinium
HLA	Human leukocyte antigen
ICD	International Classification of Diseases
Ig	Immunoglobulin
IL	Interleukin
IQ	Intelligence quotient
IU	International Unit
MS	Multiple Sclerosis

MRI	Magnetic resonance imaging
N	Number
NEDA	No evidence of disease activity
NPR	National Patient Registry
OR	Odds ratio
PD	Parkinson's disease
PPMS	Primary progressive multiple sclerosis
RIS	Radiologically isolated syndrome
RR	Relative risk
RRMS	Relapsing-remitting multiple sclerosis
SD	Standard deviation
SPMS	Secondary progressive multiple sclerosis
T_H	T-helper lymphocyte
T_{reg}	Regulatory T-lymphocyte
VDR	Vitamin D receptor
WAIS	Wechsler Adult Intelligence Scale
WHO	World Health Organization
25(OH)D	25-hydroxyvitamin D

Abstract

Background: Multiple sclerosis (MS) is a chronic demyelinating disorder of the central nervous system that can lead to severe disability. It is a complex disease likely caused by genetic and environmental factors combined. Epstein-Barr virus (EBV) infection, low vitamin D, smoking, and being overweight are the environmental factors, which have most consistently been associated with an increased MS risk. However, detailed aspects of their involvement are not entirely resolved. Timing of exposure appears to be important, but whether the effect of vitamin D on susceptibility varies by age is unclear. Further, while a link between being overweight and MS risk has consistently been reported among women, it is less clear among men. Still, as the cause of MS is ultimately unknown, research on new potential etiologic factors is also warranted. One attractive candidate is physical exercise, as it is modifiable and could prevent disease if proven effective. Etiologic research can be facilitated if the natural history of MS is well characterized and understood. However, the nature and timing of prodromal MS, i.e. subclinical disease activity before the onset of classic neurologic symptoms, is largely unknown, and is thus a challenge to studies of risk factors and relevant timing.

Objectives: The main objectives in this thesis were to gain knowledge on susceptibility periods and prodromal MS and advance research on established and putative new environmental risk factors. In detail, we intended to 1) investigate the association between postnatal timing of cod liver oil use, an important oral vitamin D source in Norway, and MS risk, 2) compare cognitive performance of men who later in their life developed MS to those who did not, to capture potential differences indicative of disease processes prior to first symptom and therefore prodromal MS, and 3) examine the association between being overweight and MS risk in men and whether fitness, as a proxy of exercise, is independently related to disease risk.

Methods: For the first objective, we used the Norwegian data of the multi-national population-based case-control study Environmental Factors in Multiple Sclerosis (EnvIMS). We included, in total, 953 MS cases with neurologist-verified diagnosis

recruited from the Norwegian MS registry and with disease duration of maximally 10 years, and 1,717 controls randomly selected from a population registry, frequency-matched on sex and age. Participants reported their cod liver oil use from childhood to adulthood and other relevant age-specific information using a validated questionnaire (EnvIMS-Q). The association between exposure to vitamin D through cod liver oil use at different ages and MS risk was estimated as odds ratio (OR) and 95% confidence intervals (CI) using logistic regression. Apart from age and sex, we adjusted the analyses for outdoor activity during the summer, a proxy of sun exposure, dietary intake of vitamin D-rich fatty fish, history of mononucleosis, smoking, body size during adolescence, education, and MS family history. For the second and third objectives, we conducted population-based nested case-control studies within the historical cohort of all men born in 1950-1995 who underwent the mandatory Norwegian conscription examination at age 18-19 (about 90% of all Norwegian men). We identified men who went on to develop MS later in life through linkage of the Conscript Service Database to the Norwegian MS registry and selected controls randomly from the same database frequency-matched on year of birth to all the cases in the MS registry. For article 2, we included 924 men who later developed MS and 19,530 controls with information on cognitive performance at conscription. We compared their cognitive scores (standard nine scale, mean=5, standard deviation (SD)=2), standardized on 5-year birth cohorts, overall and according to initial disease course, relapsing-remitting (RRMS) and primary progressive MS (PPMS), using Student's t-test. We also assessed the risk of MS in the years following conscription among men who scored lowest (>1 SD below the controls' mean) compared to the rest using Cox regression to estimate relative risk (RR) and 95% CI. For article 3, we included 854 men who later developed MS and 14,563 controls, all born in 1950-1975, with information on a) weight and height, from which we determined body mass index (BMI), and on b) physical fitness test result (score on standard nine scale). We estimated the independent effect of BMI, as a measure of body size, and fitness, as a measure of regular vigorous exercise, at age 19 and MS risk later in life using Cox regression and reported RR and 95% CI.

Results: In article 1, we found that cod liver oil use during adolescence was significantly associated with a decreased MS risk compared to no supplementation during adolescence (OR=0.67, 95% CI: 0.52-0.86), whereas there was no association between use reported during childhood or adulthood. The estimates were mutually adjusted for each other and did not materially change after adjustment for other potential confounders. A dose-response relationship was suggested between higher cod liver oil doses during adolescence and lower MS risk peaking at 600-800 international units/ day of vitamin D consumed through cod liver oil (OR=0.46, 95% CI: 0.31-0.70, p trend=0.001). In article 2, we found that only men who developed MS within 2 years after conscription scored cognitively significantly lower at age 18-19 than controls, equivalent to 6 IQ-points. There was, however, no overall difference in cognitive scores between the comparison groups. Results were similar when we assessed men who went on to develop RRMS, while men who went on to develop PPMS scored significantly lower than controls at that age, by an equivalent of 4.6-6.9 IQ-points, although they would not develop first symptoms up to 20 years later. Men who scored lowest had an increased RRMS risk within 2 years from conscription (RR=2.69, 95% CI: 1.41-5.16), and an increased PPMS risk within 20 years. Finally, in article 3, we found that higher BMI ($\geq$25kg/m^2) was associated with higher MS risk (RR=1.36, 95% CI: 1.05-1.76) compared to normal BMI, and that higher aerobic fitness was independently associated with lower MS risk (RR=0.69, 95% CI: 0.55-0.88, p trend=0.003).

Conclusions: The findings of this thesis add to the evidence linking low vitamin D to MS risk and further point to adolescence as the crucial postnatal period, in which adequate levels should be ensured. Further, these findings suggest that MS has a prodromal phase with subtle but detectable signs. RRMS could start years prior to first relapse, while PPMS could potentially start decades prior to onset of progressive symptoms. Lastly, these findings add weight to evidence linking being overweight to MS risk among men and suggest, further, that vigorous exercise or a factor strongly associated with high cardiorespiratory fitness may be an additional modifiable protective factor for MS that warrants further investigations.

List of publications

1. Cortese M., Riise T., Bjørnevik K., Holmøy T., Kampman M.T., Magalhaes S., Pugliatti M., Wolfson C., Myhr K.-M. (2015): “Timing of use of cod liver oil, a vitamin D source, and multiple sclerosis risk: The EnvIMS study”, Multiple Sclerosis Journal, 21(14): 1856-64.

2. Cortese M., Riise T., Bjørnevik K., Bahn A., Farbu E., Grytten N., Hogenesch I., Midgard R., Smith Simonsen C., Telstad W., Ascherio A., Myhr K.-M. (2016): "Preclinical disease activity in multiple sclerosis: a prospective study of cognitive performance prior to first symptom", Annals of Neurology, 80(4): 616-24.

3. Cortese M., Riise T., Bjørnevik K., Myhr K.-M., Multiple Sclerosis Conscript Service Database Study Group (2017): “Body size and physical exercise, and the risk of multiple sclerosis”. Multiple Sclerosis Journal, *Epub ahead of print*, DOI: 10.1177/1352458517699289.

Contents

The aim of medicine is to prevent disease and prolong life, the ideal of medicine is to eliminate the need of a physician.

William James Mayo (1928)

Proceedings of the National Education Association, Volume 66

1. Introduction

1.1. Multiple sclerosis- an overview

Multiple sclerosis (MS) is a chronic immune-mediated neurologic disorder of the central nervous system (CNS) and can lead to severe disability.[1] It is thought to develop in genetically predisposing individuals under the additional influence of environmental triggers.[1] The underlying causes are, however, ultimately unknown.[2] The socioeconomic burden of MS is high as it commonly afflicts young adults and is one of the most common disability causes in this group.[3] The disease course is difficult to predict, as MS is heterogeneous on the radiological, clinical, and pathological level.[4-6] Patients are thus faced with a chronic unpredictable disease in a stage of life in which they are establishing themselves privately and professionally.[3] MS reduces life expectancy moderately (7-14 years),[7] and most recent studies report improved survival over the last decades.[8] However, high morbidity due to MS and comorbid conditions markedly reduce quality of life,[9] in part mediated through alteration of employment status.[10, 11] While disease-modifying treatment can be offered to many though not all patients, MS remains a feared disease, so far without any curative treatment.[12-14]

1.2. Occurrence and distribution

It is estimated that about 2.3 million individuals worldwide suffer from MS with substantial geographical differences in incidence rates and prevalence.[15] This updated estimation from a recent survey suggests that the global burden of MS has been increasing in prevalence from 2008 to 2013 by about 9%.[16] The median global prevalence was estimated at 30 in 2008 and 33 in 2013 per 100 000 individuals, including high-risk areas like North-America and Europe (>100 per 100,000) to low-risk areas including Central America, Sub-Saharan Africa and East Asia (≤5 per 100,000) (Figure 1).[16] Kurtzke's once suggested geographical MS distribution into

three zones of high, medium and low frequency (>30, 5-25, <5 per 100,000) seems therefore still applicable.[17] The prevalence of MS is determined by disease incidence, detection, and duration and it is unclear to which extent the higher MS burden is due to an increase in disease frequency, awareness, reporting, diagnosis, or prolonged patient survival.[18] Studying incidence rates may be more insightful in this respect.

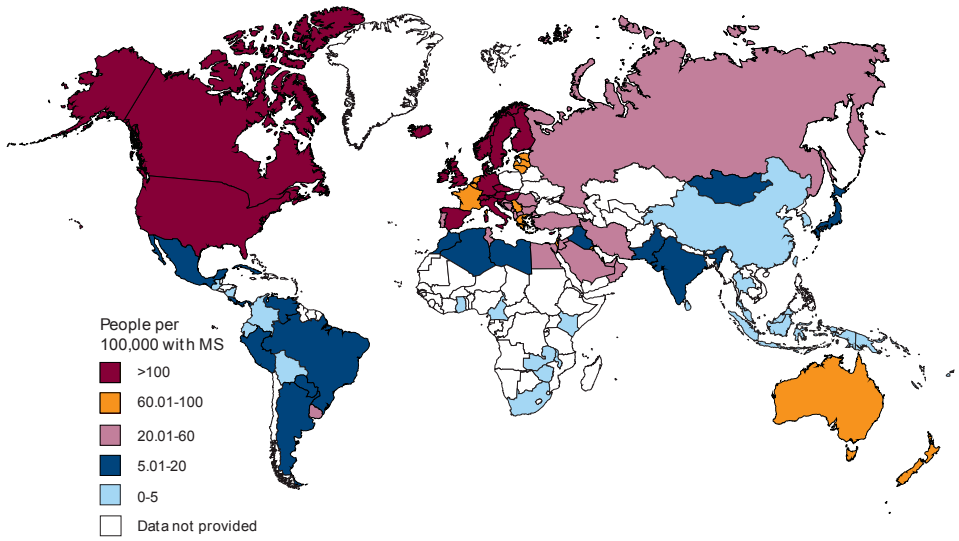

Figure 1: The prevalence of multiple sclerosis in 2013 by country.

Reprinted by permission from the Multiple Sclerosis International Federation: The Atlas of MS 2013 report, page 8,[16] *https://www.msif.org/about-us/who-we-are-and-what-we-do/advocacy/atlas/, © Copyright 2013*

The median MS incidence is estimated globally at 5.2 per 100,000 per year during the last decades with substantial regional variation ranging from 0.5 in Panama to 20.6 in Alberta, Canada.[19] The lowest annual incidence rates were reported for Central American, Caribbean, North African, and East Asian countries, while the highest rates were seen in the US and Canada followed by most European countries and Australia.[20-25] Interestingly, disease occurrence has been associated with latitude implying that MS is more frequent in areas further away from the equator.[26, 27] However, a recent systematic review of incidence studies published over several decades suggests that the latitude gradient is disappearing due to increased disease

occurrence in lower latitudes.[28] Ethnic differences in incidence rates, with blacks, Hispanics, and Asians traditionally considered low-risk groups,[29] may be leveling out as well, with rates among black individuals similar to or surpassing those among white individuals.[30, 31] The underlying causes of these changes are unclear.[32]

The lifetime risk of MS is about 1 in 200 to 400 in high-risk areas depending on sex.[33, 34] MS affects more commonly women than men with a ratio of about 2:1 to 3:1,[35] potentially due to an increased female susceptibility to certain risk factors.[36, 37] Recent findings suggest a further divergence between the incidence rates among males and females, especially in the most northern latitudes.[38, 39] This could either be due to an increased MS incidence among women, or a reduced incidence among men, or a combination of both. Many studies support the idea that there has been a genuine increase in disease occurrence among women.[35, 40] In addition, there is some evidence that this trend might be, at least partially, related to changes in habits of smoking,[41] a risk factor for MS.[42] A decline in smoking rates the last decades has been more marked in men and this could contribute to relatively higher MS rates in women.[41]

Norway is among the countries with highest MS prevalence (203 per 100,000) and annual incidence rate (8.0 per 100,000).[43] While Swank reported lower MS rates in coastal regions and higher ones inland in the 1950ies,[44] rates are comparable in different regions today and there is no latitude gradient within the country.[43, 45] A four-fold increase in incidence over six decades has been reported regionally, but the trend has more recently been stabilizing along with the increase in sex-ratio.[46, 47]

Differences by latitude and sex gave rise to hypotheses regarding MS etiology. Exposures like ethnicity, vitamin D and sun exposure, diet, infectious agents, and hormonal triggers became hereby of interest.[48]

1.3. Disease presentation

1.3.1 Clinical and radiological features

The incidence of MS increases during adolescence with very few cases manifesting during childhood.[49, 50] About 80% of MS patients experience clinical onset at ages 20 to 50 years.[51] The two initial core MS phenotypes are relapsing-remitting (RRMS) and primary progressive MS (PPMS) (Figure 2),[5] and RRMS is the most common form comprising about 85% of all cases.[52] Affected individuals experience a sudden clinical MS onset peaking around age 30, characterized by an acute episode with neurological deficits (relapse) due to focal inflammatory processes in the CNS,[53] but remission of function usually follows the more or less frequent relapses in RRMS.[54] RRMS presents commonly with motor, visual, sensory, or brain stem related functional deficits,[1, 55] Most RRMS patients eventually enter a progressive stage, secondary progressive MS (SPMS),[56] with gradual irreversible deterioration due to neurodegenerative processes possibly with superimposed relapses after a median of 15 to 20 years, as observed in mainly untreated patients.[57-61]

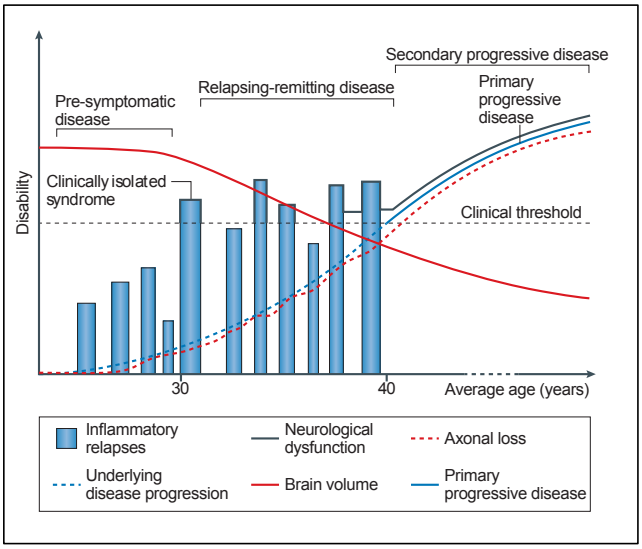

Figure 2: The clinical phenotypes of multiple sclerosis.

The figure shows the MS phenotypes Clinically isolated syndrome, Relapsing-remitting, Secondary progressive, and Primary progressive MS with mean onset age and disability

resulting from inflammation and degeneration in the CNS. It also implies sub-clinical MS, disease activity below the clinical threshold, prior to first specific neurologic symptoms. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Immunology 15: 546,[62] http://www.nature.com/nri/index.html, © Copyright 2015

Individuals developing the less frequent form, PPMS, affecting about 15% of all cases, usually experience a less acute, more insidious clinical onset with the first neurologic symptoms at a median age of 40 years, as illustrated in Figure 2.[52] This phenotype is characterized by neuronal degeneration and accumulation of irreversible functional deficits over time.[63] PPMS manifests for most cases as spastic paraparesis.[64] While RRMS is more frequent among women with an incidence ratio of about 1:2 to 1:3, PPMS affects men and women more or less equally.[47, 65]

Today, decades after an expert panel agreed on the first diagnostic criteria in a time before magnetic resonance imaging (MRI) became available,[66] MS remains a mainly clinical diagnosis made retrospectively.[5, 67] It is based on the detection of dissemination in time and space of clinical CNS involvement, explicitly proposed by Schumacher and colleagues in 1965.[68] Diagnostic criteria of PPMS were only specified in 2000.[64] However, MRI is today incorporated into the diagnostic process. The simultaneous presence of gadolinium (Gd)-enhancing and non-enhancing asymptomatic lesions on the first scan indicates dissemination in time as well as space, and allows the diagnosis of clinically definite RRMS after only one clinical event.[67, 69] MRI has helped to decrease diagnostic uncertainty in early MS and shorten the time from manifest disease to diagnosis.[70] Identifying predictors of conversion to definite MS after a single clinical event suggestive of MS contributed to these developments.[71]

Clinical parameters are also important to assess the MS course.[5] While relapses are a sign of active disease, a higher Expanded Disability Status Scale (EDSS) score indicates worsening or progression, especially when confirmed with reasonable time apart.[5, 72] EDSS is a score ranging from 0 (normal) to 10 (death due to MS) given based on a thorough neurologic evaluation of different functional systems, with important landmarks like an EDSS of 7 indicating restriction to wheelchair.[72]

Cognitive impairment, which is frequent in MS, seems to most commonly affect patients with PP- and SPMS and is thus also considered a clinical sign of progressive disease.[73] It is better captured by validated neuropsychological tests than EDSS,[73, 74] including sensitive clinical measures for the most common cognitive deficit in MS, i.e. slowing of processing speed.[75, 76] The radiological measures of disease activity and progression include Gd-enhancing T1-, new/enlarging T2-weighted MRI lesions on the one hand and brain volume loss on the other.[77-80] A newer ambitious outcome measure, "no evidence of disease activity" (NEDA), is a composite score of clinical and radiological freedom from MS activity and progression as a treatment response to potent disease-modifying agents aiming at long-term remission.[81-83]

In spite of these measures, predicting conversion and progression remains, however, challenging.[84-86] In RRMS higher initial relapse rate, shorter time elapsed to the second clinical episode, and higher EDSS several years after onset have been suggested to be negative prognostic factors.[53, 84] Relapses appear to be more predictive of short-term progression, especially in younger patients.[87, 88] In PPMS a fast initial disability accumulation speed has been associated with worse prognosis.[84]

1.3.2 Pathogenetic mechanisms

In MS, immunological processes fuel pathological changes manifesting as either neuronal inflammation or degeneration.[62] An imbalance between pro- and anti-inflammatory cellular and humoral immune components plays an important role in initiating and sustaining a cascade of pathognomonic processes, although the triggers and chronology of events are not entirely understood.[1] The immune system is probably directed against the myelin sheath that facilitates saltatory propagation of nerve impulses to the target organ and is important for the homeostasis of the neuron.[62]

Autoreactive $CD4^+$ T-lymphocytes, insufficiently inhibited by $CD4^+$ regulatory T (T_{reg})-lymphocytes,[89-91] can differentiate to neurotoxic T helper (T_H) cells type 1 and 17 and cross the blood-brain barrier.[92] They play a central role in promoting and perpetuating, along with activated CNS-resident microglia and astrocytes,

inflammation and demyelination targeting oligodendrocytes, the cells that build the myelin sheath.[92-94] Monocytes and other antigen-presenting cells are recruited and produce pro-inflammatory neurotoxic and chemotactic cytokines potentiating the immune response.[95, 96] B-lymphocytes, activated to complement-producing plasma cells, are also involved in the immune response.[97] They produce oligoclonal antibodies that have been of diagnostic and prognostic value, and might target myelin proteins, though it is unclear which antigens specifically.[67, 98, 99] The loss of the isolating protective myelin sheath leads to functional deficits due to disturbed electric impulse propagation[94, 100] and to axonal transection.[101, 102] However, at an early stage compensatory mechanisms like re-myelination can restitute the neuro-axonal integrity and function.[103, 104] Focal inflammation and demyelination is prominent in RRMS,[105] leading to the typical multifocal periventricular, infra-tentorial, and spinal lesions in the white matter, rich in $CD4^+$ T_H17- and $CD8^+$ T-cell infiltrates if acute.[93]

Inflammation and demyelination are also present in PPMS and SPMS, although typically in a more diffuse pattern, even within normal-appearing white matter.[63, 105, 106] Cortical lesions are a hallmark of progressive disease[105] and seem to correlate more strongly with physical and cognitive disability.[107] These lesions may explain, to some degree, the discordance between white matter lesion location/extent and clinical symptoms known as clinico-radiological paradox.[108] Neurodegenerative processes are associated with more CNS-intrinsic chronic inflammation sustained by chronically activated microglia and astrocytes and potentiated by exhaustion of compensatory mechanisms.[62, 109] Chronic inflammation favors the formation of reactive oxygen species provoking mitochondrial injury and hence energy inefficiency, metabolic stress, and ionic imbalance, detrimental to the neuron.[110] Accumulating irreversible neuro-axonal loss leads to brain volume loss that is associated with disability.[111, 112]

While these changes are present from clinical onset in PPMS, they indicate progressive disease in RRMS. Still, there is no clear cutoff for the onset of SPMS and neurodegenerative processes may evolve as a continuum from early MS.[113]

1.3.3 Points of debate

The distinction of two core initial disease courses[114] animated a discussion on whether RRMS and PPMS are phenotypes of the same disease or distinct diseases,[115] further highlighting the complexity of MS. Genetic,[116] clinical,[117] and pathological evidence[105] argue against, and the inconsistent immunological evidence not compellingly for fundamentally different diseases.[118-121] Differences between relapsing and progressive MS are thus probably rather quantitative than qualitative.[115] PP- and SPMS share core characteristics like higher onset age, progressive character, and speed of disability accumulation. The spinal and cortical lesion load is also comparable.[105] PPMS may thus be MS "amputated" from the relapsing stage.[122] RR- and PPMS are today rather considered two phenotypes of the same disease.[5, 63, 122]

Despite an ongoing quest for prognostic factors, evidence suggests that MS progression and the speed of disability accumulation is dependent on age and not on initial clinical features,[51, 88, 117, 122-125] explaining maybe the difficulties in predicting progression. This means that disability milestones are commonly reached at a certain age rather than after a certain time from clinical onset.[117] Individuals with disease onset later in life will reach disability milestones faster than those with earlier onset. This seems to also apply to pediatric onset MS patients who, interestingly almost exclusively, develop relapsing disease.[126]

Whether focal inflammation in the CNS triggers neuronal degeneration or whether both are mutually independent processes manifesting simultaneously but in an opposed waxing and waning manner has long been debated in MS.[63] How exactly diffuse inflammation and re-myelination affect this interplay is also unresolved.[1, 63] The first disease-modifying drugs for MS were approved in the early 1990s and several more potent ones followed over the years.[127-131] These drugs were shown to reduce (focal) inflammatory MS activity.[132] However, it is not entirely clear how effectively they delay neuronal degeneration and hereby disability progression,[133, 134] as they proved ineffective for PPMS (with the exception of one agent approved recently[135]) and SPMS.[136, 137] Brain volume decrease seems unaffected[138] and

evidence on preservation of cognitive function is inconclusive.[139] Still, early treatment to slow progression is advocated by many studies, but their relatively short follow-up time may not reflect long-term outcomes.[140] Confavreux and colleagues suggested that inflammatory and degenerative processes proceed independently from onset because of the low effect of modifying drugs on long-term irreversible disability accrual and cerebral atrophy, despite potent immunosuppression.[88] On the other hand, Leray et al. suggested MS to be a two-staged disorder, in which the progressive stage does not depend on events in the initial stage.[141] Prognostic factors like relapse rate may predict worsening and duration of the first stage but not be able to predict progression and disability in more chronic disease. There is some agreement on that once processes pertaining to progressive disease are set in motion, they run independently from inflammatory disease components.[63, 142]

Knowledge on these aspects was gained in population-based cohorts of treatment-naïve MS patients with a long follow-up, ideal to study the natural MS history.[143] They help us understand the MS evolution and course, facilitate prognostic considerations, treatment decisions, and inform the design/interpretation of clinical trials.[84, 144-146] However, it has been more difficult to learn about prodromal MS, and identify when MS starts, which could also facilitate the search for causal factors.[147] In most studies, date of first symptom(s) has been used to define MS onset.

