The impact of lifestyle factors on disease risk and long-term disability progression in multiple sclerosis

Kristin Wesnes

Thesis for the degree of Philosophiae Doctor (PhD)
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Scientific environment

This work was partially carried out at the Department of Global Public Health and Primary Care, University of Bergen, as a member of the Lifestyle epidemiology research group, and partially at the National Multiple Sclerosis Competence Center, and later at Neuro-SysMed Research Center, Department of Neurology, Haukeland University Hospital.

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Contents

Abbreviations

ARR	Annual relapse rate
BMI	Body mass index, kg/m^2
CI	Confidence interval
CIS	Clinically isolated syndrome
CNS	Central nervous system
DMT	Disease modifying therapy
EAE	Experimental autoimmune encephalomyelitis
EBV	Epstein Barr Virus
EDSS	Expanded Disability Status Scale
EnvIMS	Environmental Risk Factors in Multiple Sclerosis
EnvIMS-Q	The questionnaire in the EnvIMS study
FRS	Figure rating scale
GWAS	Genome wide association study
HLA	Human leucocyte antigen
IFN-β	Interferon beta
IL	Interleukin
IM	Infectious mononucleosis
ITT	Intention to treat
IV	Instrumental variable
MHC	Major histocompatibility complex
MR	Mendelian randomization
MRI	Magnetic resonance imaging
MS	Multiple sclerosis

NEDA	No evidence of disease activity
NHS	Nurses Health Study
NO	Nitric oxide
OFAMS	Omega-3 Fatty Acids in Multiple Sclerosis
OR	Odds ratio
PA	Physical activity
PASAT	Paced Auditory Serial Addition Test
PPMS	Primary progressive multiple sclerosis
RCT	Randomized placebo-controlled trials
RIS	Radiologically isolated syndrome
RR	Relative risk or rate ratio
RRMS	Relapsing-remitting multiple sclerosis
SD	Standard deviation
SNP	Single nucleotide polymorphism
SPMS	Secondary progressive multiple sclerosis
SZA	Solar zenith angle
Th	T helper lymphocyte
TNF	Tumor necrosis factor
Treg	T regulatory cell
UVB	Ultraviolet B
WHO	World Health Organization
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D

Abstract

Background: Multiple sclerosis (MS) is a disabling inflammatory disease of the central nervous system (CNS) likely caused by genetic susceptible variants and environmental triggers. Low vitamin D levels and smoking are already established risk factors for MS, while obesity and physical activity may also influence the risk. In addition, some of these factors are associated with disease course in MS, but less is known about their potential long-term effects on MS.

Objectives: In this thesis, we examined (i) the association between body size and MS risk across different geographical areas (Paper 1), (ii) whether frequency and intensity of physical activity in adolescence may be an independent risk factor for MS (Paper 2) and (iii) whether vitamin D levels, tobacco use and body mass index (BMI) can influence long-term disability progression in MS (Paper 3).

Methods and materials: In Paper 1 and 2, we used retrospective self-reported data from a large multinational population-based case-control study on environmental and lifestyle factors in MS (the EnvIMS study). The study on body size and MS risk in Paper 1 was based on self-reported body sizes on a 9-figure scale, at 5-year intervals, from age 5 to age 30 years in Norway and Italy. The study on physical activity (PA) and MS risk in Paper 2 was based on reported average weekly amounts of light and vigorous PA during adolescence in Norway, Sweden and Italy. We used logistic regression models to examine the associations between lifestyle factors and the risk of MS, with adjustment for relevant covariates.

For Paper 3, we had available baseline and 10-year follow-up data from 80 patients who initially participated in a randomized study on omega-3 fatty acids treatment in MS (the OFAMS study). In linear regression models, we examined the association between mean baseline levels of serum 25-hydroxyvitamin D (25(OH)D), serum cotinine (a nicotine metabolite) and BMI, and 10-year disability progression given by

the 10-year change in Expanded Disability Status Scale (EDSS) score. We also examined the importance of seasonal fluctuations of 25(OH)D on this association.

Results: In Paper 1, a large body size (body figure 6-9) was significantly associated with increased MS risk in Norway from age 15- 25 years. The association was strongest at age 25, with an age-adjusted odds ratio (OR) of 2.10 (95% confidence interval (CI): 1.08-4.09) for men and 1.48 (95% CI: 0.94-2.32) for women, compared to a "normal weight" body size 3. Further adjusting for smoking and outdoor activity gave similar estimates. In Italy we found no clear association between body size and the risk of MS, but after disease onset, the controls in both countries reported larger body sizes relative to the cases.

In Paper II, the pooled analyses for Norway, Sweden and Italy showed that vigorous PA $\geq$ 3 hours compared to < 1 hour per week was associated with a reduced risk of MS with an age- and sex-adjusted OR of 0.74 (95% CI: 0.63-0.87). We found similar estimates in country-specific analyses, also after adjusting for other established risk factors. No clear evidence of reverse causation explaining this association was observed in a subgroup analysis, excluding participants with disease onset within 10 years from reported PA.

In Paper 3, one standard deviation (SD; 18.7 nmol/L) increase in seasonally adjusted 25(OH)D levels during the OFAMS baseline study was associated with 0.45 point (95% CI: -0.75 to -0.16) less change in EDSS score after 10 years, in a model adjusting for sex, age and baseline EDSS score. There was a significant dose-response relationship across quartiles of 25(OH)D levels (p for trend = 0.024). The association was mainly driven by low 25(OH)D levels during spring and seasonally adjusted levels below 80 nmol/L. For BMI and tobacco use, no significant associations were observed, but we found a trend towards less progression with higher BMI.

Conclusions: A large body size during childhood and young adulthood was associated with increased risk of MS among men and women in Norway, but less so in Italy. Higher amounts of regularly vigorous PA were associated with lower MS risk across different geographical areas, also after adjustment for potential confounders. Higher levels of 25(OH)D during a two-year period were associated with less 10-year disability progression, which appeared to be driven by low spring levels. Our findings suggest that healthy lifestyle changes during young ages may influence the risk of developing MS in a beneficial way, and that better long-term outcomes can be achieved by maintaining 25(OH)D levels above 80 nmol/L throughout the year.

List of Publications

1. Wesnes K, Riise T, Casetta I, Drulovic J, Granieri E, Holmøy T, Kampman MT, Landtblom AM, Lauer K, Lossius A, Magalhaes S, Pekmezovic T, Bjørnevik K, Wolfson C, Pugliatti M, Myhr KM. *Body size and the risk of multiple sclerosis in Norway and Italy: the EnvIMS study*. Multiple Sclerosis Journal 2015;21:388-395.

2. Wesnes K, Myhr KM, Riise T, Cortese M, Pugliatti M, Boström I, Landtblom AM, Wolfson C, Bjørnevik K. *Physical activity is associated with a decreased multiple sclerosis risk: The EnvIMS study*. Multiple Sclerosis Journal 2018;24:150-157.

3. Wesnes K, Myhr KM, Riise T, Kvistad SS, Torkildsen Ø, Wergeland S, Holmøy T, Midgard R, Bru A, Edland A, Eikeland R, Gosal S, Harbo HF, Kleveland G, Sørenes Y, Øksendal N, Bjørnevik K. *Low Vitamin D, but not tobacco use or high BMI, is associated with long-term disability progression in multiple sclerosis*. Multiple Sclerosis and Related Disorders 2021; https://doi.org/10.1016/j.msard.2021.102801

1. Introduction

1.1 Multiple Sclerosis- prevalence and distribution

Multiple sclerosis (MS) is a chronic, immune-mediated, demyelinating disease of the central nervous system (CNS). It typically affects young adults with a peak incidence from 25 to 35 years of age,[1] and a female to male ratio of around 2-3:1.[2] Worldwide, there are around 2.2 million prevalent cases of MS, with the highest age-standardised prevalence (>120 per 100 000) in North-America and some northern European countries, moderate (60-120 per 100 000) in other European countries and Australasia, and lowest (<60 per 100 000) in countries closer to the equator, and Asia (Figure 1).[3] The distribution shows a clear latitude gradient in some, but not all parts of the world,[4] while an inverse or absent gradient has been observed at higher latitudes,[5,6] including Norway.[7] These geographical and latitudinal variations likely reflect both genetic and environmental contributions to the disease.[5]

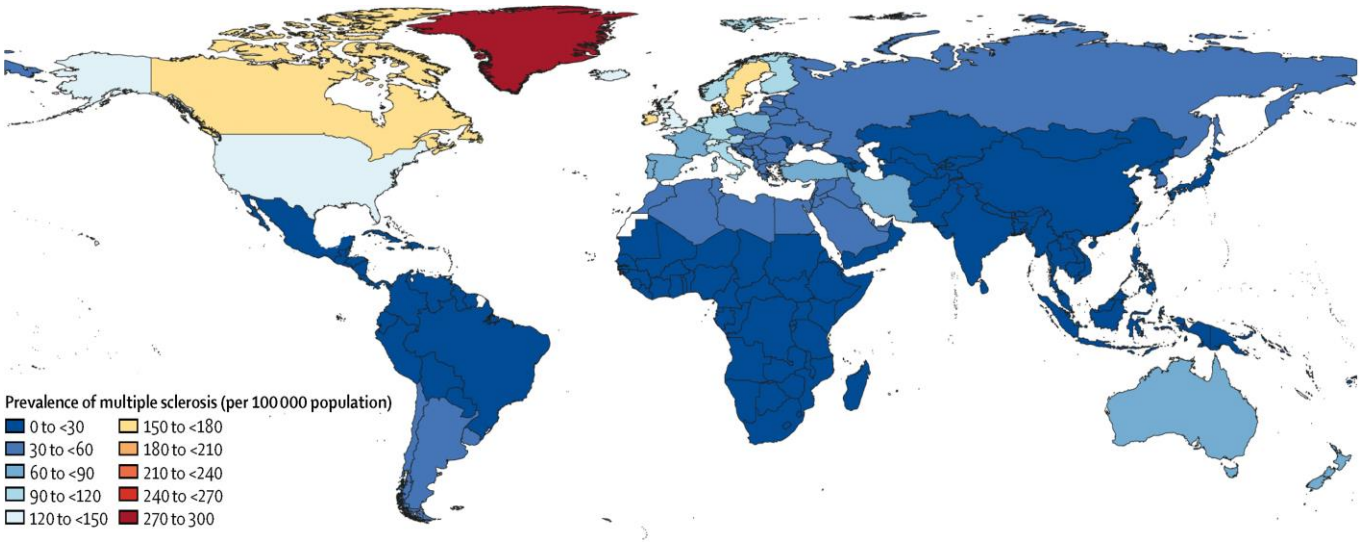

Figure 1. Age-standardised multiple sclerosis prevalence per 100 000 population in 2016; men and women combined. *Reprinted by permission from the Creative Commons CC-BY license: Adapted from GBD 2016 Multiple Sclerosis Collaborators, Lancet Neurology 2019; 18: 281.*[3]

Over the years, both prevalence and incidence rates have been rising in many parts of the world,[3,8-10] predominantly observed in women compared to men.[9,10] The increased prevalence rates may be explained by earlier diagnosis through changes and revisions of diagnostic criteria,[11,12] longer survival,[13] and better case ascertainment through improved diagnostic tools, such as magnetic resonance imaging (MRI) (affect both prevalence and incidence).[14] The increased incidence in women relative to men is more challenging to explain by sex-independent or genetic factors, and is more likely to reflect changes in environmental exposures or nutrition.[2]

1.2 Pathology and immunological mechanisms in MS

The pathology of MS involves demyelinated white and grey matter lesions,[15] axonal injury, and progressive neuronal loss.[16] While demyelination is a likely consequence of inflammation, neurodegeneration seems to be driven by oxidative stress and mitochondrial injury.[17] Although most observations suggest that inflammation likely precedes neurodegeneration,[18] the immunopathogenic mechanisms that trigger and maintain MS are complex and not fully understood.[19] Inflammation and neurodegeneration probably coexist at all stages of the disease,[20] and some neurodegenerative processes may even appear independent of inflammation.[18,19] Further, there is an ongoing debate whether MS is initiated by an extrinsic event outside the CNS (the outside-in theory), or an intrinsic event within the CNS (the inside-out theory).[18] In either way, both genetic[21] and experimental evidence points towards a contribution of both adaptive (autoreactive T and B cells and defective T regulatory (Treg) cells) and innate immune cells (microglia, macrophages and astrocytes) in the pathogenesis of MS.[19,22] The T cells are dominated by a shift towards pro-inflammatory CD4+ T helper (Th) 17 and Th1 cell pools.[22] The beneficial effect of anti-CD 20 therapies[23,24] for MS suggests that antigen-presenting B cells and their interaction with pathogenic T cells may be the main inducer of the immune cascade in MS.[25] Epidemiological and experimental evidence also suggests that environmental risk factors may be crucial for disease onset through various immunological pathways.[19]

1.3 Disease course and diagnosis

Traditionally, MS has been divided into two distinct clinical phenotypes[26] from onset: The majority of patients (85-90%) develop a relapsing-remitting MS (RRMS) characterized by symptomatic relapses of neurologic dysfunction with full or partial recovery between the relapses. The remaining 10-15% have a primary progressive MS (PPMS) with gradual disease progression and no distinct relapses.[27,28] Typical MS symptoms include visual disturbances, weakness, dyscoordination, sensory loss, and changes in bowel and bladder control, as well as more vague symptoms such as cognitive impairment and fatigue.[29] Subclinical activity can be seen as white matter lesions on MRI scans of the brain and spinal cord with typical distribution, morphology, evolution and signal abnormalities.[14] The disease progression can be monitored by the validated and widely used Expanded Disability Status Scale (EDSS) ranging from 0, which refers to no symptoms, to 10, which refers to death due to MS.[30] The lower EDSS scores from 0 to 3.5 are mainly determined by ratings in the Functional System Scores which includes seven "functional systems" of neurological deficits, [30] while the EDSS scores from 4 to 7 are mainly based on walking impairment. The highest scores from 7 to 9.5 represent severe disability that affects activity of daily living (Figure 2). Before the treatment era, the distribution of EDSS scores in MS populations had a typical bimodal shape.[31,32]

Many RRMS patients eventually develop secondary progressive MS (SPMS) dominated by progression with or without occasional relapses and plateaus.[26] A diagnosis of MS requires "dissemination in time and space", which in earlier days was mainly based on clinical course and symptoms, as described in the Poser criteria.[33]

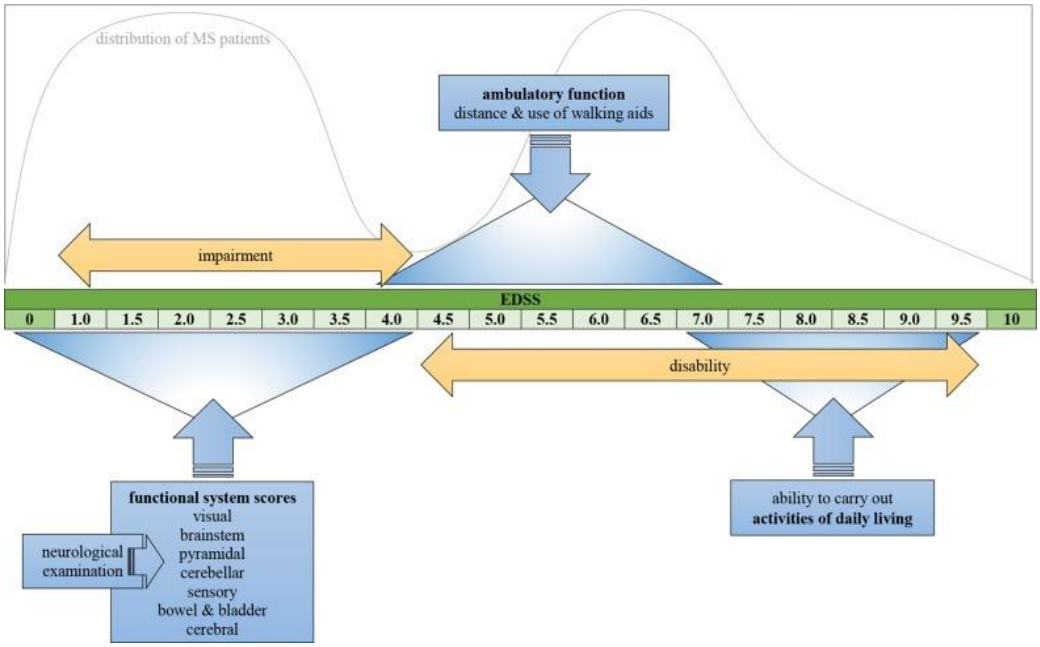

Figure 2. The Expanded Disability Status Scale (EDSS) and the factors that determine overall score; a typical bimodal distribution over the EDSS have been observed in natural history MS populations. *Reprinted by permission from the Creative Commons Licence: CNS Drugs.2017; 31(3):2017-236.*[34]

In 2001, these criteria were replaced by the McDonald criteria[35] with the latest revisions made in 2017,[12] where clinical and paraclinical evidence (MRI lesions and oligoclonal bands in the cerebrospinal fluid) of disease activity are of equal importance to confirm the dissemination in time and space needed for a definitive diagnosis of MS.[12] In addition, the paraclinical evidence of demyelinating activity not explained by other conditions has introduced two pre-clinical MS entities that may progress to definitive MS with time; clinical isolated syndrome (CIS) and radiologically isolated syndrome (RIS).[36] After onset, the disease course is unpredictable and varies highly between individuals. Important demographic, clinical and radiological prognostic factors for earlier irreversible disability are older age, male gender, progressive disease from onset, number of relapses during the first five years, pyramidal onset symptoms, spinal cord lesions and MRI lesion load.[37-41]

1.4 Disease-modifying therapies and prognosis

Before the treatment era of disease-modifying therapies (DMT) for MS, most RRMS patients developed SPMS within 10-20 years of time,[31] and around 50% in general MS populations needed walking aid around 15 years after onset;[42] this interval was considerably shorter in patients with PPMS.[27,31] Since interferon beta-1b (IFN-β) was approved as the first DMT in 1993, a large number of DMTs with various immuno-modulatory or immune-suppressive mechanisms,[43] have improved short-term, and most likely long-term prognosis for patients with inflammatory relapsing disease.[44] Along with more high-efficacy DMTs for MS, the term "No Evidence of Disease Activity" (NEDA) has been introduced as an ideal outcome for shorter or longer periods. The NEDA-3 term includes (i) no relapses, (ii) no disability progression and (iii) no MRI activity.[45] For non-inflammatory progressive disease, the DMT options are still limited, with only one approved drug (ocrelizumab), showing a modest effect on disease progression.[46] Still, even the most efficacious treatments are not able to ultimately halt or cure the disease, and therefore more knowledge about other modifiable factors that may alter the disease course is needed.

