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FACULTY OF HEALTH SCIENCES
DEPARTMENT OF CLINICAL MEDICINE

Can vitamin D_3 supplementation prevent bone loss in persons with MS?

Linn Hofsøy Steffensen

A dissertation for the degree of Philosophiae Doctor

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Contents

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Linn Hofsøy Steffensen
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Norsk sammendrag- Norwegian summary

Multippel sklerose (MS), en betennelsessykdom i nervesystemet og en av de hyppigste årsakene til kronisk nevrologisk funksjonsnedsettelse hos unge i den vestlige verden, er også en mulig årsak til beinskjørhet. Lav beintetthet er hyppigere hos personer med MS enn hos friske personer og flere studier har vist at beintetthet hos personer med MS reduseres etter hvert som funksjonsnedsettelse hemmer fysisk aktivitet. Beintetthet er i hovedsak bestemt av genetiske og hormonelle faktorer, kropps masse indeks, fysisk aktivitet og inntak av kalsium og vitamin D.

Det amerikanske og kanadiske "Insitute of Medicine" og det norske Helsedirektoratet har definert vitamin D nivå i blod ≥ 50 nmol/l som tilstrekkelig, mens flere eksperter anbefaler ≥ 75 nmol/l med tanke på beinhelse. Hovedkildene for vitamin D er sollys, fet fisk og berikede matvarer som margarin og melk. I tillegg finnes det som kosttilskudd. De amerikanske og kanadiske anbefalingene er inntak på 600 IU vitamin D daglig for de mellom 1 år og 70 år. De norske anbefalingene er inntak av 300 IU vitamin D daglig hos personer mellom 2 år og 60 år.

Studier utført hos personer med MS har påvist gjennomsnittlig vitamin D nivå i blod fra omtrent 40 nmol/l til 80 nmol/l. Vitamin D mangel kan bidra til dårlig beinhelse allerede før funksjonsnedsettelsen hemmer fysisk aktivitet. Vi ønsket å se om en ukentlig dose med høydose vitamin D_3 (20.000 IU) tatt i 96 uker kunne hindre beintap hos gangføre personer med MS i alderen 18 til 50 år. 71 personer med MS fikk enten høydose vitamin D_3 eller placebo ukentlig. 68 ble inkludert i analysen. Ved studiestart om vinteren var gjennomsnittlig vitamin D nivå i blod 56 nmol/l og halvparten av deltagerne hadde tilstrekkelige nivåer (≥ 50 nmol/l). Soleksponering i form av solferie eller solarium og jevnlig vitamin D inntak økte sjansen for å ha adekvate verdier. Andelen av deltagerne i placebogruppa som hadde tilstrekkelig nivå økte fra 55 % om vinteren til 92 % om sommeren. Alle deltagerne i gruppa som fikk vitamin D tilskudd oppnådde tilstrekkelig vitamin D nivåer om vinteren, og 91 % oppnådde 75 nmol/l eller høyere.

Ved studiestart hadde 25 % av deltagerne lavere enn forventet beintetthet (z-score ≤ -2.0). Der var ingen sikker forskjell i endring i beintetthet fra studiestart til studieslutt mellom behandlingsgruppene, heller ikke etter justering for vitamin D nivå i blod ved studiestart eller forandring i vitamin D nivå i løpet av studieperioden. I hoftene var der i hele gruppen et gjennomsnittlig beintap på 1 %.

Resultatene fra denne studien med unge gangføre personer med MS kan tyde på at både kvinner og menn med MS har tidlig forhøyet risiko for å utvikle lav beintetthet. Økt funksjonsnedsettelse vil videre kunne bidra til utvikling av beinskjørhet, samtidig som risikoen for fall med påfølgende fraktur tiltar. Det kan være hensiktsmessig å utføre beintetthetsmålinger på alle MS pasienter, mulig like etter diagnosen, men i alle fall når der oppstår påvirkning av gangfunksjonen. Selv om denne studien ikke kunne vise at vitamin D hindret utvikling av beintap, så er vitamin D inntak i anbefalte doser et viktig forebyggende tiltak mot beinskjørhet. Halvparten av deltagerne hadde vitamin D mangel i løpet av vinteren. Alle MS pasienter som i løpet av vintermånedene ikke får soleksponering og som har lavt vitamin D inntak bør bli anbefalt å ta vitamin D tilskudd for å unngå vitamin D mangel.

English summary

Multiple sclerosis (MS), a neuroinflammatory disease and one the most common causes of chronic neurological dysfunction in young adults in the Western world, is also a possible cause of secondary osteoporosis. Several studies have shown that in persons with MS, bone mineral density (BMD) decreases as disability increasingly limits physical activity. In persons with MS low BMD is more prevalent when compared with healthy controls. BMD is primarily determined by genetic and hormonal factors, body mass index, physical activity and intake of calcium and vitamin D.

Serum vitamin D(25[OH]D) level ≥ 50 nmol/L is classified as sufficient by the US and Canadian Institute of Medicine and the Norwegian Directorate of Health, however, optimal serum 25(OH)D level for bone health is according to many experts at least 75 nmol/L. The main source of vitamin D for most people is vitamin D generated in the skin during sun exposure. The dietary intake of vitamin D is usually limited, but vitamin D is especially found in fatty fishes and may also be obtained by fortified foods and in supplements. The recommended dietary intake of vitamin D is by the Institute of Medicine 600 IU daily for ages 1-70 years and the Norwegian recommendation is 300 IU daily for ages 2-60 years.

Studies performed in persons with MS have reported mean serum 25(OH)D values ranging from approximately 40 nmol/L to 80 nmol/L. Low vitamin D levels may contribute to poor bone health, even before disability limits physical activity. In this 96 week randomized controlled clinical trial, we aimed to asses whether a weekly dose of 20,000 IU vitamin D_3 could prevent bone loss in fully ambulatory persons with MS age 18-50 years. 71 persons with MS where randomized to receive either a weekly dose of 20,000 IU vitamin D_3 or placebo. 68 were included in the final analysis. At the study start in winter, mean serum 25(OH)D level was 56 nmol/L, and half of the participants had sufficient levels (≥ 50 nmol/l). Serum vitamin D levels were strongly predicted by sun exposure through sun vacation or solarium and by vitamin D intake. In the placebo group, the proportion of study participants with sufficient levels of vitamin D increased from 55% during winter to 92% during summer. All participants in the intervention group achieved sufficient winter levels. Winter levels of 75 nmol/L or higher were achieved by 91% of the participants.

BMD was lower than expected (z-score ≤ -2.0) in 25% of the participants at screening. Percentage change in BMD from baseline to study end did not differ between participants

who had received vitamin D_3 supplementation and those who had received placebo. This result was not altered by adjustment for baseline serum 25(OH)D level or change in serum vitamin D over the study period. However, we found that mean hip BMD decreased significantly in the whole study population by 1.0%.

Findings in our limited sample of young ambulatory persons with MS indicate that men as well as women with MS are at increased risk of low BMD. The results strengthen the suspicion that MS may be a cause of secondary osteoporosis. Progressing physical handicap poses persons with MS at further risk of developing osteoporosis and at the same time increases the risk of falls and fractures. It might be appropriate to perform BMD measurements in all persons with MS, perhaps shortly after diagnosis, but at least when the disease affect the ambulatory function. Even though this small study could not prove any effect of vitamin D supplementation on BMD loss, intake of vitamin D is an important factor in preventing osteoporosis. Half of the participants in this study were vitamin D insufficient during winter months. In order to achieve adequate vitamin D levels, MS patients who have no vitamin D efficient sun exposure and low dietary vitamin D intake during the winter months should be recommended to take vitamin D supplements.

List of papers

Paper I

Steffensen LH, Mellgren SI, Kampman MT (2010) Predictors and prevalence of low bone mineral density in fully ambulatory persons with multiple sclerosis. *J Neurol 257:410-418*

Steffensen LH, Mellgren SI, Kampman MT (2010) Erratum to: Predictors and prevalence of low bone mineral density in fully ambulatory persons with multiple sclerosis. *J Neurol 257(3):497-498*

Paper II

Steffensen LH, Jorgensen L, Straume B, Mellgren SI, Kampman MT (2011) Can vitamin D supplementation prevent bone loss in persons with MS? A placebo-controlled trial. *J Neurol 258:1624-1631*

Paper III

Steffensen L.H, Brustad M, Kampman M.T. (2012) What is needed to keep persons with multiple sclerosis vitamin D-sufficient throughout the year? J. Neurol. Doi: 10.1007/s00415-012-6611-6

Abbrevation

APC: Antigen presenting cell

BBB: Blood brain barrier

BMD: Bone mineral density

BMI: Body Mass Index

CFS: Cerebrospinal fluid

CIS: Clinically isolated syndrom

CNS: Central nervous system

CPBA: Competitive protein binding assay

DBP: Vitamin D binding protein

DMTs: Disease modifying treatments

DXA: Dual X-ray absorptiomatry

EAE: Experimental autoimmune encephalomyelitis

EBV: Epstein Barr Virus

EDSS: Expanded Disability Status Scale

FGF-23: Fibroblast growth factor -23

FS: Functional System

Gd: Gadolinium

HLA: Human leukocyte antigen

HPLC: High-performance liquid chromatography

iCa: Ionized calcium

INF-β: Interferon-β

IRR: Incidence rate ratio

IU: International Unit

LRP5: Low-density lipoprotein receptor-related protein 5

LS/MS: Liquid chromatography coupled with mass spectometry

MBP: Myelin basic protein

MHC: Major histocompatobility complex

MRI: Magnetic resonance imaging

MS: Multiple sclerosis

MSFC: Multiple Sclerosis Functional Composite

OPG: Osteoprotegerin

PBA: Protein binding assay

PPMS: Primary progressive multiple sclerosis

PTH: Parathyroid hormone

RANK/ RANKL: receptor activator of nuclear factor-kappa B/ RANK ligand

RCT: Randomized controlled trial

RIA: Radioimmunoassay

RRMS: Relapsing remitting multiple sclerosis

SEP: Somatosensory evoked potential

SPMS: Secondary progressive multiple sclerosis

TBBM: Total body bone mineral

TGF: Transforming growth factor

UD: Ultradistal

UVR: Ultraviolet radiation

VDR: Vitamin D receptor

VDRE: Vitamin D responsive element

VEP: Visual evoked potential

WHO: World Health Organization

1,25(OH)2D: 1,25-dihydroxyvitamin D

25(OH)D: 25-hydroxyvitamin D

1. Introduction

1.1 Multiple Sclerosis

1.1.1 Introduction

Multiple sclerosis (MS) is one the most common cause of chronic neurological dysfunction in young adults in the Western world. In MS, inflammation and chronic degeneration leads to demyelination and axonal destruction in the central nervous system (CNS) and it is a multifactorial disorder, believed to be a result of both genetical and environmental factors and has a female predominance [Compston and Coles 2008].

1.1.2 Epidemiology MS

The estimated worldwide prevalence of MS is between 1-1 and 2.5 million cases [Miller 2012]. Norway is a high risk area of MS, with the lowest prevalence in the northernmost counties Finnmark and Troms (86-104 per 100.000) and the highest in the southernmost part (180-190 per 100.000) [Kampman *et al.* 2007;Vatne *et al.* 2011].

1.1.3 Classification and clinical manifestations of MS

The majority of the patients (80-90 %) present during their second or third decade of life with the relapsing remitting form of MS (RRMS) [Noseworthy *et al.* 2000;Tremlett *et al.* 2010] which is characterised by clinical exacerbations of neurological symptoms, followed by complete or incomplete remission [Miller 2012]. After 10-20 years about 65% -70% of the RRMS patients enter a secondary progressive phase, secondary progressive MS (SPMS) [Compston and Coles 2008]. The remaining 10-20% of the patients experience a primary progressive form of MS (PPMS) with progressive clinical deterioration from the onset of the disease, without the characteristic exacerbations. Age at onset is around 40 years or later and there is a male predominance [Miller and Leary 2007;Compston and Coles 2008].