1.4. Evidence of prodromal multiple sclerosis

1.4.1 Clinically isolated syndrome

Clinically isolated syndrome (CIS) is the first clinical presentation of focal inflammatory CNS demyelination suggestive of MS and is part of the MS phenotype spectrum (Figure 2).[5, 148] An individual with CIS can be diagnosed with MS at a second independent relapse or if MRI shows dissemination in time according to current diagnostic criteria, as described above.[67] Conversion rates to definite MS depend on length of follow-up but vary markedly between CIS patients with normal

(20-25%) and abnormal (70-80%) MRI scans over the course of 15-20 years.[149, 150] Early treatment initiation delayed conversion in different clinical trials.[151-153]

CIS marks the time, at which previously subclinical MS exceeds the clinical threshold and becomes apparent (Figure 2).[1] Indeed, CIS can be associated with signs of long-lasting disease processes. A high lesion burden on the first MRI scan,[71] including old inactive lesions, and fatigue[154] are common among CIS patients and predict conversion. Further, cognitive impairment may be similarly prevalent among CIS and RRMS patients, although it was traditionally considered a late symptom associated with progression.[73, 155, 156] Presence of cognitive impairment has also been suggested to predict conversion to clinically definite MS.[157] Even brain atrophy, a correlate of neuronal degeneration, can be present at first clinical presentation.[158, 159] What triggers the clinical MS onset is unknown.

1.4.2 Radiologically isolated syndrome

The radiologically isolated syndrome (RIS) can be a sign of MS activity prior to clinical onset.[160] Individuals with RIS are detected by coincidence when a brain MRI, performed for other reasons than suspicion of MS, reveals lesions in an MS-typical pattern.[161] The existence of clinically silent MS was suggested as early as in the 1960s based on autopsy findings.[162] Depending on the study, 30-45% convert to CIS over the course of 2 to 5 years,[163] and spinal cord in addition to brain lesions are a strong predictor of conversion to CIS/RRMS or PPMS.[164, 165] RIS patients usually remain untreated due to the absence of specific neurologic symptoms suggestive of MS.[166] Interestingly, at closer examination they may display cognitive impairment,[167-169] fatigue,[170, 171] and brain volume changes[172, 173] to a similar extent as CIS and MS patients. RIS can be detected many years prior to the clinical MS onset according to case reports,[174] but time of true disease onset is unknown.

1.4.3 The neuroepidemiological challenge

It can be challenging to study the causes of diseases with a long prodromal phase, like neurodegenerative disorders including Alzheimer's and Parkinson's disease (PD).[175,

[176] Even though there is agreement that MS also starts prior to clinical presentation, discussed as early as in 1965 by Kurtzke,[177] the duration and nature of prodromal MS activity are largely unknown beyond knowledge about RIS.[178, 179] If knowledge about the onset of a disease is limited, the Hill criterion of temporality that is essential (though not sufficient) to evaluate whether an association is more likely to be causal in observational studies, might be violated.[180] The cause needs to precede the effect but an incorrect conclusion about the putative cause-effect direction is possible if a yet undetected sub-clinically active disease leads to changes in behavior, biological parameters, or proneness to certain events.[181, 182] This needs to especially be assessed for new putative risk factors. The association between head trauma and PD risk illustrates the problem of reverse causation potentially underlying an association.[175] Whether head trauma is a cause of PD or incipient postural instability in a yet unidentified PD patient increases the risk of falls and thus head injury, is difficult to decipher.[175] Further, the distinction between classic causes and triggers adds to the complexity, as additional triggers might be necessary during the latent period for the disease to manifest altogether or at an earlier time, potentially providing a window of opportunity for intervention.[18, 178] Moreover, knowledge about the true disease onset can facilitate the detection of susceptibility periods for important exposures (see next section).[18] All in all, knowledge about latency periods is difficult to gain prospectively.[178] Studies on RIS are valuable but are not prospective regarding prodromal MS, as these individuals are already aware of a potential disease. RIS patients might, further, not be representative of all prodromal MS patients.

1.5. Etiology

MS is likely caused by a combined effect of genetic and environmental factors.[1] As so far known, positive family history of MS is the strongest predictor.[183] Studies on familial risk assessing different degrees of kinship between MS patients and their relatives suggest that genetic predisposition increases with the proportion of shared genes (Figure 3).[184, 185] MS concordance is about 25% among monozygotic twins and about 1% among cousins, which is still 3-5 times higher than the lifetime MS risk in

the normal population.[186-188] Alleles coding human leukocyte antigen complex (HLA) class II cell surface proteins involved in antigen-presentation on immune cells convey the highest risk,[189-191] especially HLA-DRB1*1501,[192] but there are also protective genotypes, HLA-A*02 (HLA class I).[193] Genome-wide association studies have revealed further independent susceptibility genes, single nucleotide polymorphisms, and mutations in immunologically relevant genes involved in MS.[194, 195]

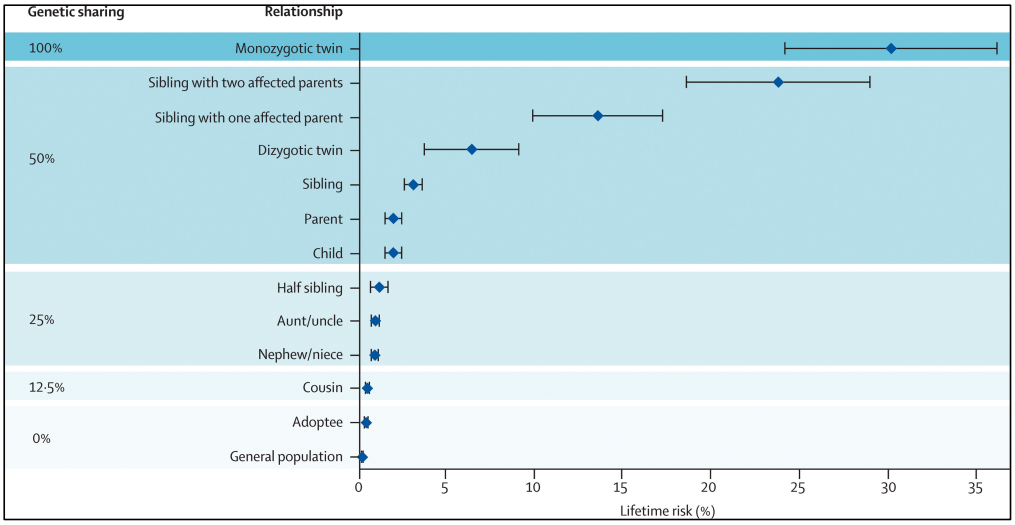

Figure 3: Recurrence risk of multiple sclerosis within families.

The figure shows the lifetime risk of individuals with positive family history of multiple sclerosis according to degree of kinship. Reprinted by permission from Elsevier: The Lancet 372: 1506,[1] http://www.thelancet.com/, © Copyright 2008

Although these findings support that genetic factors are crucial, the incomplete concordance among identical twins and implication of immune-regulating genes also involved in other autoimmune diseases and altogether explaining not more than 30% of the risk, indicate, at the same time, a major role of the environment.[62] Findings from migration and space-time cluster studies and incidence trends over the last decades provide further evidence for an important environmental influence.[27, 196] Gene-environment interaction studies try to integrate both components and explain MS risk more comprehensively,[197, 198] and epigenetic mechanisms may underlie these

interactions.[199] However, it is clear that some major undiscovered determinant(s) are likely involved to more comprehensively portray the picture of MS susceptibility.

1.6. Environmental risk factors and susceptibility periods

The environmental factors most consistently associated with the risk of developing MS are Epstein-Barr virus (EBV) infection, low vitamin D levels, tobacco smoking, and obesity in early life.[32, 200] Evidence is most consistent for RRMS and there is a lack of studies focusing on PPMS.[201] An involvement of modifiable factors in the etiology of MS represents an opportunity, as prevention of MS can be regarded as the highest potentially achievable goal provided that evidence from different fields converges.[32, 48] Studies on the population attributable risk of potential factors are theoretical examples of that approach.[202, 203]

To strive for this goal, apart from identifying relevant exposures, it is essential to determine when these influence MS susceptibility.[196] Migration studies gave some clues about the timing of environmental exposures suggesting that an individual's MS risk is determined during the first two decades of life.[204] Individuals migrating from high- to low-risk areas showed disease rates similar to those at their destination only if they moved before age 15, but retained the higher risk of their country of origin if migrating after that age.[205, 206] Further studies hinted to a continuous risk decrease with earlier age at migration.[207, 208] The fact that MS risk in the host country is not entirely adopted after migration could be due to genetic predisposition linked to ethnicity or a susceptibility period in utero.[209-211] Studies of individuals migrating from low- to high-risk areas indicate an inverse trend, but were based on very small samples.[212] Space-time cluster studies provided further clues for a critical age at which environmental factors act, reporting a statistically significant clustering of cases during early life.[213, 214]

1.6.1 Epstein-Barr virus

Infectious agents have long been suspected to play a role in MS with major attempts to explain the nature of their involvement.[215] The hygiene hypothesis for MS evolved in the 1960s after Poskanzer proposed that MS could be a rare consequence of an infection contracted at higher age in areas further away from the equator due to better sanitary conditions. [216] He compared it to clinical poliomyelitis, which was common in regions with better and rare in regions with worse sanitation, where the poliovirus was ubiquitous from childhood. For the first time, age at exposure was suggested to modify MS risk.[217] An early exposure to various infectious agents, which is more common in regions with poor hygiene, was later suggested to be important for the development of an immune response away from a pro-inflammatory T_H1- to a more T_{reg}- and T_H2-cell milieu.[218] Reports of MS epidemics after local deployment of foreign soldiers on the Faroe Islands, where MS had previously not been observed, prompted Kurtzke in the 1990s to another hypothesis.[219] A contagious infectious agent more prevalent away from the equator may lead to an asymptomatic infection in most, and occasionally to MS in some individuals years later. These hypotheses initiated a search for an infectious MS cause, which is still ongoing today.[215, 220] However, so far only the herpes virus EBV, first suggested in 1981 as a potential candidate,[221] has consistently been related to MS.[215]

Striking similarities have been noted between the epidemiology of MS and symptomatic EBV infection, referred to as infectious mononucleosis, which is more common when EBV is contracted later in life, during adolescence or adulthood.[221] The EBV-seroprevalence is about 95% by adulthood,[215] but a latitude gradient, inverse to that observed for MS distribution, was noted for EBV-antibody prevalence during childhood with a higher share of seropositive individuals in (sub)tropical (developing) regions and a lower one in more temperate (developed) regions.[222] In areas with poorer hygienic conditions EBV is most often acquired asymptomatically during childhood, whereas in more affluent areas many individuals remain uninfected up to adolescence, when the exposure likelihood to EBV in saliva increases again

substantially.[200] Infectious mononucleosis is thus considered a marker of high hygienic conditions during childhood.

The evidence suggesting that EBV is involved in MS etiology is compelling and MS could be considered a rare complication of an EBV infection.[200] Findings from prospective studies support a strong monotonic relationship between elevated EBV-specific serum antibody titers, especially IgG against Epstein-Barr nuclear antigens (EBNA), and MS risk.[223-227] Individuals with late primary infection resulting in mononucleosis were at 2.3 times higher and EBV-negative individuals at about 15 times lower risk of MS compared to individuals acquiring EBV during childhood/without mononucleosis history (Figure 4).[200, 228] A prospective study among US military personnel also found that EBV-negative individuals can practically not develop MS and importantly, observed, that all EBV-negative individuals who later developed MS seroconverted several years prior to clinical MS onset.[229] According to the hygiene hypothesis seronegative individuals ought to have the highest MS risk, but they appear to, instead, have a very low MS risk. These studies seem thus to suggest a more direct EBV involvement in MS pathogenesis rather than reflecting the hygiene hypothesis.[215]

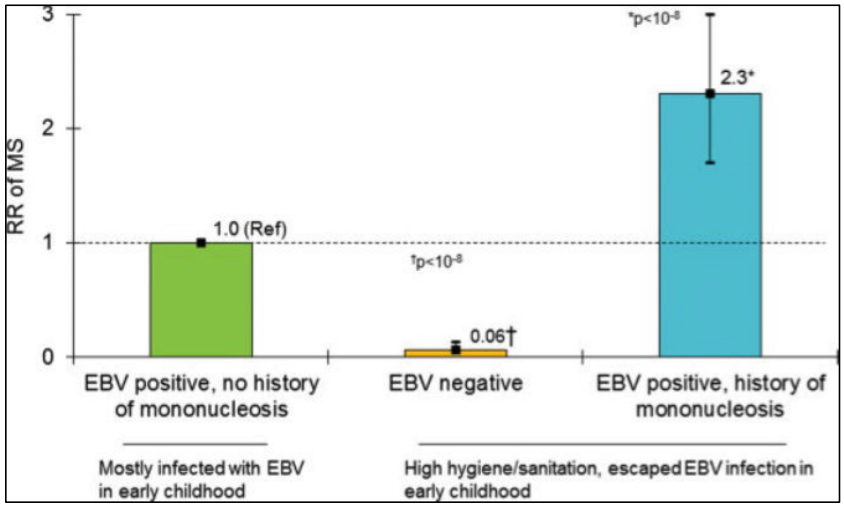

Figure 4: Epstein-Barr virus infection and multiple sclerosis risk.

The figure shows the relative risk (RR) of MS for EBV-negative individuals and individuals

who had infectious mononucleosis compared to EBV-positive individuals without history of mononucleosis (Ref) and the corresponding p-values. Reprinted by permission from Georg Thieme Verlag KG: Seminars in Neurology 36(2): 108,[200] *http://www.thieme.com/books-main/neurology/product/2163-seminars-in-neurology, © Copyright 2016*

The mechanisms underlying this strong association remain unresolved.[200] Direct CNS infection has been proposed, but the findings are overall inconsistent and the virus has not consistently been detected in the CNS.[230-234] Trials on antiviral treatment of MS patients were not successful, although this cannot exclude that EBV could initiate MS.[235, 236] The immune response to EBV could also induce cross-reactivity to self-antigens (molecular mimicry).[237-239] These mechanisms need to be elucidated before EBV can be seen as a modifiable factor for MS, supporting approaches like early intentional infection to prevent mononucleosis or the development of a vaccine.[200]

1.6.2 Vitamin D

Exposure to sunlight is the major vitamin D source.[181] Cholecalciferol (vitamin D_3) is synthesized in the skin with the aid of ultraviolet B rays (Figure 5).[240] With higher latitude the sun dose decreases and cholecalciferol intake through diet (fatty fish, fortified foods) and supplements (vitamins, cod liver oil) gains relatively in importance, especially during winter,[181] when the sun-induced production diminishes considerably (36-43°, Spain) or ceases completely (58-71°, Norway).[241] Apart from geographical, seasonal, and phenotypical factors (e.g. skin tone), sun-avoidance is an important reason for the worldwide common vitamin D deficiency.[242, 243]

In the liver cholecalciferol is enzymatically converted to 25-hydroxyvitamin D (25(OH)D), the most commonly used serological marker of vitamin D status (Figure 5).[181] Through further hydroxylation in the kidney, 25(OH)D is converted to the hormonally active metabolite, calcitriol (1,25-dihydroxyvitamin D), that can bind to vitamin D receptors in the nucleus and on the plasma membrane, affecting gene expression by functioning as transcription factor and signal transduction.[244]

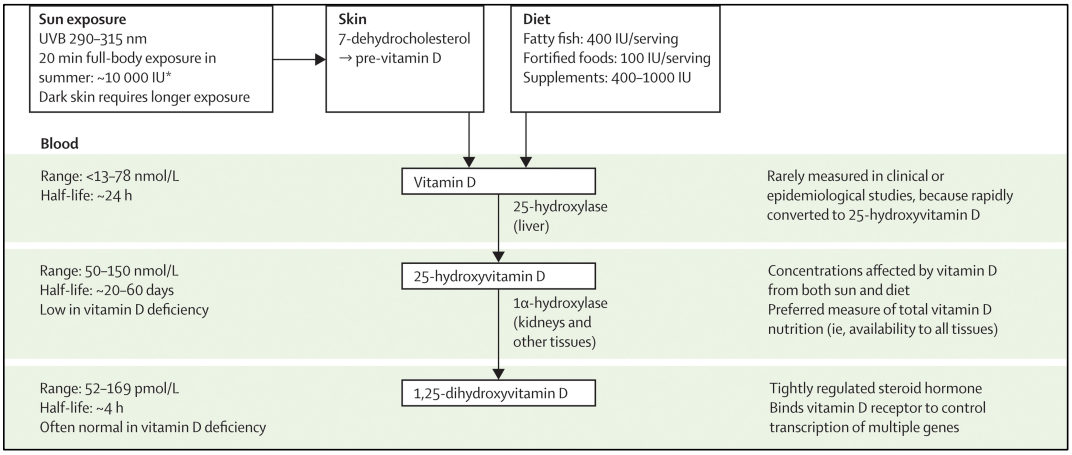

Figure 5: Vitamin D metabolism in humans.

The figure shows the sources, the metabolism, and the bioavailability of vitamin D. Reprinted by permission from Elsevier: The Lancet Neurology 9: 600,[181] *http://www.thelancet.com/journals/laneur/issue/current, © Copyright 2010*

Vitamin D deficiency was first discussed as a potential factor in MS etiology in the 1970s,[245] supported by an observed link between geography, sunlight exposure, diet, and MS prevalence in ecological studies.[44, 246, 247] Today there is converging evidence to support the vitamin D hypothesis, including four major prospective studies (three are illustrated in Figure 6).[200] Munger et al. examined data from two large cohorts and found that women reporting an intake of >400 international units (IU) of vitamin D from multivitamins had an about 40% lower MS risk.[248] The same group reported that vitamin D levels >98nmol/l, as measured in blood samples from military personnel were associated with an about 60% lower MS risk compared to levels <63nmol/l.[249] The association was most marked for intake during adolescence and modified by ethnicity. Salzer and colleagues, an independent group, reported a decrease in MS risk of similar magnitude when comparing levels of 75 and higher to levels below 75nmol/l.[250] Further support for the vitamin D hypothesis comes from recent findings in a prospective study within the Finnish Maternity Cohort including serum samples from about 1000 cases and 2000 controls. Vitamin D deficient women (<30nmol/l) had a 43% higher MS risk compared to women with levels ≥50nmol/l.[251] These prospective findings substantiate the validity of the results from different case-control

studies suggesting a protective effect of vitamin D.[252-256] An intrinsic beneficial effect of sunlight not mediated through vitamin D was proposed in a study assessing specifically outdoor-activity independent of vitamin D status.[257] Still, there is otherwise little evidence to support a purely independent mechanism, especially given positive results from diet studies.[248, 252] Recently, the results of Mendelian randomization studies using genetic predictors of vitamin D levels as an instrumental variable, argued against confounding bias underlying the association between low vitamin D and MS risk and provides thus, so far, the most causally interpretable evidence.[258, 259] Whether vitamin D modifies disease activity is not completely resolved.[260] Vitamin D supplementation trials for MS management, although alleviating safety concerns, are so far inconclusive regarding an effect, potentially due to small sample sizes,[261-263] but further trials are underway.[264]

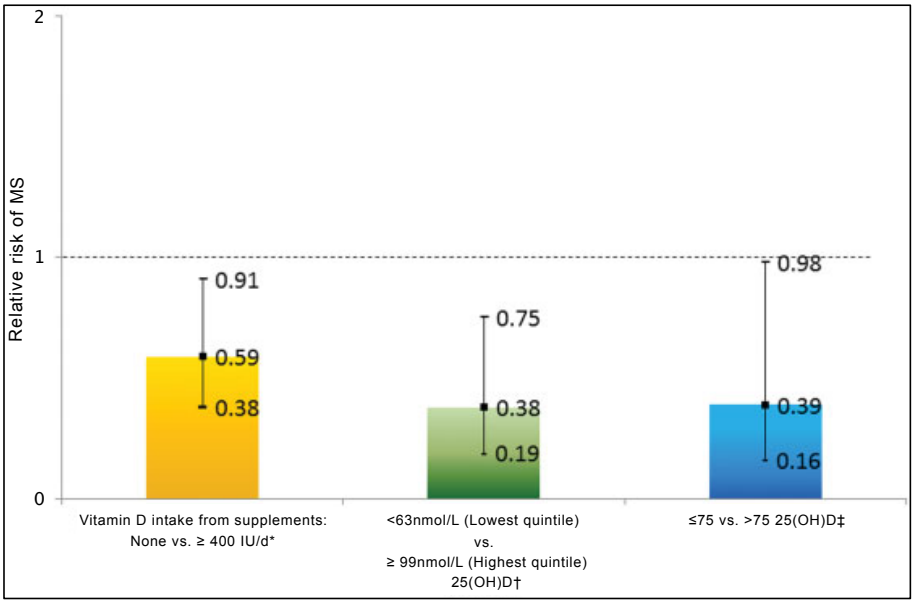

Figure 6: Multiple sclerosis risk in three major prospective studies on vitamin D.

The figure shows the relative risk of MS for exposure to vitamin D comparing supplemental intake of ≥400 international units (IU)/ day (d) versus none, serum levels of ≥99 to <63nmol/l, and >75 to ≤75nmol/l. Reprinted by permission from Georg Thieme Verlag KG: Seminars in Neurology 36(2): 104,[200] http://www.thieme.com/books-main/neurology/product/2163-seminars-in-neurology, © Copyright 2016

The immune-modulatory properties of vitamin D may be mediated through the vitamin D and the interleukin (IL)-10 receptor,[265, 266] and potentially result in a promotion of the T_{reg}-cell function,[267] as well as a range of other anti-inflammatory effects.[260] Experimental studies suggest a disease-preventive and -modulating effect of calcitriol in experimental autoimmune encephalomyelitis (EAE),[268, 269] a commonly used MS mouse model, but prevention seemed to be limited to female animals when administering cholecalciferol.[266, 270]

At which age an adequate vitamin D level is most important or whether cumulative exposure matters is not entirely understood.[196] For the design of future trials and potential implementation of public health measures knowledge about optimal timing of interventions is important.[260] Different age periods have been suggested[254, 255, 271, 272] and the prenatal phase might represent an independent susceptibility period.[209-211] No study compared all postnatal periods systematically and prospective studies are often underpowered to investigate timing of environmental factors in detail.[249, 272]

1.6.3 Tobacco smoking

The evidence suggesting a detrimental effect of smoking on MS risk is strong and comes from prospective,[273-275], retrospective,[203, 276-279] cross-sectional,[280] and biomarker data.[37, 281] Most of the reported effect estimates indicate a 1.4-1.8 times higher risk among exposed individuals and a dose-dependent effect between self-reported smoking and MS, with increasing risk in the order passive, ever, light, and heavy, compared to never smokers.[282] Elevated cotinine levels, a biomarker of current smoking, have also been linked to an increased MS risk, and the association was most pronounced for exposed individuals who were younger at blood sampling,[281] suggesting that smoking in early life might be the relevant exposure.[283] In a small investigation on gestational exposure the authors found, however, no link to MS.[283] In recent meta-analyses, the effect on MS risk was estimated to be about 1.5 among ever compared to never smokers, with a significantly higher risk in men as to women.[284]

Which of the compounds in smoke might adversely affect MS susceptibility is not clear, but a systemic immunologic effect seems likely considering that smoking has

been associated with increased risk of different autoimmune diseases like rheumatoid arthritis or systemic lupus erythematosus.[285] Chewing tobacco and snuff use was not related to an increased risk of MS. Snuffing was instead surprisingly associated with a decreased risk, and a neuro-protective effect of nicotine was proposed.[277, 286, 287] These studies indicate that combustion metabolites or lung-specific mechanisms may be involved in altering the immune response. Interestingly, a passage through the lungs seems to be important for peripheral T-cells to acquire the ability to migrate across the blood brain barrier and induce autoimmune processes.[288] The effect might also be, partially, mediated through vitamin D as smoking is associated with lower levels.[289] Further studies on a potential interaction with other genetic and environmental factors for MS might help revealing underlying mechanisms and deserve further investigation.[290]

1.6.4 Obesity

Overweight has been linked to MS risk in both prospective[291-293] and retrospective[294-296] studies using body mass index (BMI) or validated figure rating scales as measure of body fat mass.[200] A detrimental effect of excess adipose mass has consistently been suggested in women, while associations are more inconsistent and suggest a weaker effect in men.[200] Further investigations are needed to elucidate these discrepancies.