1.5 Factors associated with MS risk

MS is most likely a multifactorial disease, triggered by environmental exposures in genetically susceptible individuals. The disease has since the 1970s and for a long time been referred to as the "white man's burden",[47] based on the typical geographical distribution and partly lack of research in other ethnic populations. However, a study from 2013 observed a higher incidence of MS in Afro-Americans compared to Whites in a multiethnic population,[48] which may reflect local environmental exposures rather than their genetic background. Genetic resistance is likely more relevant in individuals of Asian ancestry, where low incidence rates repeatedly have been reported, also among migrants.[48,49] The next paragraphs will first give an overview of the current

knowledge about the main genetic and environmental contributions to the disease, before lifestyle-related factors relevant for this thesis will be discussed in more detail.

1.5.1 Heritability and genetic factors

In Western countries, the lifetime risk for MS is estimated to 0.1- 0.5% for the general population[9,50] and 2.5-2.8% among first-degree relatives.[50,51] The age-adjusted risk for monozygotic twins has been reported to be 17-18%,[51,52] which strongly suggests that non-genetic factors have an additional and important role in MS susceptibility.[52] In the 1970s, it was recognized that the immune-related human leucocyte antigen (HLA) gene cluster[53] within the major histocompatibility complex (MHC) on chromosome 6 was associated with MS risk.[54] A threefold increased risk, and by that the strongest effect, has been reported for the specific HLA-DRB1*15:01 gene variant in the HLA class II genes[54] (important for antigen recognition by T cells[22]). The genetic research has also confirmed the HLA class 1 allele HLA-A*02:01 as a protective gene variant for MS.[55]

Genome-wide association studies (GWAS) have now identified more than 200 risk loci linked to both adaptive and innate immune cells, of which MHC contains 32 of the variants, and one even detected in chromosome X, which all together explains almost half of the disease's heritability.[21] Further, potential interactions between genetic risk variants and environmental exposures have been discovered,[56,57] and epigenetic alterations may also contribute to risk modulation in susceptible individuals.[20]

1.5.2 Environmental risk factors and their timing in MS

Migration studies from the 1960s and onwards have provided strong clues for an environmental influence on MS risk. Some decades ago, there was more convincing evidence for a *decrease* in MS risk when moving from a high-risk area to a low-risk area, than for an *increase* in MS risk when moving in the opposite direction to a high-risk area.[49] Later, a clearly increased risk was found in a large population-based study among immigrants moving from their low-risk country of origin to a high-risk country (Denmark).[58] The change in risk among first-generation immigrants seems to be age-

dependent, largely occurring during the first two decades of life.[49,58] Also, the risk appears to change between generations, with a substantially higher risk observed among second-generation compared to first-generation immigrants in high-risk countries.[58,59] These findings strongly suggest that *timing* of environmental exposures also plays a likely role, with childhood and adolescence being critical ages.[60] Even exposures in utero and in neonates have been associated with increased MS risk later in life.[20,61]

Based on early migrant studies and geographical distribution, an infectious agent was strongly suspected in MS pathogenesis,[47] with age at infection as a likely contributor.[49] In particular, several viruses have been variably linked to the disease,[20] but the evidence is most consistent for Epstein Barr virus (EBV), especially seropositivity for EBV nuclear antigen (EBNA) IgG and infectious mononucleosis (IM),[62,63] typically occurring in adolescence.[63] In a meta-analysis, the overall odds ratio (OR) for MS among anti-EBNA seropositive individuals was 4.5 (95% confidence interval (CI) 3.26-6.11), while for seronegative individuals, the overall OR was 0.13 (95% CI 0.05-0.33).[64] Since EBV seropositivity is highly prevalent in the general population and only a few develop MS, complex genetic interactions or alterations are of likely relevance in the relationship.[20]

Over the years, the associations between several environmental exposures and the risk of MS have been explored in numerous studies. An umbrella overview of selected meta-analyses reported strongest and least heterogenous evidence across studies for EBV and tobacco smoking.[62] For vitamin D, higher serum levels of 25-hydroxyvitamin D (25(OH)D) have consistently been associated with decreased risk of MS in three prospective studies in White populations,[65-67] although there is weaker evidence in other ethnic groups.[65,68] Many studies have also confirmed a likely role for sun exposure as well as obesity during childhood and adolescence, on the risk of MS.[20,69] A number of other potential risk factors have also been studied, including dietary sodium intake,[70] polyunsaturated fatty acids,[71] breastfeeding,[72] air pollutants,[73] organic

solvents,[74] vaccinations,[62] gut microbiota[75] and physical activity (PA),[76] but it remains unclear to which extent they contribute to MS risk. The environmental exposures may influence MS pathogenesis through diverse biological pathways (Figure 3).[20]

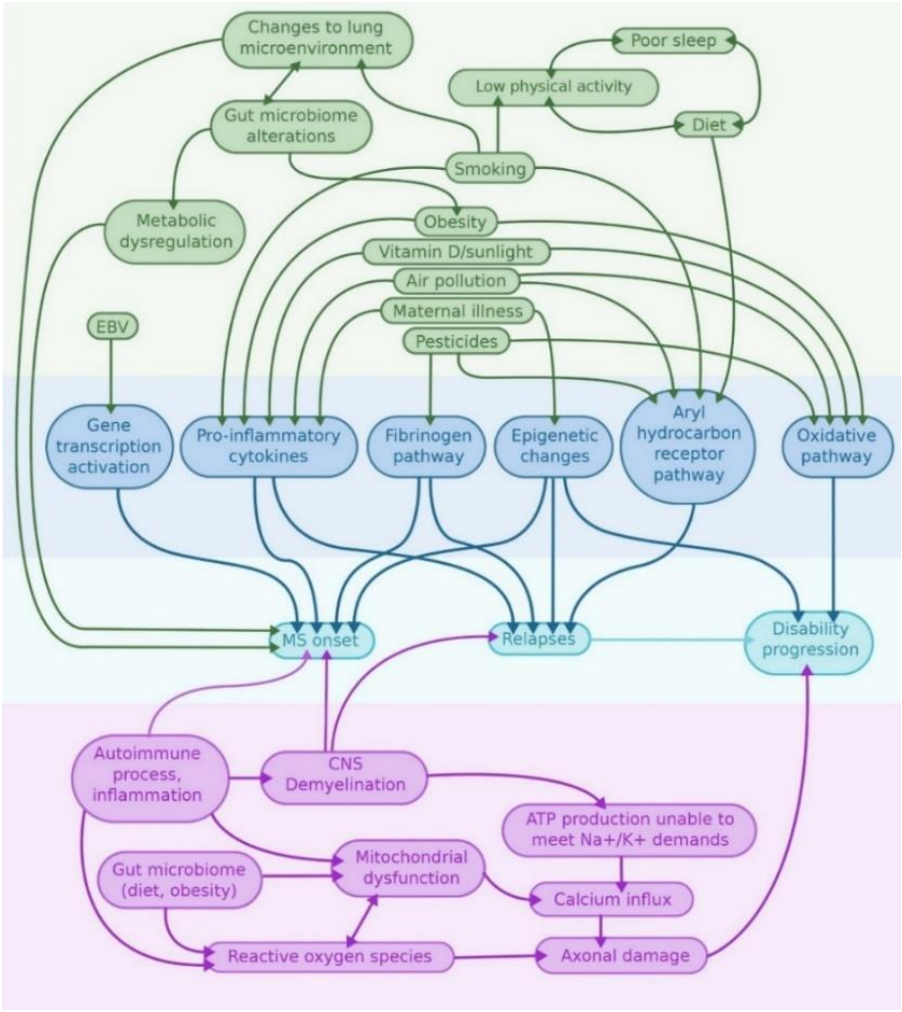

Figure 3. Possible biological pathways linking different environmental risk factors to MS pathogenesis. *Reprinted by permission from the Creative Commons Attribution License*: *Annals of Clinical and Translational Neurology 2019; 6(9): 1913.*[20]

1.6 Lifestyle factors related to MS risk and disease course

Several of the environmental exposures of likely importance to MS risk have also been examined for a potential role in MS disease course.[20] Most of these factors can be considered as modifiable *lifestyle* factors, such as levels of vitamin D, obesity, PA, and tobacco use/smoking. Thus, gaining more knowledge about these factors may provide an opportunity to prevent some cases of MS, and to reduce disease progression in those already affected by the disease.

1.6.1 Sun exposure and vitamin D

Sun exposure was early suggested as a potential etiological factor for MS, since it, like MS prevalence, varies with latitude. In the 1960s, negative correlations between average annual hours of sunshine and MS prevalence were found among U.S. Veterans in the Northern hemisphere,[77] and in Australian regions in the Southern hemisphere.[78] Both prospective cohorts[68,79] and retrospective case-control studies[68,80,81] have later reported associations between higher sun exposure and lower MS risk in different ethnic groups. In addition, indirect measures of sun exposure, such as higher levels of outdoor work,[82] more actinic skin damage[81] and less sunscreen use[83] have been associated with lower MS risk.

Sunshine contains ultraviolet B (UVB) radiation, which has likely immunosuppressive effects both directly and indirectly through the actions of UVB-induced vitamin D.[84,85] The direct effect may involve upregulation of Tregs and stimulation of anti-inflammatory cytokines such as interleukin 10 (IL-10) and other mediators.[84,86] It is therefore biologically plausible that the vitamin D pathway is not the only link between UVB exposure and MS, as recently explored in a large Swedish case-control study.[87] At higher latitudes, the strength of UVB radiation varies considerably with season and becomes weaker during the winter.[88] In MS patients, a latitude-dependent seasonal variation in relapse rates have been observed,[89] which may reflect a direct effect of UVB exposure or factors strongly related to UVB or season, such as vitamin D or seasonal infections that may also be influenced by vitamin D status.[90]

UVB radiation is the main natural source for vitamin D synthesis.[91] Several prospective studies support a likely role of 25(OH)D levels[65-67] or dietary vitamin D intake[92,93] on MS risk in different geographical areas. In addition, findings from Mendelian randomization (MR) studies suggest that low 25(OH)D levels have a causal effect on MS risk.[94,95] By using single nucleotide polymorphisms (SNPs) that are associated with vitamin D levels as an instrumental variable (IV),[96] confounding and reverse causation is unlikely because SNPs are randomly inherited at conception that temporally precedes the outcome/disease (Figure 4).[94] However, these MR studies are limited by the possibility of pleiotropy, i.e. that the SNPs may affect other pathways leading to the outcome, and that the SNPs used in the IV explain 4% or less of the total variance in 25(OH)D levels.[97,98]

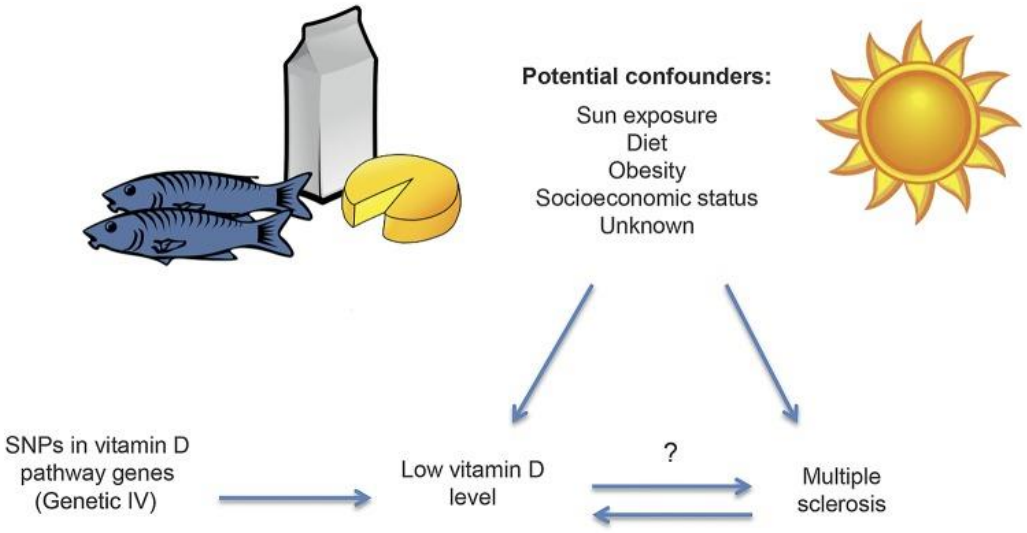

Figure 4. In MR studies, the use of a genetic instrumental variable (IV) for vitamin D levels can minimize confounding and reverse causation that often limit the interpretation of an association between vitamin D and MS in observational studies. *Reprinted by permission from the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND): Neurolol Genet. 2016 Oct; 2(5): e97.*[94]

Vitamin D has also a likely influence on MS disease activity. Observational studies have reported associations between lower vitamin D levels and higher relapse rate[99-101] and more MRI- verified inflammatory activity before[102] or during treatment with IFN-β,[101,103] and other DMTs.[104] For short-term (< 5 years) disease progression, some studies found significant associations between lower 25(OH)D levels and higher EDSS scores,[101,104] while other studies did not.[103,105] For long-term (> 10 years) disease progression, the evidence is scarce. One study found that higher baseline 25(OH)D levels over 2 years were associated with better cognitive performance in the Paced Auditory Serial Addition Test (PASAT) at year 11.[106] In another study, 25(OH)D levels did not influence long-term EDSS scores, but this study was based on infrequent measures of vitamin D once a year during the first 2 years.[107]

Randomized controlled trials (RCTs) on vitamin D and disease activity have mostly been small and short-lasting, with conflicting results regarding primary outcomes. Even the two largest RCTs on high-dose vitamin D3 versus placebo in two IFN-β treated populations failed to reach their primary endpoints in the intention-to-treat (ITT) population, i.e. NEDA-3 status at 48 weeks (SOLAR study),[108] and annual relapse rate (ARR) at 96 weeks (CHOLINE study).[109] However, in the ITT data of the SOLAR study, there was a significant reduction in cumulative new MRI lesions in the treatment group, [108] while in the CHOLINE study, analyses among the completers of the 96-week trial (69.8%) showed a significant reduction in ARR and less EDSS progression in treated patients.[109] Why RCTs have failed to confirm a substantial treatment effect of high-dose vitamin D when most observational studies have shown strong dose-dependent associations between higher 25(OH)D levels and less MS disease activity may have several explanations. These include, but are not restricted to, unmeasured confounding in observational studies, small sample sizes and short duration of RCTs,[110] and reverse causation[111] (i.e. low vitamin D being a consequence of inflammation and/or disease severity).

Vitamin D3 is primarily synthesized from UVB exposure of the skin, but additional vitamin D3 and D2 can be obtained through dietary intake of fatty fish (D3), vegetable sources (D2) and fortified food or supplements.[91,112] In the liver, solar and dietary vitamin D is converted into 25(OH)D, the main circulating form. This is also the most accurate marker for vitamin D because of its long half-life (around 3 weeks), and because the levels reflect the available sources.[113] In the kidneys, but also in immune cells and other cells, 25(OH)D is metabolized into the active compound 1,25-dihydroxyvitamin D ($1,25(OH)_2D$),[91] which has been found to have potent anti-inflammatory properties, partly through suppression of pro-inflammatory cytokines and inhibition of Th1 and Th17 differentiation.[84,91] Overall, this modulates the immune system into a more tolerable state.

In the experimental autoimmune encephalomyelitis (EAE) mouse model for MS, studies have found a protective effect of $1,25(OH)_2D$ acting on T lymphocytes.[114,115] There is also evidence for a genetic functional role of vitamin D on the MS risk allele HLA-DRB1*15 in humans.[116] In MS patients, high-dose vitamin D has shown anti-inflammatory changes on the cytokine level with up-regulation of IL-27, TGF- β1, and IL-10, [117] while another study did not detect alterations into a more regulatory profile on the lymphocyte level.[118] Still, most evidence points towards beneficial anti-inflammatory effects of vitamin D in MS, where a combination of several vitamin D related mechanisms seems plausible.[119]

Vitamin D may also be involved in remyelination and neural repair, as shown in different animal models of demyelination: In toxic cuprizone mouse models, reduced white matter demyelination[120] and less axonal loss[121] was observed after vitamin D3 supplementation. In an EAE model, injection of $1,25(OH)_2D$ elevated the number of oligodendrocyte precursor cells and oligodendrocytes in demyelinating lesions in CNS.[122] Lastly, in a recent study with lysolecithin-induced demyelination in rats, dietary vitamin D3 supplements promoted oligodendrocyte differentiation and neuroblast migration to the demyelinated lesion site.[123]

From a bone-health perspective, 25(OH)D levels <75 nmol/L is considered insufficient,[124] while vitamin D deficiency is generally defined as 25(OH)D levels <50 nmol/L.[125,126] The levels can vary considerably through the year, along with seasonal variations of UVB-induced vitamin D synthesis.[127] Residents at latitudes above 50° north or south, such as in Norway, are more prone to vitamin D deficiency during the winter months, since the weak UVB radiation leads to a "vitamin D winter" period with nearly absent cutaneous vitamin D production (Figure 5).[128] Thus, dietary sources of vitamin D become more important during this time of the year.