The patients with RRMS present with an acute episode affecting one (or occasionally several) sites in CNS which develops rapidly and gradually improves after days to weeks [Compston and Coles 2008]. The symptoms and signs generally reflect those parts of the CNS where there is a dense concentration of myelinated fibres subserving motor and sensory functions [Compston *et al.* 2006]. The first appearance are typically sensory disturbances, unilateral optic neuritis, diplopia (internuclear ophtalmoplegia), Lhermitte´s sign (trunk and limb parasthesias evoked by neck flexions), limb weekness, clumsiness, gait ataxia and neurogenic

bladder and bowel syndrome. In PPMS the disease is progressive from the onset [Compston and Coles 2008]. The most common presentation is progressive spastic paraparesis with an increasing functional impairment over months to years which do not reverse. Excessive fatigue is a common complaint in all subtypes of multiple sclerosis [Compston *et al.* 2006] and patients with MS may experience both nociceptive pain due to reduced mobility, postural changes and spasticity, and neuropathic pain due to lesion affecting the somatosensory system. Affective disturbance and cognitive deficits are also common in MS patients [Compston *et al.* 2006]. The patients may experience progressive impaired disability due to limb weakness, spasticity, ataxia, and tremor [Compston *et al* 2006]. Disability in multiple sclerosis is quantified with The Kurtzke Expanded Disability Status Scale (EDSS) from 0, with a normal neurological examination to 10, death due to MS [Kurtzke 1983]. The EDSS quantifies disability in eight Functional Systems (FS) [Fischer *et al.* 1999]: mental, visual, sensory, brainstem, pyramidal, cerebellar, bowel and bladder functions and other (includes any other neurological findings due to MS). In addition to FS score the EDSS system is based on the patient's ability to walk. With a walking distance below 500 meters, the EDSS is graded 4.5 or higher. If the patient is in need of assistance to walk (cane, cruch or brace) the EDSS is graded 6.0 or higher. If the patients is restricted to bed or chair the EDSS is graded 8.0 or higher.

1.1.4 Pathogenesis of MS

The hallmark of MS pathology is formation of the sclerotic plaque in the CNS which represents the end stage of a process involving inflammation, demyelination and remyelination, oligodendrocyte depletion and astrocytosis, and neuronal and axon degeneration [Compston and Coles 2008]. MS is considered a T-cell disease involving activated myelin-specific auto reactive T cells [Frohman *et al.* 2006]. Myelin is a protein which is synthesised by mature oligodendrocytes in the CNS. It is extending along the nerve fibres in the white matter tract and is necessary to properly propagate nerve impulses between neurons [Frohman *et al.* 2006]. The immunepathogenesis, shown in figure 1, may be described in different phases [Miller 2012]. First myelin specific reactive T cells are activated outside the CNS via a process in which cell surface receptors recognize processed antigens in association with major histocompatobility complex (MHC) class II molecules (also called human leukocyte antigen [HLA]) on antigen presenting cells (APCs). Then they become activated through one of several costimulatory molecules, such as B7/CD28 or CD40/CD40L. How these auto reactive T-cells are activated in the periphery is still a matter of debate.

Processes like molecular mimicry, where T-cells generated against non-self (virus or microbial antigens) cross-react with self-myelin and T cell activation triggered by myelin antigens presented in lymph nodes have been postulated as potential mechanism [Holmoy 2007;Comabella and Khoury 2012]. These activated T cells can proliferate and differentiate into one of several subtypes of T helper (Th) cells (also called CD4+ cells) which secrete cytokines to facilitate different types of immune response. Th1 cells are considered proinflammatory and produce proinflammatory cytokines, opposed to Th2 cells that secret anti-inflammatory cytokines. A reduction in the effector functions of regulatory T cell has also been observed [Viglietta *et al.* 2004]. After activation in the periphery the T cells adhesions molecules bind to and cross the blood brain barrier (BBB) [Dhib-Jalbut 2007]. Within the CNS the myelin-spesific CD4+ T cells may be reactivated in situ by myelin antigens presented on MHC II molecules on antigen presenting cells (APC), such as microglia or macrophages, and trigger release of proinflammatory cytokines which further disrupt the BBB and stimulate chemotaxis with a second larger wave of inflammatory cells to the CNS [Comabella and Khoury 2012]. After re-activation T cells proliferate and secrete pro-innflammatory cytokines which stimulate microglia, macrophages, astrocytes and recruited B cells and ultimately result in demyelisation.

The most studied animal model of MS is the experimental autoimmune encephalomyelitis (EAE). In 1981 Ben-Nun et al isolated myelin basic protein (MBP)-specific CD4+ T cells from rats immunized with purified MBP and cultured them in vitro. The T line cells were then injected in healthy recipients from the same rat strain that had been immunised and after 4-5 days they developed experimental autoimmune encephalomyelitis (EAE) [Ben-Nun *et al.* 1981]. EAE may also be induced by immunizing animals with other myelin derived protein or peptides [Comabella and Khoury 2012]. The EAE has exerted a major influence on MS research, although it fails to reflect some areas in the MS pathogenesis; the B-cell component and intrathecal production of IgG, how the immune-system breaks the self-tolerance and the relation between the neuroinflammation and neurodegeneration [Holmoy 2007].

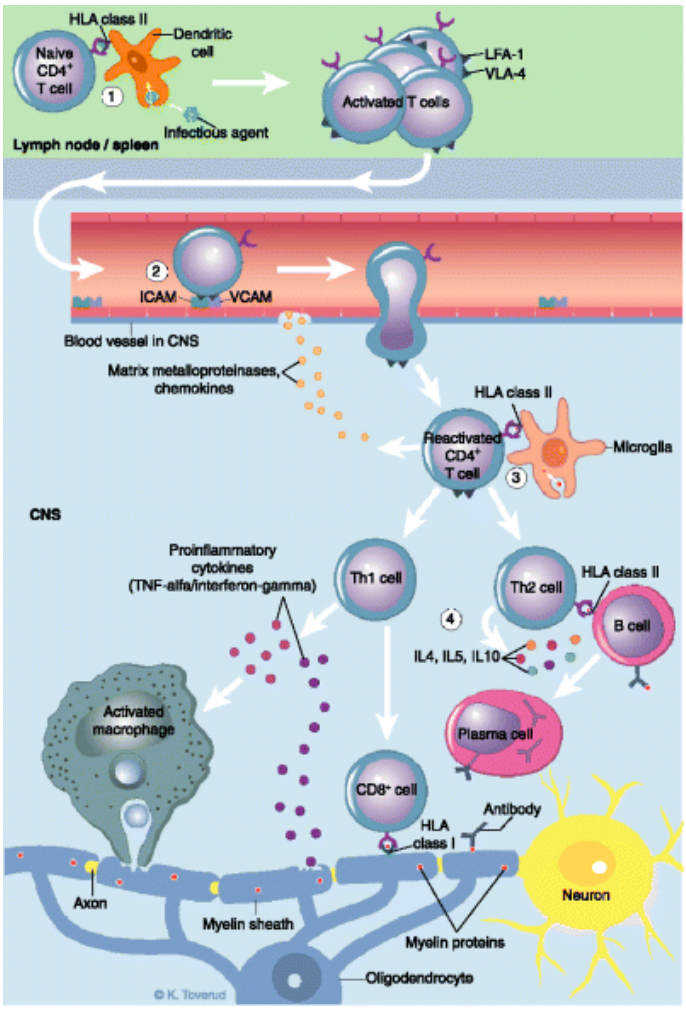


Fig. 1: The MS immunepathogenesis. Reproduced with permission from [Holmoy 2007]

1.1.5 Aetiology and risk factors for MS

The aetiology of MS remains elusive, but it is assumed that both environmental factors and a complex genetic background contribute to the disease [Comabella and Khoury 2012], the first probably accounting for as much as 80 % of the risk [Ebers 2008]. The proposed and most extensively studied environmental triggers are Epstein Barr virus, low sunlight, low vitamin D, smoking, diet and toxins [Compston and Coles 2008;Pugliatti *et al.* 2008a]. Migration of people affect the distribution of MS, and migration in childhood from a high-risk to a low-risk region is associated with a reduced MS risk and from a low-risk to a high-risk is associated with a higher MS risk. The age-at-migration was originally suggested to be before 15 years of age, but later studies from Australia found that the risk of environmental factors in multiple sclerosis may operate over a period of many years and not only in childhood and early life [Hammond *et al.* 2000]. Genetic epidemiologic studies strongly indicate that environmental

factors act at a broad population level, rather than in the familial microenvironment [Orton *et al.* 2008].

1.1.5.1 Epstein Barr virus

Several microbes have been suggested to be associated with the risk of MS, but the evidence linking MS and Epstein Barr virus (EBV) are particularly strong. EBV infection appears to be a necessary (but not sufficient) condition for adult MS to develop [Holmoy 2007] and the risk is highest among individuals first infected with EBV in adolescence or later in life, often manifested as infectious mononucleosis [Thacker *et al.* 2006].

1.1.5.2 Sun exposure and vitamin D

One of the most striking features of MS is the geographic distribution with a positive latitude gradient, showing, with some notable exceptions, a pattern of high MS frequency in areas where sunlight exposure is low and, consequently, opportunity for vitamin D synthesis in the skin is limited [Pugliatti *et al.* 2006;van der Mei *et al.* 2001;Kampman and Brustad 2008;Sloka *et al.* 2011;Vukusic *et al.* 2007;Ascherio and Munger 2007;Compston and Coles 2008] However only one prospective study so far has shown that circulating levels of 25(OH)D $\geq$ 100 nmol/L were associated with a lower risk of MS in whites [Munger *et al.* 2006].Later findings have suggested an attenuation of the latitude gradient [Alonso and Hernan 2008;Pugliatti *et al.* 2002], but MS prevalence in Australia and in France could be closely predicted by regional UVR levels [van der Mei *et al.* 2001;Vukusic *et al.* 2007], and a geospatial analysis confirmed a strong association between UVR and MS distribution [Beretich and Beretich 2009]. A recently published large case-control study from Sweden found a significant inverse relationship between exposure to ultraviolet radiation (UVR) and risk of developing MS [Baarnhielm *et al.* 2012]. Ecological studies have also shown an inverse correlation of sunlight exposure through outdoor activities during the period spanning from childhood to adolescence, but also into adulthood, with MS risk [Hammond *et al.* 2000;Freedman *et al.* 2000;van der Mei *et al.* 2003;Goldacre *et al.* 2004], even north of the Arctic Circle [Kampman *et al.* 2007]. Possible pathways for immunosuppressive effects of UVR exposure in immune-mediated disorders that are likely to be independent of vitamin D synthesis are also recognised [Beretich and Beretich 2009;Lucas and Ponsonby 2006].

The effect of dietary vitamin D on MS risk has been less studied than the correlation with UVR. Most humans are not dependent on dietary vitamin D supplies [Hollis 2005], and a

correlation of vitamin D from food with 25(OH)D is only found when UVR exposure is low [Brustad *et al.* 2004;van der Mei *et al.* 2007;Macdonald *et al.* 2008] In the Nurses' Health Study following 187,000 women aged 25 to 55 years at inclusion, total vitamin D intake and use of vitamin D supplements ≥ 400 IU/day, but not vitamin D intake from food, were associated with higher serum 25(OH)D levels and lower MS risk [Munger *et al.* 2004]. However, it must be commented that the inclusion age was quite high given that median age of onset of MS is 30 years and that environmental factors may act many years before disease onset. In addition, both vitamin D intake from food and supplement was rather low. In a MS case-control study in Norway north of the Arctic Circle, use of cod-liver oil supplements when growing up was associated with less MS in the subgroup of respondents with low summer outdoor activities [Kampman *et al.* 2007]. In a case-control study from Sweden, intake of fatty fish and vitamin D supplement use had only minor influence according to the risk of MS development [Baarnhielm *et al.* 2012].

Animal studies of EAE have shown that induction of EAE could be prevented and EAE progression blocked by whole body UV –irradiation and that vitamin D hormone supplementation completely inhibited EAE induction and progression [Hauser *et al.* 1984;Cantorna *et al.* 1996;Lemire and Archer 1991]

A role of vitamin D in the immunopathogenesis of MS is biologically plausible. Vitamin D is a potent immune modulator [Adams and Hewison 2008;Fernandes de Abreu *et al.* 2009], and a role of vitamin D has also been proposed in other immune-mediated diseases, a.o. diabetes type I, rheumatoid arthritis, and inflammatory bowel disease [Fernandes de Abreu *et al.* 2009]. Proliferation assays show an association of high 25(OH)D levels with an improved regulatory T cell function in persons with MS [Correale *et al.* 2009;Smolders *et al.* 2009b]. Also significant correlations between vitamin D status and immunological markers have been reported in cross sectional studies, which might indicate an effect on the disease activity by vitamin D, however these correlations might be caused by an effect of the disease on the vitamin D status [Ascherio *et al.* 2010]. Direct genomic signalling by active $1,25(OH)_2D$ occurs through the vitamin D receptor (VDR), which is present in multiple cells of the immune system as well as in neurons and glial cells in the human brain [Smolders *et al.* 2008a;Eyles *et al.* 2005]. Activation of the VDR by hormonal vitamin D stimulates a shift from proinflammatory Th1 responses to anti-inflammatory Th2 responses [Smolders *et al.* 2008a]. Holmøy was the first to propose that poor vitamin D status modulates the immune

response to EBV in a way that increases the risk of developing MS [Holmoy 2008;Hayes and Donald 2008].