The first longitudinal study found a significant 1.4 and 2.3 times higher MS risk, for women who reported being overweight (BMI 25-<30kg/m^2) or obese (BMI ≥30kg/m^2) at age 18 respectively, according to the definition of the World Health Organization (WHO).[293] In a different cohort, the authors found a 1.6-1.9 times higher risk of adult-onset MS for girls with a BMI≥95th percentile compared to those <85th percentile at age 7-13.[292] In boys, the association was weaker and did not reach statistical significance. Another prospective study reported a significant association between excess body fat mass and pediatric MS, especially among girls that were 12-18 years old, but no association among boys.[291] Several case-control studies followed with findings in line with those in prospective studies.[294-296] Estimates ranged from about 1.5 to 2.0 indicating higher odds of MS among those with excess body fat, with

significant results among men and women in two studies[295, 296] and only in women in the third study.[294] Interestingly, a Mendelian randomization study using genetic predictors of high BMI as an instrumental variable, reported a 41% increase in MS risk for one standard deviation (SD) increase in BMI, corresponding to 4.7kg/m^2.[297] No major differences by sex were reported. However, for causal interpretation of an association between an instrumental variable and MS, one important assumption that needs to be met is that the genetic predictors of BMI influence MS risk only through BMI and not independently through other pathways. The presence of pleiotropic effects cannot be excluded, especially for complex exposures like BMI.[298]

Previous studies point to adolescence as the important period during which excess of adipose tissue ought to be avoided to modify MS risk.[299] Childhood exposure has also been proposed, but body size during childhood correlates with that during adolescence, and could thus be a marker of adolescent exposure.[293] MS patients may experience weight loss after MS onset as suggested in a prospective study, further emphasizing the importance of exposure measurement in early life.[293]

Two main mechanisms have been proposed to underlie this link. One might be the sequestration of vitamin D, a lipophilic compound, into the adipose tissue, resulting in lower bioavailability and potentially vitamin D deficiency that could mediate the effect.[300, 301] The other relates to the low-grade systemic inflammation in obese individuals due to the endocrine activity of adipose tissue.[302] Adipocytes secrete pro-inflammatory cytokines like IL-6 and tumor necrosis factor-alpha and specifically adipokines like leptin that may induce and worsen EAE and trigger MS.[303, 304]

1.6.5 Other factors of interest

It is important to continue the search for novel, especially modifiable, factors for MS.[200] Different factors have been proposed, e.g. sodium, caffeine, polyunsaturated fatty acids, reproductive factors, and the microbiome, but evidence is limited.[200] There is also interest in the role exercise might play in MS, beyond a neuroprotective disease-modulating effect suggested in patients[305-310] and EAE.[311] However, evidence is scarce,[312, 313] maybe because capturing exercise as an exposure is challenging.

Individuals tend to over-report frequency, intensity, and duration, and objective measurements are more costly and difficult to obtain on a large scale.[314, 315] In addition, prodromal or early MS could lead to decreased exercising and reverse causation would then underlie an association between exercise and MS risk.[312] Consequently, the cause-effect direction needs to be assessed cautiously when investigating this relationship.

2. Study rationale and objectives

Vitamin D deficiency is among the factors most consistently related to MS risk. Although there is indication that this effect varies by age, it is unknown when an adequate exposure is crucial to modify MS risk. As MS is a rare disease, detailed hypotheses related to timing of relevant exposures are more efficiently assessed in case-control studies as these usually include more cases and have thus more power.

A high BMI has also been linked to MS, but results are only consistent among women and whether there is a comparable effect among men is not clear. At the same time, there has been interest in the role of physical exercise, whether it is important beyond disease modification and might affect MS risk independently of a favorable effect on body composition. Putative new etiological factors are best assessed in prospective studies, as these are less prone to bias.

Lastly, while knowledge about RIS supports the idea that MS is active prior to clinical onset, prodromal MS is not well characterized. It is unknown when disease activity starts prior to clinical MS onset and whether there are subtle signs of this activity beyond radiological changes. This might potentially be important for the clinical and the research setting. Prospective investigations provide the best evidence, but are difficult to conduct.

Considering these gaps the objectives of the project were:

a) To investigate the association between postnatal timing of cod liver oil use, an important oral vitamin D source in Norway, and MS risk.
b) To compare cognitive performance of men who later in their life developed MS to those who did not, to capture potential differences indicative of disease processes prior to first symptom.
c) To examine the association between BMI and MS risk among men and whether fitness, a proxy of exercise, is independently related to disease risk.

3. Methods

3.1. Article 1- The EnvIMS study

3.1.1 Data source, study design, and ethical approval

The multi-national case-control study Environmental Factors In Multiple Sclerosis (EnvIMS) was conducted in Canada, Italy, Norway, Sweden, and Serbia, to examine environmental risk factors for MS more in detail and potential differences in separate populations.[316] A self-administered postal questionnaire (EnvIMS-Q, see appendix), tested for acceptability, feasibility, and reliability in each involved country, inquired about age-specific past exposures such as medical history, dietary, lifestyle, occupational, and hormonal factors. The methodological details have been published elsewhere.[317] The first article of this thesis was based on the Norwegian EnvIMS data, with information on supplementation of cod liver oil, an important oral vitamin D source in Norway[318] that is relatively easy to quantify and recall and has been common in the country for several decades.

The Regional Committee for Medical and Health Research Ethics in Western Norway approved the study. Participants received an invitation and information letter and gave informed consent by returning the completed questionnaire.

3.1.2 Study endpoints

We included 953 MS cases and 1,717 controls in this study. Eligibility criteria for participation were an age of at least 18 years and among the cases a disease duration of less then 10 years at data collection. Non-responders were contacted with a second mailing within 4-6 weeks from the initial invitation to maximize response rates. The cases were recruited from the Norwegian MS registry and biobank (described more in detail in section 3.2.3).[319] The response rate among the 1,368 invited cases was 69.7% (women: 72%, men: 64.6%). The controls were randomly selected by Statistics Norway from the Norwegian National Registry (Folkeregisteret) including information on residents and their time of immigration/emigration, date of birth and

death since 1964,[320] frequency-matched in a 4:1-ratio on sex and age (within 5-year intervals) to the cases. The response rate among the 4,728 invited controls was 36.3% (women: 39.4%, men: 29.4%).

3.1.3 Exposures under investigation and covariates

We used data on cod liver oil supplementation to estimate intake of vitamin D at different ages. One teaspoon (5 ml) of cod liver oil has been the dose recommended daily for the past decades by the Norwegian Health Authorities to maintain vitamin D levels during the winter months,[321] and contains 10 μg (400 IU) of vitamin D, as well as omega-3 fatty acids (1.2 g), vitamin A (250 μg), and E (10 mg). Different questions explored the supplementation habits. Participants reported their use of cod liver oil or capsules as 'never' or at ages '0-6', '7-12', '13-15', '16-18', '19-24', and '25-30'. The age-scale was adapted to the Norwegian school system to facilitate recall. Additionally, more specific questions on quantity, frequency, and seasonality were devoted to exposure during adolescence based on previous findings.[249] The usual serving size ('no use', 'half a teaspoon', 'one teaspoon', 'half a tablespoon', 'one tablespoon or more') and supplementation frequency ('never/ seldom', '1-3 times/month', '1 time/week', '2-3 times/week', '4-6 times/week' or '7+ times/week') at ages 13-19 both during the winter and the rest of the year were quantified.

Covariate information on other environmental factors included outdoor activity in the summer as a proxy for sun exposure during the same age periods as listed above ('not that often', 'reasonably often', 'quite often', 'virtually all the time'), consumption of vitamin D-rich fatty fish (herring, mackerel, halibut/flounder, salmon/trout respectively) at ages 13-19 ('never', '1 time/month', '2-3 times/month', '1 time/week', '2 times/week', '3 and more times/week') as a proxy of additional dietary vitamin D intake, history of infectious mononucleosis ('yes', 'no', 'I don't remember'), smoking (never-ever), self-rated body size (silhouette 1-9) in 5-year age intervals using Stunkard's figure rating scale,[322] a validated measure of BMI used also in a previous study,[293] and highest education (elementary, middle, high school, college/university, unknown). Further, participants reported family history of MS

(affected parent, sibling, or child) and whether they received help to recall past exposures (by parents or other person).

3.1.4 Statistical analyses

We performed logistic regression in STATA to estimate the effect between timing of cod liver oil use and MS risk and reported odds ratios (OR) and 95% confidence intervals (CI) at a significance level of 0.05. MS cases were considered exposed if the exposure of interest occurred prior to age at disease onset. Controls were considered exposed if exposure occurred prior to index age. An index age was randomly assigned to each control to match the distribution of onset age among the cases making sure that exposure probability was equal among cases and controls.[316] Exposures after MS onset or index age were not considered relevant for our analyses (i.e. coded as non-exposed), hereby taking into account clinical disease characteristics and that exposures are not equally important throughout life.

Three age-specific variables were created to investigate the postnatal timing of exposure to vitamin D. Exposure to cod liver oil during 1) childhood (ages 0-12) compared to no use during childhood, 2) adolescence (ages 13-18) compared to no use during adolescence, and 3) adulthood (ages 19-30) compared to no use during adulthood. Supplementation during these periods was assessed regardless of use in the other periods. The age-specific exposures were first analyzed in (i) separate models adjusted for sex and age (6-year categories of year of birth to obtain balanced subgroups). Subsequently, all three exposures were included into one model that was first adjusted for only age and sex (ii) and then fully (iii).

The association between vitamin D supplementation during adolescence and MS risk was investigated more specifically. From reported frequency and quantity of supplementation at ages 13-19, we estimated cod liver oil doses (none, 1-15, 16-30, 31-45, 46-60, or ≥60 teaspoons/month) and corresponding vitamin D intake (none, ≤200, 201-400, 401-600, 601-800, or >800 IU/day). The dose-response relationship was assessed separately for cod liver oil use during winter and the remaining months.

The fully adjusted models included outdoor activity during summer (ages 0-12, 13-18, 19-30), history of mononucleosis, smoking prior to MS onset, body size at age 15, fatty fish consumption at ages 13-19, and education. We also examined whether positive family history of MS changed the association in a meaningful way.

We conducted several secondary analyses. To further increase accuracy we reassessed the association between timing of exposure to vitamin D and MS risk including only participants into the analysis who received help to recall past exposures. Moreover, to assess whether exposure duration mattered beyond timing, the association between the continuous supplementation from birth up to different ages and MS risk was assessed. Further, fatty fish consumption at ages 13-19 was analyzed as main exposure in a different model and adjusted for cod liver oil use during the same period to test the vitamin D hypothesis using other dietary sources. Finally, we also tested for effect modification by sex and age at disease onset for the association between cod liver oil use during adolescence compared to no use and MS risk by including an interaction term into an age-and-sex-adjusted model.

3.2. Articles 2 and 3- The Norwegian Conscript Service Database studies

3.2.1 Data source, study design, and ethical approval

We conducted two population-based nested case-control studies within the historical cohort of almost all Norwegian men (about 90% of the entire male population) born in 1950 to 1995 who participated in the compulsory Norwegian conscription examination at age 18 or 19 (n=1,308,872) to be evaluated for military service. Information collected during this examination is registered in the Conscript Service Database and was made available to us by the Norwegian Armed Forces through the governmental agency Statistics Norway. The database was linked to the Norwegian MS registry to identify men developing MS later in life. The unique national identification number of each Norwegian citizen/resident was used to conduct the linkage. As the conscription data is sensitive and classified, we were not allowed to use the entire cohort as a control population. In order to maximize power, six male

controls were randomly selected from men registered in the Conscript Database, who did not go on to develop MS during life, frequency-matched to all the cases in the MS registry born in 1950-95 (n=3,526). Since women were, however, granted access to the military in more recent years and we only had male controls, we finally included only male cases from the MS registry in this study, resulting in an about 1:20-ratio and a similar distribution of year of birth between male cases and controls.

The Regional Ethics Committee for Medical and Health Research in Western Norway approved these studies. At inclusion in the MS registry, MS patients gave their written informed consent for their data to be used for research. The committee waived the need for controls' consent due to major public interest in the study questions and under the condition that Statistics Norway would perform the registry linkage and issue anonymized data files for analyses, which we complied with.

3.2.2 The Norwegian Conscript Service Database

Norwegian men were conscripted at age 19 if born before 1976 and age 18 if born after that. Among non-conscripted men are physically and mentally disabled individuals, prison inmates, nationals living abroad or working at sea.[323] The examination routinely included a physical examination with measurement of height and weight, a cognitive performance, and a physical fitness test.

Cognitive performance was assessed in men born in 1950-95 at conscription by a comprehensive validated test timed at in total 53 minutes for 120 questions including a 1) mathematical, 2) word synonym, and 3) figure subtest, assessing 1) logical reasoning, arithmetic, algebraic abilities, 2) verbal ability, abstract reasoning, memory, and 3) logical and abstract reasoning.[323, 324] Subtests 1) and 2) resemble the arithmetic and vocabulary components of the Wechsler Adult Intelligence Scale (WAIS), one of the most commonly used intelligence quotient (IQ)-tests for adults, while subtest 3) is similar to Raven's Progressive Matrices, a non-verbal intelligence test. After integrating the standardized scores of the equally weighted subtests, the result of the cognitive test was issued as a normally distributed single-digit overall score on a nine-point standard scale, ("Stanine", Standard Nine), i.e. a score from 1 to

9 for lowest to highest performance with a mean of 5 and SD of 2. This scale is readily used by the military in different countries. The test-retest reliability is high (0.84 for the math, 0.90 for the vocabulary, 0.72 for the figure subtest), and the overall Stanine score correlates well with the IQ-score as measured by WAIS (r=0.75).[323, 324]

As part of the physical check, weight and height were recorded in this cohort, with which we determined BMI (weight in kg/ height in m^2). Further, the majority of men born in 1950-75 underwent a physical fitness test at conscription, while a smaller part (about 20%) underwent it during the military service and these scores were not available to use. The fitness test consisted of a timed 3000 m run assessing maximal endurance.[325] The test result, also converted to a score on the Stanine scale, correlates well with personal 8 km cross-country racing (r=0.79) and 30 km marching (r=0.59) times. The fitness score at conscription can therefore be considered a measure of aerobic endurance and cardiorespiratory fitness.

3.2.3 The Norwegian MS registry and biobank

The MS registry, a valuable tool started in 2001 to promote MS research and facilitate patient care, was used to identify MS cases, the year of clinical MS onset, and initial disease course.[319] At the time of linkage for these studies in 2015, approximately 50-60% of all MS patients in Norway were included in the database, and all neurologic departments in the country had at least a proportion of their patients registered (26-100%). The coverage was estimated through the National Patient Registry (NPR) in Norway including International Classification of Diseases (ICD)-10 codes of MS registered for patients at hospital admission, treatment at day-units, and outpatient clinics (100%). Care for patients with a chronic disease like MS is almost always provided in one of the bigger medical centers of the country, at least at some point during the disease course, and not exclusively in private neurologist practices. The NPR could have been used to identify MS cases, however, diagnoses are unverified, it does not include information on year of onset and MS course, which was crucial information for the conduct of our studies. While coverage of the MS

registry has increased to 70% in most recent assessments, efforts are made to eliminate the regional registration differences completely by establishing registration routine at all neurologic departments in Norway. At inclusion in the registry neurologists verify the MS diagnosis according to diagnostic criteria (Poser and McDonald).[66, 67, 326] Since consent is necessary for inclusion, patients who died before 2001 are not included in the registry.

3.2.4 Article 2- Statistical analyses

From the 3,526 individuals in the MS registry born in 1950-95, only the male cases were included in the study (n=1,109), as mentioned above. From the Conscript Service Database we randomly selected six-times as many controls frequency-matched on year of birth to all the cases (including female cases) (n=21,156).

We used the overall Stanine score on the cognitive conscription test as a measure of cognitive performance. Since it was a timed test we especially captured information on processing speed, the cognitive area most commonly affected in MS-typical cognitive impairment.[75, 76] To adjust for trends across decades we standardized the score of each individual according to mean and SD of overall performance within 5-year birth cohorts between 1950 and 1995. The standardized scores were then centered on a mean of 5 and a SD of 2.

Analyses were performed in STATA. We investigated whether there were potential differences due to low or lower than expected cognitive performance among men who went on to develop MS later in life compared to peers from the male Norwegian population, potentially indicating MS activity prior to clinical onset. Firstly, we calculated the difference (Δ) in mean standardized cognitive scores between future cases and controls using unpaired two-sided Student's t-test at a significance level of 0.05. We compared the performance among cases 1) overall, 2) in subgroups according to initial disease course, RR- and PPMS, and 3) stratified further according to time from conscription to clinical onset, each to the performance among the control group as a whole. For 3) we used 2-year strata from 1-2 to 33-34 and ≥35 years for cases overall and those developing RRMS, and 10-year groups, 1-10, 11-20, 21-30,

>30 years for those developing PPMS (to account for lower prevalence and later age at clinical onset than RRMS). Secondly, using Cox proportional hazards models, we assessed the risk of developing MS in the years following conscription among men with lowest cognitive score, defined as a result more than 1 SD below the mean among the control population, i.e. a score under 3 on the Stanine scale. Low-scoring individuals contributed to time at risk from the year of the cognitive test to the year of MS onset or 2013, and were compared to the remaining individuals scoring higher than that. We performed separate analyses within the strata of years to MS onset and did not assume constant hazard ratios across strata. Hazard ratios were interpreted and reported as relative risk (RR) together with a 95% CI. In a second step we performed a conservative Bonferroni correction for multiple testing within strata of time to MS onset.

3.2.5 Article 3- Statistical analyses

For this study, we included all the controls (n=19,230) randomly selected frequency-matched on year of birth to the 3,205 cases in the MS registry born in 1950-75, and male cases (n=1,016) born in the same years for the outcome. This period was chosen since data on fitness score was not available for those born after 1975.

We investigated the independent association between the exposures BMI, as measure of excess body fat mass, and fitness scores, as proxy of regular vigorous exercise, determined in Norwegian men at age 19 during conscription and the risk of MS later in life using Cox proportional hazards models. Individuals contributed to time at risk from conscription to the year of clinical MS onset or 2013, whichever came first. We reported, again, relative risks (RR) and 95% CI at a two-sided significance level of 0.05. The effect estimates were adjusted categorically for year of birth in 5-year birth cohorts between 1950 and 1975. The exposures were assessed both continuously and categorically in 1) separate models and 2) then in one model to estimate the independent, meaning mutually adjusted, effect on MS risk.

BMI categories of underweight, normal weight, overweight, and obesity (<18.5, 18.5-<25, 25-<30, and ≥30 kg/m^2) were created according to WHO definitions for the

categorical analyses using normal weight men as reference group. Overweight men were further subdivided depending on their BMI (25-<27 and 27-<30 kg/m^2) and compared to normal weight individuals separately. Fitness scores at conscription were examined both on the original Stanine scale continuously/categorically and in categories of low, medium, and high fitness (score of 1-3, 4-6, and 7-9), using those ranked lowest as a reference.

Sensitivity analyses included, among others, an extension of the analyses of BMI. Information on BMI was available for men born in 1975-95 as well, so we examined the association to MS risk including later birth cohorts in a simple model adjusted only for year of birth. Further, for both exposures we excluded individuals developing MS within 10 years from conscription and in a second step, also those developing PPMS after conscription with intent to examine whether reverse causality would explain an association between our exposures of interest and MS risk.

4. Results

4.1. Article 1

In the first article, we found a marked inverse significant association between cod liver oil use during adolescence and MS risk after adjusting for sex, age, and supplementation during the other periods, while neither use during childhood nor adulthood were associated with MS in the same model. The results were similar when we also adjusted for sun exposure, history of mononucleosis, smoking, body size, fatty fish consumption, and education. No significant differences by sex or MS onset age were suggested. The results were similar for those who had asked for help to complete the questionnaire suggesting again a protective effect of cod liver oil only when it had been used during adolescence. The inverse relationship between longer continuous supplementation and MS was not incrementally stronger, and use from birth to age 30, the longest assessable period, did not suggest the lowest risk.

During adolescence, higher vitamin D doses in the winter were associated with a reduced MS risk suggesting a significant dose-response relationship peaking at 600-800 IU/day. The findings were similar when we adjusted the model for all covariates mentioned above and MS family history. There was no association for adolescent intake during the other seasons and MS in the fully adjusted model, additionally including supplementation during winter. Higher vitamin D intake from fatty fish in adolescence was associated with a significantly reduced MS risk adjusted for all covariates. The estimates did not materially change when including supplementation during adolescence into the model, but results were no longer significant.

4.2. Article 2

In the second article, we found that only men with clinical MS onset within two years from the conscription examination at age 18 or 19 scored significantly lower on the cognitive test compared to the controls, by an equivalent of 6 IQ-points, while there was overall no difference in cognitive scores between men who went on to develop

MS later in life, regardless of years to MS onset, and those who did not. Accordingly, we found an about 2.8-times higher risk of MS, statistically significant also after Bonferroni correction, in the two years following conscription among lowest-scoring men compared to men who obtained a Stanine score of 3 or higher. However, there was no association between scoring lowest and the risk of developing MS beyond two years after the cognitive test at conscription.

In the analyses according to initial MS course, we observed similar results for men who went on to develop RRMS after conscription compared to controls with significantly lower cognitive scores only among those who developed first symptoms within 2 years and a higher MS risk only the first years following conscription among men with lowest scores. However, men developing PPMS with first progressive symptoms up to 20 years after conscription scored significantly lower at the cognitive test, equivalent to a 4.6-6.9 IQ-point difference. The risk of developing PPMS was 2-3 times higher within the 20 years following conscription among individuals who obtained Stanine scores of below 3 compared to those with a score of 3 or higher on the cognitive test. The association was no longer significant after applying the Bonferroni method to correct conservatively for multiple comparisons.

4.3. Article 3

In the third article, we found that a BMI ≥25 kg/m^2 among 19-year-old men was associated with a significantly elevated risk of MS later in life, independent of fitness. Overweight individuals had an up to 2 times higher disease risk, while the results among obese individuals were difficult to interpret due to low sample size that was, however, in line with the sex- and age-specific obesity prevalence in those decades in Norway. The associations were more marked among overweight individuals when we excluded individuals who might have been in a prodromal MS phase.

Interestingly, we observed a significant inverse association with significant p for trend between fitness and MS risk that was independent of an effect through BMI. The physically fittest men at conscription showed a statistically significant 31%

lower risk of MS compared to the most unfit ones according to the endurance test at conscription at age 19. The protective effect was suggested to be stronger in the sensitivity analyses when we excluded men developing MS within 10 years, and further, those who went on to develop PPMS.

5. Discussion

5.1. Interpretation and contribution of the findings

The findings in this thesis suggest that ensuring adequate vitamin D levels to decrease MS risk is most relevant during adolescence and there is a dose-response relationship indicating the most protective effect at supplemental doses of 600-800 IU/day during winter, when sun-induced production is insufficient. Although several previous papers suggested that adolescence is the crucial period, the overall findings on timing of vitamin D exposure are inconsistent proposing exposure during childhood,[271] adolescence,[254, 272] both,[255] or adulthood[248] to matter most. In utero exposure may, in addition, be of importance for MS risk modification.[209, 210, 327] However, no previous study could compare all the different postnatal ages systematically using one methodology and adjust for the mutual effect of vitamin D supplementation in each period as was possible here. We were, further, able to adjust for relevant risk factors.

The design and size of this study allowed testing hypotheses related to vitamin D more in detail. As MS is a rare disease, prospective studies usually lack power to examine timing aspects in detail and would need to, ideally, assess exposures from birth and follow participants for decades for prospective analyses. In fact, even the large Nurses' Health Study cohorts had limited power to detect a significant effect using recalled information on exposure to dietary vitamin D during adolescence.[272] To our knowledge, further studies on this topic have, so far, not been published.

The findings in the first article indicate an effect of supplemental vitamin D intake only in the winter and a previous study based on the EnvIMS data in the same region observed an inverse association of sun exposure and MS risk only in the summer.[253] Taken together these findings support that the suggested protective effect is likely mediated through vitamin D and not through other sunlight-related factors, at least not exclusively. Which mechanisms underlie the heightened susceptibility during early life exactly, is unknown.

These findings contribute with knowledge regarding the stage in life, in which it may be crucial to ensure adequate vitamin D levels. Intervention trials would need to consider this knowledge for a valid design. It is difficult to make concrete public health recommendations based on this study only, but evidence on susceptibility periods is converging and overall these findings support ensuring healthy vitamin D levels during early life, which might be especially important for individuals at higher risk of MS, like those with positive family history.