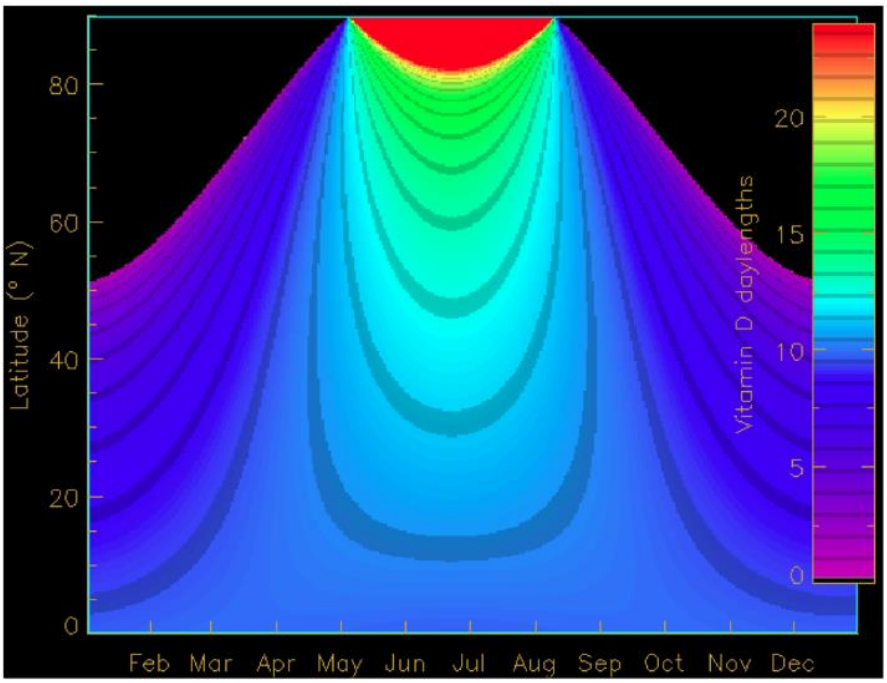

Figure 5. Daily vitamin D production (in hours) is dependent on latitude: The black area indicates the "vitamin D winter" at high latitudes when UVB exposure (from a clear atmosphere) is too weak for vitamin D production. *Reprinted by permission from an open access Creative Common CC BY License: Nutrients 2010, 2(5), 489.*[127]

1.6.2 Body size and obesity

Obesity is currently considered an epidemic among both children and adults in most parts of the world.[129] The World Health Organization (WHO) defines overweight as body mass index (BMI) 25-29.9 kg/m^2, and obesity as BMI ≥ 30 kg/m^2.[129] Since childhood and adolescence appear to be critical ages for MS susceptibility,[60] and obesity has been associated with lower levels of circulating vitamin D,[130,131] Munger and colleagues explored the association between obesity at age 18 and 20 years, and the risk of MS in two large cohorts of American female nurses (Nurses Health studies (NHS) I and II). In this study, both BMI ≥ 30 kg/m^2 at age 18 years and a large self-reported body size at age 20 years were associated with a 2-fold increased risk of MS, compared to a reference "normal weight" value.[132] Similar results were found for overweight young men in a Norwegian cohort,[76] and for both male and females at age 20 years in a Swedish population-based case-control study and in a pediatric cohort in Germany.[133,134] However, the association may be stronger among females than men, as reported in three other studies exploring the association between obesity and pediatric[135] and adult-onset MS.[136,137]

Hedström and colleagues showed that the risk may be driven by adolescence rather than childhood (age < 10 years) obesity in a Swedish population.[138] In case-control data from Sweden and California, striking interactions between HLA risk variants and overweight/obesity (BMI ≥ 27kg/m^2) in the 20s were seen, with ORs > 13 for individuals of greatest genetic MS susceptibility (positive HLA-DRB1*15 and negative protective HLA-A*02 status).[56] A likely causative role for high BMI on MS risk has been demonstrated through MR studies,[139,140] after adjusting for MS susceptible risk alleles,[140] and also for genetically determined childhood BMI.[141] Even though MR is a useful tool to investigate the causality of an association, the SNPs used in these MR analyses account for less than 6% of the total variance of BMI.[142] Therefore, the total effect of all BMI-related factors on MS risk cannot be evaluated from MR studies.

Obesity may also be relevant after disease onset. Two studies have suggested that high BMI reduces therapy response on injectible DMTs: In a Norwegian adult MS population, a lower proportion of overweight (BMI > 25 kg/m^2) patients achieved NEDA-3 during IFN-β treatment (13% versus 26% in the normal-weight group),[143] and in a large German cohort of pediatric MS patients, obese (BMI > 97th percentile) children had more relapses on low-potent injectable DMTs, and more commonly used high-potent DMTs.[134] Further, higher BMI has been associated with reduced brain volume, including grey matter loss,[144] the latter being a predictor for disability progression.[145] Also, comorbidities related to obesity[146], such as hypertension, dyslipidemia and other vascular conditions, have been associated with faster disability progression in MS.[147-149] However, evidence for a direct relationship between obesity and disability has been conflicting. Several cross-sectional studies reported associations between disability scores and general or abdominal obesity,[150,151] and in a CIS population[148] and a small MS population,[152] higher BMI was associated with short-term disease activity and EDSS disability progression, also irrespective of therapy.[152] Contrary to these results, other studies observed no significant associations between BMI and cross-sectional EDSS scores[153] or self-reported [154] or objective verified disability progression.[155] In general, MS populations tend to be leaner than their age-matched controls, as shown in several studies.[156-158]

Vitamin D levels have been proposed as a potential biological link between BMI and MS. Vitamin D deficiency is common among obese children and adults,[159] likely due to decreased bioavailability of vitamin D from cutaneous and dietary sources,[160] and greater total body adipose stores for this fat-soluble vitamin.[161] However, evidence from recent MR studies has demonstrated causal effects of BMI-associated SNPs on MS risk either independent of,[162] or with only minor attributions from genetically determined vitamin D levels, suggesting that other factors than vitamin D can be more relevant for the association between BMI and MS.

These other factors may be related to a chronic inflammatory state observed in obese individuals.[163] Obesity creates pathogenic adipose tissue with infiltration of activated innate and adaptive immune cells and dysregulated secretion of pro-inflammatory substances referred to as adipokines (Figure 6), including tumor necrosis factor (TNF), leptin and IL-6.[164] Specifically, the appetite-controlling hormone leptin has been investigated for a role in MS, since it has receptors in the CNS,[165] and has been shown to polarize T cells into a pro-inflammatory Th1 phenotype,[164] which are considered central in MS pathogenesis.[19] Although leptin-deficient mice did not develop symptoms of EAE,[166] the importance of leptin on MS in humans is less clear: One case-control study suggested that leptin may be a risk factor for MS, but the analyses were not adjusted for BMI.[167] In contrast to this, a prospective study found no association between leptin levels and clinical or MRI disease activity over 2 years,[168] and no causal effect of genetic estimates of leptin on MS risk was observed in a recent MR study.[169]

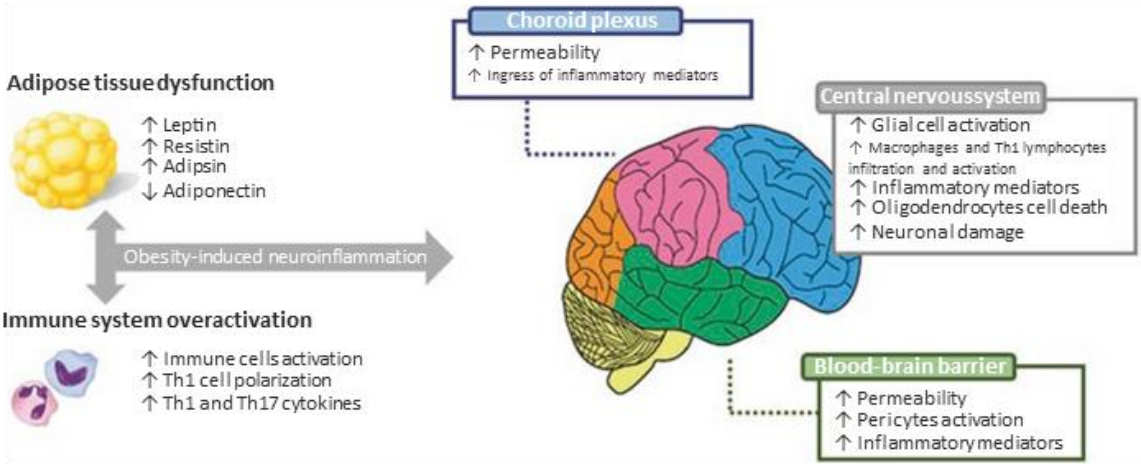

Figure 6. Pathophysiological events that may contribute to obesity-associated neuroinflammation. *Reprinted by permission from Springer International Publishing AG: Obesity and Brain Function, Advances in Neurobiology, vol 19, 195.*[170] *Copyright © 2017*

Further, dietary aspects of obesity have been examined for a role in MS: In mouse models, a more severe EAE was observed in mice on a high-fat diet, possibly induced by increased immune cell infiltration of the CNS,[171] and expansion of pro-inflammatory Th17 cell pools.[172] On the other hand, chronic calorie restriction was found to promote anti-inflammatory mechanisms and attenuated EAE.[173] In MS patients, beneficial effects were reported for the low-fat, long-lasting, "Swank diet",[174] but this study had many limitations, and interventional RCTs of good quality are needed.[175] A prospective study among 219 pediatric patients with MS showed that each 10% increase in saturated fat intake was associated with a threefold increased risk of a relapse in a model also adjusting for BMI and vitamin D levels.[176] Interestingly, ceramide species partly derived from saturated dietary fat may be relevant for DNA alterations and activation of monocytes in obese MS patients.[152] Overall, the link between BMI and MS appears to be a puzzle of many immunological pathways and mediators, and we still need more studies to fully determine the role of high BMI on disease activity and disease progression.

1.6.3 Physical activity

It is well-known that regular PA provides substantial health benefits, likely reduces all-cause mortality,[177] and has been found to decrease the risk of a number of conditions, including coronary heart disease,[178] diabetes type II,[179] various cancers,[180] Alzheimer's disease[181], and several autoimmune diseases.[182] In MS, it may also modify the disease risk and improve fatigue, mobility and quality of life,[182] although evidence for a direct effect on disease activity and progression is less clear.[183]

In animal models, a significantly delayed onset of chronic-relapsing EAE was observed in exercised rats, [184] while another study among voluntarily exercised mice showed an attenuated course of EAE.[185] In humans, only a few studies have investigated the association between PA and the risk of MS. In a case-control study, newly diagnosed MS cases reported to be more physically active than their controls in the 1-year period immediately prior to the diagnosis of MS.[186] However, this study was limited by the

subjective, qualitative nature of the PA question, and that only PA during a short time period before diagnosis was assessed.[186] Two large registry-based cohort studies among Swedish and Norwegian 18-19 year-old men eligible for Military Service found that better physical fitness assessed by a cycle ergonomic test in Sweden and a maximal endurance running test in Norway, was associated with significantly lower MS risk, also after adjusting for BMI.[76,187] In Norway, the relative risk (RR) was 0.69 (p-trend= 0.003) for the most fit compared to the least fit men. The estimates remained similar after excluding cases with disease onset within 10 years after conscription, arguing against any premorbid symptoms (i.e. reverse causation) explaining the association.[76] Dorans and colleagues examined whether the risk of MS in the female cohorts of NHS I and II was influenced by recent or cumulative amounts of PA at adult ages, or by recalled early life PA at ages 12-22 years. They found a weak association between higher categories of adult PA and lower MS risk, but the trend disappeared in lagged analyses with exclusion of the first 6 years of follow-up after reported PA. For PA during adolescence, no consistent associations between different measures of PA and MS risk were found.[188]

MS patients are in general less physically active than non-diseased individuals.[189] Engaging in different sports and physical activities appear to be favourable for muscle strength, mobility and fatigue,[190,191] but it remains unclear whether PA has beneficial effects on the disease *itself*. In general, interventions of various exercise modalities of until 6 months duration have not shown any clear associations with clinical disability scores.[183] At least, no harmful effects of exercise on MS disease, including no increased relapse rates, have been observed.[191,192]

A possible link between higher PA and lower risk of MS may be related to immunomodulatory actions of PA. While immediate exercise produces an acute-phase inflammatory response,[193] higher levels of PA over some time is associated with significantly reduced levels of CRP and a decrease in pro-inflammatory cytokine production in adipose, skeletal and vascular tissue.[194] In addition, endurance training is

associated with higher levels of anti-inflammatory IL-10 and Treg cells.[195] Cortisol and catecholamines are released during acute bouts of exercise of some intensity,[196] and both substances have anti-inflammatory properties: Cortisol has been found to suppress IL-12 and TNF-α and may thus inhibit activation of Th1 cells, while catecholamines may create a shift towards an anti-inflammatory Th2 profile by suppressing IL-12 and induce IL-10 production.[197,198] Altogether, these favourable inflammatory changes can potentially prevent immune-mediated events which eventually trigger MS.

1.6.4 Smoking and tobacco use

Tobacco smoking has consistently been associated with increased risk of MS in different populations and studies, with the evidence presented in several meta-analyses and reviews during the last decade.[62,199] Smoking is associated with approximately 1.5 times higher MS risk[199] and there is evidence for a dose-response relationship.[200,201] Past smoking,[200,201] passive smoking[202,203], and indirect measures of smoking, such as serum cotinine levels,[203] a nicotine metabolite,[204] have also been associated with increased risk of MS. However, studies on nicotine-containing oral snuff use and MS risk have reported no[205] or even a possible protective effect for MS,[201,206] indicating that nicotine may not be the main driver of the association between tobacco smoke and MS. Further, interactions between smoking and HLA risk gene variants for MS have been observed in several populations,[207] which strengthens a causal role for smoke in MS, since inherited genes in smokers and non-smokers are not affected by reverse causation, and genes are unlikely to regulate smoking behaviour.[208]

In MS disease, some studies,[209-211] but not others,[212-214] have observed faster disease progression and earlier transition to SPMS among smokers and ever-smokers compared to never-smokers. The rate of disease progression seems to be dependent on the number of pack-years,[215] and conversion to SPMS appears to be delayed by smoking cessation. [216] In addition, smoking has been associated with higher MRI lesion load and greater brain atrophy compared to never-smokers.[217] The evidence is more conflicting for smoking and inflammatory disease activity. Two studies based on

cotinine levels reported no association between tobacco use and subsequent relapses or MRI activity,[214,218] while two Danish cohort studies showed a significant association between cigarette smoking and higher relapse rate in patients treated with IFN-β[219] and natalizumab,[220] respectively.

Since a burning cigarette generates more than 4500 chemical compounds,[221] the biological links between smoking and MS are likely diverse and complex. Potential explanations include demyelination caused by chronic cyanide intoxication,[222] dysregulation of the blood-brain barrier by nicotine[223] and other compounds, different inflammatory effects, and neurotoxic actions of nitric oxide (NO).[221] Some inflammatory effects may be mediated by down-regulation of indoleamine 2,3-dioxygenase activity in T cells in combination with activation of the renin-angiotensin system, which in cells isolated from smoking MS patients led to increased production of pro-inflammatory cytokines and reduced numbers of Treg cells.[224] Since there is no evidence of an increased risk of MS among oral snuff users, inflammatory alterations of the lung tissue from cigarette smoking may be an important mechanism. [207] Of note, it has been shown that the lung tissue has the ability to stimulate and activate T cells and give them CNS migratory properties.[225] Lastly, experimental rat models have demonstrated that NO can cause axonal damage and degeneration,[226] especially in demyelinated axons,[227] and thus be a promoter for faster disability progression in smokers with MS.

2. Study rationale and objectives

2.1 Rationale

During decades of epidemiological research, it has been recognized that modifiable environmental factors are of likely importance for both MS risk and disease course. Since 1993, an increasing number of DMTs have become available,[43] but none of them have proven to cure the disease. It is therefore important to gain more knowledge about factors that can reduce the risk of MS or disease progression in MS. For MS risk, a large body of evidence has established EBV, smoking and low vitamin D as likely risk factors,[228] whereas less research regarding a potential role for obesity and PA had been conducted prior to this thesis. For MS disease course, low vitamin D[110] and obesity[143,148] have been associated with short-term inflammatory activity, but there is limited evidence on the potential long-term effects of these factors. Smoking has been associated with more rapid disease progression and earlier transition to SPMS in many,[209-211,216] but not all studies,[212-214] and the findings have been mostly based on self-reported measures. In this thesis, the overall aim was to explore the influence of lifestyle factors on both MS risk and disease progression, and by this provide better evidence-based recommendations on what may and may not prevent MS disease and reduce long-term progression.

2.2 Main objectives

The main objectives of this thesis were:

1. To examine whether self-reported body size at different ages during childhood, adolescence and young adulthood were associated with MS risk, and if so, whether this association was limited to a certain age or time-lag before disease onset.

2. To examine whether higher average amounts of light and vigorous PA during adolescence (13-19 years) were associated with lower risk of MS, and to evaluate the role of possible reverse causation.

3. To examine whether repeated measures (over two years) of serum levels of vitamin D, cotinine, and BMI were associated with long-term (10 years) disability progression in MS; and for vitamin D, to further determine the importance of seasonal fluctuations on this association.

3. Materials and methods

3.1 Paper 1 and 2: The EnvIMS study

3.1.1 The study design

The Environmental Risk Factors in Multiple Sclerosis (EnvIMS) study is a large population-based multinational case-control study designed to explore associations between age-specific environmental exposures selected from previous etiological MS research, and MS risk.[229] Disease onset among cases was defined as year of first reported MS symptoms, since symptoms may precede diagnosis by several years. The study was conducted in several European countries (Norway, Sweden, Italy and Serbia) mainly between 2009 and 2011, and later in Canada (2012-2013). All data was obtained through a mailed questionnaire, the EnvIMS-Q. The mailing package included an information brochure, the EnvIMS-Q, and a prepaid return envelope. If no response was received after 4-6 weeks, a second mailing was performed. The EnvIMS design made it possible to evaluate the consistency of associations between exposures and MS risk across different geographical areas, and to investigate interactions between selected environmental risk factors.

3.1.2 The study population

The studies based on the EnvIMS study in this thesis used available data from cases and matched controls in Norway and Italy (Paper 1 and 2), and also Sweden (Paper 2). Overall, cases were included if they (i) had a diagnosis of MS verified by the Poser[33] or McDonald criteria,[35,230] (ii) were ≥18 years of age, and (iii) had a symptom onset of ≤ 10 years at the time of study invitation. Based on power and sample size calculations before the study start, an enrollment of four controls per case was planned. The cases were selected from national or regional MS registries or databases, while population-based sources were used to provide controls matched on sex, age (within 5 years), and geographical residence. The eligible controls were cross-checked against the sources of cases to ensure that no controls were diagnosed with MS.

In *Norway*, cases were recruited from the whole country through the Norwegian MS Registry and Biobank.[231] Norway has a crude national prevalence of around 200 per 100 000.[232] A total of 1368 eligible cases were invited to the study, and 953 (70%) consented to participate. For each case, four matched controls were randomly selected from the Norwegian National Registry,[233] which includes core demographic information about all residents in Norway. A total of 1717 out of 4728 (36%) invited controls responded.

In *Italy*, the cases were recruited from the island of Sardinia, the province of Ferrara, and the Republic of San Marino (a little country surrounded by Italy). These areas have a high estimated prevalence of MS, with recently updated crude prevalence rates of 342 per 100 000 in Sardinia,[234] 195 per 100 000 in Ferrara,[235] and 204 per 100 000 in the Republic of San Marino.[236] In these regions, the cases were selected from regional MS registries, and 707 out of 1692 (42%) invited cases responded. The controls were randomly drawn from regional population-based registries, and the response rate among the controls was 21% (1333 among 6414 eligible controls).