1.1.5.3 Genetic influences

Family studies have revealed that first –degree relatives of a person with MS are generally at 15-35 times greater risk of developing MS compared with the general population and the risk correlates with the degree of kinship [Ramagopalan *et al.* 2009]. There are several genes that seem to be associated with MS. The association between MS and alleles of the MHC (HLA) classes I and II was identified in the early 1970s [Compston and Coles 2008], but variants so far identified explain only approximately 50 % of the inherited risk of MS [Ramagopalan *et al* 2009]. T-cell receptor, CTLA4 and ICAM1 are other genes associated with MS [Dyment *et al.* 2004]. Reports on novel gene-environment interactions continue to increase our understanding of the role of vitamin D in MS. The HLA-DRB1*1501 risk haplotype is a strong genetic predictor of MS risk and a vitamin D responsive element (VDER) has been identified in the promoter region of this haplotype [Ascherio *et al.* 2010;Ramagopalan *et al.* 2009]. Conversely, a significant genetic influence on regulation of circulating 25(OH)D concentrations has been found in MS twins [Orton *et al.* 2008]. The increasing ratio in the concordance of MS risk between monozygotic and dezygotic twins with increasing latitude also suggests that genetic effects may be stronger at lower UVR exposure and/or lower vitamin D status [Islam *et al.* 2007]. However, still little is known about the role of vitamin D related genes or specific genetic interactions with vitamin D in determining MS risk [Ascherio *et al.* 2010] and the contribution of VDR gene polymorphisms to immune regulation in MS is not fully understood [Smolders *et al.* 2009a]. First evidence has been provided that a functional variant of the VDR gene interacts with sun exposure in childhood to influence MS risk [Dickinson *et al.* 2009], implying that vulnerability to poor vitamin D status may be determined by genetic variations. The modification of the association of past sun exposure with MS risk by ”red hair colour” genotype provides further support for a causal effect of UVR/vitamin D in the aetiology of MS [Dwyer *et al.* 2008]. The disproportional increase in the incidence of MS in women that was first observed in Canada is likely to be caused by sex-specific exposure or susceptibility to environmental factors [Orton *et al.* 2006]. Data supporting an interaction between female sex, possibly mediated by oestrogen, and vitamin D in MS risk are accumulating. Protective effects of sun exposure are mainly observed in female MS patients [Islam *et al.* 2006;Woolmore *et al.* 2007;Dwyer *et al.* 2008;Kampman and Brustad 2008]. Sex differences in vitamin D metabolism were first reported in the EAE

model: A cholecalciferol containing diet inhibited severe EAE only in female mice, indicating a sex difference in vitamin D metabolism in the CNS [Spach and Hayes 2005]. The same group showed recently that 17β-estradiol is essential for VDR gene expression and function in the inflamed CNS in EAE mice [Nashold *et al.* 2009]. Stronger immunomodulatory effects of vitamin D on CD4 T cells and an increase in the number of T regulatory cell in women than in men have been shown [Correale *et al.* 2010]

1.1.5.4 Other

Several studies have described an association between smoking and MS risk, and although confounding cannot be excluded, the evidence suggests that smoking increases the risk for MS and also likely accelerates the progression of the disease [Ascherio and Munger 2007]. A possible association between toxins and MS risk and especially on occupational exposure to organic solvents has been proposed, but the results are divergent [Marrie 2004;Pugliatti *et al.* 2008a]. Estrogen in high levels appear to shift the immune response from the proinflammatory type I, to the noninflammatory type II and may be associated with reduced risk of MS [Ascherio and Munger 2007]. Evidence remains mostly thin, but diets high in saturated fat/low unsaturated fat and low in dietary antioxidant may influence MS risk [Ascherio and Munger 2007].

1.1.6 Diagnosing MS

MS is a clinical diagnosis, and the medical history and physical examination are the most important part in diagnosing the disease. However, Magnet Resonance Imaging (MRI) of the CNS is playing a significant role in the clinical and scientific investigation of MS. In at least 95 % of the patients conventional MRI typically show the characteristic demyelinated plaque, described as a T2 lesion in the white matter as a result of tissue damage [Compston *et al.* 2006]. During acute exacerbations the local increase of BBB permeability can be imaged using MRI after the administration of a contrast material as gadolinium (Gd) [Bruck *et al.* 1997]. Cerebrospinal fluid (CSF) analysis often shows intrathecal synthesis of immunglobulins, presented as oligoclonal band or increased IgG production and moderate pleocytosis. Positive CSF finding are defined as either elevated IgG index (in relation to serum) or two or more oliclonal bands [Polman *et al.* 2011], seen in 90 % of the MS patients [Compston and Coles 2008]. Dysfunctions of the optic nerve and spinal cord may be demonstrated by visual evoked responses (VEP) and somatosensory evoked potential (SEP) [Noseworthy *et al.* 2000].

According to the McDonald Criteria [Polman *et al.* 2011] for patients with two or more attacks who have objective clinical evidence of two or more lesions or objective clinical evidence of one lesion with reasonable historical evidence of a prior attack, no additional data are required. The criteria for diagnosis of RRMS are two or more clinical exacerbations with either objective clinical evidence or MRI showing the typically changes, or one clinical exacerbation with objective clinical evidence and MRI showing both T2 lesions and GD enhanced lesions [Polman *et al.* 2011]. Clinically isolated syndrome (CIS) is diagnosed in patients with one clinical exacerbation not fulfilling the MRI criteria for the MS diagnosis. The criteria for diagnosis of PPMS are one year of disease progression, and two ore more of the following findings; Both T2 and Gd enhaced lesions on MR of the brain, two or more T2 lesions in the spinal cord and positive CSF findings [Polman *et al.* 2011].

1.1.7 MS treatment

The medical treatment of MS consists of treatment of the acute exacerbations and several disease modifying therapies (DMTs). Acute exacerbations with moderate to serious disability are treated with high dose short-term methylprednisolone for 3-5 days to speed up the recovery, but the treatment has no influence on the occurrence of new relapses or long-term disability [Myhr and Mellgren 2009]. The goals of the DMTs are decrease the relapse rate, to arrest or slow the progression of disability and subclinical disease progression on MRI and to maintain or improve quality of life. The first line treatment consists of Interferon-β (INF-β) and Glatiramer acetate. The second line treatment consists at this moment of two agents, the Natalizumab and Fingolimod [Polman *et al.* 2006;Pelletier and Hafler 2012;Kappos *et al.* 2010]. A third line treatment with chemotherapy, mitoxantrone, is also possible. In addition several symptomatic treatments are available against pain, spasticity, mood changes, urinary/ bowel dysfunction and walking disabilities [de Sa *et al.* 2011].

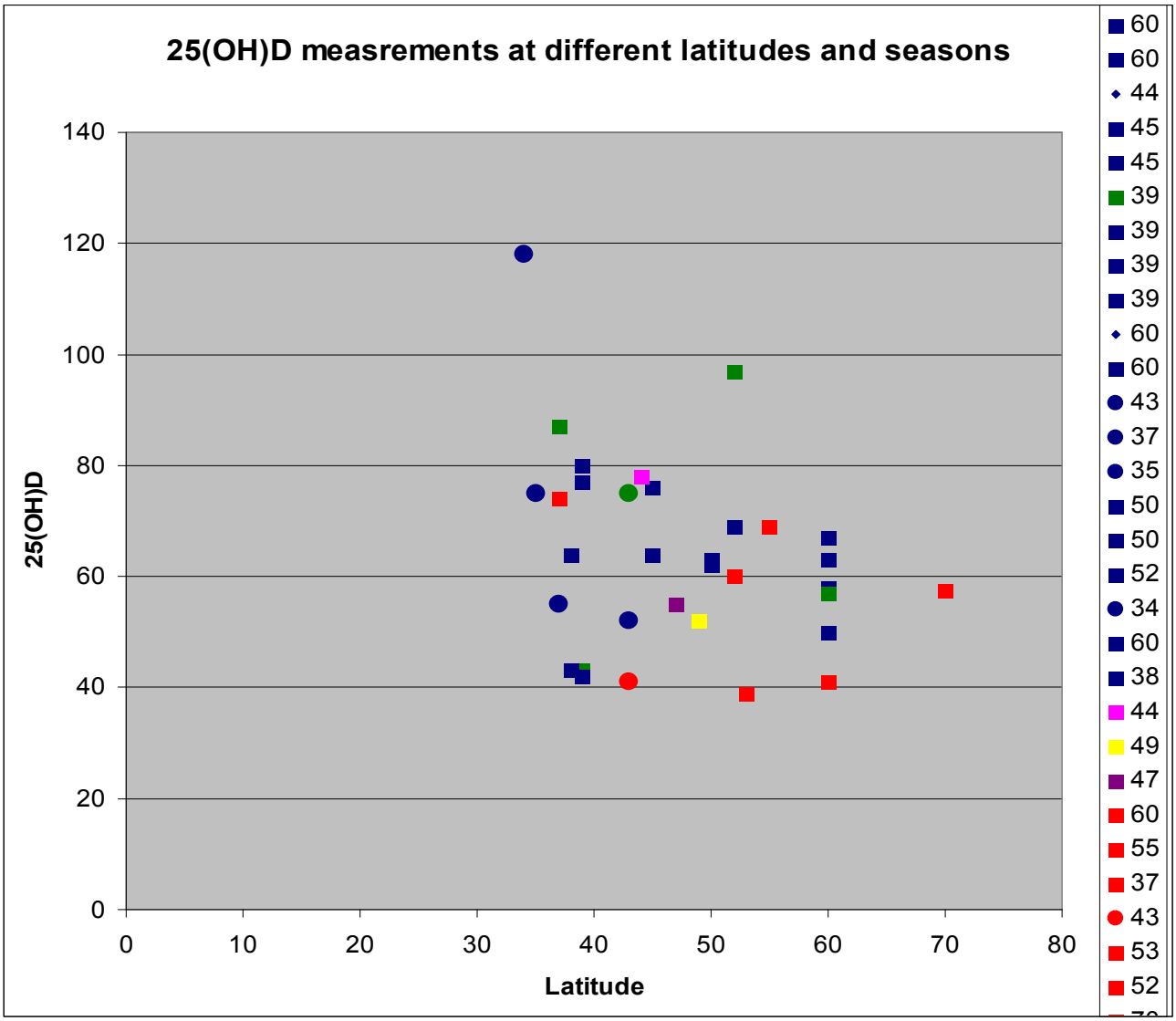
1.1.8 The clinical course of MS

Several factors have been proposed modifying the disease progression and exacerbations in MS. The relapsing form of MS is generally associated with a better prognosis and these patients are expected reaching irreversible disability later than those with a progressive disease [Confavreux *et al.* 2000;Tremlett *et al.* 2006]. MS relapse reduction during pregnancy and a rebound in the puerperium has been found [Confavreux *et al.* 1998] and the explanation has been that estrogens in high levels appear to shift the immune response from the

proinflammatory type I to the noninflammatory type II [Ascherio and Munger 2007]. However it is generally agreed that there is no difference in overall prognosis between women who have been pregnant compared with those who have not. Smoking, in addition to increase the risk of MS, also likely accelerates the progression of the disease [Ascherio and Munger 2007]. Evidence remains mostly thin but diets high in saturated fat/low unsaturated fat and low in dietary antioxidant may also influence MS progression [Ascherio and Munger 2007]. Possible due to proinflammatory responses, studies have revealed a significant association between psychosocial stress and MS exacerbations [Mohr *et al.* 2004]. However, one of the most studied prognostic factors for MS is the vitamin D, described in 1.1.9.