In this project, we also found that men who went on to develop MS within 2 years from cognitive assessment at age 18-19 performed worse cognitively compared to men who did not go on to develop MS. This suggests that MS is active with subtle CNS-specific signs beyond MRI-detectable activity years prior to the first specific neurologic MS symptom. Prodromal MS might last several years among men developing RRMS, and up to decades among men developing PPMS. These findings are novel as this is, to our knowledge, the first study that tried to prospectively quantify and assess the temporal aspect of prodromal MS. Interestingly, an Argentinian case-control study previously reported a correlation between school performance among students during the last high school year and time to first MS symptoms suggesting prodromal disease activity in those who were about to develop MS, but the study was too small to assess the timing in detail.[179] A prospective study using Swedish military data observed, consistent with our findings, no overall cognitive performance difference among future cases and controls, but the authors did not examine the temporal relationship to clinical onset.[313] Three previous studies described the presence of fatigue, depression, and subtle neurologic symptoms suggestive of demyelination prior to clinical MS onset, but all were retrospective and did not include a comparison group.[328-330] Studies on RIS give valuable insights, but those conducted were small.[161, 167, 169] Moreover, studies including RIS patients are not prospective with regard to prodromal MS. After our paper was published, two interesting studies on the MS prodrome followed. Using a cohort of relatives of MS patients, individuals considered at higher risk of MS, Xia and colleagues found significantly more subclinical abnormalities suggestive of MS in asymptomatic participants with a higher risk score.[331] In a recent prospective study, Wijnands et al.

detected significantly more health-care usage up to 5 years prior to first demyelinating event, increasing with approaching clinical onset, among MS patients compared to matched controls suggestive of a measureable MS prodrome.[332]

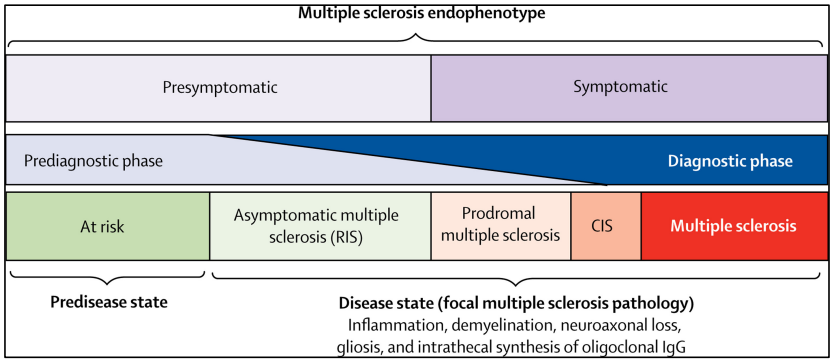

Figure 7: The natural history of multiple sclerosis.

The chart illustrates schematically the natural history of multiple sclerosis and the relationship between symptoms, phenotypes, pathology, and diagnosis. Reprinted by permission from Elsevier: The Lancet Neurology 16: 413,[333] *http://www.thelancet.com/journals/laneur/issue/current, © Copyright 2017*

These findings contribute with new knowledge about the natural history of MS, particularly latent MS and differences according to initial disease course. This field is little understood beyond RIS studies, but is starting to get more attention.[331, 332] Interestingly, the prodromal phase might be especially long among individuals developing PPMS and parallel the relapsing stage in RRMS patients that starts on average earlier in life, further supporting the unifying disease concept. Clinical PPMS is characterized by steady disability accumulation and might correspond to SPMS.[122] Knowledge about PPMS, which seems more elusive than RRMS, is needed also to understand how to best conduct research on this phenotype. As degenerative processes could be present prior to inflammatory activity, we might have to rethink the chronology of pathological events, classic onset symptoms, and phenotypes throughout the entire disease course (Figure 7). Further, these findings need to be considered when designing studies on putative etiologic factors and evaluating the likelihood of reverse causality. They might also help in the public discussion of

whether certain drugs, e.g. vaccines, might have at all been able to trigger MS considering subclinical onset and temporal aspects. Clinically, these results might contribute to the evaluation of individuals presenting with RIS. Cognitive testing might help to detect those at higher risk of developing CIS or MS within some years and requiring closer follow-up. Finally, findings on the MS prodrome open an avenue for anticipating MS diagnosis and evaluating whether earliest intervention can positively influence long-term outcomes.[333]

In the last article of this thesis, we found an independent association between higher BMI, an indicator of excess adipose tissue, and lower aerobic fitness, an indicator of little cardiorespiratory exercise, and an increased risk of MS. This not only confirms previous findings among women[293] that large body size during adolescence might be a modifiable risk factor for MS also among men, but importantly points to that exercise might potentially also be a modifiable protective factor with an effect that cannot be explained through a positive impact of exercise on body composition, and deserves further investigation beyond its effect on disease progression.[305, 306, 308, 310] In consideration of our findings in article 2, the sensitivity analyses excluding men, whose exercise level might have been affected by prodromal MS, yielded similar results and make reverse causality seem a less likely explanation for these findings. Previous studies on the role of exercise on MS risk are scarce. A prospective study using Swedish military data reported a significantly lower fitness performance among recruits who later developed MS compared to controls.[313] The findings from the Nurses' Health Studies are more challenging to interpret.[312] The significant link with self-reported physical activity weakened after excluding women who might have been in a prodromal phase, after introducing a 6-year lag, and the authors interpreted the association therefore overall as due to reverse causation. However, they reported a result for the exposure during late adolescence that was similar to our results, so they might not have captured the relevant exposure when assessing women, on average 34 and 53 years old. These inconsistencies should not be dismissed and deserve further investigation. We reported an association among young men who still have the possibility to develop MS considering average MS onset age. Recently, Wesnes and colleagues examined the role of vigorous physical activity in the context of the

EnvIMS study and reported a similar association as we did after adjusting for the established MS risk factors like sun exposure and smoking.[334]

Linking BMI to MS among men strengthens evidence for a role of this factor in MS etiology, as a biological difference by sex seemed little substantiated. However, this study also suggests that BMI is not a valid measure of body fat % among young men, corroborating prior concerns.[335] Further, we contributed with new findings on a candidate modifiable factor, exercise, for MS etiology. Overall, the last article took the findings of the previous articles into account by examining a potential modifiable risk factor for MS in late teens and evaluating the likelihood of reverse causality.

Mechanisms underlying a potential protective effect of exercise against MS are unknown and can only be speculated. Exercise could have immune-regulatory effects.[336, 337] However, it should be further investigated whether vitamin D levels or smoking habits influence fitness in a way to entirely confound the association, although so far only a small effect of vitamin D on muscle strength was most consistently reported.[338-340] The strongest predictor of higher cardiorespiratory fitness seems to be regular purposeful vigorous aerobic exercise.[341]

In summary, this thesis operated at the margin between susceptibility periods and prodromal MS, contributing with knowledge on the timing and distinction of these two aspects, which is important in order to understand and investigate MS etiology and pathomechanisms. The search for new potential modifiable risk factors ought to rigorously take into account these disease-specific aspects.

5.2. Methodological considerations

5.2.1 Merits and challenges of observational studies

Epidemiology studies the frequency, distribution, and determinants of health- and disease-related states and outcomes on a population or group level.[18, 342] Its goal is to prevent morbidity and mortality and improve public health. Non-experimental epidemiologic research, like the articles in this thesis, is observational, and reports

associations estimating the effect when the null hypothesis of no relationship is rejected.[18, 343] Still, association does not equal causation.[180] The best evidence comes from double-blinded randomized controlled trials (experimental epidemiology), as they allow if well designed and properly conducted, for causal interpretation of the findings.[18] The random allocation of participants to the exposure of interest or not, creates on average two overall comparable groups with regard to individual and extraneous factors differing, except by chance, only in the exposure.[343] Observational studies are more prone to systematic differences among the comparison groups since the exposure is not randomly assigned, but they may be a complementary source of knowledge, especially when trials are unfeasible or unethical, as the exposure cannot be manipulated or would cause harm.[18] In this thesis a trial for objectives 1 and 3 would be difficult, costly, and unethical considering the known benefits of vitamin D and aerobic exercise.[240, 341] A trial for objective 2 is not feasible.

Among non-experimental epidemiologic designs, we prefer longitudinal studies with long follow-up using prospective or historical cohorts of individuals that were disease-free at recording of exposure information, as these provide results that are less prone to bias.[18] Registry-linkage studies, as in article 2 and 3, use historical cohorts, but are prospective if records including exposure information exist independently from later disease occurrence,[18] as is the case for conscription records in Norway. As these studies rely on existing data collected independently from the study purpose, they can lack information, e.g. on potential confounders as in article 3. They are, however, less costly and knowledge becomes more readily available. Scandinavian countries have been at the forefront for these studies as they have been collecting information on demographics, exposures, and outcomes in population registries, often over decades.[18, 344, 345] The unique national identifier remaining the same throughout life can be used to identify an individual across registries and link information, but privacy issues can arise. Yet, when studying a rare disease like MS,[346] even population studies can be too small to investigate the disease in detail. Case-control studies are then more efficient to conduct.[18] Nevertheless, this design is inherently prone to bias related to the selection of cases and controls and exposure assessment after disease occurred, often relying on recalled information.

5.2.2 Causal thinking in observational studies

Causal inference would be possible in non-experimental epidemiology if we avoided all bias, i.e. systematic differences between exposed/unexposed or diseased/non-diseased.[18] Directed acyclic graphs (DAG) are tools in causal inference, a field applying causal thinking in randomized experiments to observational studies to facilitate reflection on causal networks potentially underlying the hypothesis, on study design, and model building.[347, 348] As these can help illustrate limitations in this thesis, they are introduced in brief here. DAGs denote the relationship between an exposure of interest, here denoted as E, an outcome, denoted as D, and associated factors, denoted as C, M, F. Arrows indicate the presumed cause-effect direction between these, i.e. the chronology of events. However, independent of the arrow directions, any path from E to D needs to be evaluated on whether it is an open or closed path. Open paths connecting E and D other than the direct arrow between them need to be closed to estimate an unbiased isolated relationship (d-separation). When we condition on a variable, meaning adjust for it in the model or conduct an analysis stratified on this variable, it can open or close a path, depending on the situation. Factors conditioned on are denoted with a box, e.g. C. E and D are associated when:

1) E causes D (*causality*)
2) D causes E (*reverse causality*)
3) C is a common cause of E and D that we do not condition on (*confounding*)
4) M lies on the path between E and D and we do not condition on it (*mediation*)
5) F is a common effect of E and D that we condition on (*collider stratification bias*).

5.2.3 Threats to the validity of the findings

5.2.3.1 Information bias

Exposure and outcome misclassification due to measurement error can lead to information bias, typically when they are differential. This means that there is a systematic difference in exposure misclassification among cases and controls or in outcome misclassification among exposed and unexposed that can have different reasons, for example an error was more frequent or larger in one of the comparison groups.[18] Non-differential misclassification can occur in prospective and retrospective

studies, while differential misclassification occurs more often in retrospective studies, in which outcomes are known when conducting the study and can influence error (see below). It is difficult to predict the bias direction and whether the association would over- or underestimate the true effect. Overall, measurement error can affect the validity and the reliability in a study.

It is possible that exposures in article 2 and 3 were misclassified, meaning that measures of cognitive performance, aerobic fitness, or height/weight during conscription had some error, but it is unlikely that there was a systematic difference among men who went on to develop MS and those who did not. Reassuringly, values in the comparison group followed a normal distribution with expected mean. Non-differential error was probably also limited through use of objective exposure measures.

Non-differential exposure misclassification could have arisen in article 1 if cod liver oil supplementation was not remembered correctly as it reached far back in time. Limiting eligibility to cases with maximal disease duration of 10 years, adapting the ages in the questionnaire to the country's school system, and inviting participants to ask the family for help in recalling information were aimed at reducing this problem. Non-differential exposure misclassification could also have occurred if cod liver oil use was not a good proxy of vitamin D intake, for example because it did not efficiently increase the serum levels in all. We would then have underestimated the effect of vitamin D. There is, however, currently no evidence to support this. More likely, differential exposure misclassification among cases and controls might have occurred and led to recall bias, a form of information bias case-control studies are prone to. It results from the differential reporting of past exposures among cases and controls, as diseased individuals tend to recall exposures differently due to their disease compared to controls. In article 1 we estimated the effect of cod liver oil use (CLO) at different ages on MS risk (Figure 8) by using self-reported information in EnvIMS (CLO*) as a proxy of true use. The degree of measurement error (ME) in recalling exposure information depended on the disease status (MS), indicated by the arrow from MS to ME, and may have affected CLO*. Apart from the path between

CLO* and MS through CLO, there is an open path through ME that might lead to biased estimates of the true effect between CLO and MS. However, as we found an association only for adolescence consistent with previous studies, but no association at other ages, it is unlikely that recall bias fully explains our findings. Differential recall among cases and controls would than need to only be present for the exposure during adolescence.

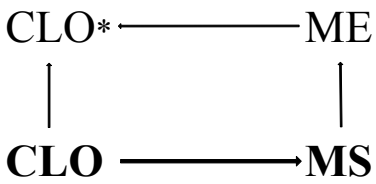

Figure 8

We used the Norwegian MS registry and other population registries to define disease status. Probability of misclassification depends on data quality in the registries, but it is unlikely that misclassification was related to exposure. There is currently no sensitive biomarker for MS and the diagnostic process includes some uncertainty. However, since neurologists verified the MS diagnoses according to diagnostic criteria reviewing the medical records, validity of the diagnoses is as high as it can possibly be considering current standards. The other population registries, controls were randomly selected from, have a (nearly) complete coverage.

5.2.3.2 Selection bias

Selection bias can occur when the comparison groups are a non-random selection of the general source populations and have characteristics related to the exposure and the outcome of interest.[263] Selection bias is more likely to arise in retrospective case-control studies as study participants are recruited after disease occurrence, which might affect the likelihood of participation. Diseased individuals are more likely to participate in research, while non-diseased are more likely to participate if they are health-conscious. This means that the outcome leads to a selective inclusion, where the controls might be less representative of the source population that gave rise to the cases in the study and their characteristics might be related to the exposure of interest.

When the majority of eligible cases but a small proportion of eligible controls agrees to participate, as indicated by the response rates in article 1, cases and controls might not be comparable. The invited controls were less likely to join and those participating had a higher socioeconomic status, were maybe more health conscious and therefore consumed more cod liver oil during life than the general population. This could explain the association in this study. The DAG (Figure 9) shows that cod liver oil use (CLO) and disease status (MS) would affect participation in the study (S) and create an open path between CLO and MS, since by being able to study only individuals actually agreeing to participate, we condition on S and create a collider bias.

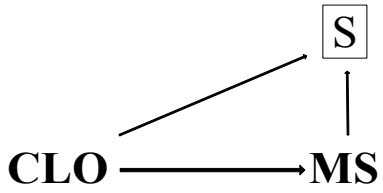

Figure 9

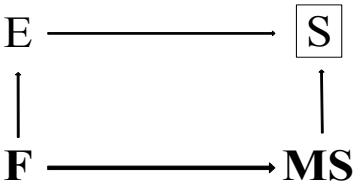

Figure 10

Selection bias is unlikely but not impossible in population-based cohort studies. Loss to follow-up could potentially lead to selection bias. During the long latency between conscription and MS onset, loss to follow-up could have occurred, for example, due to emigration out of the country before the development of MS. A bias could have arisen if likelihood of emigrating was related to exposure status and later disease risk, i.e. that men with higher fitness who went on to develop MS were more likely to emigrate prior to disease onset than those who did not develop MS. In DAG terms this would mean that fitness levels (F) affect emigration (E), and emigration, in turn, affects who could participate in the study (S) (Figure 10). There would then be an open path from F to MS through E and S, since we condition on a common effect of exposure F and outcome MS (collider bias). However, it is unlikely that loss to follow-up biased the results in articles 2 and 3 in such a way. Similarly, the incomplete coverage of the MS registry could have led to bias if only a non-representative sample of cases in the country was registered and registration was

related to exposure probability. This is, however, also unlikely, as registration did not depend, for example on individual factors, like higher BMI or lower fitness, nor were MS cases from different regions systematically different. Rather was the registration routine not yet established everywhere in the country to the same degree, though every neurologic department showed some registration activity.

5.2.3.3 Confounding

Confounding is a threat in observational studies as we cannot be sure that the comparison groups are exchangeable with regard to other characteristics except for the exposure we are interested to study, as in an experimental trial.[18] Exposure and outcome might have a common cause inducing an association between them that gives a biased estimate of the true effect. Confounding bias can be removed as long as we have information on the common causes and account for them in the analyses. Although we adjusted the models in article 1 for established MS risk factors, positive family history, and education, we cannot exclude the presence of unmeasured confounding. If there was confounding, it would need to act in an age-dependent manner to produce an association for cod liver oil use during one but not all age periods. There might also be residual confounding by sun exposure as we adjusted for the proxy outdoor activity that might imperfectly capture the exposure. The fact that we found an association between supplemental vitamin D intake during the winter, but not during the summer when sun exposure contributes most to vitamin D levels and dietary intake is negligible, is somewhat reassuring that we accurately captured the different entities.

Further, we cannot exclude the possibility that confounding underlies the findings in article 2 and 3. It would have been optimal to adjust the models in article 2 for education, as it is associated with cognitive performance and potentially also MS risk, as suggested in some previous studies,[349] but not all. However, if education were a confounder, it would need to act dependent on time to MS onset to explain away the observed association pattern conditional on time. Alternatively, education could have negatively confounded the association between cognitive performance and MS risk (regardless of time to clinical MS onset), but it is, again, difficult to explain how

negative confounding acts only among those further away from clinical disease. The fact that our findings suggest only a time-dependent effect argues against that confounding fully explains our results. Yet, in article 3 confounding is more likely to affect the observed associations for high BMI and aerobic fitness, and MS risk, as we could not adjust for potential common causes, like vitamin D (VitD) and smoking (Sm) (Figure 11). While the results on BMI are in line with previous findings including from prospective and Mendelian randomization studies, the results on exercise need verification, although measuring exercise level in a valid and reliable way is challenging. A sensitivity analysis estimating how strongly potential confounders need to be associated with exposure and outcome to remove the association, can help evaluating this threat.

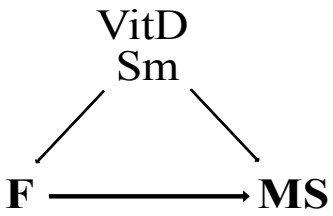

Figure 11

5.2.3.4 Reverse causality

In article 2, we intended to examine reverse causality, i.e. whether MS has an effect on cognitive performance prior to clinical onset. Since we did not find a difference in cognitive scores among later affected and non-affected men overall, but only when considering time to first MS symptom, reverse causality seems likely. In terms of a DAG, cognitive performance (C_{-2}) was only associated with clinical disease (MS) among those developing first symptoms within 2 years from cognitive testing. This means that preclinical disease activity (P) may affect cognitive performance (C_{-2}) a few years prior to first classic symptoms and results also in a higher risk of clinical disease inducing an association between C_{-2} and MS as a confounder (Figure 12). C_{-2} is at the same time a collider between C_{-4} and P, blocking the path between cognitive performance beyond 2 years prior to first symptom and risk of clinical MS through P. C_{-4} and MS will thus not be associated, given that this DAG is correct.

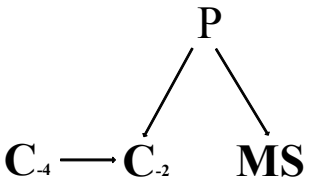

Figure 12

5.2.3.5 Generalizability

The target population of this thesis includes individuals and populations at risk of MS. While the findings in article 2 and 3 might be generalizable to Western populations, limited generalizability may be due to the fact that we were able to only study white men and further studies need to verify whether similar findings can be confirmed among women and individuals of other ethnicity. Our results extend the generalizability of previous studies reporting a more consistent link between high BMI and MS risk among women to men.

As MS is a heterogeneous disease, the MS registry needs to include patients across the distribution of disease course and severity to be representative, which seems the case even though the coverage was not complete. MS cases with very benign disease might have been less represented, as they were maybe less likely to be followed at neurologic departments. Benign MS is rare, and the large majority of MS cases receive some clinical follow-up, independently of severity. Registered MS patients are most likely a random representative sample of all MS cases in Norway and findings might be generalizable to other MS patients of similar populations.

6. Conclusions and outlook

We have contributed with knowledge on the timing of environmental risk factors and prodromal signs of MS. We investigated the relevant timing of exposure to vitamin D to modify MS risk, the nature and timing of prodromal MS according to disease course, and finally the independent association between having an excess body fat mass and exercising little or at low intensity as a young man, and the risk of MS during adulthood.

Future studies should investigate whether higher doses of vitamin D during childhood and adulthood suggest a similar protective effect as observed for the exposure during adolescence. Knowledge is needed about which pathophysiological mechanisms underlie the particular vulnerability during adolescence. Further investigations on prodromal MS are also warranted, especially on the duration of the prodromal phase, on pathological correlates characterizing subclinical disease activity, and on triggers of clinical disease. In addition, future studies on the effect of exposure to excessive adipose tissue in early life on risk of developing MS could consider using other measures than BMI among men. Finally, further studies are needed to confirm findings linking vigorous exercise to a lower MS risk.

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Article 1

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Research Paper

Timing of use of cod liver oil, a vitamin D source, and multiple sclerosis risk: The EnvIMS study

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Abstract

Background: Low vitamin D levels have been associated with an increased risk of multiple sclerosis (MS), although it remains unknown whether this relationship varies by age.

Objective: The objective of this paper is to investigate the association between vitamin D_3 supplementation through cod liver oil at different postnatal ages and MS risk.

Methods: In the Norwegian component of the multinational case-control study Environmental Factors In Multiple Sclerosis (EnvIMS), a total of 953 MS patients with maximum disease duration of 10 years and 1717 controls reported their cod liver oil use from childhood to adulthood.

Results: Self-reported supplement use at ages 13–18 was associated with a reduced risk of MS (OR 0.67, 95% CI 0.52–0.86), whereas supplementation during childhood was not found to alter MS risk (OR 1.01, 95% CI 0.81–1.26), each compared to non-use during the respective period. An inverse association was found between MS risk and the dose of cod liver oil during adolescence, suggesting a dose-response relationship (p trend = 0.001) with the strongest effect for an estimated vitamin D_3 intake of 600–800 IU/d (OR 0.46, 95% CI 0.31–0.70).

Conclusions: These findings not only support the hypothesis relating to low vitamin D as a risk factor for MS, but further point to adolescence as an important susceptibility period for adult-onset MS.

Keywords: Multiple sclerosis, vitamin D, timing, environmental risk factors, susceptibility, age, supplementation, cod liver oil

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Introduction

A low vitamin D level is one of the factors most consistently associated with multiple sclerosis (MS).[1] Yet, it is not well understood at which age an adequate exposure might be especially important and an intervention optimally timed to modify MS risk.[2]

Observational studies investigating the timing of exposure reached different conclusions regarding the possibly most susceptible postnatal period: childhood,[3] adolescence,[4] childhood and adolescence,[5] adulthood.[6] Migration and space-time cluster studies also pointed to different postnatal susceptibility periods,[7–10] though these findings could also reflect the effect of other environmental risk factors.[2] Furthermore, lower vitamin D exposure and serum levels during the prenatal phase have been associated with increased risk of MS later in life and could mark an independent susceptibility period.[11–13]

Serum vitamin D levels are influenced by sun exposure and diet.[1] Cod liver oil is an important dietary vitamin D source in high-latitude countries like Norway where there is no sun-induced vitamin D production during the winter.[14] Norwegian Health Authorities have recommended 5 ml of cod liver oil daily (400 IU of vitamin D) for more than 60 years to prevent diseases like rickets, formerly more prevalent in areas with little access to vitamin D-rich fatty fish.[15] A survey from 1997 estimated that about 35%

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of the Norwegian population and 50% of those aged 60–79 were still using the supplement on a daily basis.[16]

As the literature is inconsistent in delimiting a critical window in which vitamin D might act, we investigated the association between the postnatal timing of cod liver oil supplementation, an important oral vitamin D source in Norway, and the risk of developing MS.

Methods

The EnvIMS study

The multi-national multicenter case-control study of Environmental Factors In Multiple Sclerosis (EnvIMS) was launched to investigate environmental risk factors for MS and examine possible differences between distinct populations. A self-administered postal questionnaire (EnvIMS-Q) was designed including detailed questions about age-specific past exposures such as sun habits, diet, supplement use, past medical history, lifestyle, occupational and hormonal factors.[17] The questionnaire has been assessed for acceptability, feasibility and reliability.[17] The complete study design and methodology has been published elsewhere.[17]

The Norwegian component of the EnvIMS study was approved by the Regional Ethical Committee for Medical and Health Research for Western Norway (n. 11, 18.12.2008). All eligible study participants received an invitation to participate in the form of an information letter explaining the study objectives, relevance and instructions for participation. Completion and return of the questionnaire implied participants' informed consent.