In *Sweden*, the study population comprised cases and controls from the counties of Östergötland and Värmland. In 2011, the nationwide prevalence rate was around 190 per 100 000.[237] The Swedish MS Registry[238] provided 381 eligible cases for this study, of whom 259 (68%) consented to participate. However, 244 were finally included in the analyses, since 14 had missing on age of onset and one had more than 10 years disease duration. Matched controls were randomly selected from the Swedish Population Register,[239] and from 1734 invited controls, 644 (37.1%) were available for the analyses.

3.1.3 The EnvIMS Questionnaire

The EnvIMS-Q was a 6-page self-administered postal questionnaire divided in different sections of exposures. It was first developed in English, and then translated into the participating countries' own languages. The content of the EnvIMS-Q was identical for cases and controls and included main "core questions" similar for all

countries on environmental and lifestyle factors that covered childhood infections (including IM), vitamin D sources (outdoor activity/sun exposure, dietary habits and supplementation), tobacco smoking and passive smoking habits, body size, and PA. The EnvIMS-Q has shown cross-cultural feasibility, acceptability and reliability among both cases and controls.[240]

For the study in Paper 1, recall on past body sizes was facilitated by means of the visual Stunkard's figure rating scale (FRS)[241] which depicts nine female or male body silhouettes ranging from 1 (=leanest) to 9 (= most obese) (Figure 7). The participants were asked to report the body silhouette that best reflected their own body size every five years from age 5 years until 30 years, and at current age (at time of the study). In addition, they also reported their current height and weight, to validate their perceived body size.

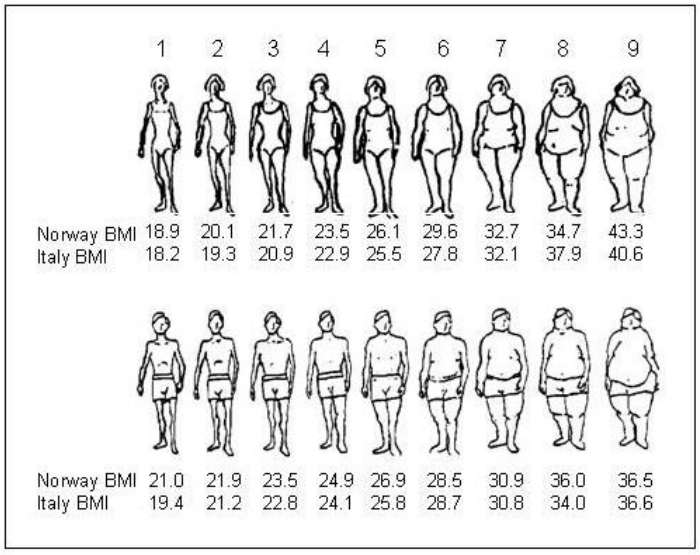

Figure 7. Stunkard's Figure Rating scale[241] with corresponding current mean BMI based on reported height and weight for females and males in the EnvIMS study (cases and controls combined). *Reprinted by permission from SAGE publications: Multiple Sclerosis Journal*. 2015;21(4):389 (Paper 1).[158] *Copyright © 2015*

Other relevant covariates included smoking habits (smokers, ever-smokers and non-smokers) and outdoor activity during summer at corresponding ages as reported body silhouettes, as a marker for sun exposure/vitamin D. The frequency of outdoor activity was reported on a four-point scale (1= not that often, 2= reasonably often, 3= quite often and 4= virtually all the time). Data on outdoor activity during the winter was omitted in the analyses, since UVB radiation is weaker and UVB-induced vitamin D synthesis is minimal in the winter months at latitudes above 60˚ where Norway is situated.[128]

For the study in Paper 2, one section in the EnvIMS-Q provided data on adolescent PA. The participants reported their average weekly amount of light PA (i.e. no increased respiratory rate or perspiration) and vigorous PA (i.e. increased respiratory rate and perspiration) between age 13-19 years on a four-point scale ("none," "less than 1," "1–2," and "3 or more" hours per week). Other covariates obtained from the the EnvIMS-Q were smoking (ever-never), IM (ever-never), cumulative outdoor activity during summer in adolescence, and body size at age 15 years (defined by Stunkard's FRS as previously described).

3.1.4 Ethical considerations and approvals

The EnvIMS study was approved by local ethical committees at each site.[229] Each participant was de-identified by a unique numerical ID printed on the EnvIMS-Q pages, and return of the questionnaire was considered informed consent.

3.1.5 Statistical analyses

To ensure that the cases and controls had the same exposure opportunities, the controls were assigned an index age corresponding to the age of disease onset for a matching case. Thus, exposures reported after the index age/age of onset were not considered as exposure and were excluded in the analyses. Logistic regression models were used to estimate the ORs with 95% CI for associations between the exposures and the risk of MS. The OR is a risk estimate that can be compared with the RR for diseases with a

low incidence in a population, such as MS.[242] A p-value for trend across categories of body size and PA was calculated by including these exposures as continuous variables in the models.

In Paper 1, the age-specific body size was either included as a categorical or a continuous variable in different regression models, since a chi-square goodness-of-fit deviance test showed no better fit of the model with body size as a categorical versus a continuous variable, suggesting a dose-response relationship. In the main analyses, the body silhouette 3 was chosen as the reference, since this corresponded to a "normal" current BMI according to WHO definitions[129] in the study population, and also allowed for comparison with a large cohort study on body sizes based on Stunkard's FRS, and MS risk.[132] The body sizes 6-9 were combined into a "large body size" category to ensure sufficient numbers in each category. We stratified on sex and adjusted for age groups, summer outdoor activity, and smoking habits at the same age as reported body size.

Thereafter, we performed similar analyses with body size as a continuous variable to estimate the OR for MS per one unit increase in body size. In these models, we adjusted for sex, since there was no significant interaction between sex and body size on the multiplicative scale. Finally, to explore whether there was a stronger association between obesity and MS risk closer to disease onset, we performed time-lag analyses of reported body sizes 1-15 years prior to onset by converting the age-specific body sizes into "year-before-onset" body sizes, as further described in Paper 1. For instance, if the age of onset was 24, then the participants' reported body size at age 20 and at age 15 represented their body sizes four and nine years before onset, respectively.

In Paper 2 we did both pooled analyses for Norway, Italy and Sweden, and country-specific analyses to assess any geographical differences. Light and vigorous PA were categorized into three levels; "< 1 hour", "1-2 hours, and "≥3 hours" of average weekly activity. The lowest level ("< 1 hour") was used as the reference in the logistic regression analyses. The main models were all adjusted for sex and age-groups, and in

multivariable models, we additionally adjusted for potential confounders, including level of summer outdoor activity during adolescence, IM, smoking, and body size at age 15 years. Further, the pooled analyses were stratified on sex to examine whether an association differed between males and females, and we also ran a sensitivity analysis where we excluded participants with an index age/age of onset of 30 years or less to evaluate the possibility of reverse causation.

The statistical analyses were performed in IBM SPSS Statistics, and the level of significance was set to < 0.05.

3.2 Paper 3: The OFAMS baseline and follow-up study

3.2.1 The study design and study population

The study in Paper 3 was based on data from a Norwegian cohort of MS patients who participated in the OFAMS (Omega-3 Fatty Acids in MS) study,[243] and then in the OFAMS 10-year follow-up study.

The OFAMS baseline study was a randomized, placebo-controlled study of marine omega-3 fatty acids in MS conducted at 13 neurological centers in Norway between 2004 and 2008. A detailed description of the study is reported elsewhere.[243] A total of 92 patients aged 18-55 years with a diagnosis of RRMS were screened for the study, of whom 88 completed more than a year. During the study period of 24 months, frequent clinical examinations, MRI scans of the brain and blood samples were done. IFN-β was given subcutaneously to the whole population during the last 18 months. The blood samples were cryopreserved at -80°C at the Neurological Department, Haukeland University Hospital, Bergen, for later within-study and post-study analyses. Overall, the OFAMS study failed to meet its primary endpoint of an effect of omega-3 in MS,[243] but later observational studies based on available OFAMS data and serum analyses showed an association between 25(OH)D levels and MRI activity *before*

initiation of IFN-β,[102] and more disease activity among overweight and obese individuals *after* initiation of IFN-β.[143]

About 10 years later, *the OFAMS 10-year follow-up study* was organized and coordinated by the author of this thesis (K. Wesnes). The OFAMS patients still alive (N=91) were invited to this study, and 85 (93.4%) gave their informed consent. Data collection was performed during 2017 by neurologists and study site personnel at the 13 collaborating neurological centers. K. Wesnes examined the OFAMS patients at St. Olav's University Hospital, Trondheim (N=12) and at Telemark Hospital Trust, Skien (N=7). The study included a clinical visit with an assessment of the EDSS score,[30] the MS Functional Composite[244] (25-Foot Walk test, 9-Hole Peg test and PASAT), the oral Symbol Digit Modalities Test, [245] self-administered questionnaires on fatigue (Fatigue Severity Scale), mental health (Hospital Anxiety and Depression Scale), and lifestyle habits during the last 10 years (questionnaire developed for this study). In addition, an MRI scan of the brain, as well as blood samples for routine analyses and cryopreservation at -80°C for later studies, were performed. Available and de-identified data with unique study-IDs was plotted in a data set by Dr. Wesnes.

3.2.2 Lifestyle exposures in the OFAMS baseline study

For the study in Paper 3, 25(OH)D levels and cotinine levels were already measured as part of previous studies within the OFAMS study population.[102,213] The 25(OH)D levels were simultaneously analysed in nine defrosted blood samples collected at baseline visit, month 1, 3, 6, 7, 9, 12, 18, and 24 with a radioimmunoassay kit at the Department of Medical Biochemistry, St. Olav's hospital, Trondheim, Norway.[102] Cotinine levels were simultaneously analysed in five defrosted blood samples from baseline visit, month 6, 12, 18, and 24 with liquid chromatography-tandem mass spectrometry at Bevital AS, Bergen, Norway.[213] Participants with cotinine levels >85 nmol/L in ≥ 60% of the samples during the OFAMS baseline study were classified as tobacco users.

BMI at each visit was calculated from the participants' reported height (in meters) and weight (in kg) at screening, and then at baseline visit, month 1, 3, 6, 7, 9, 12, 18, and 24 (N=10).

3.2.3 Outcome measure: EDSS progression

For Paper 3, we decided to focus on disability progression based on the EDSS score, since this score is globally accepted and easy to interpret and compare with other studies. The EDSS score was assessed at baseline visit, month 6, 12, 18, and 24 in the baseline study, and then repeated once at the 10-year follow-up visit (Figure 8). The EDSS progression was defined as the change in EDSS between the last score in the baseline study and the new score at follow-up. The majority had their last EDSS score at month 24, but one patient had the last score at month 12 and one patient at month 18.

OFAMS baseline study N=88																		OFAMS follow-up N=80
Inclusion period	2004-2006																	2017
Month	0	1	2	3	4	5	6	7	8	9	10	11	12		18		24	Single visit
25(OH)D[a]	x	x		x			x	x		x			x		x		x	(x)
Cotinine[b]	x						x						x		x		x	
BMI[c]	x	x		x			x	x		x			x		x		x	(x)
EDSS[d]	x						x						x		x		x	x

a. 25(OH)D: serum 25-hydroxyvitamin D, nmol/L
b. Tobacco use measured by cotinine levels, nmol/L
c. BMI: Body mass index, kg/m^2. BMI was also measured at screening (not shown)
d. EDSS: Expanded disability status scale

Figure 8. A timeline that illustrates the frequency of relevant exposures and outcome measurements during the OFAMS baseline study and the follow-up study.

3.2.4 Missing data

For the study in Paper 3, we first included 88 patients who completed more than a year in the baseline study. Since eight of these had missing on EDSS scores in the follow-up study, our study population finally comprised 80 patients (90.9%) available for the analyses.

3.2.5 Ethical considerations and approvals

Both studies were approved by the Regional Committee for Medical and Health Research Ethics in Western Norway. The participants received information and signed informed consent prior to inclusion.

3.2.6 Statistical analyses

Prior to the analyses, the crude 25(OH)D levels were seasonally adjusted by a sine function adapted to the 25(OH)D levels in the baseline study.[246] Then, a mean value for seasonally adjusted 25(OH)D, cotinine and BMI for each patient were calculated, based on all available measures during the baseline period. We used linear regression to estimate the association between the separate lifestyle factors and the change in EDSS score during follow-up. We included the exposures as standardised continuous variables (mean=0, standard deviation (SD)=1) to maximize power, and in separate models as categorical variables (quartiles) to explore possible nonlinear associations. A p-value for linear trend across the quartiles was estimated by including the median value for each quartile as a continuous variable in the regression model. All models were adjusted for sex, age and baseline EDSS score (=last EDSS score in the baseline study). We further mutually adjusted for all three lifestyle factors, MRI inflammatory activity and annual relapse rate during the baseline study, disease duration from year of diagnosis to follow-up, and the use of DMT at follow-up. We also adjusted for a cumulative sun exposure variable based on recalled summer outdoor activity during the follow-up period, but as this only had a minor influence on the estimates, the variable was omitted in the final models.

For 25(OH)D levels, we performed additional analyses as well: First, we explored whether there was a non-linear relationship between the seasonally adjusted values, and the increase in EDSS score by fitting a Locally Estimated Scatterplot Smoothing (LOESS) curve to the data. Second, we investigated the seasonal influence on the association between 25(OH)D and EDSS progression by dichotomizing the patient's mean 25(OH)D levels per season into a "< median" and "≥ median" variable. The seasons were summer (June-August), fall (September-November), winter (December-February) and spring (March-May). The dichotomized seasonal variables were then included as independent variables in linear regression models adjusted for sex, age and baseline EDSS score, with EDSS change between baseline and follow-up as the outcome variable.

The statistical analyses were done in IBM SPSS Statistics, while the plots were made in R version 3.6.0. P-values < 0.05 were considered significant.

3.3. An overview of the Papers

This table gives a brief overview of the Papers' topics, study population, main statistical methods and covariates:

Papers	Topic	Study population	Main statistical methods	Covariates
Paper 1	Body size and the risk of MS	**The EnvIMS population:** *Norway*: 953 cases, 1717 controls *Italy:* 707 cases, 1333 controls	**Logistic regression** *Exposure:* Body size modelled as a categorical and continuous variable *Outcome*: Risk of MS. Separate analyses for Norway and Italy.	*Depending on model:* - Sex - Age groups - Smoking status - Summer outdoor activity at corresponding ages
Paper 2	Adolescent physical activity and the risk of MS	**The EnvIMS population:** *Norway:* 953 cases, 1717 controls *Italy:* 707 cases, 1333 controls *Sweden:* 244 cases, 644 controls	**Logistic regression** *Exposure:* Physical activity modelled as a categorical variable in all analyses. *Outcome:* Risk of MS. Pooled and country-wise analyses.	*Depending on model:* - Sex - Age groups - IM - Summer outdoor activity during adolescence - Smoking status - Body size at age 15 years
Paper 3	Lifestyle factors (vitamin D, tobacco use, BMI) and long-term disability progression in MS	**The OFAMS population:** 80 MS patients with available EDSS scores from OFAMS baseline and follow-up study	**Linear regression** *Exposures*: seasonally adjusted 25(OH)D levels, cotinine levels, and BMI values as standardized continuous and categorical (quartiles) variables. *Outcome*: Change in EDSS score between the last score in the baseline study and the score at follow-up.	*Depending on model:* - Sex - Age - Mutually adjustments for all three lifestyle exposures - Disease duration - DMT at follow-up - Cumulative MRI activity and annual relapse rate during baseline study

BMI: Body mass index, EDSS: Expanded Disability Status Scale, 25(OH)D: 25-hydroxyvitamin D, IM: Infectious mononucleosis, DMT: Disease-modifying therapy

4. Results

4.1 Paper 1

"Body size and the risk of multiple sclerosis in Norway and Italy: The EnvIMS study"

In this study, we found that a large body size (Stunkard's silhouettes 6-9) in Norway was associated with an increased risk of MS compared to body size 3 with a significant p-trend from age 15 to age 25 years. The strongest association was found at age 25 for both males and females, (OR 2.21 (95% CI: 1.09-4.46) for men and OR 1.43 (95% CI: 0.90-2.27) for women). Further adjusting for smoking and summer outdoor activity gave similar results. In Italy, no clear trend in these analyses was found. However, a potential protective effect of body size 1 and 2 compared to body size 3 was found in both countries.

In the sex-adjusted analyses with body size as a continuous variable, each one- unit increase in body size was associated with a significantly increased risk of MS from age 10 until age 30 years in Norway, which was most pronounced at age 25. In Italy a similar, but non-significant, trend was found until age 20. Finally, we observed that a large body size was associated with increased risk of MS during the whole 15-year period before MS onset in Norway, but not in Italy. After disease onset, an inverse association was seen in both countries, with controls having larger body sizes relative to the cases.

4.2 Paper 2

"Physical activity is associated with a decreased multiple sclerosis risk: The EnvIMS study"

In this study, higher levels of vigorous PA in the pooled analyses for all countries were associated with a decreased risk of MS, with a significant p-trend across the categories. The age- and sex-adjusted OR for the highest level (≥3 hours of PA per week) was 0.74 (95% CI 0.63-0.87) compared to the lowest level (< 1 hour per week). Further adjustment for additional covariates gave similar results. The same trend was found in separate analyses for each country, although not all p-trends were significant in multivariable analyses. The association was stronger for women than men, but the difference was not significant when testing for interaction between sex and vigorous PA on the multiplicative scale (p= 0.58). In a sensitivity analysis in the pooled data with exclusion of participants with an age of onset/index age of ≤ 30 years, a similar age- and sex-adjusted OR for the highest versus the lowest level of vigorous PA was found (OR 0.79, 95% CI: 0.65-0.96 versus OR 0.74, 95% CI: 0.63-0.87 for all participants).

4.3 Paper 3

"Low vitamin D, but not tobacco use or high BMI, is associated with long-term disability progression in multiple sclerosis"

In this study, higher seasonally adjusted 25(OH)D levels during the OFAMS baseline study were significantly associated with reduced 10-year EDSS progression in the continuous model (per 1 SD increase) as well as in the categorical model (quartiles). Adjustment for potential confounders in the models did not attenuate the association. During the baseline period, 25(OH)D levels were lowest in March and highest in August. In the analyses with dichotomized 25(OH)D levels per season, low 25(OH)D levels during early spring appeared to be the main driver of the association, also after

mutually adjusting for the other seasonal levels. Finally, a fitted LOESS-curve to the measures showed a ceiling effect for seasonally adjusted 25(OH)D levels around 80 nmol/L, as little additional benefits on disease progression for higher 25(OH)D levels were seen.