1.1.9 Vitamin D status in persons with MS and the effect on the clinical activity

Vitamin D status probably declines after MS onset [Munger *et al.* 2006]. Studies that measure 25(OH)D in MS patients are therefore uninformative as to whether higher vitamin D status decreases MS risk [Ascherio *et al.* 2010]. The only prospective study is a nested case-control study among more than 7 million US military personnel that found mean serum 25(OH)D level between 70.3 nmol/L and 73.5 nmol/l before MS onset and 63.3 nmol/L after MS onset [Munger *et al.* 2006]. Most observational studies comparing 25(OH)D levels in Caucasian MS patients and controls report on less than 50 individuals in each group and the reported mean 25(OH)D levels ranging from approximately 40 to 80 nmol/l [Amezcua *et al.* 2012;Barnes *et al.* 2007;Burton *et al.* 2010;Cosman *et al.* 1998;Hiremath *et al.* 2009;Holmoy *et al.* 2009;Kimball *et al.* 2007;Knippenberg *et al.* 2011;Loken-Amsrud *et al.* 2012;Lonergan *et al.* 2011;Lucas *et al.* 2011;Nieves *et al.* 1994;Orton *et al.* 2008;Ozgocmen *et al.* 2005;Pierrot-Deseilligny 2009;Runia *et al.* 2012;Simpson S Jr *et al.* 2010;Smolders *et al.* 2008b;Soilu-Hanninen *et al.* 2005;Soilu-Hanninen *et al.* 2008;Stein *et al.* 2011;van der Mei *et al.* 2007;Weinstock-Guttman *et al.* 2011;Yildiz *et al.* 2011;Triantafyllou *et al.* 2012;Baarnhielm *et al.* 2012]. The largest study to date comparing 25(OH)D levels in 1013 incident cases of MS and 1194 matched controls found mean values of 62.9 nmol/L in cases and 66.3 nmol/L in controls, a difference that is unlikely to be of clinical significance [Baarnhielm *et al.* 2012]. Figure 2 shows measured vitamin D status in relation to latitude and season.



☐ Northern latitude ○ Southern latitude

Blue: Whole year Red: Winter Green: Summer Pink: June-March Yellow: June-February Purple: February-April

Fig. 2: Measured vitamin D status in relation to latitude and season.

Associations of 25(OH)D with a.o. disability, body mass index (BMI), and sex limit comparability of results. Studies in Hispanics and African Americans report lower serum 25(OH)D than in whites, which may be explained by darker skin tones and socioeconomic and cultural differences regarding dietary sources of vitamin D [Amezcua *et al.* 2012;Gelfand *et al.* 2011]. Results of studies comparing 25(OH)D in persons with MS and healthy individuals are inconsistent: levels in persons with MS are reported to be lower than [Ozgocmen *et al.* 2005;Correale *et al.* 2009], higher than [Holmoy *et al.* 2009;Hiremath *et al.*

2009] or equal to [Soilu-Hanninen *et al.* 2008;Barnes *et al.* 2007;van der Mei *et al.* 2007] levels in controls. Levels of 25(OH)D have also been measured in CSF and there were no significant differences between CSF 25(OH)D in 36 persons with RRMS compared with persons with other inflammatory or non-inflammatory neurological diseases [Holmoy *et al.* 2009].

Some observational studies have suggested a relationship between low serum vitamin D levels and disease activity in relapsing-remitting MS, but the causality of this relationship has not been proven. Lower vitamin D levels have been reported during relapses than remission in RRMS patients [Correale *et al.* 2009;Soilu-Hanninen *et al.* 2005;Soilu-Hanninen *et al* 2008] and serum levels of 25(OH)D were associated with both relapse rate [Smolders *et al.* 2008b;Tremlett *et al.* 2008] and disability [van der Mei *et al.* 2007;Smolders *et al.* 2008b] in MS patients. Studies among veterans in the Multiple Sclerosis Surveillance registry have found that among veterans with RRMS low sun-exposure during the ages of 6-15 years was significantly associated with earlier symptom onset and intake of cod- liver at the same age was associated with later onset of MS, and among those with the progressive forms of MS low sun exposure increased the risk of disease progression and intake of cod liver oil delayed the progression [McDowell *et al.* 2011]. One report showed that children with higher serum 25(OH)D concentrations at presentation with an acquired demyelinating syndrome had a lower risk of early MS diagnosis [Hanwell H. *et al.* 2009]. Brain MRI parameters are commonly used as surrogate markers for MS disease activity. In patients with relapsing remitting MS, no correlation was found between serum 25(OH)D and MRI parameters [Soilu-Hanninen *et al.* 2008]. More indirectly, variations in relapse rate, markers of inflammation, and number of brain lesions on MRI have, with few exceptions, shown a seasonal pattern that can be related to variation in UVR exposure and vitamin D status [Tremlett *et al.* 2008;Balashov *et al.* 1998;Embry *et al.* 2000;Killestein *et al.* 2002;VanAmerongen *et al.* 2004;Gray O. *et al.* 2009]. Although the studies from other races found lower serum 25(OH)D than in whites, they did not find that the measurements varied with increasing disability [Amezcua *et al.* 2012;Gelfand *et al.* 2011]. It is known that the vitamin D receptor polymorphisms has evolved differently among populations, but how this influences vitamin D status and MS is unknown [Amezcua *et al.* 2012].

In a pilot study, vitamin D supplementation has been associated with more favourable clinical outcomes [Burton *et al.* 2010], whereas three small randomised controlled trials did not find

any beneficial clinical effects [Stein *et al. 2*011;Kampman *et al.* 2012;Soilu-Hanninen *et al.* 2012]. One of these studies reported a reduction in the number of T1 enhancing lesions on MRI in the high-dose vitamin D group [Soilu-Hanninen *et al.* 2012]. In a 28 week safety study of increasing daily doses of vitamin D (4000 to 40,000 IU cholecalciferol), the overall number of MRI lesions decreased significantly from baseline to the end of the trial [Kimball *et al.* 2007]. Effect of vitamin D supplementation on other surrogate markers of disease activity has also been reported. Supplementation with 1,000 IU cholecalciferol increased serum levels of the anti-inflammatory cytokine transforming growth factor (TGF)-β1 [Mahon *et al.* 2003]. Randomised controlled studies with high dose vitamin D supplementation, with sufficient statistical power for clinical endpoints, have not yet been published.

1.2 Osteoporosis

1.2.1 Bone physiology

Bone strength is a function of both bone density and quality. The bone must be both stiff and flexible enough to resist deformation and fracture, and it undergoes active growth, modelling and remodelling [Bonnick 2004]. The bone tasks, in addition to provide a frame to keep the body supported, are production of blood cells in the bone marrow and it constitutes the body`s store of the minerals, calcium and phosphate [Heaney *et al.* 2000]. The bone matrix consists of type I collagen stiffened by crystals of calcium hydroxyapatite. The bone cells principally consist of three different cells, the osteoblasts which are responsible for the bone matrix formulation, the osteoclasts which are the bone resorption cells, and the most numerous, the osteocytes that are osteoblasts that have been surrounded by the bone matrix that they have synthesised [Seeman and Delmas 2006]. The skeleton is composed of two types of bone [Bonnick 2004]. Cortical bone is compact bone which typically is in the long bones and the surface of flat bones. Trabecular bone is spongious bone which is primarily in the vertebral bodies, pelvis and in the distal ends of long bones, and it is the trabecular bone that contains the bone marrow, either hematopoietic or fat. The peak bone mass is achieved during the third decade [Heaney *et al.* 2000]. About three-fourths of the variance in peak bone mass is determined by genetic factors, where important involved genes are those related to the body size, (hormones, receptors and proteins involved in the growth hormone/IGF axis), to the sex steroids, to the vitamin D receptor and to several skeletal cytokines [Heaney *et al.* 2000]. Studies have identified genetic variants that regulate bone mass, including low-density lipoprotein receptor-related protein (LRP5), osteoprotegerin (OPG) and receptor activator of

NK-κβ (RANKL) [Styrkarsdottir *et al.* 2009]. The OPG/RANK pathway and its ligand (RANKL) are the most important mediators of osteoclast activity, where binding of the RANKL to RANK stimulates the activity and binding of OPG prevents RANKL binding to the RANK. LRP5 is the most important mediator of osteoblast activity and bone formation [Sandhu and Hampson 2011]. The balance between OPG/RANKL signalling and the level of active OPG regulate the bone metabolism [Kong *et al.* 2000]. The pathogenesis of osteoporosis following decline in oestrogen levels are thought to be mediated through the OPG/RANKL system [Hearn and Silber 2010]. Three hormones play a significant role in bone development: parathyroid hormone (PTH), calcitonin and vitamin D. PTH speeds up bone breakdown, calcitonin conserves calcium and vitamin D increases calcium absorption in the intestine. The bone mass accretion is also influenced by the nutrition during years of growth, and especially the intake of calcium, vitamin D, phosphorous and protein, exercise, hormonal status and smoking [Heaney *et al.* 2000]. During the fourth or fifth decade an age related bone loss starts, resulting in a progressive decline in the bone mineral density (BMD) [Poole and Compston 2006].

1.2.2 Osteoporosis

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microartitechural detoriation of bone tissue, leading to increase in bone fragility and susceptibility to fractures [Bonnick 2004;Raisz 2005]. Figure 3 shows scanning electron micrographs of the structure of L3 vertebra in a 31 year old woman (top) and in a 70 year old woman (bottom) were many of the plate-like structures have become converted to thin rods. Osteoporosis can occur because of failure to achieve peak bone mass [Sandhu and Hampson 2011;Heaney *et al.* 2000], when there is an excessive bone resorption resulting in decreased bone mass and microarchitectural deterioration, and when there is inadequate formation response to an increased resorption [Raisz 2005;Sandhu and Hampson 2011].

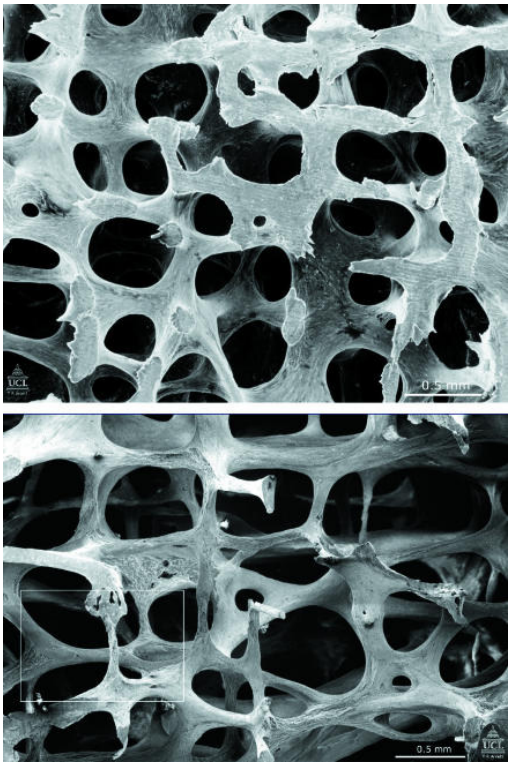


Fig. 3: The picture shows scanning electron micrographs of the structure of L3 vertebra in a 31 year old woman (top) and in a 70 year old woman (bottom). Reproduced with permission from [Poole and Compston 2006]

Figure 4 shows a diagrammatic representation of the bone mass life-line in individuals who achieve their full genetic potential for skeletal mass and those who do not. Oestrogen deficiency is critical to the pathogenesis of osteoporosis in both sexes, but postmenopausal women are at the highest risk for developing the disease [Raisz 2005]. Decreased calcium and/or vitamin D deficiency may result in secondary hyperparathyroidism and reduced mineralisation of the osteoid with low bone mass /osteoporosis as a result. Also immobility and loss of mechanical loading, cigarette smoking and alcohol consume and maternal history of hip fracture are other risk factors and possible reasons to accelerated bone loss and osteoporosis [Raisz 2005;Poole and Compston 2006]. Several underlying diseases (Endocrine, hematologic, rheumatologic and connective tissue diseases and gastrointestinal disorders, Parkinson disease, stroke with hemiplegia and Multiple Sclerosis) may lead to secondary osteoporosis [Hofbauer *et al.* 2010;Poole and Compston 2006;Kampman *et al.* 2011]. Numerous drugs (e.g. glucocorticoids, anticonvulsants, immunosuppressive agents, antidepressants and several hormonal drugs) may lead to drug-induced osteoporosis [Hofbauer *et al.* 2010]. Although low bone mass has a major role in the pathogenesis of

fracture, factors related to falling, risk of falling and protective response make important contributions [Poole and Compston 2006].