Study population

These analyses used the Norwegian EnvIMS data collected in 2008. The cases, diagnosed according to the McDonald criteria,[18] were recruited from the Norwegian MS-registry and Biobank.[19] Only patients with disease duration shorter than 10 years were eligible for participation. Of the 1368 invited cases, 953 (69.7%) returned the questionnaire. The response rate was 72% among women as compared to 64.6% in men.

Controls, frequency-matched on age and sex, were randomly selected in a 4:1-ratio from the population-based National Registry in Norway (Folkeregisteret). Of the 4728 invited controls, 1717 (36.3%) participated. The response rate in women was again higher than in men (39.4% vs. 29.4%).

Exposure, outcome and covariates

Cod liver oil is an important source of vitamin D in the Norwegian population.[16] The recommended one teaspoon (two capsules) daily of cod liver oil (5 ml) of the most commonly used Norwegian brand (Möller's, Axellus AS, Oslo) contains 10 µg (400 IU) of vitamin D_3, 250 µg of vitamin A, 10 mg of vitamin E and 1.2 g of the omega-3 fatty acids EPA and DHA. A Norwegian survey estimated that the majority of consumers use one tablespoon (15 ml) as a serving size.[20]

Given the importance of cod liver oil as a source of vitamin D in Norway, the use of this supplement was explored in several questions in the EnvIMS-Q. Participants were asked to report whether they had used cod liver oil or capsules "never" or at ages "0–6," "7–12," "13–15," "16–18," "19–24" and "25–30." The age-scale was adapted to the Norwegian school system. Additionally, the frequency of supplement use at ages 13–19 during the winter and the rest of the year was explored using two separate variables on a six-point scale including "never/seldom," "1–3 times/month," "1 time/week," "2–3 times/week," "4–6 times/week" and "7+ times/week." Another question classified the usual quantity of cod liver oil consumed at each serving during the same period into "no use," "half a teaspoon," "one teaspoon," "half a tablespoon" and "one tablespoon or more." The special interest of the investigators in this period was based on previous findings indicating that adolescence might be especially important for MS risk modification.[21]

Further, data on important covariates were retrieved from the questionnaire. The level of sun exposure was estimated by summer outdoor activity at ages "0–6," "7–12," "13–15," "16–18," "19–24," "25–30" and "in recent years," and quantified as "not that often," "reasonably often," "quite often" and "virtually all the time." The frequency of consumption of vitamin D-rich fatty fish at main meals at ages 13–19 was explored on a six-point scale with "never," "1 time/month," "2–3 times/month," "1 time/week," "2 times/week" and "3 and more times/week" for a) "herring," b) "mackerel," c) "halibut, flounder" and d) "salmon, trout," respectively. From the information elicited about infectious mononucleosis, past occurrence of the disease was used as covariate ("yes," "no" or "I don't remember"). Participants also reported their body shape at five-year age intervals based on a nine-point scale derived from body sketches,[22] a figure rating scale, which has been shown to reflect well individuals' body mass index (BMI).[23,24] Information on the smoking habits (smoking onset never or after versus before MS onset) and the participants' level of

education (elementary, middle, high school or university) was also included in the analyses.

Finally, participants reported whether they had a family history of MS (affected parent, sibling or child) and whether they had asked their parents or another person for help in recalling information.

Statistical analyses

Statistical analyses were performed using STATA 13.1 (StataCorp, College Station, TX, USA). The associations between exposure and outcome were estimated through logistic regression and reported as odds ratios (OR) with 95% confidence intervals (CIs). All estimates were adjusted for sex and year of birth (six-year categories to create balanced subgroups).

According to the distribution of age at disease onset in the cases, an index age with corresponding distribution was assigned to controls taking into account age at time of study. Participants were considered exposed only if the exposure of interest occurred before the index postnatal age or age at disease onset. Participants exposed only after this period were considered unexposed.

Based on reported cod liver oil use at different ages, three variables were created: 1) cod liver oil use during childhood (ages 0–12) regardless of use during the other periods compared to no use during childhood, 2) cod liver oil use during adolescence (ages 13–18) regardless of use during the other periods compared to no use during adolescence and 3) cod liver oil use during adulthood (ages 19–30) regardless of use during the other periods compared to no use during adulthood. The effect of these variables was estimated in i) separate models adjusted for age and sex, ii) simultaneously in the same model and finally iii) also adjusted for different covariates.

Additionally, MS risk was compared for cod liver oil use continuously from birth up to different ages to investigate whether the duration of exposure was important. Information about frequency and quantity of supplement use at ages 13–19 was analyzed both as a categorical and a continuous variable using those who reported never having taken cod liver oil or capsules during a certain season as reference.

Consumption frequency of fatty fish during adolescence was examined by assigning scores between 0 and 5 to each participant to account for how frequently on the six-point scale "herring," "mackerel," "halibut, flounder" and "salmon, trout," respectively, were consumed. These scores were added up to an overall score ranging from 0 to 20 reflecting the fatty-fish consumption. To facilitate analyses of the overall score, a quintile-inspired five-point scale from 1 to 5 was created grouping overall scores of 0, 1–2, 3–4, 5–6, $\geqslant$7 and analyzed as a continuous variable.

Further, reported sun exposure during the summer at ages 0–12, 13–18 and 19–30, history of infectious mononucleosis, smoking prior to MS onset, body size at age 15, frequency of consumption of oily fish at ages 13–19 and years of education were added to the model to adjust for possible confounding. In a second step interaction terms were created to test for effect modification by sex and age at disease onset.

Results

Mean study age and sex distribution were similar in both groups (Table 1). Cases were significantly more likely than controls to have smoked before MS onset, experienced infectious mononucleosis, reported a lower educational level, a large body size at age 15 and infrequent summer sun exposure during adolescence.

Timing of cod liver oil use

Supplementation habits did not vary with sex, but with age at the time of study. Supplementation during childhood, adolescence and adulthood was more common among participants born before 1962.

Cod liver oil use was reported by 54.4% of cases and 55.9% of controls during at least one of the age ranges of interest between birth and age 30. Information on the age-specific supplementation was missing for 11.6% of participants. The association between the risk of developing MS and cod liver oil use varied considerably depending on the timing of the supplementation (Table 2). A marked inverse association was observed for intake at ages 13–18 after adjusting for age, sex and supplementation during the other periods. Neither cod liver oil use during childhood nor adult life was associated with reduced disease risk compared to non-use during those periods.

The association between cod liver oil supplementation during adolescence and MS risk was not meaningfully altered after adjusting for sun exposure, infectious mononucleosis, smoking, body size, oily fish consumption and education. No significant differences were seen in the effect between men and women and according to age at disease onset (data not shown).

Table 1. Selected characteristics of the Norwegian participants in EnvIMS[a,b].

	Cases (n = 953)	Controls (n = 1717)
Age at study, mean (SD)	44.8 (10.5)	46.0 (10.8)
Male, n (%)	286 (30.0)	461 (26.9)
Age at disease onset, mean (SD)	37.6 (10.2)[g]	n.a.
Disease duration, mean (SD)	7.2 (2.7)[g]	n.a.
Smoking before MS onset[c], n (%)	545 (58.9)	853 (50.7)
Infectious mononucleosis, n (%)		
"Yes"	160 (17.3)	155 (9.3)
"No"	729 (78.7)	1486 (88.8)
"I don't remember"	37 (4.0)	33 (2.0)
Educational level, n (%)		
High school or lower	538 (57.1)	802 (47.3)
University career	402 (42.7)	890 (52.5)
Body size at age 15, n (%)[d]		
Normal (silhouette 1–4)[e]	794 (87.0)	1486 (89.5)
Large (silhouette 5–9)[f]	119 (13.0)	174 (10.5)
Summer sun exposure, n (%)		
Lower vs. higher at age 13–15	302 (32.7)/623 (67.4)	456 (27.4)/1210 (72.6)
Lower vs. higher at age 16–18	487 (52.4)/443 (47.6)	779 (46.8)/885 (53.2)

EnvIMS: Environmental Factors In Multiple Sclerosis; SD: standard deviation; n: count; n.a.: not applicable.
[a]Missing data for covariates ranging from 0% to 3.6%.
[b]Cases asked significantly more often for help than controls to recall information when filling out the questionnaire (mother: 34.5 vs. 17.2%, father: 6.9 vs. 2.5%, other person: 8.3% vs. 4.2%).
[c]Only 0.9 % in this group smoked less than three years.
[d]Based on Stunkard's figure rating scale.
[e]Body mass index (BMI) ranges from 18.9 to 23.5 kg/m^2.
[f]BMI ranges from 26.1 to 43.3 kg/m^2 including overweight and obesity.
[g]Based on data from the Norwegian MS-registry and Biobank.

We observed that 404 cases (42.4%) and 368 controls (21.4%) asked for help in completing the questionnaire. Of these, 34.1% vs. 42.2% used cod liver oil during childhood, 19.8% vs. 30.2% during adolescence and 15.2% vs. 19.4% during adulthood, comparing cases and controls, respectively. When restricting the analysis of the fully adjusted model 3 (Table 2) to those asking for help, the pattern of association remained similar. The respective OR (95% CI) were 0.99 (0.66–1.49) for childhood, 0.67 (0.42–1.06) for adolescence and 0.99 (0.64–1.54) for adulthood use.

Continuous supplementation from birth up to a certain age was increasingly more strongly associated with a reduced MS risk the longer the supplementation lasted, except for the longest period from birth to age 30 (Figure 1).

Supplementation during adolescence

Supplementation during adolescence was further analyzed for seasonality, frequency and habitual serving size.

The habit of using (liquid) cod liver oil during adolescence was more common during the winter (33.7% and 43.6%) than the rest of the year (19.7% and 23.9%), both in cases and controls. Information was missing for 15.9% of participants for intake during winter and 29.3% during the rest of the year. The frequency of intake most often reported during adolescence in the winter, if any, was "7+ times a week" both for cases (41.8%) and controls (39.3%). The serving size most commonly reported was "1 tablespoon" for cases (58.7%) and controls (64.3%).

Table 3 shows the association between MS risk and increasing doses of vitamin D_3 as estimated from the frequency and quantity of supplementation in the winter during adolescence, suggesting a dose-response relationship (p trend = 0.001) and the strongest protective effect for 600–800 IU/d. The estimates did not substantially change after adjusting for a selection of confounders (Table 3), nor after also adding family history of MS into the model.

The association was significant for supplementation only during the winter. For the supplement use in

Table 2. Association between cod liver oil use at different ages and the risk of MS.

	Use compared to no use of cod liver oil during		
	Childhood (0–12 y)[a]	Adolescence (13–18 y)[a]	Adulthood (19–30 y)[a]
Cases *n* (%)	301 (31.8)	196 (20.6)	165 (17.4)
Controls *n* (%)	631 (36.9)	473 (27.6)	309 (18.0)
	OR (95% CI)[b]	*OR (95% CI)*[b]	*OR (95% CI)*[b]
Model 1[c]	0.82 (0.69–0.97)[f]	0.70 (0.58–0.85)[g]	1.00 (0.81–1.24)
Model 2[d]	1.01 (0.81–1.26)	0.67 (0.52–0.86)[h]	1.17 (0.93–1.47)
Model 3[e]	1.01 (0.79–1.29)	0.72 (0.55–0.96)[f]	1.18 (0.92–1.51)

MS: multiple sclerosis; y: years; *n*: count; OR: odds ratio; CI: confidence interval.
[a]Continuous or periodical use regardless of prior and subsequent use.
[b]OR of MS for age at disease onset after the exposure period of interest by cod liver oil use during specific age periods compared to no use during the same period.
[c]Model 1: Separate model for each age period. Adjusted for age and sex.
[d]Model 2: All three age periods included in the same model. Adjusted for age and sex.
[e]Model 3: All three age periods included in the same model. Adjusted for age, sex, smoking before disease onset, history of infectious mononucleosis, sun exposure, body shape at age 15, education, and consumption of fatty fish.
[f]*P* value < 0.05.
[g]*P* value < 0.0001.
[h]*P* value < 0.005.

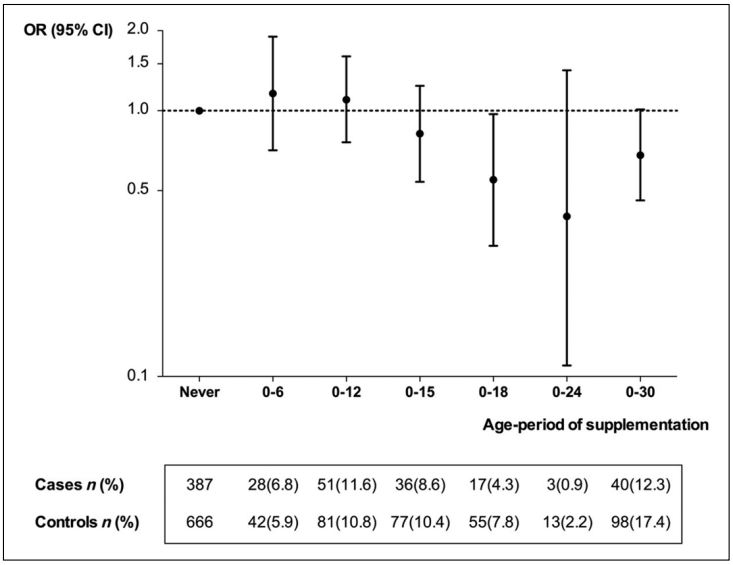

Figure 1. Association between cod liver oil use during increasingly longer age-periods and MS risk.
MS: multiple sclerosis; OR: odds ratio; CI: confidence interval. n: count.
OR of MS in groups with cod liver oil use continuously from birth up to a specific age compared to those who reported no use in the past and number of cases and controls.

other seasons we found no evidence of an association between doses of cod liver oil during adolescence and MS (OR 1.03, 95% CI 0.84–1.26, *p* trend = 0.773, adjusted for supplementation during adolescence in the winter and the covariates listed in the methods).

Fish consumption

Consumption of fatty fish during adolescence was associated with a significant reduction in risk for later MS development (OR 0.92, 95% CI 0.86–0.99, *p* trend = 0.047, adjusted for all covariates mentioned in

Table 3. Association between average daily intake of vitamin D_3 through monthly supplemented cod liver oil in the winter during adolescence (ages 13–19) and MS risk for age at disease onset >19.

Cod liver oil (ts/month)	*Vitamin D_3 (IU/day)*	Cases *n* (%)	Controls *n* (%)	OR[a]	95% CI
None[b]	–	525 (66.0)	784 (56.1)	1.00	–
1–15	⩽ 200	79 (9.9)	160 (11.5)	0.74	0.55–0.99[c]
16–30	201–400	55 (6.9)	125 (9.0)	0.68	0.48–0.95[c]
31–45	401–600	14 (1.8)	38 (2.7)	0.58	0.31–1.08
46–60	601–800	32 (4.0)	104 (7.4)	0.46	0.31–0.70[d]
>60	>800	90 (11.3)	186 (13.3)	0.77	0.58–1.02

MS: multiple sclerosis; *n*: count; ts: teaspoons; IU: international units; OR: odds ratio; CI: confidence interval.
[a]All estimates adjusted for age and sex; p-trend for association = 0.001, OR (95% CI) = 0.91 (0.87–0.96). Adjusting in addition for smoking before disease onset, history of infectious mononucleosis, sun exposure, body shape at age 15, education, and consumption of fatty fish: *p* trend = 0.025, OR (95% CI) = 0.94 (0.89–0.99). The effect estimate did not materially change when adjusting in addition for supplementation during adolescence in the summer.
[b]Reference group consists of those who reported neither an intake of cod liver oil liquid nor capsules.
[c]*P* value < 0.05.
[d]*P* value < 0.0001.

the methods). This OR was related to one step on the five-point scale and is equivalent to an OR of 0.72 comparing most frequent with no fish consumption. When further adjusting for cod liver oil use during adolescence, the result did not meaningfully change, but was no longer significant (OR 0.93, 95% CI 0.86–1.01, *p* trend = 0.085).

Discussion

We found an inverse association between MS risk and cod liver oil supplementation during adolescence and a dose-response protective effect suggested for higher dosages of vitamin D consumed through cod liver oil. However, no association was observed for supplementation during childhood or adulthood. These findings suggest that adolescence might be an especially susceptible period for disease risk modification through dietary vitamin D and are in line with previous observational[4,21] and experimental[25] studies. Other environmental risk factors like infectious mononucleosis,[26] high BMI[24] and other lifestyle factors[27] have also been suggested to act mainly during adolescence.

Previous studies in the same area found that sun exposure through outdoor activity during adolescence was associated with a decreased disease risk only when exposure occurred during the summer,[4,28] while the association between MS and cod liver oil use during adolescence in the present study was only significant for intake during the winter when sun-induced vitamin D-production ceases. These seasonal differences suggest that the risk-modifying effect is vitamin D mediated.

A low vitamin D level is one of the risk factors most consistently associated with an increased risk of MS. A prospective study reported a decreased MS risk among adult nurses comparing supplemental intake of ⩾400 IU/d of vitamin D to no intake.[6] However, it is unclear how vitamin D levels in adulthood correlate with those during adolescence.

Our findings indicate an especially sensitive period during adolescence for MS risk modification but probably not the only one.[21] Higher doses of vitamin D may be needed during childhood and adulthood to reach the same degree of risk modification as during adolescence.[21,25] Even though we did not find an association between overall cod liver oil use during childhood or adulthood and MS risk, we could not evaluate whether a protective effect was restricted to high-dose users at these ages, as this information was not collected.

A prospective study in the United States (US) did not find an association between total recalled dietary vitamin D intake during adolescence and MS risk.[29] Intake of ⩾400 IU/d of vitamin D from multivitamins during adolescence showed, however, a nonsignificant reduced MS risk of an order of magnitude similar to the findings in our study under the rare-disease-assumption.[29] Power might not have been optimal to yield significant results particularly since diet contributes only to a small extent to the vitamin D status compared to sun exposure in the studied area.[29] In the area of the present study there is virtually no contribution of sun exposure during winter.

There are alternative explanations to our findings. Adolescence might be the most sensitive period in which vitamin D unfolds its observed immunomodulatory effects[30] or is of importance in the terminal phase of brain development. Another explanation could be that vitamin D supplementation during different periods in life might not lead to comparable serum levels. No age-dependent difference was, however, experimentally found in how dietary vitamin amounts translate into serum levels.[25]

Our results might also be due to chance, but this is unlikely considering the strong association and conformity with previous findings. We adjusted the estimates for other known environmental risk factors, but we cannot exclude the possibility of residual confounding.

Alternatively, the inverse association between cod liver oil use during adolescence and MS risk might be due to a longer period of exposure rather than the right timing. Adolescent cod liver oil users may be more likely to have additionally consumed the supplement during the other periods resulting in a longer exposure. However, when analyzing continuous supplementation from birth over increasingly longer periods, the strength of association did not steadily increase. The longest supplementation period was less strongly associated with a reduced MS risk than the shorter ones.

Lastly, our findings might be due to the protective effect of other cod liver oil ingredients. Vitamin A and E as well as omega-3 fatty acids have become of interest as possible disease-modifying candidates of MS.[31–33] Fewer studies focused on a risk-modulating potential and results are not consistent. A U-shaped pattern of association between serum vitamin A levels and MS risk was reported in a registry-based smaller cohort study.[34] However, intake of carotenoids, vitamin E and omega-3 fatty acids assessed by food frequency questionnaires was unrelated to disease risk in two large cohorts of women.[35,36] We cannot exclude the possibility that vitamin A in cod liver oil contributed to our findings. The dose-response relationship observed for use during adolescence might be attributed to a protective effect of vitamin A, which increases along with vitamin D in higher doses of cod liver oil. It is unknown whether this nutrient acts age-dependently and could thus explain the findings on the timing of exposure to cod liver oil. Salzer and colleagues reported similar associations for younger participants (16–26 years) as in the entire cohort.[34] Even if age at exposure was not addressed in detail, this observation contradicts somewhat the presence of a strong age-dependent effect for vitamin A. Moreover, evidence for an age-varying action of vitamin D exists beyond studies focusing on dietary sources, and thus independent of vitamin A.[4]

The MS prevalence is lower in populations with consumption of fish,[37] and earlier studies suggested an inverse association between fish consumption and MS.[4,38] Focusing on vitamin D-rich fatty fish, we found that consuming it during adolescence might be protective against the disease. The association was similar but no longer significant after adjusting for cod liver oil use. Our study might be underpowered to show subtle associations.

Previous studies investigating and comparing associations in different age periods in humans were smaller and could not account for all of the known risk factors of MS in the analyses.[4,5] In this present large case-control study we analyzed cod liver oil supplementation, widely used in the Norwegian population, as a dietary proxy for vitamin D intake and serum levels. Investigating and comparing various age periods we found evidence of clear age differences in how vitamin D might affect MS risk even after adjusting for possible confounding.

Case-control studies, although efficient to conduct and suited to study past exposures in detail, are subject to methodological limitations. Despite the population-based sampling of both cases and controls, selection bias could, for instance, be an issue considering the different response rates between both groups. Compared to those included in the study, individuals not responding could have a different distribution of some of their main characteristics affecting the correlation between exposure and disease. We do not have any information on such a possible relation, but we found a higher proportion of controls with the highest level of education compared to the cases, indicating a higher socioeconomic status in controls. We accounted for this by adjusting for confounding by education.

Recall bias is another potential threat to the validity of findings in case-control studies. Nevertheless, the overall proportion of participants reporting cod liver oil use did not differ among cases and controls, and it is unlikely that participants were biased in recalling the timing of this supplementation. Knowledge about the period in life most susceptible to MS risk modification is not yet established.

In order to reduce the risk for misclassified responses, non-differential due to memory issues in general and

differential due to deteriorating cognitive function in MS patients, only cases with a maximum disease duration of 10 years were eligible for the study. Furthermore, the age-period scale used to explore supplementation habits was adapted to the Norwegian school system to facilitate recall. In addition, participants were encouraged to ask their parents for help to correctly reconstruct past exposures if needed.

In conclusion, our findings suggest that adolescence might be an important postnatal age-period for an MS risk-reduction. Commonly used doses of vitamin D contained in cod liver oil might contribute to modify MS risk when supplemented throughout adolescence. Further studies are needed to confirm our findings and to investigate whether higher doses might potentially be as protective during childhood or adulthood.

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Conflicts of interest

M. Cortese has nothing to declare.

T. Riise has nothing to declare.

K. Bjørnevik has nothing to declare.

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Article 2

II

RESEARCH ARTICLE

Preclinical Disease Activity in Multiple Sclerosis: A Prospective Study of Cognitive Performance prior to First Symptom

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Objective: To prospectively investigate potential signs of preclinical multiple sclerosis (MS) activity and when they are present prior to first symptom using data from a historical cohort.
Methods: We linked the cognitive performance of all Norwegian men born 1950–1995 who underwent conscription examination at age 18 to 19 years to the Norwegian MS registry to identify those later developing MS, and randomly selected controls frequency-matched on year of birth from the Norwegian Conscript Service database. In this nested case–control study, cognitive test scores were available for 924 male cases and 19,530 male controls. We estimated mean score differences among cases and controls (Student *t* test) and the risk of developing MS comparing lower to higher scores (Cox regression) in strata of years to clinical onset.
Results: Men developing first clinical MS symptoms up to 2 years after the examination scored significantly lower than controls ($\Delta = 0.80$, $p = 0.0095$), corresponding to a 6 intelligence quotient (IQ)-point difference. Those scoring lowest, that is, >1 standard deviation below the controls' mean, had an increased MS risk during the 2 following years (relative risk = 2.81, 95% confidence interval = 1.52–5.20). Whereas results were similar for relapsing–remitting MS cases (RRMS), those developing primary–progressive MS (PPMS) scored a significant 4.6 to 6.9 IQ points lower than controls up to 20 years prior to first progressive symptoms.
Interpretation: RRMS may start years prior to clinical presentation, and disease processes in PPMS could start decades prior to first apparent progressive symptoms. Cognitive problems could be present in both MS forms before apparent symptoms. Apart from potential implications for clinical practice and research, these findings challenge our thinking about the disease.

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Multiple sclerosis (MS) is a demyelinating disease of the central nervous system that is thought to be caused by an interplay of genetic and environmental factors, but whose etiology is ultimately unknown.[1] It is the most common nontraumatic disabling neurologic disease of young adults, constituting a considerable burden for the individual and the health care system.[2,3]

In MS, substantial lesion load on brain magnetic resonance imaging (MRI), cognitive dysfunction, and disabling fatigue can be detected as early as at the first clinical MS event, known as clinically isolated syndrome (CIS).[4–7] These early findings may indicate long-lasting disease processes. Disease activity on MRI can occasionally even be detected before the first neurological symptoms become apparent. In these individuals with radiologically isolated syndrome (RIS), brain lesions suggestive of the disease may be incidentally identified on MRI performed for reasons other than suspicion of MS.[8] Although 30 to 45% of these cases proceed to develop CIS over several years,[9,10] RIS is currently not recognized as a separate MS phenotype due to the absence of clinical symptoms and remains therefore usually untreated.[10,11] RIS is, however, associated with cognitive impairment detectable by thorough cognitive testing,[12,13] suggesting that maybe not all RIS cases are asymptomatic.