For tobacco use (cotinine levels), no clear association with long-term disability progression was observed, neither in the continuous model, nor in the categorical model. For BMI, no significant association was found, but we observed a trend towards less EDSS progression among participants with the highest BMI values.

5. Discussion

5.1 Contribution of the findings

5.1.1 Paper 1

Our study on body size and the risk of MS was among the first to demonstrate that the observed association is evident at young adult ages beyond adolescence,[132,133] and in both men and women.[132,135] Further, we could explore associations between reported body sizes at different ages from early childhood (age 5 years) until young adulthood (age 30 years), and also assess whether a lean body size might be of relevance. The EnvIMS design made it possible to compare results from two different geographical areas, and to adjust for relevant environmental exposures at corresponding ages that could confound the results. Since we had information on reported body sizes in the years before and after MS onset, we could also evaluate (i) whether an association between body size and MS risk could depend on the time interval before diagnosis, and (ii) whether the disease itself changed the body composition of MS cases relative to controls. Contrary to a similar analysis in the female cohorts of NHS I and II,[132] our data showed that a large(r) body size in Norway was associated with an increased risk of MS during at least 15 years prior to diagnosis. However, in our analyses, we only included reported body sizes at likely susceptible ages up to 30 years (with the latest MS onset at age 45 years), while the NHS I and II included baseline information on weight and height at any age before MS diagnosis over the whole age spectre.[132] On the other hand, both studies showed a decline in weight among cases relative to controls after disease onset, consistent with other studies that have reported lower BMI in MS populations compared to the general population.[132,157]

In the EnvIMS data, we only observed a significant association between a large body size and MS risk in Norway, but not in Italy. There could be several explanations for this finding. First, obese Italians may differ from obese Norwegians with regard to dietary factors or other lifestyle behaviours, or their genetic profile may include

protective variants interacting with BMI-related risk genes.[139] Second, the current mean BMI was significantly lower among both cases and controls in the Italian compared to the Norwegian study population, which could have influenced the results. Nevertheless, the findings in Norway are consistent with observations in prospective cohort studies suggesting an increased risk of MS among overweight and obese teenagers in California,[135] young men (18-19 years) in Norway[76] and young women (18-20 years) in USA.[132] The potential biological mechanisms explaining this relationship could be related to lower vitamin D levels among overweight individuals,[159] or perhaps more likely to chronic inflammatory changes in obese individuals,[163,170] as discussed in the introduction of this thesis.

5.1.2 Paper 2

PA and exercise have been mostly examined in patients diagnosed with MS. Prior to our study, only a few studies had examined the role of PA and the risk of MS. Our results in Paper 2 are consistent with two large prospective nested case-control studies that found significant associations between better physical performance and lower MS risk among 18-19 year-old men in Norway and Sweden.[76,187] However, a prospective study among female American nurses argued that a weak association between adult PA and MS risk could be due to pre-diagnostic MS-symptoms, since the trend disappeared when excluding the immediate 6 years of follow-up after reported PA.[188] We extended on these previous findings by including data from several countries, both sexes, and adjusted for a larger set of established risk factors in the analyses.

Since less vigorous PA may be caused by pre-diagnostic prodromal symptoms of MS and thus result in reverse causation, we performed a sensitivity analysis where we excluded participants with MS symptom onset/ index age $\leq$ 30 years of age. In this analysis, similar effect estimates compared to the whole population were found, which indicates that reverse causation is less likely to fully explain our findings. This is consistent with a prospective study on physical fitness and MS risk in young men, using a similar sensitivity analysis to evaluate the direction of the association.[76]

Lastly, we found no association between light PA and the risk of MS, indicating that not only the amounts, but also a certain *intensity* of PA may be of importance for the observed relationship. This is not unexpected, since the potential link between PA and MS may go via anti-inflammatory mechanisms that require exercise of moderate to high intensity, such as increased levels of cortisol,[247] and a subsequent rise in anti-inflammatory cytokines.[193] The definition of vigorous PA in the EnvIMS-Q did not differentiate moderate from higher intensity levels, and therefore other studies are needed to examine any additional influence of high-intensity exercise on MS risk.

5.1.3 Paper 3

In Paper 3, we examined whether vitamin D could influence long-term disease progression in MS, since most studies on vitamin D and MS course have focused on short-term outcomes.[110] One cohort study in California found no significant association between baseline de-seasonalized 25(OH)D levels and long-term (10 years) EDSS progression, but in this study, seasonal variations of 25(OH)D could not be captured from the infrequent annual measures during the 2-year baseline period.[107] Another prospective study of participants originally included in the BENEFIT trial on IFN-β versus placebo, showed that higher 25(OH)D levels measured every six months during the 24-month baseline period were significantly associated with better 11-year cognitive performance on the PASAT test.[106] In contrast to this study, we focused on mainly physical disability progression assessed by the EDSS score. To our knowledge, such a significant association between 25(OH)D levels and 10-year EDSS progression, has not been demonstrated before.

Our study benefited from frequently measured 25(OH)D levels during two years, making the participants' 25(OH)D levels less prone to extreme values in single observations, and also gave us the possibility to evaluate the influence of seasonal fluctuations of 25(OH)D levels on our main findings. Indeed, we showed that low spring levels appeared to be the main driver of the observed association, also after adjusting for 25(OH)D levels during other seasons. This extends on previous findings

of a seasonal pattern of relapse rates.[89,248] Lastly, the non-linear observation of a ceiling effect for 25(OH)D levels above 80 nmol/L in combination with only a modest effect of high-dose vitamin D3 treatment in recent RCTs,[108,109] and a diminished relapse rate when 25(OH)D levels reached 110 nmol/L in an observational study,[249] suggest that MS patients do not need to aim for supraphysiological 25(OH)D levels.

We found neither a clear association between indirect tobacco measures and 10-year disability progression, nor significant more SPMS after 10 years among classified tobacco users. This contrasts the findings of more rapid disease progression and earlier conversion to SPMS among smokers in larger cohorts. Compared to our data, the MS populations in these studies had longer mean disease duration at baseline (10-15 years versus 1.9 years),[215,250] or smoking data was retrospectively collected through cross-sectional surveys,[209,216] which are more prone to misclassification errors than objective measures. In fact, our results are in line with two cotinine-based prospective studies by Munger et al.[214] and Kvistad et al.[218] conducted in RCT populations of the BENEFIT trial[251] and the baseline OFAMS trial,[243] respectively. In these studies, the participants had mainly short disease duration and early initiation of DMT, and no association between tobacco use and disease activity or disease progression was found during 2-5 years. While these studies dichotomized the tobacco variable into tobacco use versus non-tobacco use based on pre-defined cut-off values for cotinine, we included all available mean levels of cotinine during the baseline period in our analyses, thus minimizing misclassification of a light smoker or intermittent smoker as a non-smoker. In the categorical analyses, the lowest quartile of cotinine had a range of 0.0- 1.2 nmol/L, which makes smoking and other tobacco use in this group extremely unlikely. Still, we have to bear in mind that higher cotinine levels could represent oral snuff use, which has been associated with a decreased risk of MS.[201,206] However, in the follow-up study, only three participants classified as tobacco users in the baseline study (cotinine > 85 ng/ml in ≥ 60 % of the samples[218]), reported a history of solely snuff use.

Lastly, our findings could have been affected by an overall low disability progression (mean EDSS change of 0.9 points) in the population, and/or beneficial effects of smoking cessation,[216] since 21.3% of the classified tobacco users reported no longer tobacco intake at follow-up. Unfortunately, our small sample size made subgroup analysis of continuous smokers not feasible. In summary, we were not able to detect any adverse effects of tobacco use in our population, which may apply to other populations with more active disease.

Different measures of obesity have been associated with disease activity and/or worse disability (progression) in some,[134,148,151] but not all[154,155] studies. In our study, we found a non-significant trend of less disability progression among the patients with the highest BMI, also after adjusting for relevant covariates. In general, BMI is a challenging exposure since it can be a proxy for many other factors/comorbidities as well as a consequence of the outcome of interest (i.e. it may be prone to reverse causation). This means that any observed association between BMI and MS may be due to other (unmeasured) confounders, or a result of MS-related behavioural or dietary changes. Several other studies have shown that MS populations in general have lower BMI compared to an age-matched population.[132,156-158] Although an interpretation of non-significant results should be made cautiously, our results may indicate that lower BMI reflects a more severe disease, while higher BMI reflects a more benign MS. This is supported by the observation of more prevalent use of potent DMT after 10 years in the lowest (54.5%) compared to the highest quartile (31.6%) of BMI.

5.2 Methodological considerations and limitations

5.2.1 Observational studies and their quality of evidence

The three studies included in this thesis are observational of nature, which means that the investigator passively observes the population without making any specific interventions.[252] In a hierarchal ranking of the level of evidence from different studies (Figure 9) observational studies are placed beneath the gold standard of RCT,[253] in which randomization ensures that the groups are similar in all aspects except the exposure/intervention of interest; thus allowing for a causal interpretation.

TABLE 1. GRADES OF EVIDENCE FOR THE PURPORTED QUALITY OF STUDY DESIGN.*

I	Evidence obtained from at least one properly randomized, controlled trial.
II-1	Evidence obtained from well-designed controlled trials without randomization.
II-2	Evidence obtained from well-designed cohort or case–control analytic studies, preferably from more than one center or research group.
II-3	Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin treatment in the 1940s) could also be regarded as this type of evidence.
III	Opinions of respected authorities, based on clinical experience; descriptive studies and case reports; or reports of expert committees.

*The grades are those of the U.S. Preventive Services Task Force.[7]

Figure 9. The hierarchal model of evidence. Observational studies are ranked below RCT and other well-designed controlled trials. *Reprinted by permission from N Engl J Med 2000; 342:188.*[253] *© Massachusetts Medical Society.*

In observational studies, one should be careful to interpret a significant association as causal, as these studies are more prone to various types of bias, especially confounding, but also different types of selection bias and measurement bias.[254] A bias can be defined as "a systematic error in any type of epidemiologic study that results in an incorrect estimate of the association between exposures and outcome."[255] Any bias can threaten

the *validity* of the study; internal validity refers to how well you can rely on an observed association between a defined exposure and outcome within a study, while external validity refers to how well the findings can be generalized to other populations.[256]

5.2.2 The EnvIMS study: Advantages of the study design

MS is a relatively rare disease with a likely long latent period between a potential exposure of interest and disease onset. In such situations, cohort studies that prospectively follow a group of exposed and non-exposed individuals until the eventual outcome/disease occurs, require large samples, a long follow-up period, and are expensive to conduct.[257] Therefore, a more feasible, less expensive and rapid approach is to design a case-control study where exposures among cases with the defined outcome (e.g. MS) are compared to controls from the same source population without the outcome. The EnvIMS study is such a case-control study, where the relevant exposures were retrospectively collected through the self-administered EnvIMS-Q. While cohort studies are often limited to the exposures included at study start and may lack information about relevant exposures detected at a later stage, the case-control design allows for a more rapid evaluation of different exposures of interest, since the outcome is already known.

In the EnvIMS study, any misclassification of cases as non-cases were minimized by including only cases with a verified diagnosis of MS, cross-checking the controls for negative MS diagnosis, and including a question about MS diagnosis in the EnvIMS-Q. To ensure a representative sample of the controls, they were frequency-matched to the cases by age, sex and area, as well as randomly selected from the general population which also produced the cases.[229] Further, the EnvIMS study benefited from a large sample size which increased the precision in the statistical analyses, and by this reduced the risk of a type II error (i.e. to falsely accept a null hypothesis of no association due to wide confidence intervals in the estimates).[258] In addition, by including several populations, the EnvIMS study could evaluate the consistency of findings across different geographical areas using the same methodology.

5.2.3 The EnvIMS study: Selection bias

To induce a selection bias in an epidemiological study, the selection of participants has to be related to both the exposure and the outcome as a common effect.[254] In case-control studies, a selection bias can typically occur if the exposure distribution in the control group systematically differs from the exposure distribution in the source population where the cases were drawn from.[255] The EnvIMS study was designed to minimize selection bias by using a population-based approach. However, no matter how optimal a selection procedure is by design, the subsequent response rates may induce selection bias. In the EnvIMS study, the response rates among controls were (as expected) lower than cases, with the lowest response rates in the Italian EnvIMS data (42% among cases and 21% among controls). This can be a problem, if the selection of participants into the study is related to both exposure and outcome. While responding cases are likely motivated to take part in a study due to the disease itself (and not the exposures of interest), the controls who participate in such studies often have a higher socioeconomic status,[259] and may therefore have characteristics related to the exposures of interests that differ from the source population.

A previous study based on the Norwegian EnvIMS data reported that the controls had a higher level of education compared to the cases, which may be a result of selection bias.[260] Since higher education is associated with better health,[261] the EnvIMS controls may have been more physically active and had lower BMI during their childhood than the source population. Still, our findings of a likely influence of obesity and vigorous PA on MS risk are in line with prospective studies of large cohorts, which are less prone to selection bias by the study design, since selection into the study is not affected by the future outcome.[255] Further, another study in a complete cohort of Norwegian workers linked to the Norwegian MS registry observed an inverse association between level of education and MS risk,[262] again arguing against a systematical difference between the EnvIMS controls and the source population with respect to education and related lifestyle behaviours.

5.2.4 The EnvIMS study: Measurement errors and misclassification

Measurement errors of the exposure and/or the outcome are virtually always present to a greater or lesser extent in any study, and may introduce measurement bias if it affects the association between an exposure and outcome.[254] A measurement error or misclassification of an exposure is *nondifferential* if it is unrelated to the outcome; otherwise it is said to be *differential*; i.e. when measurement error of an exposure is affected by the disease status.[254,255] Both the magnitude and the direction of any type of measurement bias are difficult to predict in most studies when the true value is not known.[254]

In the EnvIMS study, non-differential misclassification of some non-diagnosed prodromal MS cases as controls could exist, but since MS is a rare disease, such misclassification is likely of minimal importance. Misclassification of controls as MS cases is even more unlikely, since the cases were recruited from reliable sources (registries and databases) dependent on a verified MS diagnosis.

Retrospective case-control studies are prone to recall bias, a type of differential misclassification of exposure that occurs when the participants' recall of a past exposure is affected by their disease status.[254] For instance, MS cases will likely seek for etiological causes to their disease, and may therefore recall and report past exposures differently than the controls. The likelihood of recall bias is larger when the cases are already familiar with a known risk factor for the disease. Since body size and physical inactivity were not among the established risk factors for MS at the time of study enrollment, it is less likely that the retrospective reporting of these factors could have led to recall bias. Another possible recall bias could arise if the cases and the controls differed systematically in the way they perceived their body size. However, the correlation between calculated BMI and reported body size at the time of the study was strong and not significantly different between the groups, which argues against a recall bias related to this phenomenon. Rather, the results could have been influenced by non-differential misclassification errors, i.e that the controls and cases misclassified

their past body sizes and PA in a similar way (not affected by their outcome status), since it is challenging to remember details about exposures that took place many years ago. To reduce such non-differential errors, the EnvIMS-Q tried to facilitate recall by adapting the ages for exposures to the countries' school system, and by encouraging the participants to ask close relatives/parents if their own memory on a topic was limited. In Paper 1 and 2 of this thesis, we did not compare the results between those who asked a close relative, and those who did not, which could have detected some meaningful differences.

5.2.5 The EnvIMS study: Confounding and reverse causation

In observational studies, the probability of being exposed versus not being exposed is likely affected by common causes of the exposure and outcome; also known as *confounding*.[254] Causal interpretation of an observed association can only be made if there were no counfounders, or if the association has been adequately adjusted for a sufficient set of confounding variables, which in real life may be impossible.[254] In our three Papers, we have used traditional statistical regression models to adjust for measured confounders to reduce the risk of confounding bias by observed variables, but there may always be unmeasured confounding, or imperfectly measured variables that may affect the results from these analyses. In any circumstances, it is important to avoid adjustments for variables that can be a common effect (and not cause) of the exposure and outcome, as adjusting for such a variable may induce "collider bias" in the estimates and lead to wrong conclusions.[254]

In Paper 1 (on body size) we adjusted for the potential confounders sex, age, smoking habits and summer outdoor activity. Since the outdoor variable does not equal the exact amount of sun exposure, there is likely some residual confounding related to this variable. Further, we *could* have adjusted for level of education and amount of PA during adolescence, but we decided to omit them, since these factors are considered less specific and may include factors we had already adjusted for. In retrospect, it could be relevant to adjust for PA, since PA has later been associated with MS risk[76,263] and

may also act as a mediator[264] on the pathway between body size and the risk of MS, as explained in this figure:

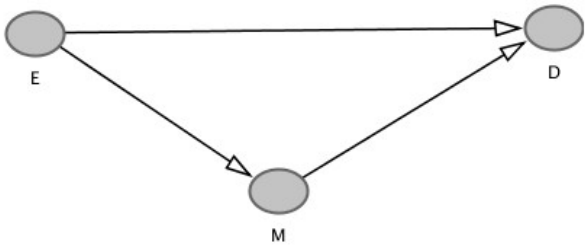

Figure 10. A directed acyclic graph showing the direct effect from exposure E to disease D (direct arrow from E to D), and the indirect effect via mediator M (arrow from E to M and arrow from M to D).

For example, a large body size can lead to lack of energy and less PA, which in turn can affect the risk of MS.[260,263] Adjusting for potential mediators can help us to detect important pathways between body size and MS risk,[264] which could be of value in the interpretation of the findings. However, it is unlikely that adolescent PA is a major mediator or confounder for the association between body size and MS risk in our study, since adjusting for body size in the multivariable model in Paper 2 did not influence the estimates between PA and MS risk in any meaningful way. We therefore believe that we have adequately adjusted for the most relevant confounders among the observed variables in Paper 1.

In Paper 2 on PA and MS risk, we adjusted for established risk factors for MS (IM, sun exposure via summer outdoor activity, smoking, and body size), since all these factors could likely affect both levels of PA and the risk of MS. The EnvIMS-Q also obtained information about autoimmune diseases (and their ages of onset) which could possibly influence the adolescent level of physical activity and the risk of MS, but it is more common to be diagnosed with MS without any co-existing autoimmune disorder,[265] or

they tend to develop *after* MS onset.[266] In both Paper 1 and Paper 2, we should also be aware of unmeasured and residual confounding that we could not account for.