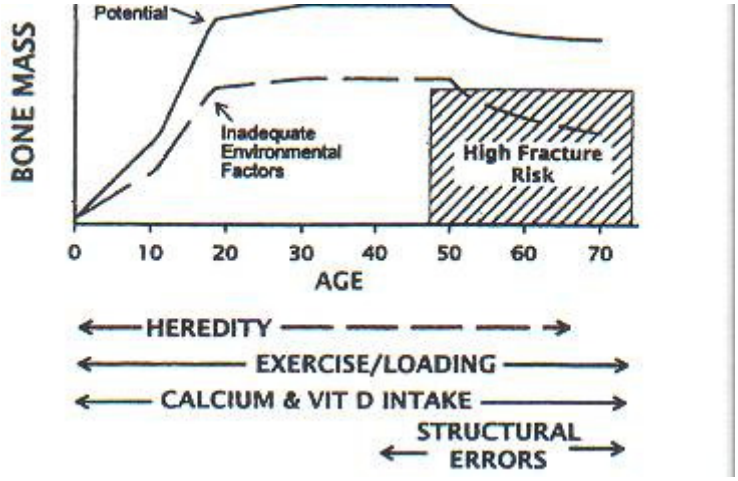


Fig. 4: Diagrammatic representation of the bone mass life-line. Reproduced with permission from [Heaney *et al.* 2000]

1.2.3 Definition of low bone mass and osteoporosis

Diagnosis of osteoporosis is based on measurements of the BMD in the spine and hip, using dual X-ray absorptiometry (DXA) which is considered the gold standard. The examination is non-invasiv and the radiation dose is extremely low. Quantitative ultrasound, based on the measurements of speed of sound is also applied. BMD is reported as a comparison to the peak population mean in sex-matched young healthy adults, the T-score, or a sex-matched and age-matched healthy population, the Z-score. The World Health Organization (WHO) has defined osteoporosis as a T-score of less or equal to -2.5 and osteopenia as a T-score between -1.0 and -2.5. The T-score criteria are used for postmenopausal women and men aged 50 years and older [Bonnick 2004;Poole and Compston 2006]. T-scores are designed to predict fracture risk in postmenopausal women. Age is an important predictor of fracture risk. At the same BMD, an older woman is more likely to have a fracture compared with a younger woman of reproductive age, most likely because of lower bone quality [Kanis 2002]. Therefore, T-scores do not predict the same fracture risk in younger women of reproductive age as they do in older, postmenopausal women. A position statement by the International Society for Clinical Densitometry recommends that Z-scores, not T-scores, should be preferred in women before menopause and males under age 50 [Leslie *et al.* 2006]. A Z-score of ≤ 2.0 is "below the expected range for age". Substitution of DXA Z-scores for T-scores gave significant

diagnostic disagreement and significantly fewer persons being diagnosed with low BMD [Carey *et al.* 2009]. For premenopausal women and men younger than 50 years of age the diagnosis of osteoporosis requires in addition to Z-score of ≤ 2.0, the presence of a fragility fracture or a recognized risk factor for secondary osteoporosis [Writing group for the ISCD position development conference 2004].

1.2.4 Prevention and treatment of osteoporosis and fractures

The primary risk of osteoporosis is fragility fracture, and the degree of mortality and morbidity related to the fracture are dependent on the localisation and the severity of the fracture. Hip fractures have the most serious impact, with high mortality and morbidity, spinal fractures are associated with pain and loss of height and both cause loss of mobility and autonomy which represent a loss of quality of life [Sambrook and Cooper 2006]. Most fractures will occur in the relatively large group of persons with osteopenia, rather than in the smaller group with osteoporosis, although the risk of fracture is higher in the osteoporosis group [Siris *et al.* 2004]. Both prevention and the non-pharmacological intervention in treatment of osteopenia and osteoporosis includes general lifestyle factors, such as a balanced diet containing calcium and vitamin D, smoking cessation and avoidance of heavy alcohol use and regular exercise including weight-bearing and muscle strengthening exercise [Sandhu and Hampson 2011]. Exercise programmes incorporating balance, gait and strength training do reduce risk and rate of falls [Gillespie *et al.* 2009]. The basis of all osteoporosis treatment is supplementation with calcium and vitamin D, in addition pharmacological intervention includes medications that inhibit the bone resorption and stimulate the bone formulation [Poole and Compston 2006]. Oestrogen hormone replacement is regarded as second line therapy because the fracture prevention is outweighed by the higher risk of breast cancer, coronary heart disease, stroke and thromboembolism [Poole and Compston 2006;Sandhu and Hampson 2011]. Evidence does not support treatment with calcium and vitamin D alone, except in institutionalised elderly people and people that have low levels in the blood [Gillespie *et al.* 2009;Poole and Compston 2006].

1.3 Vitamin D

1.3.1 Vitamin D sources

Vitamin D generally refers to two fat soluble prohormones, vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) [O'Mahony *et al.* 2011]. The dietary intake of vitamin D is

usually limited, but vitamin D_3 is found in fatty fishes, raw egg yolk, and vitamin D_2 comes from yeast and plants [O'Mahony *et al.* 2011;Diehl and Chiu 2010]. Both vitamin D_2 and vitamin D_3 may also be obtained by fortified foods like milk, butter, yogurt and bread [O'Mahony *et al.* 2011] and by vitamin D supplements, like cod-liver oil. Since vitamin D_2 metabolites make up only a minor part of the total circulating vitamin D content of the body [Heaney *et al.* 2011], vitamin D_3 is presumably the most relevant. The main source of vitamin D for most people is vitamin D_3 generated by photosynthesis in the skin in which UVR converts cutaneous 7-dehydoxycholesterol into pre-vitamin D_3, which is quickly converted by heat isomerisation to vitamin D_3 [Pearce and Cheetham 2010;Diehl and Chiu 2010]. Cutaneous syntheses of vitamin D depend on pigmentation, age, gender clothing and sunscreen use, in addition to latitude and local weather conditions [Rosen 2011]. The contribution from the different vitamin D sources are in addition to latitude and season dependent of living condition and outdoor activities, genetic traits and culture behaviour [Mithal *et al.* 2009]. A metanalysis has shown that measured values of 25(OH)D decreased with latitude in Caucasians, but not in non-Caucasians [Hagenau *et al.* 2009]. Because of seasonal variation of UVR, cutaneous vitamin D production may be absent during part of the winter and the length of this period increases with latitude [Brustad *et al* 2004]. In Tromsø, North-Norway at 70˚N, cutaneous vitamin D synthesis may be absent from early October to mid-March [Engelsen *et al.* 2005]. People living in areas with no vitamin D effective UVR for long periods are dependent on dietary sources of vitamin D. Sun holidays and the use of sun beds have been found to be strong predictors of their 25(OH)D levels [Brustad *et al. 2*004].

1.3.2 Vitamin D metabolism

Vitamin D are lipophilic molecules and in the circulation most vitamin D metabolites are transported bound to the vitamin D binding protein (DBP) [Jones 2008]. Once in the circulation, vitamin D is either transported to be stored in fat tissues or to the liver to be converted to 25(OH)D by one or several cytochrome P450s [Adams and Hewison 2010]. CYP2RI appears to have the highest affinity for vitamin D [Adams and Hewison 2010], and to be the most important 25-hydroxylase involved [Dusso *et al.* 2005]. Mutations in CYP2RI genes have been identified in patients with low 25(OH)D [Dusso *et al.* 2005]. The conversion of vitamin D to 25(OH)D is hardly regulated and the levels of 25(OH)D reflect the solar and dietary exposure [Rosen 2011;Dusso *et al.* 2005]. The half-life of 25(OH)D is about 15 days [Jones 2008] and because of its stability and its reflection of vitamin D from all sources, it is the preferred metabolite for measuring nutritional vitamin D status [Ross *et al.* 2011].

25(OH)D is a prohormone to the active form of vitamin D, the hormone 1,25-dihydroxyvitamin D (1,25$[OH]_2$D) [Adams and Hewison 2010], which is synthesised principally in the kidney by the enzyme 1α-hydroxylase [Dusso *et al.* 2005]. The renal 1α-hydroxylase is highly regulated due to its potent activity regulating the calcium homeostasis, and the half-life of 1,25$(OH)_2$D is only 10–20 hours [Jones 2008]. Hypocalcaemia may either directly, but especially through stimulating PTH production, enhance the synthesis 1,25$(OH)_2$D [Rosen 2011;Dusso *et al.* 2005;Adams and Hewison 2010]. Enhanced synthesis is also promoted by low phosphate concentration. The resulting increase in 1,25$(OH)_2$D promotes intestinal calcium and phosphate absorption, and mobilizes calcium and phosphate from bone matrix [Adams and Hewison 2010]. When the mineral concentration is corrected, fibroblast growth factor 23 (FGF-23) secreted by osteocytes in the bone matrix down-regulates the synthesis of 1,25$(OH)_2$D [Adams and Hewison 2010;Rosen 2011]. There is also a negative feedback regulation of PTH by 1,25$(OH)_2$D, minimizing the potential for vitamin D intoxication [Dusso *et al.* 2005]. Figure 5 illustrates the synthesis and metabolism of vitamin D. Activation of vitamin D does not only occur in the kidneys. Extrarenal hydroxylation also occurs in other tissues; bone, colon, breast, uterus, ovary, prostate, lung, pancreas, monicytes/macrophages, blood vessels and synovial cells, and parathyroid cells [Dusso *et al.* 2005;Peterlik and Cross 2005]. This system, where 1,25$(OH)_2$D locally acts as a cytokine, is not regulated by PTH, but by the supply of the substrate 25(OH)D [Adams and Hewison 2010]. The different vitamin D metabolites and especially the most potent 1,25$(OH)_2$D, requires a mechanism to attenuate its activity. Vitamin D is potentially toxic, and the toxicity results from hypercalcemia, nephrocalcinosis, aortic calcification and other unwanted deposits of calcium and phosphorous in soft tissue [DeLuca 2008]. When adequate amounts of vitamin D is available, both 1,25$(OH)_2$D and 25(OH)D are catabolised to biologically inactive, water-soluble calcitroic acid [Holick 2007] and this is carried out in virtually al target cells [Dusso *et al.* 2005].

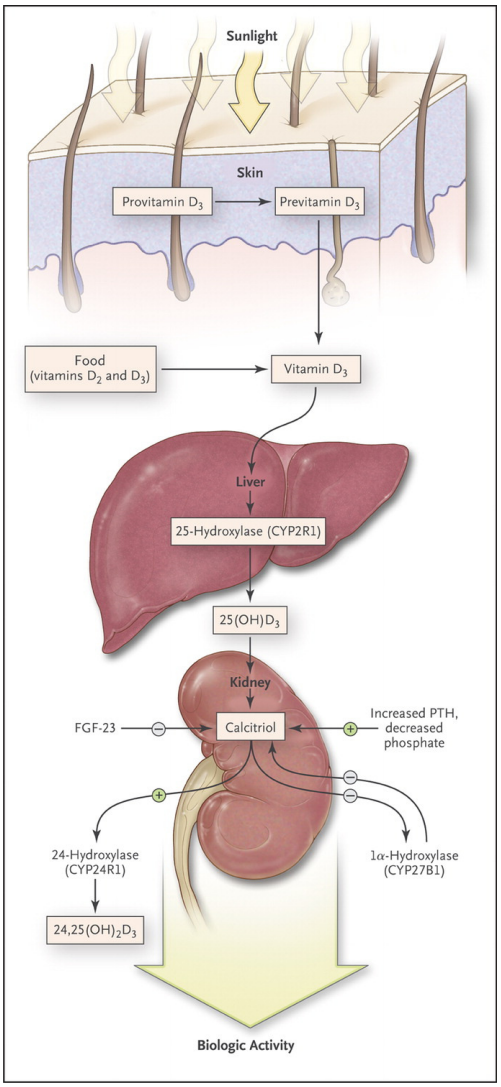


Fig. 5: Synthesis and Metabolism of Vitamin D. Reproduced with permission from [Rosen 2011], Copyright Massachusetts Medical Society

1.3.3 Vitamin D effects

Most of the biological activities of vitamin D require a VDR on the target cells, which acts as a ligand-activated transcription factor and regulates transcription of vitamin D- responsive genes [Dusso *et al.* 2005]. Such VDR are found in many genes, mainly related to bone metabolism, but 1.25$(OH)_2$D is also involved in controls of more than 200 genes responsible for regulation of cellular proliferation, differentiation, apoptosis and angiogenesis [Holick 2007]. The main effect is in the vitamin D endocrine system, where vitamin D acting as a hormone is an essential component maintaining and regulating the mineral homeostasis and especially keeping extracellular calcium levels within narrow limits: a process vital for normal cellular physiology and critical for skeletal mineralisation [Dusso *et al.* 2005;Rosen

2011]. 1.25$(OH)_2$D is also a potent immunomodulator, especially modulating the immune response to invading microbial agents [Adams and Hewison 2010]. Essentially al published studies report inhibition of Th1-associated cytokine production and a modulation of the Th2 response, affecting cytokines such as IL-4, IL-5 and IL-13 [Lange *et al.* 2009]. It has also been shown to upregulate T-regulatory cells, leading to an increase in the anti-inflammatory cytokine IL-10 [Lange *et al.* 2009]. In the past years, attention has also turned to non-skeletal effect of vitamin D, particular in relation to cardiovascular disease, cancer and immune dysfunction [Pearce and Cheetham 2010;Holick 2007].