Although these early findings suggest that MS begins prior to clinical onset, the nature and timing of a possible preclinical disease activity beyond radiological signs is largely unknown. Most studies on the natural history of the disease have focused upon detecting predictors of conversion to CIS or MS,[6,9,14] and disability progression.[15,16] Two studies investigating cognitive function in the preclinical phase of MS reported inconsistent findings.[17,18] One of these studies described a positive correlation between school performance in future patients and time to MS onset.[18]

As little is known about the preclinical phase of MS, we prospectively investigated potential early MS signs and their temporal occurrence in individuals who later developed MS. For this purpose, we used cognitive test scores of almost the whole male Norwegian population collected over several decades of conscription examinations.

Subjects and Methods

Study Design

We conducted a registry-based nested case–control study within the historical cohort of almost all Norwegian men born 1950 through 1995 who participated in the mandatory Norwegian conscription examination at age 18 or 19 years (n = 1,308,872). To identify those who subsequently developed MS, the Norwegian Conscript Service database was linked to the Norwegian MS registry.[19] The linkage was done by a Norwegian governmental agency, Statistics Norway, using the national identification number unique to every Norwegian citizen and resident. Six male controls were randomly selected from the Conscript Service database frequency-matched on year of birth to the cases in the MS registry born in the years 1950 to 1995 (n = 3,526). This resulted in a control population of 21,156 individuals. In this study, we only included male MS cases from the MS registry born in that period (n = 1,109). Hence, this corresponded to a ratio of about 1:20 and a similar distribution of year of birth between male cases and controls.

The study was approved by the Regional Committee for Medical and Health Research Ethics for Western Norway (reference number 2013/1307). MS patients' consent for the MS registry includes use of data for research purposes. The data set issued for analyses contained anonymized information.

The Norwegian MS Registry and Biobank

For the current study, we used information on year of onset and initial MS course from patients registered in the Norwegian MS Registry, which was established to facilitate patient care and promote MS research.[19] At the time of the linkage for this study, approximately 50 to 60% of all MS patients in Norway were registered. This is estimated based on the Norwegian National Patient Registry (NPR) containing International Statistical Classification of Diseases, 10th Edition (ICD-10) MS codes given to every patient at hospital admission, treatment at day units or outpatient clinics (100% coverage), and to some extent to the small proportion of MS patients treated only by neurologists at private practices. The NPR does not include information on year of onset or diagnosis and disease course. The responsible authorities aim at a complete coverage of the MS registry with focused registration efforts in some regions. However, there are still some regional differences. Whereas some neurological departments actively register patients, some others have not developed this routine yet.

The Norwegian Conscript Service Database

The Norwegian Conscript Service database includes information on about 90% of all Norwegian men collected at the mandatory conscription examination before military service. Physically and mentally disabled individuals, prison inmates, and Norwegians living abroad or working at sea are commonly among those not attending.[20] The conscription examination has been compulsory for women only since 2010. There were, consequently, too few women to be considered in this study. Age at testing shifted from predominantly 19 years for those born before 1976 to 18 years for the birth cohorts thereafter.[21]

Cognitive performance was assessed by a comprehensive validated timed test (53 minutes, 120 questions) including a mathematical part (25 minutes, testing logical reasoning, arithmetic, algebraic ability), a word synonym test (8 minutes, testing verbal ability, abstract reasoning, memory), and a figure test (20 minutes, testing logical and abstract reasoning).[20,22] The math and word synonym subtests are not identical, but quite similar to the arithmetic and vocabulary parts in the commonly

used intelligence quotient (IQ) test, the Wechsler Adult Intelligence Scale (WAIS), and the figure subtest resembles Raven's Progressive Matrices, a nonverbal intelligence test.[20,22] The overall cognitive performance was rated on a 9-point standard scale, the Stanine (Standard Nine) scale, with normally distributed single-digit scores ranging from 1 to 9 for lowest to highest performance (mean = 5, standard deviation [SD] = 2). This score combines the standardized scores of the equally weighted subtests. In the present study only the overall scores, not the subtest scores, were available to us. The test–retest reliability has been estimated to be 0.84 for the mathematical, 0.90 for the vocabulary, and 0.72 for the figure test.[20,22] The overall Stanine score correlates well with the IQ score as measured by WAIS ($r = 0.75$). The format of the mathematical test was changed in the 1990s from answers in prose to multiple choice, whereas the test content remained the same.[20,22]

Statistical Analyses

Statistical analyses were performed using Stata 14 (StataCorp, College Station, TX).

We determined the mean and corresponding SD of the cognitive Stanine scores within birth cohorts of 5 years from 1950 to 1995, which we used to derive standardized cognitive scores for each studied individual to adjust for variations in score means over the decades. The standardized scores were subsequently centered on a mean of 5 and SD of 2 and used for all the analyses. Missing cognitive scores were not imputed. The difference (Δ) in mean standardized cognitive scores between cases and controls was assessed using unpaired 2-sided Student t test at a significance level of 0.05. All comparisons were drawn to the control group as a whole. Cases were assessed overall and in subgroups according to initial disease course: relapsing–remitting MS (RRMS) and primary–progressive MS (PPMS). Additionally, MS patients were stratified into years from examination to first clinical MS symptom (2-year groups from 1–2 to 33–34 and $\geq$35 years, for cases overall and the subgroup of RRMS and 10-year groups, 1–10, 11–20, 21–30, $>$30 years, for PPMS to account for lower prevalence and later age at clinical onset than RRMS[23]). Misclassification of time to clinical disease onset is possible in some patients born in the mid-1970s who were affected by the shift in conscription age from 19 to 18 years. In a sensitivity analysis, we excluded cases born in the years 1972 to 1978 to estimate the effect of a potential misclassification due to this change in testing age. Furthermore, we calculated the proportion of men scoring lowest during the examination to capture future patients, who may have performed lower than expected. This was defined a priori as a score $>$1 SD below the mean score among controls, which corresponded to a score of $<$3 on the Stanine scale. The cutoffs 1 to 2 SD are often used to differentiate between cognitively impaired and unimpaired individuals in MS research and served as orientation for the cutoff selection in this study.[4,5,12] We used Cox proportional hazards models to estimate the risk of developing MS in the years following the conscription examination, comparing those scoring lowest to the remaining individuals. Individuals contributed with time at risk from the year of the cognitive test to the year of MS onset or end of follow-up (2013), whichever occurred first. We performed separate analyses within the strata of years to clinical MS onset without assuming constant hazard ratios across strata. Men developing MS after the period of interest were included in the analyses and censored. We reported relative risk (RR) and 95% confidence interval (CI). Additionally, we adjusted the probability value conservatively for multiple comparisons by applying a Bonferroni correction.

Results

Cognitive scores were available for 924 male cases and 19,530 male controls and were approximately normally distributed in both groups. Median year of birth was similar across quartiles of cognitive performance among cases and controls (Table 1). Mean age at clinical onset in RRMS cases was similar across quartiles of cognitive performance. Cases with PPMS had first clinical symptoms on average later in life than cases with RRMS. Those with lower scores experienced the first symptoms of PPMS at a younger age compared to those with higher scores.

Cognitive Performance prior to Clinical MS Onset

Only men with clinical onset of MS up to 2 years after the conscription examination scored significantly lower in the stratified analyses compared to the control group ($n = 42$, $\Delta = 0.80$, 95% CI = 0.20–1.41, $p = 0.0095$). This difference corresponds to 6 points on the IQ scale (mean = 100, SD = 15). The results were similar when we excluded cases born 1972–1978 from this group ($n = 34$, $\Delta = 1.04$, 95% CI = 0.36–1.71, $p = 0.0025$, 7.8 IQ points). There was no significant overall difference in cognitive scores between the controls and men who later developed MS (Table 2). Furthermore, the risk of developing MS after the examination was significantly higher the following 2 years among those scoring lowest, that is, $>$1 SD below the controls' mean (40.5% of cases vs 19.4% of controls, RR = 2.81, 95% CI = 1.52–5.20, $p = 0.001$, Bonferroni correction for 18 tests: $p = 0.018$), compared to the remaining individuals (Fig 1). However, there was no association between scoring this low (19.9% of cases vs 19.4% of controls) and the risk of MS beyond 2 years after conscription (RR = 1.02, 95% CI = 0.86–1.21).

Cognitive Performance according to Initial Disease Course

When repeating the analyses in individuals later developing MS, stratified for initial disease course, findings in those who developed RRMS were consistent with the results in all cases. Whereas future patients showed

TABLE 1. Characteristics of Males Who Developed MS in Their Life and Male Controls Born 1950–1995, by Quartiles of Their Cognitive Test Scores at Conscription at Ages 18 to 19 Years in Norway[a]

	Quartiles of Cognitive Stanine Scores,[b,c] Mean			
	2.53, n = 5,410	4.39, n = 4,923	5.74, n = 5,732	7.75, n = 4,389
Year of birth, median (SD)				
Controls	1961 (8.7)	1964 (8.9)	1960 (8.2)	1962 (8.8)
Cases	1961 (8.4)	1966 (8.8)	1960 (7.2)	1963 (8.5)
Age at clinical onset, mean yr (SD)				
RRMS	30.2 (7.8)	31.1 (8.0)	31.9 (8.0)	30.4 (8.6)
PPMS	35.3 (8.4)	33.3 (9.6)	38.3 (7.9)	38.3 (8.1)

[a]Including all cases and controls with cognitive score assessed at conscription.
[b]Standard scale scores: 1–9 (lowest to highest performance), mean = 5, SD = 2, standardized on 5-year birth cohorts.
[c]Missing data: 7.7% for cognitive score among controls and 16.7% among male cases in the Norwegian MS registry born 1950–1995; 0% for year of birth; 1.2% for age at clinical onset among cases with cognitive score. 4.4% have unknown status or missing data on the MS form among cases with cognitive score.
MS = multiple sclerosis; Stanine = Standard Nine scale; SD = standard deviation; RRMS = relapsing–remitting MS; PPMS = primary–progressive MS.

overall no cognitive difference compared to the controls (see Table 2), we observed significantly lower scores only in those developing first symptoms up to 2 years after the examination (n = 38, $\Delta = 0.78$, 95% CI = 0.14–1.42, $p = 0.016$). In line with the findings in all cases, the risk of MS was significantly higher only in the 2 years following the examination in those performing >1 SD below the controls' mean (RR = 2.69, 95% CI = 1.41–5.16, $p = 0.003$, Bonferroni correction for 18 tests: $p = 0.054$). There was no association beyond the 2 following years (RR = 0.98, 95% CI = 0.80–1.18). However, among men later developing PPMS we observed significantly lower mean scores compared to the controls in those who experienced the first progressive symptoms up to 20 years later (Fig 2). The differences corresponded to 4.6 to 6.9 IQ points. There were only 6 cases developing PPMS > 30 years after the examination (mean score = 5.46, $p = 0.58$). The risk of PPMS was

TABLE 2. Comparison of Cognitive Performance at Conscription of Male Individuals Who Later Developed MS and Male Controls[a]

	Cognitive Test Scores, Stanine[b]			
	No.	Mean ± SD	Δ (95% CI)[c]	p[d]
Controls	19,530	5.00 ± 2.00	—	—
Cases				
All	860	4.91 ± 1.99	0.10 (−0.04 to 0.23)	0.17
RRMS	710	4.94 ± 1.98	0.06 (−0.09 to 0.21)	0.42
PPMS	110	4.59 ± 2.03	0.41 (0.04 to 0.79)	0.03

[a]Including future cases with conscription examination prior to clinical MS onset and all controls.
[b]Standard scale from 1 to 9 indicating lowest to highest performance, with mean = 5 and SD = 2. Scores are standardized on 5-year birth cohorts. One point on the scale corresponds to 7.5 intelligence quotient points.
[c]Mean score difference Δ between controls (all) and cases (all and stratified according to initial MS course).
[d]Probability value of 2-sided unpaired Student t test.
MS = multiple sclerosis; Stanine = Standard Nine scale; SD = standard deviation; CI = confidence interval; RRMS = relapsing–remitting MS; PPMS = primary–progressive MS.

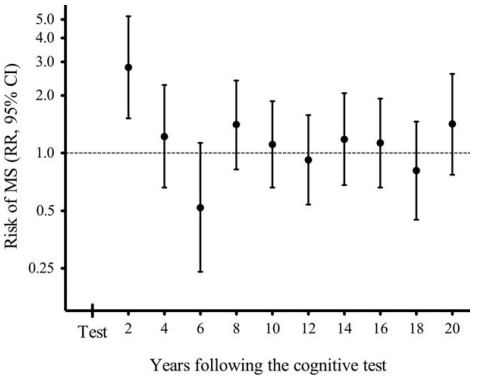

FIGURE 1: Association between low cognitive scores at conscription in men aged 18 or 19 years and the risk of developing multiple sclerosis (MS) in the following years, reflecting the risk of developing MS in the years following the conscription examination with cognitive testing comparing those scoring >1 standard deviation below the controls' mean to the remaining individuals. CI = confidence interval; RR = relative risk.

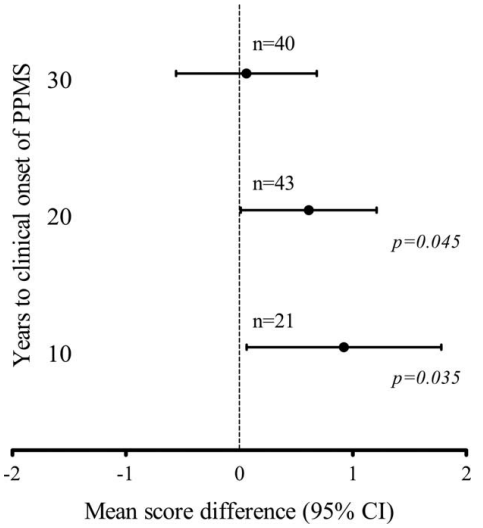

FIGURE 2: Relative mean cognitive Stanine (Standard Nine scale) scores at conscription in men aged 18 or 19 years who later developed primary–progressive multiple sclerosis (PPMS), stratified in 10-year groups to clinical onset, compared to controls, indicating the difference between the overall mean score among the 19,530 controls (mean = 5.00, 95% confidence interval [CI] = 4.98–5.03) and mean scores in strata of future PPMS cases according to time to clinical onset, determined using unpaired Student *t* test. Stanine scores are on a standard scale from 1 for 9, indicating lowest to highest performance (mean = 5, standard deviation = 2) and are standardized on 5-year birth cohorts. One point on the scale corresponds to 7.5 intelligence quotient points.

significantly elevated during 20 years following the cognitive test in men scoring >1 SD below the mean performance among controls compared to men with relatively higher scores (Fig 3). The estimates were no longer significant after applying a conservative Bonferroni correction for 4 tests (1–10 years: $p = 0.15$; 11–20 years: $p = 0.14$).

Discussion

In this prospective study, we found that men who experienced their first clinical MS symptom up to 2 years after their conscription examination at age 18 to 19 years scored significantly lower on the cognitive test than the controls by an equivalent of 6 IQ points. This finding was consistent for cases with RRMS. However, in future cases of PPMS we observed significantly lower scores in those with clinical onset up to 20 years later. Our findings imply that MS may start with not easily detectable symptoms prior to the apparent clinical MS onset. Cognitive dysfunction may, thus, be an early sign of disease.

Only a few studies have examined possible signs of disease activity prior to clinical MS onset. Consistent with our observations, a prospective study using the Swedish Military Conscription Registry found overall no difference in cognitive function score.[17] Case identification was, however, based on nonvalidated ICD MS diagnosis codes from the Swedish National Patient Registry,

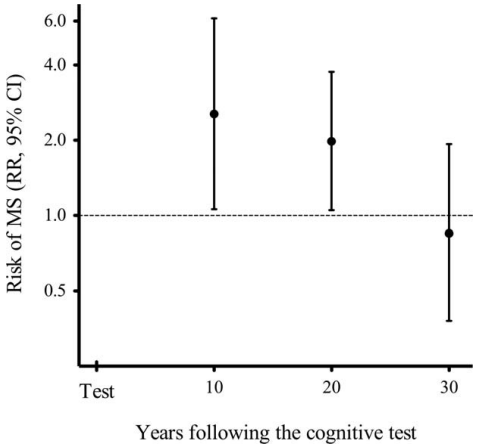

FIGURE 3: Association between low cognitive scores at conscription in men aged 18 or 19 years and the risk of developing primary progressive multiple sclerosis (MS) in the following years, reflecting the risk of developing primary progressive MS in the years following the conscription examination with cognitive testing comparing those scoring >1 standard deviation below the controls' mean to the remaining individuals. CI = confidence interval; RR = relative risk.

and the authors could not estimate differences according to time to clinical MS onset, as we present in our analyses. The authors concluded therefore that cognitive function was not indicative of early disease processes. Another study found a significantly worse high school performance during final years at school in students who later developed MS compared to those who did not.[18] Interestingly, performance during the last year was correlated with the time interval to clinical onset of MS, suggesting prodromal disease activity in those closest to onset.[18] However, because this was a small case–control study, the power was probably insufficient to examine this aspect in detail. Furthermore, 2 smaller studies suggested that individuals with RIS have a similar cognitive impairment profile to that of patients with RRMS, but the temporal relationship was not assessed.[12,13] As we investigated the presence of subtle MS symptoms prior to the apparent disease onset, our findings do not exclude the possibility that disease activity detected on MRI and laboratory abnormalities might precede clinical onset of MS by an even longer period.[8,9]

Our finding in the total MS population of worse cognitive performance in those developing MS up to 2 years after the conscription examination was driven by RRMS. Intriguingly, those later developing PPMS performed worse than the controls during cognitive tests at age 18 to 19 years, although they did not experience progressive symptoms until up to 2 decades after the conscription examination. RRMS and PPMS are thought to be different phenotypes of the same disease rather than different disease entities[23] and might start at a similar but present clinically at different periods in life. Hence, disease processes in patients with PPMS might start many years prior to onset of progressive symptoms around the age of 40 years. Characteristics of PPMS like the clinical onset later in life, its progressive nature, and the speed at which disability accumulates, seem to parallel properties of secondary progressive MS (SPMS),[23] which evolves eventually in many patients with initially relapsing disease. Accordingly, a long subclinical phase with not easily noticeable signs might precede the clinical onset of PPMS and parallel the first stage of RRMS.

Men developing PPMS showed an impact on cognitive function farther from clinical onset than those developing RRMS. Although cognitive impairment is a common MS symptom, independent of the disease course,[24] many studies have tried to identify in which form it is most prevalent, yielding inconsistent findings.[25] Although it appears that patients in their secondary progressive disease phase tend to be most affected, patients with PPMS seem to be more frequently and severely affected than those with relapsing MS, independent of disability status.[26,27] Although motor symptoms are predominantly present in PPMS often due to pronounced diffuse spinal cord pathology, including atrophy, patients also appear to suffer widely from marked cortical brain atrophy, which is a correlate of cognitive dysfunction.[25] Moreover, cortical demyelination, which is also associated with cognitive impairment,[28] seems to be more pronounced in PPMS and SPMS compared to RRMS patients.[29]

There are different possible explanations for our findings on cognitive impairment. Cognitive dysfunction might precede first obvious MS symptoms. Diffuse pathological processes in the brain[13,28] might increasingly disrupt neuronal connectivity and lead to subtle cognitive disturbances that are compensated for in daily life but unveiled by thorough testing. Considering research on the role of cognitive reserve, our study might even have underestimated the rate of those revealing preclinical disease processes.[30] Men with higher efficiency in meeting cognitive demands, thanks to higher education and cognitively stimulating occupational or leisure activities, might compensate for potential ongoing brain pathology and still score higher in the test. Lower cognitive scores before clinical onset may also result from symptoms associated with cognitive disturbances, such as depression or fatigue, and preceding the first focal neurologic attack.[24,31] We cannot exclude the possibility that some future cases were affected by such symptoms during the examination, which might have negatively influenced their test performance by lack of drive and concentration. In that case, cognitive impairment may be considered an indirect early disease sign, especially because we did not find any overall performance difference. There is currently no evidence that depression or fatigue may be risk factors of MS and require adjustment as possible confounders. Furthermore, we need to consider the possibility that our findings might be due to confounding by established or potential MS risk factors. Higher education can influence cognitive performance and has been associated with lower MS risk in previous studies. It could therefore confound the observed associations.[32,33] However, it is again unlikely that differences in schooling or other factors can explain that we only found evidence of lower performance when considering the temporal relationship, but no difference overall. If preclinical disease processes influenced schooling and school performance in a negative way,[18] adjusting for differences could lead to a false-negative result. Alternatively, the observations might be biased due to competing risks, like emigration or premature death, occurring with higher probability in controls with lower or in cases with higher cognitive performance before they can develop MS. This

risk constellation and systematic difference is, however, unlikely. Lastly, it cannot be excluded that our findings might be due to chance, but statistical significance and evidence from previous studies on early cognitive problems in MS argue against this.[4,14]

Our study has several strengths. It is a large prospective study based on objective test measures of almost the whole male Norwegian population over several decades. As the controls were randomly selected from this cohort, selection bias is unlikely. The MS registry, used to identify future cases, contains diagnoses of MS patients from all over the country verified by neurologists according to the McDonald criteria.[34] Hence, misclassification was probably rare in this study. We could prospectively investigate a potential impact on cognitive function and, for the first time, when it was detectable prior to disease manifestation. Our results unveiled a possible latent MS phase with subtle symptoms and relativize the idea of a clinical threshold in MS.

Our findings confirm the importance of early diagnosis[34] and initiation of disease-modifying treatment.[35] They may also justify screening for cognitive dysfunction in early disease phases, allowing prognostic considerations and prompt interventions to maximize cognitive reserve if cognitive rehabilitation proves effective.[30,36,37] Screening for cognitive impairment could also be important for the evaluation of RIS cases regarding their risk of conversion to MS and approaches to their follow-up including potential disease-modifying treatment.[12,13] In addition, our observations could bring us further in understanding differences between RRMS and the less-well understood PPMS, which is today resistant to standard treatment.[25] Our findings should, furthermore, be considered for the design of future MS risk factor studies to minimize reverse causation issues. Taken as a whole, these findings might challenge our thinking about MS and reignite the discussion about classification of different disease stages, possible onset symptoms, and diagnostic criteria.[11]

Potential limitations need to be considered when interpreting these results, because the disease is more prevalent in women,[24] one weakness is that the study was restricted to men. However, external validity for white men is high, as this study is based on the whole male population of a Western country over several decades. Apart from a potentially higher prevalence in men, there is currently no evidence that cognitive impairment in male and female MS patients displays different characteristics.[38] Nevertheless, further studies are needed to exclude major differences. Furthermore, it is possible that there is misclassification of 1 year regarding time to clinical MS onset in some future patients born around 1975, for whom testing age was shifted from 19 to 18 years. However, the results in cases closest to clinical MS onset remained unchanged, when excluding those born 1972 through 1978 from the analysis. Another limitation may be that our findings were based on one performance measurement per person. We could, therefore, not examine the individual development of cognitive function over time. Furthermore, additional information on level of education and prevalence of depression in the participants would have been ideal, but it was not available to us. Moreover, the unavailable subtest scores could have provided further information on the potentially affected cognitive area. However, all subtests are specifically timed and probably we would therefore primarily obtain, with all of them, a measure of processing speed efficiency, as we do with the composite score, although a specific neuropsychological test of information processing speed was not performed. As processing speed is the cognitive ability most commonly affected in MS,[24] tests specifically assessing this skill predict best cognitive impairment in different neuropsychological test batteries and are thus recommended as a screener tool in clinical time-limited settings.[39,40] Furthermore, the incomplete MS registry coverage might also have led to bias. Differences in registration are, however, due to regional hospital compliance, and a systematically differential registration according to specific disease characteristics is unlikely. It is furthermore unlikely that there are regional differences in characteristics of the MS patient population and cognitive scores that could create a major bias in the estimation of the true impact on cognition. Moreover, we chose, a priori, 1 SD as cutoff to investigate the MS risk in those with low performance; lower cutoffs, used in some previous studies on cognitive impairment in MS patients, might have yielded different results. However, we intended to capture future cases performing lower than expected, in other words, men who began to be affected cognitively, but were not necessarily or not yet cognitively impaired. Lastly, the cognitive test was not validated specifically for individuals developing MS and is supposed to screen and rank cognitive function compared to the norm. Consequently, it is possible that cognitive tests tailored for MS, and thus more sensitive for MS-specific areas of cognitive impairment, might detect even more subtle differences between future cases and controls.