The possibility of reverse causation- a form of confounding[254]- needs to be addressed when interpreting the association between physical activity and MS, since prodromal symptoms before MS onset may affect PA and the risk of definitive MS. We tried to evaluate this by excluding participants with symptom onset ≤ age 30 years in a sensitivity analysis, ensuring an interval of at least 10 years between reported PA and MS symptoms. Later published studies investigating clinical,[267,268] cognitive,[269] and biochemical data[270] on prodromal MS have confirmed that this interval is a reasonable choice. Reassuringly, no apparent reverse causation was found in our study, nor in a similar sensitivity analysis conducted in a large prospective male cohort exploring the association between physical performance and the risk of MS.[76]

5.2.6 The OFAMS studies: Sample size and selection bias

The OFAMS population was originally recruited for an RCT on omega-3 fatty acids, and the sample size was based on the power calculations and effect assumptions made in advance for this purpose. However, for later observational studies in the same cohort, the characteristics and small size of the population could challenge the interpretations of the findings for several reasons: First, the OFAMS population may be less representative of a general MS population, since the specific inclusion and exclusion criteria in the study[243] excluded patients with severe comorbidities, and/or patients with active disease who could not delay the initiation of DMT. Indeed, we found that the OFAMS population had a low mean EDSS progression over 10 years (mean progression of 0.9 points), and 23.3% among those with an inflammatory RRMS phenotype (N=73) did not use any DMT at the follow-up visit. Second, small sample sizes are prone to type II error (i.e. falsely accepting a null hypothesis),[258] as a small sample generally leads to increased random variation and decreased precision. In addition, cohort studies of any size can suffer from a selection bias known as attrition bias; a systematic difference related to the exposures and outcome between those who

are lost to follow-up and those remaining in the study.[257] For example, if heavy smokers with a more severe disease were less likely to participate in the follow-up study, this could have led to an attrition bias in the estimates. Fortunately, EDSS scores at follow-up were obtained from 90.9% of the eligible participants from the baseline study, making attrition bias less likely.

5.2.7 The OFAMS studies: Reverse causation

Lifestyle factors are somewhat challenging to examine in the context of a disease, since the disease itself may modify the factors and lead to reverse causation. For vitamin D, most prospective studies and larger trials with the exposure measured before the outcome, have shown that vitamin D supplements and/or higher 25(OH)D levels likely reduce inflammatory activity and may delay progression.[110] On the other hand, there is some research arguing that low vitamin D can be a consequence of inflammation or poor health in patients with MS, based on minor to no effect in small randomized trials on vitamin D supplementation in MS .[111] In our study, the vitamin D levels preceded the follow-up EDSS score by a long period, and it is therefore unlikely that reverse causation could explain the findings in our study. Although we adjusted for possible disease-related confounders during the baseline period, this cannot tell us the direction of the remaining association between vitamin D and long-term disease progression. Still, our results could have been attenuated by a large number of participants taking vitamin D containing supplements in the follow-up period, possibly motivated by disease severity in some patients.

The lack of an association between tobacco use and disease progression in our study could be explained by reverse causation in a setting where more severe disease at baseline affected the tobacco use/smoking at follow-up. Indeed, the OFAMS data show (i) higher EDSS score (corresponding to more severe disease) in the highest compared to the lowest quartile of cotinine levels in the baseline study (mean EDSS at the last visit 2.5 versus 1.8, respectively), and (ii) as many as 50% (11/22) of the patients in the highest quartile reported no longer tobacco use at the follow-up visit. Therefore, our

results could reflect the "effect" of disease severity on smoking cessation, which in turn may reduce disease progression in MS.[216] Finally, reverse causation may also play a role in the observed non-significant trend between higher BMI and less EDSS progression, as previously discussed. For example, dietary and nutritional changes due to more severe MS disease or co-existing depression may lead to weight loss, resulting in an inverse relationship between BMI and MS disability.

5.2.8 The OFAMS studies: Confounding and other limitations

In Paper 3, we adjusted for a number of potential confounders related to lifestyle and disease status available in the OFAMS baseline study. Most of them were objectively measured, which reduces the possibility of under-reporting and measurement errors. At the follow-up visit, the patients received a questionnaire on lifestyle which inquired about past and current vitamin D-related dietary habits, use of vitamins and other supplements, amounts of summer outdoor activity (a proxy for sun exposure), smoking and snuff habits, and frequency of vigorous PA during the last 10 years. Although it could be tempting to include some of these retrospective measures as covariates in our regression analyses, they are less precise and could also introduce recall bias into the analyses. We therefore decided to keep most of this additional information outside our statistical analyses. Instead, we used these self-reported data to explain some of the findings in our study. In addition, there may still be some unmeasured and residual confounding affecting our estimates.

As already discussed, cotinine is an imperfect marker of tobacco smoking, since it reflects nicotine intake of *any* source. However, this is likely of less importance in our study, since only three participants classified as tobacco users in the baseline study [218] reported a history of solely snuff use in the follow-up study. Although we categorized cotinine levels differently in our analyses, the self-reported data obtained at follow-up confirm no history of smoking or snuff use in the first quartile of cotinine (makes it valid as a reference group). Further, all the cotinine levels in the highest quartile represent classified tobacco users, with only one cotinine value from a participant with

a tobacco history of solely snuff use. Surprisingly, for two other values in the highest quartile, the participants reported neither previous smoke, nor oral snuff at follow-up. Whether this represents measurement errors or cotinine levels related to other nicotine sources is unknown.

Finally, the EDSS score as an objective measure of disability has some limitations as well, due to its inter-rater variability, the dominant focus on ambulatory dysfunction for EDSS scores of 4 and above, and that it is a better tool for physical than cognitive disability.[271] However, since the EDSS score is validated, widely used and accepted, and since most OFAMS participants did not progress to scores above 4, it is still a valuable measure that allows for comparisons with many other studies using the same disability scores.

6. Conclusions and future perspectives

In this thesis, we found that overweight and obesity in adolescence and young adulthood is associated with an increased risk of MS in Norway, and we further observed that larger amounts of regular vigorous PA in different geographical areas may reduce the risk of MS. Although our studies based on the EnvIMS data have some limitations due to the retrospective study design, the results are consistent with findings from large prospective cohorts. In our third study, we observed that higher 25(OH)D levels may reduce long-term disability progression, and that seasonal fluctuations with 25(OH)D levels below 80 nmol/L during winter and early spring at higher latitudes seem to drive this association. Based on our research and the results from other studies on vitamin D in MS, we recommend that MS patients should aim for 25(OH)D levels above 80 nmol/L throughout the year and use supplements when needed. For tobacco use and BMI, no clear associations with disability progression were found in our data, although they may still be of relevance in other populations.

Since young adulthood is a period that has been less explored in the studies on obesity and MS, more studies are needed to confirm that excess body weight in young adulthood may also be of importance for MS risk. Further, we need some more knowledge about the specific (biological) factors related to BMI/body size that are of greatest importance in the pathogenesis of MS. A randomized controlled study on calorie restriction among obese teenagers is unfortunately not feasible since MS is a rare disease with a likely long latent period. In Italy, preferably prospective studies on obesity and the risk of MS should be performed to assess whether the negative findings in the EnvIMS data represents a true lack of association in this country. The published studies on PA and the risk of MS have shown somewhat conflicting results, and large prospective studies with more detailed and accurate information on amounts and intensity of PA in adolescence and adult years should be conducted to determine the role of different levels of PA on MS risk.

For vitamin D, large cohort studies with a representative population-based sample and global assessment of disability progression with clinical, cognitive and MRI measures (i.e. atrophy rate) should be performed to further explore whether vitamin D has a true impact on long-term prognosis in MS. Since the evidence on BMI and disease course is conflicting and may be prone to reverse causation, a randomized study with a specific dietary intervention in overweight MS patients could better clarify whether body composition has adverse effects on MS inflammation and disease course. For smoking and tobacco intake, a combination of objective cotinine measures along with detailed reports of tobacco habits, should be used to increase the validity of the findings.

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Errata

Paper 1: In the last sentence in the second paragraph of the Discussion, the incorrect reference 14 (“Temporal trends in the incidence of multiple sclerosis: A systematic review) is added. The correct reference is 12 (“Childhood body mass index and multiple sclerosis risk: A long-term cohort study”).

Paper 3

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Original article

Low vitamin D, but not tobacco use or high BMI, is associated with long-term disability progression in multiple sclerosis

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ABSTRACT

Background: Low vitamin D levels, tobacco use and high body mass index (BMI) have been linked to adverse disease outcomes in multiple sclerosis (MS), but their influence on long-term disability progression remains unclear. Therefore, we explored whether these modifiable lifestyle factors were associated with 10-year clinical disability progression in patients with MS.

Methods: In this prospective study, a cohort of 88 patients with relapsing-remitting MS completed a randomized controlled study on ω-3 fatty acids between 2004 and 2008. During 24 months, serum 25-hydroxyvitamin D (25(OH)D), serum cotinine (nicotine metabolite), and BMI were repeatedly measured. In 2017, a follow-up study was conducted among 80 of the participants, including disability assessment by the Expanded Disability Status Scale (EDSS). Linear regression was used to explore associations between the lifestyle factors and the EDSS change over 10 years.

Results: Higher seasonally adjusted 25(OH)D levels were associated with lower 10-year EDSS progression (change in EDSS per 1 SD increase in 25(OH)D in a model adjusted for sex, age and baseline EDSS: -0.45 point, 95% CI: -0.75 to -0.16, p=0.003). Further adjustments for potential confounders related to lifestyle and disease status gave similar results. The association was mainly driven by low 25(OH)D levels during spring, as well as seasonally adjusted levels below 80 nmol/L. No clear association was found for BMI and cotinine.

Conclusion: Lower 25(OH)D levels, but apparently not tobacco use or higher BMI, were significantly associated with worse long-term disability progression in MS.

Abbreviations: DMT, disease-modifying treatment; 25(OH)D, 25-hydroxyvitamin D; RCT, randomized controlled trial; IFN-β, interferon beta 1a; aHSCT, autologous hematopoietic stem cell transplantation; CUA, combined unique activity.

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1. Introduction

Multiple sclerosis (MS) is a disabling chronic disease with several disease-modifying treatment (DMT) options, but so far, no curable treatment exists (Dobson and Giovannoni, 2019). Established risk factors related to lifestyle such as vitamin D deficiency, tobacco smoking, and obesity may also affect disease course (Waubant et al., 2019). Higher serum levels of 25-hydroxyvitamin D (25(OH)D) have been associated with less radiological inflammatory activity and lower relapse rate in observational studies (Smolders et al., 2019). However, two larger randomized controlled trials (RCTs) on high dose vitamin D supplementation failed to demonstrate a clear effect on relapse rate and disability progression in the intention-to-treat population (Hupperts et al., 2019, Camu et al., 2019). Further, several (Hernan et al., 2005, Healy et al., 2009, Manouchehrinia et al., 2013), but not all (Koch et al., 2007, Kvistad et al., 2016, Munger et al., 2015) studies, suggest that smoking increases the risk of a faster disease progression and earlier transition to secondary progressive MS (SPMS). For obesity, some studies indicate that higher body mass index (BMI) leads to more disease activity through weaker therapy response (Kvistad et al., 2015, Huppke et al., 2019), and may affect brain volume loss (Mowry et al., 2018), whereas other studies have failed to demonstrate any association between BMI and disease progression (Pilutti et al., 2012, Bove et al., 2016).

Only a few studies have examined associations between lifestyle factors and long-term disability progression in MS (Cortese et al., 2020, University of California, San Francisco MS-EPIC Team, 2016). To address this, we conducted a study to examine whether 25(OH)D levels, tobacco use, and BMI were associated with disability progression over 10 years, using prospective data from a well-defined Norwegian cohort of patients with MS.

2. Methods

2.1. Study population and design

2.1.1. The OFAMS baseline study

A total of 92 patients with relapsing-remitting MS (RRMS) aged 18-55 years were enrolled in an RCT on marine ω-3 fatty acids versus placebo (the OFAMS study) between 2004 and 2006, and then closely followed for 24 months. A detailed description of the study is reported elsewhere (Torkildsen et al., 2012). In the following text, we will refer to this study as "the baseline study". Frequent clinical examinations, blood samples and MRI scans of the brain were performed during the study period. No particular advice on lifestyle changes or vitamin D supplementation was given to the patients. Overall, the study demonstrated no significant effect of ω-3 fatty acids on disease activity (Torkildsen et al., 2012). However, in subsequent analyses, lower 25(OH)D levels were associated with more inflammatory MRI-activity *before* initiation of subcutaneous interferon beta 1a (IFN-β) at study month 6 (Loken-Amsrud et al., 2012), and higher BMI was associated with more disease activity *after* initiation of IFN-β (Kvistad et al., 2015).

2.1.2. The OFAMS follow up study

In 2017, the OFAMS population was invited to a 10-year follow-up study to evaluate disease progression and current disability status. A trained neurologist at each participating centre performed a clinical examination of the patients. In addition, the patients answered a questionnaire regarding lifestyle habits, including sun exposure and tobacco use (smoking and/or snuff use) during the last 10 years.

2.2. Ethical approvals and Patient Consents

The OFAMS baseline study and the OFAMS follow-up study were approved by the Regional Committee for Medical and Health Research Ethics in Western Norway. All participants gave their written informed consent prior to the studies.

2.3. Assessment of lifestyle factors in OFAMS baseline study

2.3.1. Vitamin D measurement

Serum samples were collected at the baseline visit, and then at month 1, 3, 6, 7, 9, 12, 18, and 24. The samples were stored at -80°C until simultaneous analysis of all nine samples from each patient at the Department of Medical Biochemistry, St. Olav's University hospital, Trondheim, Norway (Loken-Amsrud et al., 2012). 25(OH)D levels in nmol/L were measured by radioimmunoassay (RIA kit; ImmunoDiagnostic Systems, Boldon, UK). The coefficient of variation was 5.4% at 29 nmol/l and 6.3% at 112 nmol/l.

2.3.2. Cotinine measurement

Cotinine levels, a sensitive and specific biomarker for nicotine intake (SRNT Subcommittee on Biochemical Verification, 2002), were measured simultaneously in serum samples collected at baseline visit, month 6, 12, 18, and 24 (Kvistad et al., 2016). The analysis was performed by liquid chromatography-tandem mass spectrometry (Bevital AS, Bergen, Norway). The within-day coefficient of variation was 2.0% to 6.6%, and the between-day coefficient of variation was 3.9%. The cut-off value for recent tobacco use was set to cotinine levels $>$ 85 nmol/L, with tobacco users defined as having $>$ 85 nmol/L in $\geq$ 60% of the samples.

2.3.3. Body mass index

The participants' height (in meters) and weight (in kg) were measured at screening, and then at baseline visit, month 1, 3, 6, 7, 9, 12, 18, and 24. From these values, BMI at each visit was calculated as kg/m^2.

2.4. Other relevant covariates

Current use of DMT at follow-up was categorized as "none", "less potent" (IFN-β, glatiramer acetate, teriflunomide, and dimethyl fumarate) and "potent" (fingolimod, natalizumab, autologous hematopoietic stem cell transplantation (aHSCT), and rituximab). For disease activity, we included two variables from the baseline study: the cumulative number of combined unique activity (CUA) lesions (Torkildsen et al., 2012) on subsequent MRI brain scans, and the annual relapse rate.

At the follow-up visit, the participants were asked about the frequency of outdoor activity in summer season (April-September) 10 years ago, 5 years ago and last year, categorizing this into "$<$ 1 time per week", "1-2 times per week", "3-4 times per week" and "approximately daily". From these data, we created a cumulative sun exposure variable.

2.5. Outcome measure

2.5.1. EDSS progression

The disability status was assessed by the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) at baseline visit, month 6, 12, 18, and 24 during the baseline study and repeated once in the follow-up study 10 years later. The EDSS progression was defined as the EDSS change from the last score in the baseline study until the score at follow-up. For all patients but two the last EDSS score was at month 24; one patient had the last score at month 12 and the other one at month 18.

2.6. Missing values

92 patients were screened to participate in the baseline study, but four were lost to follow-up during the first six months of the study. In the follow-up study, 85 of the 91 patients still alive (93.4%) gave their consent to participate, including 81 of the 88 patients who completed at least 12 months of the baseline study. However, EDSS score at follow-up was missing for one of these 81 patients, leaving 80 patients eligible for the main analyses.

2.7. Statistical analyses

For each lifestyle factor, we estimated the mean value per patient based on all available measurements during the baseline study. Since vitamin D levels vary with season in Norway, the 25(OH)D levels were seasonally adjusted by a sine function modelled within the baseline study, as previously described (Saltyte Benth et al., 2012).

We used linear regression models to estimate the association between the lifestyle factors and the EDSS progression from the last score in the OFAMS baseline study to the assessment in the follow-up study. All exposures were modelled as both categorical (quartiles) and continuous variables to maximize power and to explore possible nonlinear associations. In continuous analyses, we standardized the variables (mean = 0, standard deviation (SD) = 1) to estimate the change in EDSS per 1 SD increase in the exposure variable. To test for a linear trend across the quartiles, the median value of each quartile was included in the regression model as a continuous variable. All available measurements in the OFAMS baseline study were used to standardize and categorize variables. All models were adjusted for sex, age and baseline EDSS score (= last score in the baseline study). In multivariable models, we mutually adjusted for all three lifestyle factors, disease activity (CUA and annual relapse rate) in the baseline study, disease duration (from year of diagnosis until follow-up), and use of DMT at follow-up. We also adjusted for cumulative sun exposure in the follow-up period, but as this only had a minor influence on the effect estimates, we omitted this variable in the final models.

To illustrate the monthly fluctuations of 25(OH)D levels in our population, a Locally Estimated Scatterplot Smoothing (LOESS) curve was fitted to the available measures, with corresponding 95% confidence intervals (CI). To evaluate whether an association between 25(OH)D levels and EDSS progression varied by season, we computed a dichotomized variable of < median and ≥ median 25(OH)D levels per season based on each patient's mean 25(OH)D level for that season. The four seasons were summer (June-August), fall (September-November), winter (December-February), and spring (March-May). We then included the dichotomized seasonal variables (< median or ≥ median) as independent variables in linear regression analyses, with the change in EDSS score as the dependent variable, adjusted for sex, age and baseline EDSS score. Finally, to investigate whether there was a nonlinear relationship between seasonally adjusted 25(OH)D levels and disease progression, we plotted a LOESS-curve to the available data.

All the statistical analyses were performed in IBM SPSS Statistics, version 25.0 (SPSS Inc., Chicago, Ill., USA). The plots were made in R version 3.6.0 (The R Foundation) using the *ggplot2* package. P-values were considered significant at values <0.05. All tests were two-sided.