1.3.4 Vitamin D levels and intake

Vitamin D deficiency is defined by 25(OH)D < 25 nmol/l [Meyer *et al.* 2006;Ross *et al.* 2011]. It may manifest as a medical condition with skeletal deformities known as rickets in children, and osteomalacia in adults. It causes pains in bone and muscles and muscle weakness. Affected children have difficulty standing and walking, and elderly more frequent falls and fractures [Holick *et al.* 2011]. The optimum level of 25(OH)D however, is more frequently debated. The US and Canadian Institute of Medicine (IOM), based on bone health outcomes, have suggested that 25(OH)D levels of at least 50 nmol/l are sufficient [Ross *et al.* 2011] and this is also the recommended 25(OH)D level by the Norwegian Directorate of Health [Meyer *et al.* 2006]. Being highly hydrophobic and tightly protein-bound, measuring serum 25(OH)D is challenging [Hollis 2008]. There are several 25(OH)D assays available, and they can mainly be divided in two groups; protein-binding assays (PBA) including competitive protein-binding (CPBA) assay and radioimmunoassay (RIA) and direct physical detection methods including high-performance liquid chromatography (HPLC) and liquid chromatography coupled with mass spectrometry (LC/MS) [Hollis 2008]. In the immunoassays, an antibody will recognize 25(OH)D, and the quantification relies on enzymatic, radioactive or electrochemilumnicent marker coupled to the antibody. The RIA have been used in the majority of the studies worldwide to define "normal" 25(OH)D levels, but both PBA may have difficulties with matrix effects and cross-reactions between the various vitamin D metabolites [Binkley *et al.* 2009]. The latest addition to automated 25(OH)D assay is from Roche Diagnostic, their test is an RIA and can be performed on their Elecsys and Cobas system [Hollis 2008]. Results from the Tromsø study have revealed an impact of smoking on the results of serum 25(OH)D analysis using this assay [Grimnes *et al.* 2010]. The HPLC separate the vitamin D_2 and Vitamin D_3 and is by most people considered the gold standard [Hollis 2008]. However, the equipment is very expensive and the through-

put is much lower than for the automated immunoassays [Hollis 2008]. To define the optimal 25(OH)D levels, several markers for skeletal and extraskeletal outcome have been used. There is an inverse association between 25(OH)D and PTH, and the 25(OH)D level needed to maximally suppress the PTH concentration has been investigated in several studies [Dawson-Hughes *et al.* 2005].Threshold points of 25(OH)D ranging from 25 nmol/l to 125 nmol/l have been reported, a recent three-face study in a cohort of 387 healthy Caucasian men and women aged 65 and above showed a rapid-change phase up to 25 nmol/l, a slow-change phase up to 70 nmol/l and a no change phase at 25(OH)D of 70 nmol/l and higher [Durazo-Arvizu *et al.* 2010]. Because of its partial dependence upon vitamin D, calcium absorption is another functional indicator of vitamin D adequacy. At 25(OH)D level of 50 nmol/l calcium absorption was found significantly reduced relative to that at 25(OH)D level of 86 nmol/l [Heaney *et al.* 2003]. Vitamin D, through its effect on the calcium metabolism, is linked to bone health outcomes (BMD, fracture, fall and muscle strength). Higher 25(OH)D have been associated with reduced rates of bone loss, falls and fracture [Dawson-Hughes *et al. 2*005] and significant positive association between 25(OH)D levels up to 90 nmol/l and BMD have been found [Bischoff-Ferrari *et al.* 2004]. Therefore some experts recommend serum 25(OH)D values of at least 70- 75 nmol/L for bone health [Dawson-Hughes *et al.* 2005;Holick *et al.* 2011]. For non-skeletal outcomes the available scientific evidence is found insufficient to support any recommendation of sufficient 25(OH)D level [Ross *et al.* 2011]. Recommended dietary intake corresponding to the recommended level of serum 25(OH)D level of 50 nmol/L is according to the IOM 600 International Units (IU) daily for ages 1-70 years, 400 IU for infants and 800 IU for ages 71 and older [Ross *et al.* 2011], while the Norwegian recommendation is 400 IU daily for ages 6-23 months and those older than 60 years and 300 IU for ages 2-60 years [Meyer *et al.* 2006]. In the report by the IOM, the limit for upper tolerable daily intake is 4000 IU and there may be reason for concern at serum levels ≥ 125 nmol/L [Ross *et al.* 2011]

1.4 Multiple sclerosis, osteoporosis and vitamin D

1.4.1 Shared pathogenic and aetiological factors for MS and osteoporosis

Low circulating serum 25(OH)D levels predispose to low BMD and osteoporosis, and in severe form it may lead to vitamin D-dependent rickets [Meyer *et al.* 2006;Ross *et al.* 2011]. The hormone 1,25(OH)D_2 is, in addition to be the main regulator of the calcium homeostasis, also a potent immune regulator [Smolders *et al.* 2008a] and through this effect

hypovitaminosis is a candidate risk factor for MS [Correale *et al.* 2009]. Since vitamin D status may be a risk factor for MS, skeletal consequences of hypovitaminosis D could be apparent from the onset of the disease [Moen *et al.* 2011b]. Osteoimminology and the immunepathogenesis of MS also share several proinflammatory cytokines (IL-1, TNF-α, IL-6, IL-11) [Altintas *et al.* 2009]. Abnormalities in the OPG/RANKL system regulating the osteoclastogensis have been reported in various immune diseases and a small study reports significantly higher OPG and RANKL in MS patients than in healthy controls [Kurban *et al.* 2008]. High serum levels of the proinflammatory cytokine, osteopontin (OPN), predict the development of osteoporosis in post-menopasusal women [Chang *et al.* 2010] but increased levels have been reported in MS brain lesions and in plasma and CSF in MS patients [Braitch and Constantinescu 2010;Vogt *et al.* 2003]. Smoking is also regarded as a risk factor for both MS and osteoporosis [Compston and Coles 2008;Poole and Compston 2006]. Limited physical activity and low level of exercise due to immobility in persons with MS may interfere with the bone mass acquisition and cause a higher bone loss than in healthy individuals. Daily use of corticosterioids during three or more months is a known risk factor for osteoporosis also in MS patients [Stepan *et al.* 2004]. Treatment with pulsed intravenous metylprednisolon does not seem to confer the same risk [Dovio *et al.* 2004;Zorzon *et al.* 2005], although Triantafyllou found that BMD measurements was negatively associated with increasing dosage of intravenous corticosteroids [Triantafyllou *et al.* 2012].

1.4.2 BMD and osteoporosis in persons with MS

Due to the relationship between immobility and osteoporosis, Nieves and coworkers first proposed that MS patients may be at increased risk for osteoporosis and fracture. In a study of female MS patients they found that both total body bone mineral (TBBM), BMD in the lumbar spine and in the femoral neck were related to the severity of the MS [Nieves *et al.* 1994]. Later several studies have measured BMD in MS patients. A comprehensive review of 20 published studies and one unpublished study retrieved on 5 April 2011 was published by Gibson and Summers [Gibson and Summers 2011]. Five out of six case-control studies showed reduced BMD in people with MS in either lumbar spine, femoral neck or total body. Only one study found no reduction [Zorzon *et al.* 2005]. In studies comparing BMD in persons with MS with reference data, the overall picture is that BMD is reduced at the lumbar spine and hip. A recently published case-control study reports a statistically significant lower BMD at femoral sites compared to healthy controls [Sioka *et al.* 2011]. A consistent finding with a strong negative correlation with EDSS where found, and the correlation was strongest

at the femoral neck. Most studies report BMD measurements as mean average T- or Z-scores, but some studies report the prevalence of osteopenia and osteoporosis. One study of men with MS found osteopenia in 43% and osteoporosis in 38 % of the participants [Weinstock-Guttman *et al.* 2004]. In a group of 70 persons with MS on long-term treatment with glucocorticoids, 46% had osteopenia and 45% osteoporosis [Stepan *et al.* 2004]. In a small case-control study, respectively 9.6% and 38.4% of ambulatory premenopausal women with MS had osteoporotic and osteopenic BMD values, in the control group the respective values were 0% and 26.8% [Terzi *et al.* 2010]. The latest study of 119 RRMS patients, osteopenia in lumbar spine and femoral neck was present in 26% and 50%, and the number for osteoporosis was 3% and 11% respectively [Triantafyllou *et al.* 2012].

A likely explanation for the association with EDSS is the immobility and loss of mechanical loading which may accelerated bone loss [Raisz 2005;Poole and Compston 2006], however a recently published case-control study in people with CIS or MS shortly after MS onset, also found that 50.5% of the persons with CIS or MS shortly after onset had either osteopenia or osteoporosis, compared to 37.1% of the controls, even though the persons with MS had no or minor physical disability [Moen *et al.* 2011b].

1.4.3 Fracture risk in persons with MS

The incidence of fractures and the risk factors for fractures during the course of MS have been described in several studies, and the results presented until April 2011 have been reviewed by Gibson and Summer [Gibson and Summers 2011]. They describe four observational studies with several limitations where the annual fracture risk was between 1.3% and 6.2 % [Weinstock-Guttman *et al.* 2004;Troiano *et al.* 1992;Logan, Jr. *et al.* 2008;Sibley *et al.* 1991]. Only one of the studies which included 170 persons with MS reported an increased annual fracture incidence compared to the general population annual fracture incidence, which has been estimated at 3.6% by Donaldson and coworkers [Donaldson *et al.* 2008]. Case-control studies, however, suggest an increase in annual fracture incidence in persons with MS, and cross sectional data have shown an increase in fracture rate with increasing levels of disability [Gibson and Summers 2011]. A population-based case control study where one aim was to compare the history of fracture in people with CIS or MS shortly after MS onset compared to the general population found, in spite of enhanced self-reported fall tendency, no difference in the number of fractures reported [Moen *et al.* 2011a]. In a population-based cohort study in the United Kingdom, using data from the UK General Practice Research Database linked to the National Hospital Registry, they found that compared to controls, MS patients had an

almost three fold increased risk of hip fracture and a 1.4 fold increase in osteoporotic fracture in general [Bazelier *et al.* 2011]. In a case control study where the case population was retrieved from the Danish MS Registry linked to the National Hospital Discharge Register, incidence rate ratio (IRR) between MS patients and controls was 1.4, where in particular the risk of tibia fracture (IRR 3.36), femur fracture (IRR 6.66) and hip fracture (IRR 3.20) were elevated [Bazelier *et al.* 2012b]. From the same Danish register incident MS persons with MS were indentified, and the study showed that incident MS patients had an increased risk of femur/hip fracture, a risk that was almost doubled compared to controls [Bazelier *et al.* 2012a].