In conclusion, our study provides evidence that MS might be preclinically active with not readily detectable symptoms several years prior to the first classic MS attack or decades prior to the first progressive MS symptoms. Cognitive function may be affected even before the first focal symptoms occur. Monitoring changes in cognitive function in individuals with CIS and especially RIS may contribute to identifying those at risk of MS.

Acknowledgment

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Author Contributions

M.C., T.R., K.B., A.A., and K.-M.M. contributed to study concept and design. M.C., T.R., K.B., A.B., E.F., N.G., I.H., R.M., C.S.S., W.T., and K.-M.M. contributed to data acquisition and analysis. M.C., T.R., K.B., and K.-M.M. contributed to drafting the manuscript and figures.

Potential Conflicts of Interest

Nothing to report.

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Appendix A: EnvIMS-Q in Norwegian

A

Skjemaet skal leses av en maskin. Det er derfor viktig at du legger vekt på følgende ved utfyllingen:

- Bruk blå eller sort kulepenn.
- I de små avkrysningsboksene setter du *et kryss* for det svaret som du mener passer best, slik: ☒
- Hvis du mener at du har satt kryss i feil boks, kan du rette det ved å fylle boksen helt, slik: ■
- Der du ikke kan svare på et spørsmål vennligst bruk "Vet ikke" eller "Husker ikke" avkrysningsboksene.

SEKSJON 1: BAKGRUNNSDATA

1. Hvilket år er du født?

19 ☐☐

+

2. Hvilken utdanning er den høyeste du, faren din og moren din har fullført?
(sett ett kryss for hver av dere tre)

	Du selv	Far	Mor
7-årig folkeskole eller mindre	☐	☐	☐
Grunnskole 9-10 år	☐	☐	☐
Gymnas/ Videregående skole (11-13 år)	☐	☐	☐
Høgskole/Universitet (mer enn 14 år)	☐	☐	☐
Vet ikke	☐	☐	☐

3. Hvilken etnisk gruppe tilhører dine foreldre

	Far	Mor		Far	Mor
1. Norsk/europeisk/annen vestlig	☐	☐	4. Afrikansk	☐	☐
2. Samisk	☐	☐	5. Midtøsten	☐	☐
3. Asiatisk	☐	☐	6. Latinamerikansk	☐	☐

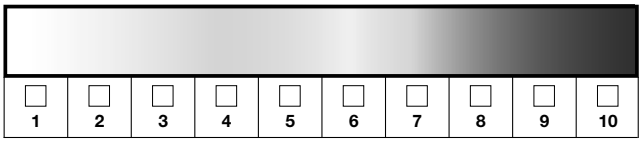
+

4. Fyll ut kjønn og fødselsår for hvert søsken (inkludert halvsøsken og adoptivsøsken): Jeg er enebarn ☐

	1	2	3	4	5	6
Fødselsår:	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐
Kjønn (M/K)	M ☐ K ☐	M ☐ K ☐	M ☐ K ☐	M ☐ K ☐	M ☐ K ☐	M ☐ K ☐

SEKSJON 2: SOLVANER

1. Sett ett kryss på det tallet under fargen som best passer din naturlige hudfarge ved å sammenligne med huden på innersiden av overarmen.



☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10

+

2. Hvordan reagerer huden din første gang du soler deg om sommeren hvis du ikke bruker krem med solfaktor?

1. Jeg blir alltid solbrent og jeg blir aldri brun ☐
2. Jeg blir vanligvis solbrent og blir mindre brun enn andre ☐
3. Jeg blir av og til solbrent og blir brun omtrent som de fleste andre ☐
4. Jeg blir sjeldent solbrent og blir lett brun ☐

3. Hva er din opprinnelige hårfarge?
(sett ett kryss)

1. Svart ☐
2. Mørkbrun ☐
3. Brun ☐
4. Blond, gul ☐
5. Rød ☐

4. Hvilken øyefarge har du?
(sett ett kryss)

1. Svart ☐
2. Brun ☐
3. Grå, grønn ☐
4. Blå ☐

+ +

5. Om sommeren: Hvor mye utendørsaktiviteter (lek, idrett, tur, hagearbeid, jobb) hadde du?

	Lite	Middels	Ganske mye	Ute stort sett hele tiden
0-6 år	☐	☐	☐	☐
7-12 år (barneskolen)	☐	☐	☐	☐
13-15 år (ungdomsskolen)	☐	☐	☐	☐
16-18 år (videregående)	☐	☐	☐	☐
19-24 år	☐	☐	☐	☐
25-30 år	☐	☐	☐	☐
I de siste tre årene	☐	☐	☐	☐

6. Om vinteren: Hvor mye utendørsaktiviteter (lek, idrett, tur, hagearbeid, jobb) hadde du?

	Lite	Middels	Ganske mye	Ute stort sett hele tiden
0-6 år	☐	☐	☐	☐
7-12 år (barneskolen)	☐	☐	☐	☐
13-15 år (ungdomsskolen)	☐	☐	☐	☐
16-18 år (videregående)	☐	☐	☐	☐
19-24 år	☐	☐	☐	☐
25-30 år	☐	☐	☐	☐
I de siste tre årene	☐	☐	☐	☐

7. Hvor mye tid har du tilbrakt utendørs i forbindelse med arbeidet ditt eller studiene dine?

Alder	Inne stort sett hele tiden	Ute stort sett hele tiden	Samme tid inne og ute
16-20 år	☐	☐	☐
21-25 år	☐	☐	☐
26-30 år	☐	☐	☐

8. Hvor ofte var du på badeferie i "syden"?

Alder	Aldri/sjelden	1 uke i året eller mindre	2-3 uker i året	4 uker eller mer i året
0-6 år	☐	☐	☐	☐
7-12 år (barneskolen)	☐	☐	☐	☐
13-15 år (ungdomsskolen)	☐	☐	☐	☐
16-18 år (videregående)	☐	☐	☐	☐
19-24 år	☐	☐	☐	☐
25-30 år	☐	☐	☐	☐
I de siste tre årene	☐	☐	☐	☐

9. Hvor ofte brukte du krem med solfaktor (forsøk å tenke deg et gjennomsnitt)?

Alder	Aldri/sjelden	Av og til	Ganske ofte	Nesten alltid
0-6 år	☐	☐	☐	☐
7-12 år (barneskolen)	☐	☐	☐	☐
13-15 år (ungdomsskolen)	☐	☐	☐	☐
16-18 år (videregående)	☐	☐	☐	☐
19-24 år	☐	☐	☐	☐
25-30 år	☐	☐	☐	☐
I de siste tre årene	☐	☐	☐	☐

10. Hvor ofte har du solt deg i solarium?

Alder	Aldri/sjelden	Mindre enn 1 gang pr. år	Mindre enn 1 gang pr. måned	En gang pr. måned eller oftere
16-20 år	☐	☐	☐	☐
21-25 år	☐	☐	☐	☐
26-30 år	☐	☐	☐	☐

SEKSJON 3: KOSTHOLD

Vi er interessert i å få kjennskap til hvordan kostholdet ditt vanligvis var i tenårene **(fra 13 til 19 års alder)** før du eventuelt flyttet hjemmefra. Først vil vi vite hvor ofte du spiste fisk og ber deg fylle ut spørsmålene så godt du kan.

1. Tilgangen på fisk kan variere gjennom året. Vær vennlig å markere i hvilke årstider du vanligvis spiste de ulike fiskeslagene (sett gjerne flere kryss).

	Vinter	Vår	Sommer	Høst	Aldri/sjelden
Torsk, sei, hyse, lyr	☐	☐	☐	☐	☐
Kveite, flyndre	☐	☐	☐	☐	☐
Laks, ørret	☐	☐	☐	☐	☐
Makrell	☐	☐	☐	☐	☐
Sild	☐	☐	☐	☐	☐

2. Med tanke på de periodene av året der du spiste fisk, hvor ofte pleide du å spise følgende til middag? (sett ett kryss pr. linje)

	Aldri/sjelden	1 pr.mnd.	2-3 pr.mnd.	1 pr.uke	2 pr.uke	3+ pr.uke
Torsk, sei, hyse, lyr	☐	☐	☐	☐	☐	☐
Kveite, flyndre	☐	☐	☐	☐	☐	☐
Laks, ørret	☐	☐	☐	☐	☐	☐
Makrell	☐	☐	☐	☐	☐	☐
Sild	☐	☐	☐	☐	☐	☐

3. Hvor ofte spiste du fiskelever fra du var 13 til 19 år gammel?

Aldri	1-3 pr.år	4-6 pr.år	7-9 pr.år	10+ pr.år	Vet ikke
☐	☐	☐	☐	☐	☐

4. Da du var 13 til 19 år gammel, hvor ofte spiste du følgende matvarer: (sett ett kryss for hver linje)

	Aldri	Mindre enn 1 pr.mnd.	1-3 pr.mnd.	1 pr. uke	2-3 pr.uke	4+ pr.uke
Kjøtt, (biff, stek, koteletter) og kjøttprodukter (kjøttkaker, kjøttpudding, pølser)	☐	☐	☐	☐	☐	☐
Røkt kjøtt	☐	☐	☐	☐	☐	☐
Røkte pølser (wienerpølser)	☐	☐	☐	☐	☐	☐
Røkt fisk	☐	☐	☐	☐	☐	☐
Røkt ost	☐	☐	☐	☐	☐	☐

5. Da du var 13 til 19 år gammel, hvor mange brødskiver med følgende pålegg spiste du i gjennomsnitt: (sett ett kryss for hver linje)

	0 pr.mnd.	1-3 pr.mnd.	1 pr.uke	2-3 pr.uke	4-6 pr.uke	7-9 pr.uke	10+ pr.uke
Makrell i tomat, røkt makrell	☐	☐	☐	☐	☐	☐	☐
Kaviar/"Svolvær postei"	☐	☐	☐	☐	☐	☐	☐
Sardiner, sild, ansjos	☐	☐	☐	☐	☐	☐	☐
Laks (gravet/røkt)	☐	☐	☐	☐	☐	☐	☐
Annet fiskepålegg	☐	☐	☐	☐	☐	☐	☐

6. Hvor mange brødskiver spiste du hver dag i gjennomsnitt? ☐☐

7. Hva slags fett brukte du vanligvis på brødet? (sett gjerne flere kryss)

Brukte ikke fett på brødet	Plantemargarin	Smør	Vet ikke
☐	☐	☐	☐

8. Dersom du brukte fett på brødet, hvor tykt lag pleide du å smøre på? (En kuvertpakke med margarin veier 12 gram) (sett ett kryss)

Skrapet (3 g)	Tynt lag (5 g)	Godt dekket (8 g)	Tykt lag (12 g)
☐	☐	☐	☐

9. Hvor ofte brukte du kosttilskudd da du var 13 til 19 år gammel? For flytende tran og tranpiller, vær vennlig å markere i hvilke årstider du brukte dem. Dersom du brukte dem hele året, sett ett kryss for vinter, og ett kryss for resten av året.

	Aldri/sjelden	1-3 pr. mnd.	1 pr. uke	2-3 pr. uke	4-6 pr. uke	7+ pr. uke
Tran						
Om vinteren	☐	☐	☐	☐	☐	☐
Resten av året	☐	☐	☐	☐	☐	☐
Tranpiller						
Om vinteren	☐	☐	☐	☐	☐	☐
Resten av året	☐	☐	☐	☐	☐	☐
Fiskeoljekapsler	☐	☐	☐	☐	☐	☐
Multivitaminer eller annet kosttilskudd slik som Sanasol, Vitaplex, Biovit, Kostpluss og Vitamineral	☐	☐	☐	☐	☐	☐

10. Hvor mye tran pleide du å ta hver gang?

Brukte ikke tran	½ ts.	1 ts.	½ ss.	1+ ss.
☐	☐	☐	☐	☐

11. Hva slags multivitamin/kosttilskudd brukte du i følgende aldere? (sett gjerne flere kryss)

	Aldri	0-6 år	7-12 år (barneskolen)	13-15 år (ungdomsskolen)	16-18 år (videregående)	19-24 år	25-30 år
Multivitaminer	☐	☐	☐	☐	☐	☐	☐
Kalsium	☐	☐	☐	☐	☐	☐	☐
Vitamin D	☐	☐	☐	☐	☐	☐	☐
Vitamin B12	☐	☐	☐	☐	☐	☐	☐
Tran/Tranpiller	☐	☐	☐	☐	☐	☐	☐
Fiskeoljekapsler	☐	☐	☐	☐	☐	☐	☐

12. Ble du ammet?

Nei	Vet ikke	Ja →	Hvor mange måneder? 1-3 mnd.	4-6 mnd.	7-9 mnd.	10+ mnd.
☐	☐	☐ →	☐	☐	☐	☐

SEKSJON 4: HELSE

1. Har du hatt noen av følgende sykdommer eller kirurgisk behandling? Prøv å huske hvilken skoleklasse du gikk i da du hadde sykdommen. (sett gjerne flere kryss)

	Nei	Vet ikke	Ja	Alder ved diagnose/sykdom: 0-6 år	7-12 år (barneskolen)	13-15 år (ungdomsskolen)	16-18 år (videregående)	19-24 år	25-30 år
Fjernet mandlene	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Meslinger	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Kusma	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Røde hunder	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Vannkopper	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Lungebetennelse	☐	☐	☐→	☐	☐	☐	☐	☐	☐

2. Har du hatt kyssesyken (mononukleose)? Ja ☐ Nei ☐ Husker ikke ☐

Hvis, ja ble det tatt blodprøve for å stille diagnosen? Ja ☐ Nei ☐ Husker ikke ☐

Hvilken skoleklasse gikk du i da du hadde sykdommen? 0-6 år ☐ | 7-12 år (barneskolen) ☐ | 13-15 år (ungdomsskolen) ☐ | 16-18 år (videregående) ☐ | 19-24 år ☐ | 25-30 år ☐

3. Husker du hvilken måned du hadde kyssesyken (01-12)? ☐☐

Hvis ikke, husker du ihverfall hvilken årstid det var? Vår ☐ Sommer ☐ Høst ☐ Vinter ☐ Husker ikke ☐

4. Har du hatt urinveisinfeksjon (blærekatarr)? I så fall, prøv å huske når.

Nei	Vet ikke	Ja	Alder (sett gjerne flere kryss): 0-6 år	7-12 år	13-15 år	16-18 år	19-24 år	25-30 år
☐	☐	☐→	☐	☐	☐	☐	☐	☐

5. Har du noen gang hatt infeksjon med innvollsormer eller andre parasitter (amøber, bendelorm, mark i magen)

Nei	Vet ikke	Ja	Alder ved start: 0-6 år	7-12 år	13-15 år	16-18 år	19-24 år	25-30 år
☐	☐	☐→	☐	☐	☐	☐	☐	☐

6. Har du hatt allergiske reaksjoner (øyekatarr, eksem, høysnue, astma) mot noen av det som er nevnt under? I så fall, angi omtrent hvilken alder du først merket disse symptomene

	Nei	Vet ikke	Ja	0-6 år	7-12 år	13-15 år	16-18 år	19-24 år	25-30 år
Pollen	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Husstøv	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Allergi mot kjæledyr og husdyr	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Mat	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Annen allergi	☐	☐	☐→	☐	☐	☐	☐	☐	☐

7. Har du eller har du hatt noen av følgende sykdommer?

	Nei	Ja	Vet ikke	Alder ved første diagnose		Nei	Ja	Vet ikke	Alder ved første diagnose
Systemisk lupus erythematosus (Lupus)	☐	☐	☐	☐☐ år	Diabetes mellitus type 1	☐	☐	☐	☐☐ år
Reumatoid artritt (leddgikt)	☐	☐	☐	☐☐ år	Cøliaki	☐	☐	☐	☐☐ år
Hypotyreose (lavt stoffskifte)	☐	☐	☐	☐☐ år	Psoriasis	☐	☐	☐	☐☐ år
Hypertyreose (høyt stoffskifte)	☐	☐	☐	☐☐ år	Leukemi (blodkreft)	☐	☐	☐	☐☐ år
Multippel sklerose	☐	☐	☐	☐☐ år	Hodgkins lymfom	☐	☐	☐	☐☐ år
Synsnervebetennelse	☐	☐	☐	☐☐ år	Annen type lymfom	☐	☐	☐	☐☐ år
Crohns sykdom	☐	☐	☐	☐☐ år	Føflekkkreft	☐	☐	☐	☐☐ år
Ulcerøs colitt	☐	☐	☐	☐☐ år	Annen type hudkreft	☐	☐	☐	☐☐ år
Annet,	☐	☐	☐	☐☐ år	Nyresykdom	☐	☐	☐	☐☐ år

presiser: _____

8. Har noen i familien din hatt noen av følgende sykdommer?

	Nei	Far	Mor	Søsken	Barn	Vet ikke
Systemisk lupus erythematosus (Lupus)	☐	☐	☐	☐	☐	☐
Reumatoid artritt (leddgikt)	☐	☐	☐	☐	☐	☐
Hypotyreose	☐	☐	☐	☐	☐	☐
Hypertyreose	☐	☐	☐	☐	☐	☐
Multippel sklerose	☐	☐	☐	☐	☐	☐
Synsnervebetennelse	☐	☐	☐	☐	☐	☐
Crohns sykdom	☐	☐	☐	☐	☐	☐
Ulcerøs colitt	☐	☐	☐	☐	☐	☐
Psoriasis	☐	☐	☐	☐	☐	☐
Diabetes mellitus type 1 (insulinkrevende sukkersyke)	☐	☐	☐	☐	☐	☐
Cøliaki	☐	☐	☐	☐	☐	☐
Leukemi	☐	☐	☐	☐	☐	☐
Hodgkins lymfom	☐	☐	☐	☐	☐	☐
Annen type lymfom	☐	☐	☐	☐	☐	☐

SEKSJON 5: RØYKEVANER OG LIVSSTIL

1. Har du noen gang røykt daglig?

Ja ☐ Nei, aldri ☐ Hvis nei, gå til spørsmål 5

2. Hvis ja, hvor mange sigaretter røykte du igjennomsnitt pr. dag?

	Røykte ikke	Antall sigaretter hver dag: 1-4 sig.	5-10 sig.	11-20 sig.	21+ sig.
11-15 år	☐	☐	☐	☐	☐
16-20 år	☐	☐	☐	☐	☐
21-25 år	☐	☐	☐	☐	☐
26-30 år	☐	☐	☐	☐	☐

3. Hvor gammel var du da du begynte å røyke daglig?

Alder: ☐☐ år

4. Hvor mange år har du røykt tilsammen?

☐☐ år

5. Da din mor var gravid med deg, pleide hun å røyke?

Nei ☐ Vet ikke ☐ Ja ☐→ Hvor mange sigaretter røykte hun pr. dag? < 10 ☐ 10 + ☐

6. Da du var barn, pleide faren din å røyke inne i huset?

Han var en ikke-røyker ☐ Nei, han røykte ikke inne ☐ Vet ikke ☐ Ja ☐→ Hvor mange sigaretter røykte han inne huset pr. dag? < 10 ☐ 10 + ☐

7. Da du var barn, pleide moren din å røyke inne i huset?

Hun var en ikke-røyker ☐ Nei, hun røykte ikke inne ☐ Vet ikke ☐ Ja ☐→ Hvor mange sigaretter røykte hun inne huset pr. dag? < 10 ☐ 10 + ☐

8. Har du bodd sammen med noen andre som pleide å røyke inne i huset før du var 21 år?

Nei ☐ Ja ☐→

Hvem?	Hvor mange sigaretter røykte de inne huset pr. dag?	
Bror ☐	< 10 ☐	10 + ☐
Søster ☐	< 10 ☐	10 + ☐
Annen ☐	< 10 ☐	10 + ☐

9. Har du bodd sammen med en partner eller noen andre som pleide å røyke inne i huset fra du var 21 til 25 år?

Nei ☐ Ja ☐→ Hvor mange sigaretter røykte han/hun inne i huset pr. dag? < 10 ☐ 10 + ☐

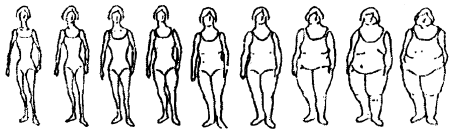
10. Har du bodd sammen med en partner eller noen andre som pleide å røyke inne i huset fra du var 26 til 30 år?

Nei ☐ Ja ☐→ Hvor mange sigaretter røykte han/hun inne i huset pr. dag? < 10 ☐ 10 + ☐

11. Har du jobbet med noen som pleide å røyke på din arbeidsplass?

Nei ☐ Ja ☐

12. Hvilket diagram illustrerer best din figur på de forskjellige alderstrinn?



5- år	☐	☐	☐	☐	☐	☐	☐	☐	☐
10-år	☐	☐	☐	☐	☐	☐	☐	☐	☐
15-år	☐	☐	☐	☐	☐	☐	☐	☐	☐
20-år	☐	☐	☐	☐	☐	☐	☐	☐	☐
25-år	☐	☐	☐	☐	☐	☐	☐	☐	☐
30-år	☐	☐	☐	☐	☐	☐	☐	☐	☐
I dag	☐	☐	☐	☐	☐	☐	☐	☐	☐

13. Hva er din nåværende vekt? ☐☐☐ kg

14. Hva er høyden din? ☐☐☐ cm

15. Hvordan var din fysiske aktivitet i fritiden da du var 13 til 19 år gammel? Tenk deg et ukentlig gjennomsnitt for året. Skolevei regnes som fritid. besvar begge spørsmålene.

	timer per uke			
	Ingen	Under 1	1-2	3 eller flere
Lett aktivitetet (ikke svett eller andpusten)	☐	☐	☐	☐
Hard fysisk aktivitet (svett og andpusten)	☐	☐	☐	☐

SEKSJON 6: ARBEIDSMILJØ

1. Har du på din arbeidsplass vært betydelig eksponert for:

	Nei	Vet ikke	Ja	Hvor gammel var du da eksponeringen startet?	Hvor mange år har du vært eksponert?	Hva slags arbeid hadde du da du ble eksponert?
Motorolje	☐	☐	☐	☐☐ år	☐☐ år	_____
Skjæreolje	☐	☐	☐	☐☐ år	☐☐ år	_____
Formolje	☐	☐	☐	☐☐ år	☐☐ år	_____
Hydraulikkolje	☐	☐	☐	☐☐ år	☐☐ år	_____
Turbinolje	☐	☐	☐	☐☐ år	☐☐ år	_____
Asfalt	☐	☐	☐	☐☐ år	☐☐ år	_____
Boreslam	☐	☐	☐	☐☐ år	☐☐ år	_____
Råolje	☐	☐	☐	☐☐ år	☐☐ år	_____
Narkosegasser	☐	☐	☐	☐☐ år	☐☐ år	_____
Organiske løsemidler*	☐	☐	☐	☐☐ år	☐☐ år	_____

*F.eks. avfettingsmidler, trikloroetylen, tetrakloroetylen, white spirit, tynnere, toluen, styren, xylen el. liknende

SEKSJON 7: HORMONELLE FAKTORER

1. Hvor gammel var du da du fikk din første menstruasjon? ☐☐ år

2. Er du gravid nå? Nei ☐ Ja ☐

3. Har du vært gravid? Nei ☐ Ja ☐ Om svaret er ja, vennligst oppgi utfallet og årstallet for graviditetene.

	Graviditet 1	Graviditet 2	Graviditet 3	Graviditet 4	Graviditet 5	Graviditet 6
Levende født	☐	☐	☐	☐	☐	☐
Ammet du barnet minst i en måned?	☐	☐	☐	☐	☐	☐
Abort (spontan abort eller provosert abort	☐	☐	☐	☐	☐	☐
Dødfødsel	☐	☐	☐	☐	☐	☐
År	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐

4. Har du noen gang fått hormonbehandling p.g.a. infertilitet? Hvis ja, når skjedde dette første gang? År ☐☐☐☐

5. Har du brukt P-piller (ikke mini-piller) av typen som kan tas i 3 uker og deretter tas sukkerpiller i 1 uke, P-plaster eller vaginal P-ring?

Nei ☐ Ja ☐ →

Hvor gammel var du første gang du brukte slike prevensjonsmidler? ☐☐ år

Hvor lenge brukte du slike prevensjonsmidler?

< 1 år ☐ 1-3 år ☐ 4-5 år ☐ 6-9 år ☐ 10+ år ☐

1. Helt til slutt vil vi gjerne vite om du har fått informasjon fra andre ved utfylling av dette skjemaet, f.eks. din mor?

Hvis ja, hvem? Mor ☐
Far ☐
Andre ☐

Takk for at du ville delta i undersøkelsen!

Appendix B: EnvIMS-Q in English

B

This Questionnaire will be read by an automatic optical reader

- Please use a blue or black pen to indicate your answer choice.

Participant ID: ☐

- Put an X in the box which corresponds to your correct answer choice : ☒
- If you put an X in the wrong box, please fill in the whole box completely ■ and then select the correct answer by placing an X in the correct box ☒

By filling out this form and sending it back to us, you consent to be a part of the study.