3. Results

3.1. Patient characteristics

The study population comprised 80 participants who completed more than 12 months in the baseline study and had an available EDSS score in the follow-up study. Table 1 gives the main baseline characteristics of this population. The mean EDSS score increased from 1.9 (SD: 0.84) at the baseline visit to 2.8 (SD: 1.6) at the follow-up visit, and seven (8.8%) of the patients converted to SPMS during the follow-up period. At follow-up, 72.5% received any kind of DMT, including seven patients still on IFN-β and two patients on past aHSCT treatment. Fewer used tobacco (40.0% vs. 61.3% in the baseline study), and 76.3% used vitamin D containing supplements in various doses and formulas. For most patients, BMI remained stable over the years, with mean BMI 25.6 kg/m^2 (SD: 4.2) and 25.7 kg/m^2 (SD: 4.6) during the baseline and follow-up study, respectively.

Table 1
Characteristics of the study population at OFAMS baseline visit or during the baseline study.

Variable	Values
Patients, N	80
Females, N (%)	52 (65)
Age, mean (SD)	38.3 (8.3)
Years from diagnosis, mean (SD)	1.9 (3.2)
EDSS score, mean (SD)	1.9 (0.84)
Seasonally adjusted 25(OH)D during baseline study, mean (SD)	74.1 (18.1)
Tobacco users during baseline study, N(%)[a]	49 (61.3)
BMI in kg/m^2 during baseline study, mean (SD)	25.6 (4.2)

SD: standard deviation; 25(OH)D: 25-hydroxyvitamin D nmol/L; BMI: body mass index.
[a] Tobacco users defined as serum cotinine levels > 85 nmol/L in ≥60% of five consecutive samples.

3.2. Vitamin D

Higher 25(OH)D levels were significantly associated with lower 10-year EDSS progression (Table 2). In the continuous model adjusted for sex, age and baseline EDSS score, 1 SD increase in seasonally adjusted average 25(OH)D levels was associated with 0.45 point (95% CI: 0.16-0.75, p=0.003) lower progression in EDSS scores at follow-up. Further adjustment for other covariates, including mean cotinine levels, mean BMI values and disease activity during the baseline study, did not influence the results. In the categorical analyses, there was a significant dose-response relationship between 25(OH)D and change in EDSS score with a p-trend of 0.024 in the simplest model (Table 2). The effect estimates and the p-trend remained similar when more covariates were added to the model.

Fig. 1 illustrates the seasonal fluctuation of repeated measures of 25(OH)D throughout the baseline study, with the highest levels seen in August and the lowest levels seen in March. In the model that included dichotomized 25(OH)D variables for all four seasons, only higher (≥ median) 25(OH)D levels during the spring, when the levels were lowest, were significantly associated with 10-year EDSS progression (Fig. 2).

When exploring the possible nonlinear relationship between 25(OH)D and disease progression with a LOESS-curve (Fig. 3), an increase in seasonally adjusted 25(OH)D levels from around 50-60 nmol/L to 80 nmol/L was associated with approximately one point decrease in EDSS progression, whereas little additional benefit was seen for higher 25(OH)D levels.

3.3. Cotinine levels

Tobacco use based on cotinine levels showed no significant association with EDSS progression, neither in the simple model adjusted for sex, age, and baseline EDSS score, nor in the models adjusted for additional variables (Table 2). Although five of seven patients (71%) who converted to SPMS were classified as tobacco users during the baseline study, this finding was not significant (p= 0.70) according to Fisher's exact two-sided test for small samples.

3.4. BMI

For BMI, there was a tendency towards a beneficial effect for the patients with BMI values in the highest quartile, but no significant dose-response curve was present (Table 2). We found a similar non-significant trend in the continuous model.

4. Discussion

In this prospective study, we found a significant and consistent association between higher 25(OH)D levels and lower 10-year disability progression independent of potential confounders related to lifestyle

Table 2
The association between mean values of lifestyle factors during the baseline study and the 10-year EDSS progression from last EDSS score in the baseline study.

Lifestyle factors	Quartile 1	Quartile 2 Change in EDSS(95%CI)	Quartile 3 Change in EDSS(95%CI)	Quartile 4 Change in EDSS(95%CI)	p-trend	Per 1 SD increase[a] Change in EDSS (95%CI)	p-value
25(OH)D[b]							
Patients, N	20	18	21	21			
Median (range), nmol/L	54.9 (36.4- 60.1)	66.9 (60.3- 70.7)	77.5 (71.1- 83.8)	97.6 (84.0- 118.4)			
Model 1[c]	Reference	0.13 (-0.67- 0.93)	-0.61 (-1.41- 0.19)	-0.78 (-1.59- 0.03)	0.024	-0.45 (-0.75- -0.16)	0.003
Model 2[d]	Reference	0.29 (-0.53- 1.11)	-0.59 (-1.38- 0.21)	-0.76 (-1.56- 0.05)	0.022	-0.46 (-0.75- -0.17)	0.002
Model 3[e]	Reference	-0.06 (-0.93- 0.82)	-0.86 (-1.72- 0.00)	-0.99 (-1.83- -0.15)	0.010	-0.49 (-0.79- -0.20)	0.002
Cotinine[b]							
Patients, N	20	19	19	22			
Median (range), nmol/L	0.4 (0.0- 1.2)	123.8 (1.2- 400.8)	738.9 (407.7- 946.6)	1140.6 (980.3- 2443.6)			
Model 1[c]	Reference	0.55 (-0.25- 1.35)	0.30 (-0.51- 1.10)	-0.17 (-0.98- 0.64)	0.353	-0.09 (-0.38- 0.20)	0.557
Model 2[d]	Reference	0.48 (-0.28- 1.24)	0.16 (-0.62- 0.94)	-0.11 (-0.88- 0.66)	0.393	-0.07 (-0.34- 0.20)	0.618
Model 3[e]	Reference	0.34 (-0.45- 1.12)	-0.07 (-0.88- 0.75)	-0.19 (-0.98- 0.60)	0.296	-0.09 (-0.37- 0.20)	0.538
BMI[b]							
Patients, N	22	20	19	19			
Median (range), kg/m^2	21.5 (17.7- 22.9)	23.8 (22.9- 25.2)	26.3 (25.3- 28.2)	31.1 (28.8- 38.3)			
Model 1[c]	Reference	0.04 (-0.75- 0.82)	-0.10 (-0.90- 0.69)	-0.51 (-1.31- 0.28)	0.157	-0.20 (-0.48- 0.08)	0.160
Model 2[d]	Reference	0.11 (-0.64- 0.86)	-0.13 (-0.89- 0.63)	-0.43 (-1.19- 0.33)	0.182	-0.20 (-0.47- 0.06)	0.134
Model 3[e]	Reference	0.02 (-0.74- 0.78)	-0.15 (-0.91- 0.62)	-0.40 (-1.16- 0.36)	0.247	-0.18 (-0.44- 0.09)	0.189

SD: standard deviation; CI: confidence interval; 25(OH)D: 25- hydroxyvitamin D; BMI: body mass index.
[a] 1 SD for seasonally adjusted 25(OH)D =18.7 nmol/L, 1 SD for mean cotinine= 523.8 nmol/L, 1 SD for mean BMI= 4.2 kg/m^2
[b] Mean values for the baseline period based on N consecutive samples, where N= 9 for seasonally adjusted 25(OH)D, N=5 for cotinine and N=10 for BMI.
[c] Model 1: Adjusted for sex, age and EDSS score at last visit in the baseline study.
[d] Model 2: Model 1 + mutually adjusted for 25(OH)D, cotinine and BMI as standardized continuous variables.
[e] Model 3: Model 2 + further adjusted for disease duration from year of diagnosis until follow-up (2017), use of disease-modifying treatment at follow-up (none, less potent, potent), brain MRI activity (cumulative Combined Unique Activity) and relapse rate during the baseline study.

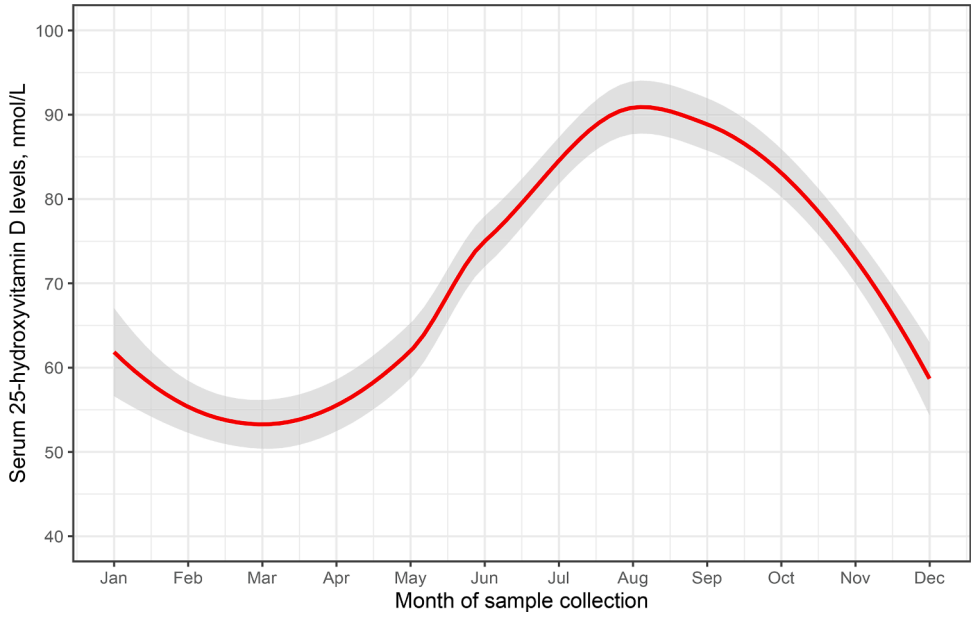

Fig. 1. The seasonal fluctuation of 25-hydroxyvitamin D levels based on sample analyses in the baseline study shown by a fitted LOESS curve with 95% confidence intervals.

and disease status. The association was mainly driven by levels during spring when 25(OH)D reached its seasonal nadir. Further, a ceiling effect in the association appeared around 80 nmol/L, as there were only minor changes in disease progression for 25(OH)D increases above this level. Tobacco use and BMI were not significantly associated with long-term disability in our study.

Our findings on vitamin D are consistent with previous findings on a likely role of vitamin D on disease course in MS. While several studies have shown a significant relationship between vitamin D levels and inflammatory activity in MS over a few years, few have demonstrated any significant association between vitamin D levels and disease progression (Smolders et al., 2019). This may be due to shorter follow-up time, as use of DMTs delay disability progression and the time to secondary progressive MS (Claflin et al., 2018, Brown et al., 2019). A recent study found poorer long-term (11 years) cognitive performance in the Paced Auditory Serial Addition Test in patients with lower 25(OH)D levels at baseline (Cortese et al., 2020), which in part supports our results. Thus, a longer observational period may be necessary to detect a potential effect of vitamin D levels on physical and cognitive disability scores.

In our data, a ceiling effect appeared in the association between 25 (OH)D and disability progression as there was almost no additional

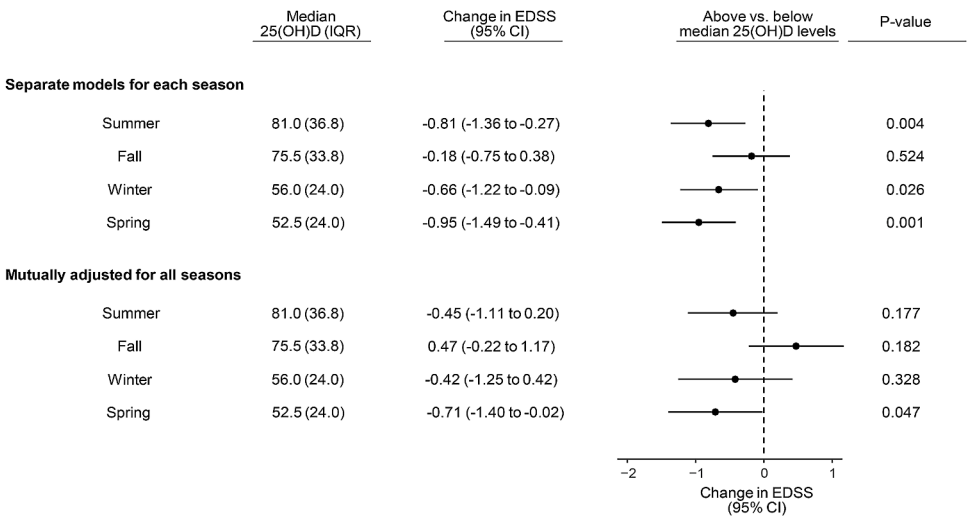

Fig. 2. The association between dichotomized seasonal 25-hydroxyvitamin D levels and long-term EDSS progression.
The seasonal 25-hydroxyvitamin D levels are dichotomized into "< median" and "≥ median" values and further adjusted for sex, age and EDSS score at last visit in the baseline study. Change in EDSS is the difference between the EDSS score at follow-up and the last EDSS score in the baseline study. The plots on the right side illustrate the estimates. 25(OH)D: 25-hydroxyvitamin D; IQR: interquartile range; CI: confidence interval.

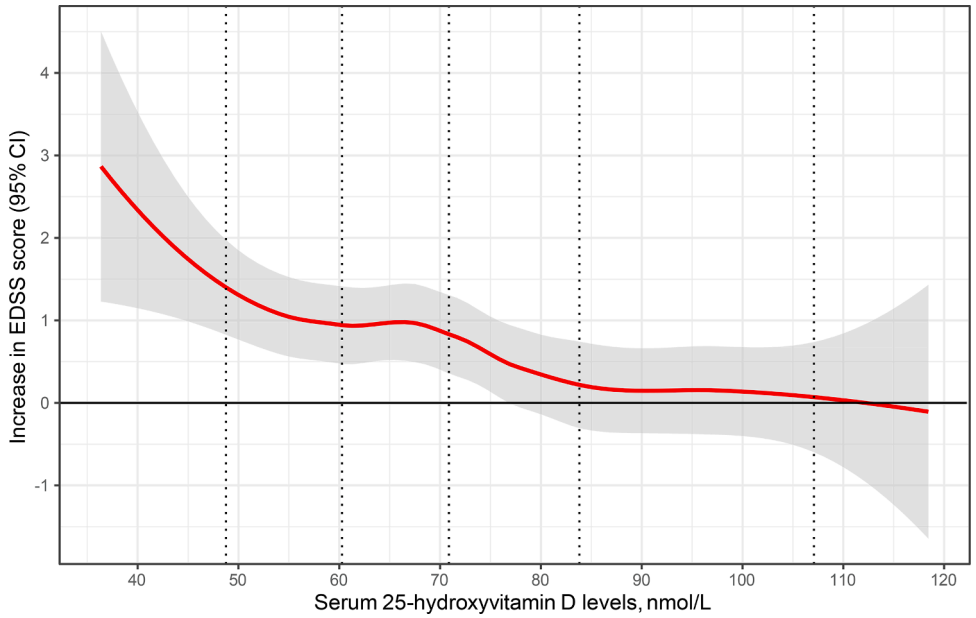

Fig. 3. Seasonally adjusted 25-hydroxyvitamin D levels and the increase in EDSS score fitted by a LOESS curve.
The increase in EDSS score is defined as the follow-up EDSS score subtracted by the last EDSS score in the baseline study. The vertical lines correspond to the fifth, 25th, 50th, 75th, and 95th percentile of serum 25-hydroxyvitamin D levels.

benefit for levels above 80 nmol/L. This finding is in line with a previous observational study among 156 RRMS patients on IFN-β or glatiramer acetate who were supplemented with vitamin D3. During follow-up, the relapse incidence rate significantly decreased until 25(OH)D levels reached 110-120 nmol/L - above this, the relapse rate stabilized (Pierrot-Deseilligny et al., 2012). Overall, this may suggest that the optimal 25(OH)D level for MS patients could lay within a high normal range of 80-120 nmol/L.

In our study population, 25(OH)D levels during spring had the strongest association with long-term disability. This may be explained by the "vitamin D winter" (Engelsen et al., 2005) period at latitudes above 50° when UVB radiation, the main natural source of vitamin D (Prietl et al., 2013), is too weak to induce any meaningful cutaneous synthesis of pre-vitamin D (Engelsen et al., 2005). This lack of synthesis cannot be fully compensated by a 15-25 days half-life of 25(OH)D in non-supplemented individuals (Martinaityte et al., 2017), making early spring extra prone for insufficient levels. Other studies have similarly found higher relapse rate during (early) spring (Miclea et al., 2017, Spelman et al., 2014). Vitamin D supplementation can compensate for the seasonal UVB-related variations in 25(OH)D (Miclea et al., 2017), and may also increase the half-life through storage in adipose tissue (Martinaityte et al., 2017), thus likely avoiding the lowest levels during the winter months at high latitudes.

The association between vitamin D and MS can be explained through plausible biological mechanisms. Both antigen-presenting cells of the innate immune system and T- and B-lymphocytes of the adaptive immune system express vitamin D receptors and are able to synthesize the active vitamin D compound calcitriol (Prietl et al., 2013, Hart et al., 2011). Through various mechanisms, calcitriol modulates the immune system into a more tolerogenic and anti-inflammatory state, thus likely

preventing and down-scaling autoimmune actions (Prietl et al., 2013). On the other hand, UVB radiation itself has likely immunomodulatory effects independent of the vitamin D pathway (Hart et al., 2011). However, when adjusting for cumulative sun exposure in our models, only a minor influence on the estimates was seen, suggesting that our results likely represent effects of vitamin D rather than UVB radiation.

In contrast to other cohorts (Healy et al., 2009, Manouchehrinia et al., 2013), we found no significant association between tobacco use and EDSS progression. Our results may have been affected by generally low disease progression in the population and beneficial effect of smoking cessation (Ramanujam et al., 2015) during follow-up (21.3% fewer tobacco users at follow-up visit). Since we used a nicotine metabolite to classify tobacco use in the baseline study, the results could potentially have been influenced by snuff use, which also contains nicotine and has been associated with a decreased risk for MS (Hedstrom et al., 2009). However, only three tobacco users in the baseline study reported a history of solely snuff use at follow-up, making it unlikely that our results can be explained by many snuff-users relative to smokers.