The increased risk of fracture in persons with MS may be caused by the underlying conditions such as hypovitaminosis D, limited physical activity, use of corticosteroids and other medication, and smoking (se section 1.4.1). In addition it is generally accepted that MS increases the risk of falling due to the symptoms of the disease including imbalance, muscle weakness, blurred vision, dizziness and cognitive impairment [Bazelier *et al.* 2012c;Moen *et al.* 2011a]. In the review by Gibson and Summers fall frequency ranged from 32% reporting ≥ two falls in the past 2 month to 64% reporting ≥ two falls in 1 year, which are high compared to fall frequency in elderly of whom approximately 30% fall annually [Gibson and Summers 2011]. Moen et al found that 20% of the persons with CIS or recently diagnosed with MS actually had reported a tendency to fall, against only 3% in controls. Propensity to fall was associated with EDSS [Moen *et al.* 2011a].

2. Aims of the study

The aim of this phase II 96 week randomized clinical trial (RCT) was to assess whether a weekly dose of 20,000 IU vitamin D_3 could prevent bone loss in fully ambulatory persons with MS age 18–50 years.

The primary objective of the study was to determine changes in BMD over the study period comparing the treatment group and the placebo group.

The secondary objectives were to assess:

- The prevalence of low BMD at baseline (compared with standard laboratory reference values)
- Which past and current exposures that were associated with baseline BMD

- The vitamin D status at baseline and its predictors
- The effect of supplementation of 20,000 IU Vitamin D_3 per week on vitamin D status
- The seasonal variations of 25(OH) vitamin D in the placebo group

3. Methods and materials

3.1 Trial design

3.1.1 Participants

This was a single centre, randomised, double-blinded, placebo-controlled, 96 week trial designed to assess the effect of supplementing 20,000 IU vitamin D_3 weekly on bone mineral density in fully ambulatory patients with relapsing-remitting multiple sclerosis. The study was conducted at the Department of Neurology, University Hospital of North Norway, the only neurology service in Troms and Finnmark, the northernmost counties of Norway. The department manages approximately 200 persons aged 18-50 years with a diagnosis of MS according to the McDonald criteria [McDonald *et al.* 2001] in a population of 250,000 people from Troms, Finnmark, and northern Nordland above the Arctic Circle. From this population, 153 patients who were assumed to be able to walk without rest for 300 m, were invited to participate. Patients were eligible for inclusion if they were 18 to 50 years of age with clinical definite MS according to McDonald criteria and EDSS score ≤ 4.5 [Kurtzke1983].

3.1.2 Exclusions criteria

Exclusion criteria were in paper I: Not able to walk 300 m; postmenopause; other autoimmune illness; history of conditions or diseases affecting bone; pregnant or lactating during the past 6 months; use of bone-active medications

Exclusion criteria were in paper II and III: As in paper I except inability to walk 500 m or more and unwillingness to use appropriate contraception. A pregnancy test (HCG in serum) was performed in all female participants at screening.

3.1.3 Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics. All participants provided written informed consent. The trial was registered in ClinicalTrials.gov (ID NCT00785473).

3.2 Randomisation and intervention

A statistician at the Clinical Research Centre, who was not otherwise involved in the study, performed the randomisation by blocks of six, stratified by sex, with a concealed, computer-generated randomisation procedure. An identification number and a randomisation number were created for each participant. All study personnel and participants were blinded to treatment assignment for the duration of the study. The active treatment was 20,000 IU vitamin D_3 (cholecalciferol) once a week, administered as a capsule of Dekristol™ (Mibe GmbH Arzneimittel, Brehna, Germany). The average cholecalciferol content measured in 10 capsules was 18,120 IU per capsule, which is within the 10% limit accepted by EU authorities. Identical placebo capsules were provided by the manufacturer of Dekristol™ (SWISS CAPS AG, Kirchberg, Switzerland). Participants were allowed to continue vitamin D supplements they used at baseline. All participants received 500 mg elemental calcium daily, administered as a chewable tablet of Weifa Kalsium™ (calcium carbonate, Weifa AS, Oslo, Norway). Participants who had gastrointestinal side effects attributed to Weifa calcium discontinued the calcium supplement if they had an estimated dietary calcium intake of ≥ 800 mg/day. If calcium intake was lower, they switched to Calcium Sandoz™ effervescent tablets (calcium lactate-gluconate and calcium carbonate, Sandoz A/S, Odense, Denmark) which were better tolerated.

3.3 Adherence and safety

The participants were offered a reminder to take the study medication by weekly mobile phone text messages. Adherence to study medication was evaluated by capsule counting (Adherence [%] = [number of capsules consumed/number of capsules that should have been consumed] x 100). The participants were considered adherent if they had taken at least 80 % of the study medication. Participants were educated regarding clinical signs of hypercalcaemia, and ionised serum calcium was determined every 12 weeks. Adverse events were assessed every 12 weeks via phone consultation, at study visits week 48 and week 96, and in the event of hospital admission. Serious adverse events and unexpected medical events were reported to the Norwegian Medicines Agency and to the Regional Committee for Medical and Health Research Ethics.

3.4 Clinical examination

Full physical examination was performed and complete neurological status from which EDSS was determined at each study visit by a neurologist. Erect height and body weight were

measured with participants wearing light clothing and no shoes. The MS functional composite (MSFC) was assessed by trained nurses [MSFC administration and scoring manual, 2001]. Strength of handgrip (kPa) was determined using a Martin vigorimeter (Elmed Inc., Addison, IL, USA). Variables that were measured at study visits or retrieved from charts are listed in table 1.

3.5 BMD measurements

Measurement of BMD by DXA was performed by trained technicians using a Lunar Prodigy advanced densitometer (Lunar Radiation Corp., Madison, WI, U.S.A.). The long-term precision was 0.26-0.28%, obtained by daily calibration of the densitometer. BMD was determined at the hip (mean of left and right total hip), the spine (anterior-posterior spine L1-L4), and the non-dominant ultradistal (UD) radius. In clinical practice, BMD is commonly measured at the hip and the lumbar spine. We added BMD at the forearm in non-dominant hand, which also is a relevant fracture site, to assess possible effects of MS on a peripheral skeletal site.

Table 1 Variables measured at study visit, retrieved from charts, or collected by questionnaire

Demographic and anthropometric	**Physical function and activity**	**MS-related**	**Childhood & adolescence (retrospective)**	**Nutritients, medications, hormones, lifestyle habits**
Age Sex Years lived above the Arctic Circle before age 20 Family history (1st degree relatives) of low energy fractures* (yes/no) Personal history of low energy fractures* (yes/no) Body mass index (BMI, kg/m^2)	Ambulatory function without rest or aid (>500 m/≤500 m) Timed 25-foot walk (sec)[2001] 10-foot timed tandem walk (sec)[Herbert 2006] Strength of grip non-dominant hand (kPa) 9-hole peg test non-dominant hand (sec)[MSFC administration and scoring manual, 2001] Leisure time physical activity†	Age at MS onset Disease duration EDSS [Kurtzke1983]	Physical activity† Consumption of milk and yoghurt (glasses or cups/d) Cod-liver oil supplement use (no/winter only/all year) Summer outdoor activities age 16-20‡ Body shape at age 15§ Eating disorder (yes/no)	Serum 25(OH)D (nmol/L) Vitamin D intake from diet and supplements (µg/d)¶ Solarium past year (yes/no) Calcium intake from diet and supplements (mg/d) ¶ Cumulative dose of intravenous methylprednisolone (g) Ongoing beta-interferon treatment (yes/no) Age at first menstruation Oral contraceptive use (years) Alcohol (U/week) Smoking (years daily smoking)

*Fracture resulting from low energy trauma (fall from standing height or less, or trauma that in a healthy individual would not give rise to fracture); † Physical activity was categorised as: (a) mostly sedentary; (b) walking or bicycling at least 4 h/week; (c) exercising at least 4 h/week; and (d) participating in competitive sports several times a week; ‡ In summer, how much did your activities (playing, sports, walking, working) take you outside? (a) not that often, (b) a moderate amount, (c) quite a lot, (d) virtually all the time [van der Mei *et al.* 2007]; §rated on nine-level figure drawing [Michels *et al.* 2007]; ¶assessed by food frequency questionnaire [Hjartaker *et al.* 2007].

3.6 Blood tests

Every 12 weeks blood was drawn and haematology, liver enzymes, ionised calcium and PTH were analysed at the Department of Clinical Chemistry, University Hospital of Northern Norway. For interpretation of these blood tests, laboratory reference ranges were used. At week 96 serum testosterone was measured in men and FSH in women. FSH values of ≥ 40 IU/L were defined as a marker for perimenopause [Harlow *et al.* 2007;Recker *et al.* 2000]. Batch analyses of baseline serum 25(OH)D were first performed using an electrochemiluminescence immunoassay on a Cobas™ analyser (Elecsys vitamin D_3 reagent kit, Roche Diagnostics GmbH, Mannheim, Germany), and these results are presented in paper I. Analyses of serum 25(OH)D measurements presented in paper II and III were performed by mass spectroscopy (coefficient of variation at 75 nmol/L was 3,5%) at the Hormone Laboratory, Haukeland University Hospital, Bergen, Norway. In the placebo group, analyses were performed at baseline (winter), at study week 60 (spring), 72 (summer), 84 (autumn) and at study end (winter). In the intervention group, serum 25(OH)D levels were only measured in winter. Serum 25(OH)D levels were classified as deficient (<25 nmol/L), insufficient (25-50 nmol/L), sufficient (≥50 nmol/L), and high (≥ 75 nmol/L). We applied the level of sufficiency as recommended by the IOM and the Norwegian Directorate of Health (50nmol/L) in statistical analyses [Ross *et al.* 2011;Meyer *et al.* 2006].

3.7 Questionnaires

The information collected by self-administered questionnaires is specified in Table I. Dietary vitamin D intake was calculated from a validated food frequency questionnaire, administrated at week 0, 48 and 96 [Brustad *et al.* 2004;Hjartaker *et al.* 2007]. Use of vitamin D-containing supplements and sun exposure during the last three months (tanning bed and sun vacation) were recorded every 12 weeks. Time spent outside during the last 7 days was reported in spring, summer and autumn, but not in winter. At 69° N the sun is below the horizon from November 20th to January 20th, and ambient UVR is not sufficient for cutaneous vitamin D synthesis from early October through mid-March [Engelsen *et al.* 2005]. Physical activity was categorised as: a) mostly sedentary, b) walking/bicycling at least 4 h/week, c) exercising at least 4 h/week or d) participating in competitive sports several times a week.

3.8 Statistical analysis

Data from BMD measurements in a comparable group of persons with MS were not available when the trial was planned, and power calculation was not performed prior to the study.

Analyses were carried out according to a pre-established analysis plan. In the patient who discontinued the study medication because of pregnancy, the last measurements (week 48) were carried forward. Comparisons between groups were made using Student's T test for continuous data, Mann-Whitney U test for data that were not normally distributed and χ^2 test/Fisher exact test for categorical variables. Pearson correlation coefficients (r) were calculated for bivariate correlations. In paper II and III the modified intention-to-treat population included all participants who received at least one dose of the study medication and presented at one follow-up visit. All statistical analyses were conducted using SPSS 15.0, 16.0 and 18.0 for Windows (SPSS Inc. Chicago, IL, U.S.A.). P-values < 0.05 were considered statistically significant.

3.8.1 Statistical tests applied in paper I

Multiple linear regression models were constructed for the dependent variables "BMD femoral neck", "BMD lumbar spine", and "BMD non-dominant UD radius". Variables introduced into regression models were those with P values < 0.25 in Pearson correlations. Five initial models were built for each site of measurement. Integrated models for each site included sex, BMI and variables that were significantly associated with BMD at the particular site of measurement in the initial models. The final models were developed considering all variables that showed significant associations in at least one of the integrated models. Logistic regression was applied to the dependent variable "low BMD", according to the same principles of modelling as described for linear regression. The high number of variables available for regression analysis in relation to the number of participants in the study carries a risk of finding spurious associations. Formal corrections for multiple measurements were not applied, but results were interpreted with caution when P values were between 0.05 and >0.005. Where judgments of statistical significance were made, the biological feasibility and consistency of the findings were taken into account.

3.8.2 Statistical tests applied in paper II

Distribution and variance of BMD and vitamin D values were assessed by appropriate tests. Interactions between sex and group were tested for. The main outcome, percentage of change in BMD, was analysed by independent sample T-test. Changes in BMD from baseline to week 96 were analysed by paired samples T-test. A preplanned multiple regression model included the variables age, body mass index (BMI), baseline BMD, baseline 25(OH)D, percentage

change in 25(OH)D, and perimenopausal FSH values. Subgroup analyses were performed by sex and by treatment group.