Date: _____

SECTION 1: DEMOGRAPHICS

1. Year of birth: ☐☐☐☐ **Your age now**: ☐☐

Are you a woman ☐ **or a man** ☐

Please complete the following table with information about where you lived at the following ages: (Please print)

	Town/City	Province/State & Country
At birth	_____	_____
0-5 yrs	_____	_____
	_____	_____
6-10 yrs	_____	_____
	_____	_____
11-15 yrs	_____	_____
	_____	_____
16-20 yrs	_____	_____
	_____	_____
21-25 yrs	_____	_____
	_____	_____
26-30 yrs	_____	_____
	_____	_____

2. What is the highest level of education attained by you, your mother and your father?

	Yourself	Your mother	Your father
Some elementary school education	☐	☐	☐
Completed elementary school	☐	☐	☐
Some high school education	☐	☐	☐
Completed high school	☐	☐	☐
CEGEP or college diploma	☐	☐	☐
Technical or trade school diploma	☐	☐	☐
University degree (Bachelor's)	☐	☐	☐
Graduate studies	☐	☐	☐
►(Specify level e.g. Masters, PhD, etc)	_____	_____	_____
Don't know	☐	☐	☐

3. What are your birth parents' ethnic backgrounds?

	Your father	Your mother
White	☐	☐
Chinese	☐	☐
Latin American	☐	☐
Arab	☐	☐
Aboriginal (e.g., North American Indian, Inuit)	☐	☐
West Asian (e.g., Iranian, Afghan)	☐	☐
Black	☐	☐
Japanese	☐	☐
Southeast Asian (e.g., Vietnamese, Cambodian)	☐	☐
Korean	☐	☐
South Asian (e.g., Indian, Sri Lankan)	☐	☐
Filipino	☐	☐
Other:	☐	☐
(Specify)	_____	_____

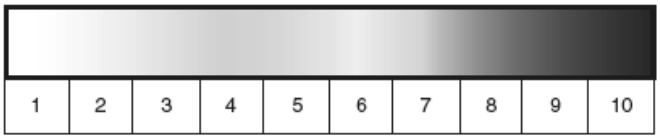
4. Please indicate in the box how many brothers and sisters you have. Include all children who lived with you during your childhood. If you are an only child, enter 0 in the box. ☐

Please indicate the years of their births and their gender.

	1	2	3	4	5	6
Year of Birth:	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐
Sex (M/F)	M ☐ F ☐	M ☐ F ☐	M ☐ F ☐	M ☐ F ☐	M ☐ F ☐	M ☐ F ☐

SECTION 2: SUN EXPOSURE

1. Please select the corresponding box below the colour that best matches the natural colour of your skin at the inner upper arm (without tanning). Set the colour chart against the inner part of your arm, between the elbow and the armpit, and select the number that corresponds best to the part of the figure that is closest to the colour of your skin.



1	2	3	4	5	6	7	8	9	10

2. What is the tanning reaction of your skin to its first sun exposure in the summer, with *no* use of sunscreen?

1. Always burn, never tan	☐
2. Usually burn, tan less than average (with difficulty)	☐
3. Sometimes mild burn, tan about average	☐
4. Rarely burn, tan more than average (with ease)	☐
5. Don't know	☐

3. What is the natural colour of your hair as a young adult?

1. Black	☐
2. Dark Brown	☐
3. Light Brown	☐
4. Blonde	☐
5. Red	☐

4. What colour are your eyes?

1. Black	☐
2. Brown	☐
3. Gray, green	☐
4. Blue	☐
5. Hazel	☐

5. In the past, in summer, how often did your activities (playing, participating in sports, watching sports, gardening, walking, work activities, etc.) take you outside at the following ages?

	Not that often	Reasonably often	Quite often	Virtually all the time	Don't know
0-5 yrs	☐	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐	
16-20 yrs	☐	☐	☐	☐	
21-25 yrs	☐	☐	☐	☐	
26-30 yrs	☐	☐	☐	☐	
In the past 3 years	☐	☐	☐	☐	

6a. In the past, in winter, how often did your activities (playing, participating in sports, watching sports, shovelling snow, walking, work activities, etc.) take you outside at the following ages?

	Not that often	Reasonably often	Quite often	Virtually all the time	Don't know
0-5 yrs	☐	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐	
16-20 yrs	☐	☐	☐	☐	
21-25 yrs	☐	☐	☐	☐	
26-30 yrs	☐	☐	☐	☐	
In the past 3 years	☐	☐	☐	☐	

6b. On weekends and holidays, how much time did you normally spend outside at the following ages:

	Never	Less than 1 hour/day	1-2 hours/day	3-4 hours/day	More than 4hours/day	Don't know
0-5 yrs	☐	☐	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐	☐	
16-20 yrs	☐	☐	☐	☐	☐	
21-25 yrs	☐	☐	☐	☐	☐	
26-30 yrs	☐	☐	☐	☐	☐	
In the past 3 years	☐	☐	☐	☐	☐	

7. At the following ages, where have your work and occupational activities (including parenting, caregiving, etc.) been carried out:

	Mainly indoors	Mainly outdoors	Equal time spent indoors and outdoors
16-20 yrs	☐	☐	☐
21-25 yrs	☐	☐	☐
26-30 yrs	☐	☐	☐

8. How often did you go on vacation to sunny places during winter months at the following ages?

	Never/seldom	1week/year or less	1-2 weeks/year	4+ weeks/year
0-5 yrs	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐
16-20 yrs	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐
In the past 3 years	☐	☐	☐	☐

9. How often did you use sun protection (sunscreen or protective clothing such as hats, long sleeves) at the following ages?

	Never/Seldom	Sometimes	Quite often	Almost always	Don't know
0-5 yrs	☐	☐	☐	☐	☐
6-10 yrs	☐	☐	☐	☐	☐
11-15 yrs	☐	☐	☐	☐	
16-20 yrs	☐	☐	☐	☐	
21-25 yrs	☐	☐	☐	☐	
26-30 yrs	☐	☐	☐	☐	
In the past 3 years	☐	☐	☐	☐	

10. How often did you use sunlamps or tanning beds at these ages?

	Never/Seldom	Less than once/year	Less than once/month	Once or more/month
16-20 yrs	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐

SECTION 3: DIET

We would like to ask you information about your diet when you were a **"teenager" (between 13 and 19 years old)**. If your diet changed substantially during this period of time, please try to report the average consumption for the period.

1. Please indicate <u>in which season(s)</u> you generally consumed the following foods while you were a *teenager (age 13-19 years)?*
(you may choose <u>more than one</u> checkbox per row)

	Winter	Spring	Summer	Fall	Never/ seldom
Cows' milk (liquid or reconstituted powdered)	☐	☐	☐	☐	☐
Other type of milk (Specify: _____)	☐	☐	☐	☐	☐
Yogurt	☐	☐	☐	☐	☐
Eggs (prepared any style)	☐	☐	☐	☐	☐
Fresh cheeses (e.g., fresh ricotta, cottage cheese, cream cheese)	☐	☐	☐	☐	☐
Aged cheeses (e.g., Parmesan, strong cheddar)	☐	☐	☐	☐	☐
Smoked cheeses (e.g., smoked gouda)	☐	☐	☐	☐	☐
Other cheeses (e.g., cheddar, marble, feta, havarti, mozzarella, Monterey Jack, gouda, pecorino, Gloucester, Cheshire)	☐	☐	☐	☐	☐
Red meat (e.g., beef, lamb, venison, bison) or cold cuts (of all types)	☐	☐	☐	☐	☐
Smoked meat & pork	☐	☐	☐	☐	☐
Hotdogs, frankfurters, weiners	☐	☐	☐	☐	☐
Fresh fish	☐	☐	☐	☐	☐
Frozen fish	☐	☐	☐	☐	☐
Preserved fish (in oil, in salt, dried)	☐	☐	☐	☐	☐
Smoked fish	☐	☐	☐	☐	☐
Shellfish:					
(i) Molluscs (cuttlefish, octopus, squid, mussels, clams, oyster, scallops, etc.)	☐	☐	☐	☐	☐
(ii) Crustaceans (prawns, scampi, lobster, shrimp, crab, etc.)	☐	☐	☐	☐	☐

2a. Please indicate how often you generally ate the following foods while you were a *teenager (age 13-19 years).*

(Please select only one box per row)

	Never	Less than once/mth	1-3 times/mth	Once/ week	2-3 times/ week	More than 3 times/ week
Cow's milk (liquid or reconstituted powdered)	☐	☐	☐	☐	☐	☐
Other type of milk (Specify: _____)	☐	☐	☐	☐	☐	☐
Yogurt	☐	☐	☐	☐	☐	☐
Eggs (prepared any style)	☐	☐	☐	☐	☐	☐
Fresh cheeses (e.g., fresh ricotta, cottage cheese, cream cheese)	☐	☐	☐	☐	☐	☐
Aged cheeses (e.g., Parmesan, strong cheddar)	☐	☐	☐	☐	☐	☐
Smoked cheeses (e.g., smoked gouda)	☐	☐	☐	☐	☐	☐
Other cheeses (e.g., cheddar, marble, feta, havarti, mozzarella, Monterey Jack, gouda, pecorino, Gloucester, Cheshire)	☐	☐	☐	☐	☐	☐
Red meat (e.g., beef, lamb, venison, bison) or cold cuts (of all types)	☐	☐	☐	☐	☐	☐
Smoked meat & pork	☐	☐	☐	☐	☐	☐
Hotdogs, frankfurters, weiners	☐	☐	☐	☐	☐	☐
Fresh fish	☐	☐	☐	☐	☐	☐
Frozen fish	☐	☐	☐	☐	☐	☐
Preserved fish (in oil, in salt, dried)	☐	☐	☐	☐	☐	☐
Smoked fish	☐	☐	☐	☐	☐	☐
Shellfish:						
(i) Molluscs (cuttlefish, octopus, squid, mussels, clams, oyster, scallops, etc.)	☐	☐	☐	☐	☐	☐
(ii) Crustaceans (prawns, scampi, lobster, shrimp, crab, etc.)	☐	☐	☐	☐	☐	☐

2b. We are particularly interested in how often you ate the following types of fish as a teenager *(age 13-19 years).*

	Never	Less than once/mth	1-3 times/mth	Once/ week	2-3 times/ week	More than 3 times/ week
Fresh or frozen salmon (*not* including smoked or canned)	☐	☐	☐	☐	☐	☐
Canned salmon	☐	☐	☐	☐	☐	☐
Fresh or frozen tuna (*not* including canned)	☐	☐	☐	☐	☐	☐
Canned tuna	☐	☐	☐	☐	☐	☐
Trout, Carp	☐	☐	☐	☐	☐	☐
Halibut	☐	☐	☐	☐	☐	☐
Sardines, anchovies	☐	☐	☐	☐	☐	☐
Fresh or frozen mackerel	☐	☐	☐	☐	☐	☐
Cod	☐	☐	☐	☐	☐	☐
Herring	☐	☐	☐	☐	☐	☐
Grouper, swordfish	☐	☐	☐	☐	☐	☐
Flounder, sole, smelt	☐	☐	☐	☐	☐	☐
Pickerel, snapper, perch	☐	☐	☐	☐	☐	☐
Other: specify _____	☐	☐	☐	☐	☐	☐

3. What type of water did you usually use when you were a *teenager (age 13-19 years)?* *(you can check more than one box per row)*

	No Consumption	For drinking	For cooking	To make coffee/ tea/ hot drinks
Well water, spring water.	☐	☐	☐	☐
Tap water	☐	☐	☐	☐
Bottled water	☐	☐	☐	☐
Don't know		☐	☐	☐

4. How often did you use the following condiments and oils as a *teenager (age 13-19 years)* including as dressings, or sauces, and for cooking?
(Please check only one box per row)

	Never	Less than once/mth	1-3 times/ mth	Once/ week	2-3 times/ week	4-5 times/ week	More than 5 times/week
Butter	☐	☐	☐	☐	☐	☐	☐
Margarine	☐	☐	☐	☐	☐	☐	☐
Lard	☐	☐	☐	☐	☐	☐	☐
Mayonnaise	☐	☐	☐	☐	☐	☐	☐
Vegetable oils:							
(i) Corn, sesame, walnut, sunflower, flaxseed, safflower oil	☐	☐	☐	☐	☐	☐	☐
(ii) Canola, peanut, olive, coconut, avocado, almond oil	☐	☐	☐	☐	☐	☐	☐
(iii) Other vegetable oils: Specify: _____	☐	☐	☐	☐	☐	☐	☐

5. Did you take any of the following dietary supplements when you were a *teenager (age 13-19 years)*?

	Yes	No	Don't know
Cod liver oil liquid	☐	☐	☐
Cod liver oil capsules	☐	☐	☐
Fish oil capsules	☐	☐	☐
Multivitamins	☐	☐	☐
Calcium	☐	☐	☐
Vitamin B12	☐	☐	☐
Vitamin C	☐	☐	☐
Vitamin D	☐	☐	☐

6. Please report what you were fed as a baby. (*You can select more than one box per column and line.*)

	Breast milk	Artificial formula	Other milk (e.g. cow, soy, etc.)	Don't know
From 1-3 mths	☐	☐	☐	☐
From 4-6 mths	☐	☐	☐	☐
From 7-9 mths	☐	☐	☐	☐
From 10 mths & older	☐	☐	☐	☐
Specify:			_____	

SECTION 4: MEDICAL HISTORY

The following questions concern illnesses that you may have had when you were younger.

1. Please indicate at what age you had the following illnesses or surgical interventions. To help you remember, think about which school grade you were in when you had the illness/surgery. Check all that apply.

	Didn't have	Don't know	Did have	Age at diagnosis: 0-5 yrs	6-10 yrs	11-15 yrs	16-20 yrs	21-25 yrs	26-30 yrs
Measles	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Mumps	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Rubella (German Measles)	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Chicken pox	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Tonsillectomy (tonsil removal)	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Pneumonia *(check as many times as applies)*	☐	☐	☐→	☐	☐	☐	☐	☐	☐

2a. Have you had infectious mononucleosis (also called "mono" or "the kissing disease")?

Yes ☐ →go to question 2b

No ☐ Don't know ☐ → If no or don't know, skip to question **#4**

2b. If yes, did have a blood test to check the diagnosis?

Yes ☐ No ☐ Don't remember ☐

2c. At what *age* did you have mononucleosis?

0-5 yrs ☐ 6-10 yrs ☐ 11-15 yrs ☐ 16-20 yrs ☐ 21-25 yrs ☐ 26-30 yrs ☐

3a. Do you remember in which *month* you were diagnosed with mono?

No ☐ Yes ☐ if yes, in which month was it? ☐☐

→ If you *know* the month, skip to question **#4**.

3b. If you don't remember the exact month, can you recall in which *season* you had mono?

Spring	Summer	Fall	Winter	Don't Remember
☐	☐	☐	☐	☐

4. Have you ever had a urinary tract infection (UTI)? If yes, please give your best estimate of the age(s) when it/they occurred.

No	Don't know	Yes	Ages when UTI occurred. *(you can check more than one box in the same row)* 0-5 yrs	6-10 yrs	11-15 yrs	16-20 yrs	21-25 yrs	26-30 yrs
☐	☐	☐→	☐	☐	☐	☐	☐	☐

5. Have you ever had a parasitic infection (e.g., Tenia or tapeworm, ossiuri, ascarides, giardia, cryptosporidium, etc.)? If yes, please give your best estimate of your age when it first occurred.

No	Don't know	Yes	Age of ***first*** infection 0-5 yrs	6-10 yrs	11-15 yrs	16-20 yrs	21-25 yrs	26-30 yrs
☐	☐	☐→	☐	☐	☐	☐	☐	☐

6. Do you have a history of allergy (such as conjunctivitis or red itchy watery eyes, rhinitis or runny nose, eczema, hives, asthma) to any of the following? If yes, please estimate the approximate age at which you experienced the first symptoms (i.e., when did the allergies begin?).

	No	Don't know	Yes	Age at ***first*** symptoms 0-5 yrs	6-10 yrs	11-15 yrs	16-20 yrs	21-25 yrs	26-30 yrs
Pollens	☐	☐	☐→	☐	☐	☐	☐	☐	☐
House dust	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Animal dander/fur	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Any food	☐	☐	☐→	☐	☐	☐	☐	☐	☐
Other allergies Specify: _____	☐	☐	☐→	☐	☐	☐	☐	☐	☐

7. Has a doctor ever told you that you had any of the following disorders?

	No	Don't know	Yes	Age at diagnosis	Age at first symptoms
Systemic lupus erythematosus (Lupus)	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Rheumatoid arthritis	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Hypothyroidism	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Hyperthyroidism	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Multiple sclerosis	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Optic neuritis	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Crohn's disease	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Ulcerative colitis	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Type I diabetes mellitus (juvenile diabetes)	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Celiac disease	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Psoriasis	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Leukemia	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Hodgkin's lymphoma	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Non Hodgkin's lymphoma	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Melanoma skin cancer	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Non-melanoma skin cancer	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Kidney disorders	☐	☐	☐→	☐☐ yrs	☐☐ yrs
Other medical disorders, specify: _____	☐	☐	☐→	☐☐ yrs	☐☐ yrs

8. To your knowledge, does anyone in your family have a history of any of the following diseases?

	No	Father	Mother	Brother/Sister	Child	Don't know
Systemic lupus erythematosus (lupus)	☐	☐	☐	☐	☐	☐
Rheumatoid arthritis	☐	☐	☐	☐	☐	☐
Hypothyroidism	☐	☐	☐	☐	☐	☐
Hyperthyroidism	☐	☐	☐	☐	☐	☐
Multiple sclerosis	☐	☐	☐	☐	☐	☐
Optic neuritis	☐	☐	☐	☐	☐	☐
Crohn's disease	☐	☐	☐	☐	☐	☐
Ulcerative colitis	☐	☐	☐	☐	☐	☐
Type I diabetes mellitus (juvenile diabetes)	☐	☐	☐	☐	☐	☐
Celiac disease	☐	☐	☐	☐	☐	☐
Psoriasis	☐	☐	☐	☐	☐	☐
Leukemia	☐	☐	☐	☐	☐	☐
Hodgkin's lymphoma	☐	☐	☐	☐	☐	☐
Non Hodgkin's lymphoma	☐	☐	☐	☐	☐	☐

SECTION 5: SMOKING HABITS AND LIFESTYLE FACTORS

1. Have you ever been a regular smoker? ("regular" = smoked one or more cigarettes per day for 6 months or longer)

Yes ☐ No ☐ → If your answer is no skip to question **#5**.

2. If yes, how many cigarettes per day on average did you smoke at the following ages?

	0 cig./day	1-4 cig./day	5-10 cig./day	11-20 cig./day	21+ cig./day
11-15 yrs	☐	☐	☐	☐	☐
16-20 yrs	☐	☐	☐	☐	☐
21-25 yrs	☐	☐	☐	☐	☐
26-30 yrs	☐	☐	☐	☐	☐

3. At what age did you start to smoke cigarettes daily? (Age) ☐☐

3a. Do you still smoke? Yes ☐ No ☐

4. How many years have you smoked in total? (Number of years) ☐☐

5. Did your mother smoke while she was pregnant with you?

No ☐ Don't know ☐ Yes → ☐ How many cigarettes per day did she smoke? Less than 10 ☐ 10+ ☐

6. Did your <u>mother</u> smoke <u>inside the house</u> when you were a child?

She was a non-smoker ☐ No, she didn't ☐ Don't know ☐ Yes → ☐ If yes, how many cigarettes per day did she smoke inside the house? Less than 10 ☐ 10+ ☐

7. Did your <u>father</u> smoke <u>inside the house</u> when you were a child?

He was a non-smoker ☐ No, he didn't ☐ Don't know ☐ Yes → ☐ If yes, how many cigarettes per day did he smoke inside the house? Less than 10 ☐ 10+ ☐

8. Did you live with anybody else who smoked <u>inside the house</u> before you were age 21?

No ☐ Yes → ☐

Who?	How many cigarettes a day did he/she smoke inside the house?	
Brother ☐	Less than 10 ☐	10+ ☐
Sister ☐	Less than 10 ☐	10+ ☐
Other ☐	Less than 10 ☐	10+ ☐

9. Did you live with anybody who smoked <u>inside the house</u> when you were between the ages of 21-25 years?

No ☐ Yes → ☐ How many cigarettes per day were smoked inside the house? Less than 10 ☐ 10+ ☐

10. Did you live with anybody who smoked <u>inside the house</u> when you were between the ages of 26-30 years?

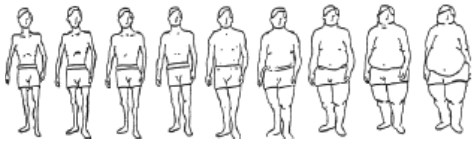
No ☐ Yes→ ☐ How many cigarettes per day were smoked inside the house?

Less than 10 ☐ 10+ ☐

11. Have you ever worked in an environment where someone regularly smoked <u>inside your workplace</u>?

No ☐ Yes ☐

12. What figure best depicts the shape of your body at the different ages.



At 5-years	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
At 10-years	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
At 15-years	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
At 20-years	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
At 25-years	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
At 30-years	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Today	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

13. What is your current weight? ☐☐☐ (Pounds) or ☐☐☐ (Kilograms)

14. How tall are you? ☐ (Feet) & ☐☐ (Inches) or ☐☐☐ (Centimetres)

15. What was your level of physical activity per week when you were a teenager (between 13 and 19 years old)? (For example, light physical activities refer to activities that require light physical effort such as walking leisurely, stretching, vacuuming or light yard work. Vigorous physical activities refer to activities that take heavy physical effort such as jogging, running, stair machine, sports (e.g. tennis, basketball, soccer, etc.)).

	None	Less than once/week	1-2 times/week	3 or more times/week
Light physical activity (your heart beats slightly faster than normal)	☐	☐	☐	☐
Vigorous physical activity (your heart rate increases a lot)	☐	☐	☐	☐

MEN – please **proceed to the last question (#14) on page 9**

SECTION 6: HORMONAL FACTORS

WOMEN ONLY. Men, please proceed to the last question (#14) on this page.

1. How old were you when you started getting your period? ☐☐ Age

2. Are you pregnant now? Yes ☐ No ☐

3. Have you ever been pregnant? Yes ☐ No ☐ → if no skip to question **#5**.

4. If yes, please provide the following information on the outcome of each pregnancy and the year(s).

	1st pregnancy	2nd pregnancy	3rd pregnancy	4th pregnancy	5th pregnancy	6th pregnancy
Born alive	☐	☐	☐	☐	☐	☐
Breastfed for at least 1 month	☐	☐	☐	☐	☐	☐
Lost pregnancy (spontaneous or induced abortion, interuterine death, still born)	☐	☐	☐	☐	☐	☐
Lost at # weeks:	_____	_____	_____	_____	_____	_____
Year of outcome:	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐	☐☐☐☐

5. Have you ever undergone hormonal treatment for infertility?
Yes ☐ No ☐ → if no skip to question **#7**

6. If yes, please indicate the year(s) you received treatment and the number of cycles per year.

Year(s): ☐☐☐☐ ☐☐☐☐ ☐☐☐☐ ☐☐☐☐ ☐☐☐☐

No of cycles/year: ☐☐ ☐☐ ☐☐ ☐☐ ☐☐

7. Have you ever used a birth control pill (not the "mini-pill" that contains progesterone only, but the type that is taken for 3 weeks, followed by 1 week replacement with "sugar-pills"), hormonal patches, vaginal hormonal rings, or *hormonal* inter-uterine devices (IUD)?
Yes ☐ No ☐ → if no skip to question **#10**

8. If yes, how old were you when you started using these contraceptives? ☐☐ Age

9. For how long did you/have you used these contraceptives?

Less than 1 year	1-3 years	4-5 years	6-9 years	10+ years
☐	☐	☐	☐	☐

10. Have you ever suffered from hirsutism, that is, from an excess of coarse hair in areas of the body where it is not normally found (e.g., face, chest, back, abdomen)?
Yes ☐ Don't know ☐ No ☐ → if no/don't know skip to last question **#14**

11. If yes, have you ever been given hormonal therapies to treat this? Yes ☐ No ☐ → if no skip to last question **#14**

12. At what age did you start these therapies? ☐☐ Age

13. For how long did you take these therapies?

Less than 1 year	1-3 years	4-5 years	6-9 years	10+ years
☐	☐	☐	☐	☐

14. Lastly, we would like to know if someone helped you fill out the questionnaire.

No ☐ Yes ☐ → Who? Mother ☐ Father ☐ Other ☐

Thank you for your participation!

If there is anything else that you would like to tell us about the survey, please do so in the space provided below.

Please return the questionnaire in the enclosed self-addressed envelope to the following address:

EnvIMS Study
Neuroepidemiology Research Unit
1025 Pine Avenue West, Suite P2.028
Montreal, QC H3A 1A1