For BMI, we observed a non-significant trend towards less EDSS progression with higher BMI. Studies on BMI and long-term outcomes in MS may be difficult to interpret, as MS itself or changes in diet and activity may affect BMI (Habek et al., 2010), making findings prone to reverse causation. Patients with MS tend to have lower mean BMI (Nortvedt et al., 2005, Dardiotis et al., 2019), and gain less weight with age as compared to the general population (Bove et al., 2016, Wesnes et al., 2015), which could suggest that maintaining a higher BMI over the years reflects a more benign MS with less chronic disease burden affecting weight. This is consistent with other observations in our study, as use of potent DMT at follow-up was more prevalent in the lowest BMI quartile (54.5%) than in the highest quartile (31.6%).

Our study has several strengths. First, it benefits from a prospective design, a well-defined cohort, and a long follow-up time. Second, the lifestyle variables are based on objective and repeated measures over 24 months, making the results less prone to extreme values in single observations. Third, we could adjust for several potential confounders and explore the importance of seasonality in the relationship between 25 (OH)D levels and disability progression.

There are also some limitations to our study. The relatively small sample size may have limited the statistical power to detect associations in our study (i.e., increasing the likelihood of a type II error). In addition, the low level of disease progression in the study group could have influenced our findings, and factors that were not associated with progression in our study (e.g., smoking and BMI) may be more relevant for patients with a more aggressive disease course. Since the baseline study and the follow-up study was separated by a long period, we did not have detailed information on lifestyle habits between the two studies. It is therefore possible that lifestyle changes, such as increasing use of vitamin D supplements, may have attenuated the associations. At follow-up, only one EDSS score per patient was available, which could have been influenced by the patients' mood and level of fatigue at the time of assessment. However, such day-to-day changes may act in both directions, and are therefore less likely to affect the results. Our study population was originally recruited for a randomized clinical trial based on specific inclusion and exclusion criteria (Torkildsen et al., 2012), and may not be fully representative of the general MS population. Still, our findings on vitamin D are consistent with previous prospective studies and are biologically plausible. Lastly, we cannot exclude the possibility that our findings may be affected by residual or unmeasured confounding that we could not account for.

5. Conclusions

In summary, we found a significant association between higher vitamin D levels and lower long-term disability progression in patients with MS, suggesting that vitamin D may have a favourable effect on long-term outcomes in MS. This association seems to be driven by seasonal low levels during late winter/early spring at latitudes above 50°. No clear association was found between tobacco use or BMI and long-term disability scores, indicating that these factors may have less relevance for long-term prognosis.

CRediT authorship contribution statement

Kristin Wesnes: Conceptualization, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Visualization, Project administration, Funding acquisition. **Kjell-Morten Myhr:** Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Trond Riise:** Writing - review & editing, Supervision, Methodology. **Silje Stokke Kvistad:** Investigation, Resources, Writing - review & editing. **Øivind Torkildsen:** Resources, Data curation, Writing - review & editing. **Stig Wergeland:** Data curation, Writing - review & editing. **Trygve Holmøy:** Investigation, Resources, Writing - review & editing. **Rune Midgard:** Investigation, Resources, Writing - review & editing. **Alla Bru:** Investigation, Resources, Writing - review & editing. **Astrid Edland:** Investigation, Resources, Writing - review & editing. **Randi Eikeland:** Investigation, Resources, Writing - review & editing. **Sonia Gosal:** Investigation, Resources, Writing - review & editing. **Hanne F. Harbo:** Investigation, Resources, Writing - review & editing. **Grethe Kleveland:** Investigation, Resources, Writing - review & editing. **Yvonne S. Sørenes:** Investigation, Resources, Writing - review & editing. **Nina Øksendal:** Investigation, Resources, Writing - review & editing. **Kjetil Bjørnevik:** Conceptualization, Formal analysis, Methodology, Validation, Formal analysis, Data curation, Writing - review & editing, Visualization, Supervision.

Declaration of Competing Interests

K. Wesnes has received unrestricted research grants from Novartis and Biogen, research grant from the Independent Order of Odd Fellows, speaker honoraria from Biogen, and PhD-grant #912020 from the Western Norway Regional Health Authority. K.M. Myhr has received unrestricted research grants to his institution, scientific advisory board or speaker honoraria from Almirall, Biogen, Genzyme, Merck, Novartis, Roche, or Teva, and has participated in clinical trials organized by Biogen, Merck, Novartis and Roche. S.S. Kvistad has received financial research support from Novartis and Biogen. Ø. Torkildsen has received speaker honoraria from and served on scientific advisory boards for Biogen, Sanofi-Aventis, Merck and Novartis. S. Wergeland has received speaker honoraria and served on scientific advisory boards for Biogen, Genzyme and Novartis. T. Holmøy has received speaker honoraria, research support/grants and participated in clinical trials for Biogen, Merck, Sanofi and Novartis, is member of the scientific board of the Norwegian MS society, and has received financial support from the Research Council of Norway (grant #250864). R. Midgard has served on scientific advisory boards for Novartis Norway and Merck and received travel funding and/or speaker honoraria from Biogen, Novartis and Sanofi Genzyme. A. Edland has received speaker honoraria from Biogen, Merck, Sanofi and Novartis. H.F. Harbo has received speaker honoraria from Biogen, Sanofi-Aventis, Merck, Novartis, and Roche. N. Øksendal has received speaker honoraria from Biogen, participated in clinical trials for Biogen and Sanofi -Aventis, and has served on a scientific advisory board for Novartis. T. Riise, A. Bru, R. Eikeland, S. Gosal, G. Kleveland, Y.S. Sørenes, and K. Bjørnevik report no relevant disclosures.

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Appendix 1: EnvIMS-Q in English

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)	_____	_____

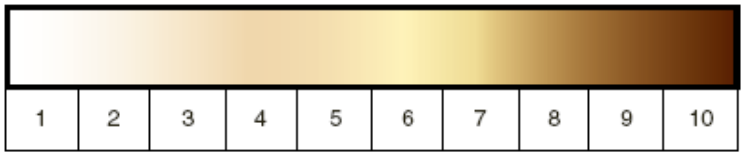
4. Please indicate in the box how many brothers and sisters you have. Include all children who lived with you during your childhood. If you are an only child, enter 0 in the box. ☐

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.



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 in which season(s) you generally consumed the following foods while you were a *teenager (age 13-19 years)?*
(you may choose more than one 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.

			Ages when UTI occurred. *(you can check more than one box in the same row)*					
No	Don't know	Yes	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.

			Age of ***first*** infection					
No	Don't know	Yes	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?).

				Age at ***first*** symptoms					
	No	Don't know	Yes	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 mother smoke inside the house 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 father smoke inside the house 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 inside the house 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 inside the house 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 inside the house when you were between the ages of 26-30 years?

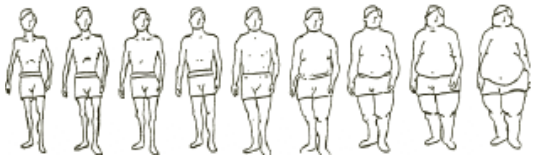
No ☐ Yes→ ☐ How many cigarettes per day were smoked inside the house?

Less than 10 ☐ 10+ ☐

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

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

Appendix 2: EnvIMS-Q in Norwegian

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

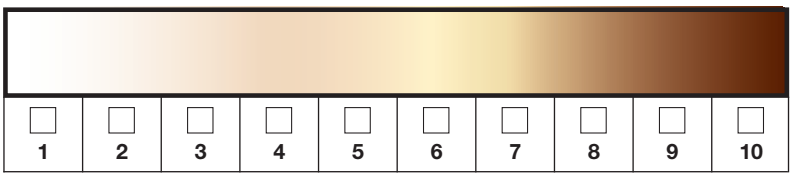
	Far	Mor
1. Norsk/europeisk/annen vestlig	☐	☐
2. Samisk	☐	☐
3. Asiatisk	☐	☐
4. Afrikansk	☐	☐
5. Midtøsten	☐	☐
6. Latinamerikansk	☐	☐

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 + ☐

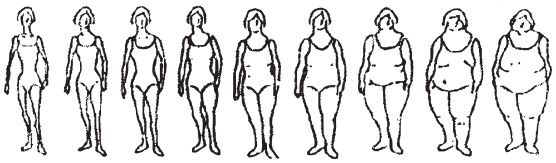
10. Har du bodd sammen med en partner eller noen andre som pleide å røyke inne i huset fra du var 26 til 30 år?

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 aktivitet (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 3: Lifestyle questionnaire in the OFAMS follow-up study

Pasient- ID: _____ Initialer: _____ Dato: _____

SPØRRESKJEMA OFAMS 10 ÅRS OPPFØLGING

Dato: _____

Sted: _____

Fødselsdato (dd.mm.yy): _____

Kjønn: ☐ Mann ☐ Kvinne

I vår studie ønsker vi å vurdere hvordan livsstilsfaktorer kan påvirke forløpet ved MS. I dette spørreskjemaet vil du derfor bli bedt om å besvare spørsmål vedrørende bakgrunnsdata og livsstil/kosthold. Vi ønsker at du besvarer spørsmålene så nøyaktig som mulig, og dersom du er usikker på noen spørsmål kan du spørre prosjektansvarlig.

SEKSJON 1: BAKGRUNNSDATA

1. Sivil status (sett kryss): ☐Gift ☐Samboer ☐Skilt ☐Enslig

2. Sett kryss for høyest *fullførte* utdannelse og oppgi alder ved fullføring. Sett et ekstra kryss dersom du har påbegynt, men *ikke fullført* en enda høyere utdannelse.

	Påbegynt	**Fullført**	**Alder fullført**
Grunnskole: 9-10 år			
Gymnas/videregående skole: 11-13 år			
Høyskole/universitet: 14-16 år			
Høyskole/universitet (masternivå): over 16 år			

3. Er du for tiden student, i jobb, arbeidssøkende og/eller ufør ? (sett kryss):

☐ Student
- Prosentandel: _____% - Nivå: ☐Bachelor ☐Master ☐PhD

☐ I jobb
- Stillingsprosent: _____%
- Type jobb:

☐ Arbeidssøkende
- Tidligere yrke:

☐ Ufør/ langtidssykmeldt (AAP) fra: **måned (0-12)**: _____ **årstall**: _____
- Prosent ufør: _____%
- Årsak ufør/langstidssykmeldt: ☐MS ☐Annen sykdom ☐Skade

Pasient- ID: _____ Initialer: _____ Dato: _____

4. Hvilken etnisk gruppe tilhører du? (sett ett kryss)

Norsk/ europeisk/ annen vestlig	
Samisk	
Asiatisk	
Afrikansk	
Midtøsten	
Latin-Amerikansk	
Blanding av flere	

SEKSJON 2: SOLVANER

Vi ønsker å kartlegge dine solvaner i løpet av siste 10 år.

1. Hvor mye utendørsaktiviteter (lek, idrett, tur, hagearbeid) har du i gjennomsnitt hatt i **sommerhalvåret** (fra april-september)? (sett kryss)

	Under 1 gang per uke	**1-2 ganger per uke**	**3-4 ganger per uke**	**Tilnærmet daglig**
For 10 år siden				
For 5 år siden				
Siste året				

2. Hvor mange uker i løpet av et år har du vært på ferie til "Syden" for soling og bading? (sett kryss)

	Ingen	**1 uke eller mindre**	**2-3 uker**	**4 uker eller mer**	**Husker ikke**
For 10 år siden					
For 5 år siden					
Siste året					

3. Hvor ofte i løpet av et år har du solt deg i solarium ? (sett kryss)

	Aldri/sjelden	**Mindre enn 1 gang per måned**	**1-4 ganger per måned**	**Mer enn 1 gang per uke**
For 10 år siden				
For 5 år siden				
Siste året				

4. Har du for det meste jobbet (sett kryss):

	Utendørs	**Innendørs**	**Like mye ute som inne**	**Har ikke jobbet**
For 10 år siden				
For 5 år siden				
Siste året				

Pasient- ID: _____ Initialer: _____ Dato: _____

SEKSJON 3: KOSTHOLD

Vi ønsker å kartlegge dine kostholdsvaner:

1. Hvor ofte har du i gjennomsnitt spist følgende produkter? (sett kryss)

For 10 år siden						
	Aldri/ sjelden	**1-3 ganger per måned**	**1 gang per uke**	**2-3 ganger per uke**	**4-6 ganger per uke**	**Daglig**
Fet fisk middag *						
Fet fisk pålegg *						
Egg (kokt eller stekt)						
Smør/ margarin i matlaging						
Helmelk						
Leverpostei						
Gulrot						
Hvitost/Gulost						
Brokkoli						
Paprika						
For 5 år siden						
	Aldri/ sjelden	**1-3 ganger per måned**	**1 gang per uke**	**2-3 ganger per uke**	**4-6 ganger per uke**	**Daglig**
Fet fisk middag *						
Fet fisk pålegg *						
Egg (kokt eller stekt)						
Smør/ margarin i matlaging						
Helmelk						
Leverpostei						
Gulrot						
Hvitost/Gulost						
Brokkoli						
Paprika						
I løpet av det siste året						
	Aldri/ sjelden	**1-3 ganger per måned**	**1 gang per uke**	**2-3 ganger per uke**	**4-6 ganger per uke**	**Daglig**
Fet fisk middag *						
Fet fisk pålegg *						
Egg (kokt eller stekt)						
Smør/ margarin i matlaging						
Helmelk						
Leverpostei						
Gulrot						
Hvitost/Gulost						
Brokkoli						
Paprika						

* **Fet fisk** = laks, ørret, kveite, flyndre, makrell, sild, sardiner

Pasient- ID: _____ Initialer: _____ Dato: _____

2. Hvordan vil du definere ditt kosthold? (sett kryss)

	For 10 år siden	**For 5 år siden**	**I dag**
1. Spiser både kjøtt, fisk og meieriprodukter			
2. Spiser fisk og meieriprodukter, men ikke kjøtt			
3. Vegetarianer som spiser meieriprodukter			
4. Veganer som ikke spiser animalske produkter			

3. Har du brukt kosttilskudd som inneholder vitaminer*? (sett kryss og oppgi evt. navn på produkt)

	Ja	**Nei**	**Husker ikke**	**Navn på produkt(er)**
For 10 år siden				
For 5 år siden				
Siste året				

* Vitamin D-tabletter, trankapsler, tran, vitaminbjørner, multivitaminer, andre vitamin-tilskudd

4. Hvilke(t) kosttilskudd i tabellen har du brukt **mest** på de ulike tidspunkt? (sett flere kryss om du har brukt flere typer like mye)

	For 10 år siden	**For 5 år siden**	**Siste året**
Vitamin D tabletter			
Trankapsler			
Tran			
Vitaminbjørner			
Andre multivitamin-produkter			
Brukte ikke slike tilskudd			

5. Hvor ofte har du brukt kosttilskudd *som oppgitt i punkt 4* **i vinterhalvåret (oktober-mars)?** (sett kryss)

	For 10 år siden	**For 5 år siden**	**Siste året**
Aldri/ sjelden			
1-3 dager per måned			
1-3 dager per uke			
4-6 dager per uke			
Daglig			
Husker ikke			

Pasient- ID: _____ Initialer: _____ Dato: _____

6. Hvor ofte har du brukt kosttilskudd *som oppgitt i punkt 4* i **sommerhalvåret (mai til august)**? (sett kryss)

	For 10 år siden	**For 5 år siden**	**Siste året**
Aldri/ sjelden			
1-3 dager per måned			
1-3 dager per uke			
4-6 dager per uke			
Daglig			
Husker ikke			

SEKSJON 4: RØYKING OG SNUSBRUK

Vi ønsker å kartlegge dine røyke- og snusvaner**:**

1. Røyker du nå? ☐Ja ☐Nei

2. Har du røykt i løpet av siste 10 år? ☐Ja ☐Nei (ved Nei- gå til punkt 6).

3. Hvis "ja" i punkt 2- hvor mange av de siste 10 årene har du røykt? _____ av 10 år.

4. Hvor ofte har du røykt/røyker du i gjennomsnitt? (sett kryss)

	1-3 dager per måned	**1-2 dager per uke**	**3-6 dager per uke**	**Daglig**	**Røykte ikke**
For 10 år siden					
For 5 år siden					
Siste året					

5. Hvor mange sigaretter har du røykt i gjennomsnitt per dag med røyking? (sett kryss)

	1- 4 sigaretter	**5-10 sigaretter**	**11-20 sigaretter**	**Over 20 sigaretter**
For 10 år siden				
For 5 år siden				
Siste året				

6. Snuser du nå? ☐Ja ☐Nei

7. Har du snust i løpet av siste 10 år? ☐Ja ☐Nei (ved Nei- gå til seksjon 5)

8. Hvis "ja" i punkt 7-hvor mange av de siste 10 årene har du snust? _____ av 10 år

Pasient- ID: _____ Initialer: _____ Dato: _____

9. Hvis du har snust- hvor ofte har du snust i gjennomsnitt? (sett kryss)

	1-3 dager per måned	**1-2 dager per uke**	**3-6 dager per uke**	**Daglig**	**Snuste ikke**
For 10 år siden					
For 5 år siden					
Siste året					

SEKSJON 5: FYSISK AKTIVITET

1. Hvor ofte har du i gjennomsnitt trent/ vært så fysisk aktiv at du har fått økt puls og blitt svett og andpusten? (sett kryss)

	Kun sporadisk	**1-2 timer per uke**	**3-5 timer per uke**	**6 timer eller mer per uke**
For 10 år siden				
For 5 år siden				
Siste året				
Siste måned				

2. Hva slags fysisk aktivitet (som har medført økt puls + svett og andpusten) har du drevet med? (oppgi en eller flere aktiviteter)

	Type aktivitet(er)	**Ingen aktivitet**
For 10 år siden		
For 5 år siden		
Siste året		
Siste måned		

Errata for
The Impact of lifestyle factors on disease risk and long-term disability progression in multiple sclerosis

Kristin Wesnes

Thesis for the degree philosophiae doctor (PhD)
at the University of Bergen

12.05.21 Kristin Wesnes
(date and sign. of candidate)

18.05.21
(date and sign. of faculty)

Errata

Page 15, third line:
The sex ratio should be included after "from 25 to 35 years of age": "*and a female to male ratio of around 2-3:1* (reference: Harbo et al, *Ther Adv Neurol Disord*, 2013, doi: 10.1177/1756285613488434)

Page 30, last sentence (before Figure 6):
Reference is missing after "no causal effect of genetic estimates on MS risk was observed in a recent MR study": Harroud et al, MSvirtual 2020 FC04.05, *Mult Scler*, 2020, doi: 10.1177/1352458520974936

Page 62:
The reference 268 is wrong, as it refers to a pre-published version of the study. The correct reference is: Cortese et al, *Ann Neurol*, 2016, doi: 10.1002/ana.24769

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