3.8.3 Statistical tests applied in paper III

Comparisons of baseline characteristics were performed between treatment groups, between dichotomised 25(OH)D levels (< or ≥ 50 nmol/L at baseline), and between women and men. Univariate analyses of associations were performed for baseline 25(OH)D values and baseline characteristics. Predictors of 25(OH)D on population level, age, BMI and sex, as well as possible predictors of 25(OH)D values (p value <0.25 in univariate analysis) were included in linear regression models. The paired Student's T-test was used to compare mean 25(OH)D values at baseline and at study end in both treatments groups, and between winter and summer values in the placebo group.

4. Summary of the results

4.1 Paper I and paper I erratum

In this paper we present the predictors and the prevalence of low bone mineral density in fully ambulatory persons with multiple sclerosis. 80 participants were included in the analysis. 24% (20% women and 34% men) had low BMD (z-score ≤ -2.0) in one or several measured sites. Mean BMD for women and men at femoral neck, lumbar spine and UD radius are shown in figure 6. BMD at femoral neck was significantly negatively associated with age, male sex, slower 10-foot timed tandem walk and positively associated with BMI, walking distance > 500 m and physical activity growing up. In female participants there was also a positive association with oral contraceptive use. According to BMD at the lumbar spine we found the same associations, except for age, slower 10-foot timed tandem walk and use of oral contraceptives. BMD at the non-dominant UD radius was positively associated with BMI, strenght of grip, age at MS onset and summer outdoors activity at age 16-20 year. Serum 25(OH)D levels were not correlated with BMD at any site.

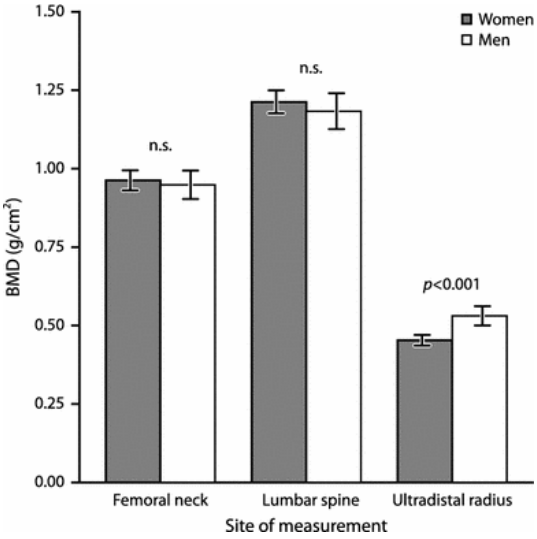


Fig. 6: Bar chart showing BMD for women and men at three sites of measurements. Error bars represent 95% confidence intervals.

4.2 Paper II

In this paper we report the results with regard to the main objective and the aim of this study whether a weekly dose of 20,000 IU vitamin D_3 could prevent bone loss in fully ambulatory persons with MS age 18–50 years. Figure 7 shows the flow of the participants in the study. Percentage change in BMD from week 0 to week 96 did not differ between participants who had received vitamin D_3 supplementation and those who had received placebo. This result was not altered by adjustment for baseline serum 25(OH)D or change in serum 25(OH)D over the study period. There were no significant associations between percentage change in BMD and possible predictors of BMD (sex, age, BMI, baseline BMD, baseline serum 25[OH]D, change serum 25[OH]D, serum testosterone in men at week 96, and EDSS). One participant in each treatment arm experienced an unexpected medical event that was not related to the study medication and one participant in the placebo group discontinued the study medication at week 94 due to nephrolithiasis and ionised calcium (iCa) during the study did not exceed the upper limit of reference values (1.34 mmol/L) in any of the participants.

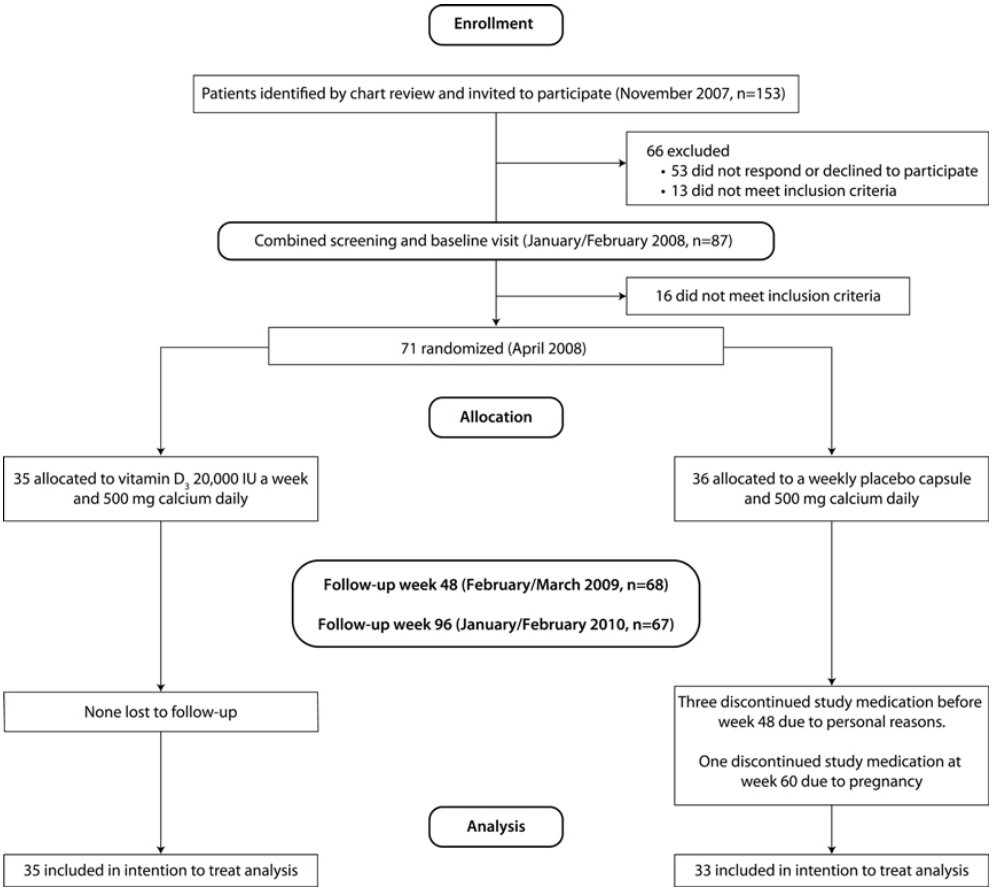


Fig. 7: Flow of the participants through the study

4.3 Paper III

In this paper we report vitamin D status and its predictors in persons with MS living at high northern latitude (68˚-71˚). At baseline during winter 10% of the patients were vitamin D deficient (serum 25[OH]D <25 nmol/L), 40% were deficient (serum 25[OH]D between 25 and 49 nmol/lL), 29 had sufficient levels (serum 25[OH]D between 50 and 74 nmol/lL) and 21% had high levels (serum 25[OH]D >74 nmol/L). Mean serum 25(OH)D was 56 nmol/L and was positively predicted by sun vacation last three months, tanning bed last three months and total vitamin D_3 intake. Mean total daily intake of vitamin D_3 for the participants with serum 25(OH)D< 50 nmol/l was 6.5 µg (95% CI 5.2;7.8) (260 IU [95% CI 208;312]) and for the participants with serum 25(OH)D≥ 50 nmol/l 13.3 µg (95% CI 9.8;16.9) (532 IU [95% CI 392;676].

In the placebo group we found a seasonal variations in serum 25(OH)D levels with a mean 25(OH)D level during baseline winter at 58 nmol/L that raised to 87 nmol/L during summer, and the proportion of the participants with sufficient 25(OH)D levels (≥ 50 nmol/L) increased

from 55% in winter to 92% during summer. In the intervention group mean serum 25(OH)D increased from 56 nmol/L at baseline to 123 nmol/L at study end. This represents a mean increase of 2.3 nmol/L per 100 IU vitamin D_3 daily supplement, and all participants achieved sufficient vitamin D levels (serum 25[OH]D > 50 nmol/L) at study end and 91% had serum 25(OH)D values ≥ 75 nmol/L. Figure 8 shows the seasonal variation in serum 25(OH)D in the placebo group and at study start and study end in the intervention group.

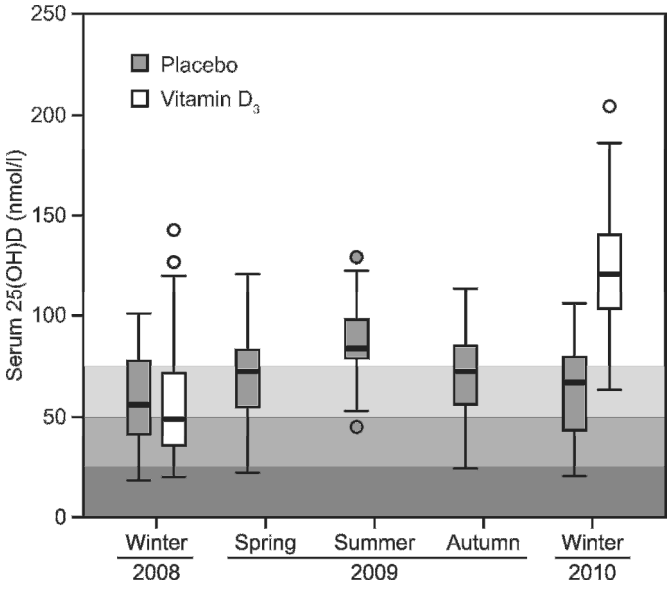


Figure 8 Seasonal variation of serum 25(OH) D levels in the placebo group and serum 25(OH)D levels at baseline and at study end in the intervention group.

5. Discussion

5.1 General discussion

This study shows that low BMD was prevalent at baseline, and a weekly dose of 20,000 IU vitamin D_3 administrated for 96 weeks could not prevent bone loss in fully ambulatory persons with MS age 18-50 year. Findings did not vary by sex and were not altered after adjustment for serum 25(OH)D. MS-related variables, as well as lifestyle exposure during growing up, differentially affect BMD at three clinically important sites: the femoral neck, the lumbar spine and the UD radius. At the study start in winter, mean serum 25(OH)D was 56 nmol/l, and half of the participants had sufficient 25(OH)D levels (≥ 50 nmol/l). Winter serum 25(OH)D levels were strongly predicted by sun exposure through sun vacation or solarium and by vitamin D intake. In the placebo group, the proportion of study participants with

sufficient levels of vitamin D increased from 55% during winter to 92% during summer. All participants in the intervention group achieved sufficient (≥ 50 nmol/l) winter levels of serum 25(OH)D.

5.1.1 Paper I

At the combined screening and baseline visit, low BMD (z score ≤−2) was present in 19 out of 80 participants. Because BMD follows a Gaussian distribution, low BMD (z score ≤−2.0) would be expected to be present in approximately 2.5% of premenopausal women and men below age 50 [Lewiecki 2005]. Prevalence of low BMD was much higher in our study, where almost 25% of the participants at screening had low BMD at one or more sites. Previous studies reporting osteopenia (t-score between -1.0 and -2.5) and osteoporosis (t-score ≥ -2.5) present even higher number [Altintas *et al.* 2009;Stepan *et al.* 2004;Weinstock-Guttman *et al.* 2004;Moen *et al.* 2011b;Triantafyllou *et al.* 2012]. We further demonstrate that MS-related variables as well as past exposures differentially affect BMD at three clinically important skeletal sites: the femoral neck, the lumbar spine, and the UD radius. BMI is a strong and well known predictor of BMD, and we found that BMI was positively associated with BMD at femoral neck, lumbar spine and UD-radius. Most published studies have reported BMD without adjustment for BMI, but in a recently published study by Sioka et al, BMI was a significant independent variable of BMD values in lumbar spine and femoral neck in persons with MS [Sioka *et al.* 2011].

BMD at the femoral neck and lumbar spine were associated with current ambulatory function and physical activity during growth, indicating a major effect of exercise and mechanical loading at these skeletal sites. In a review by Gibson and Summers a consistent finding was a strong negative correlation between EDSS and BMD level, where the correlation was strongest at the femoral neck [Gibson and Summers 2011]. However, we found that ambulatory function (>500 m/≤500 m) resulted in a better fitting model than EDSS at the femoral neck and lumbar spine. We also found that the 10-foot timed tandem walk was independently associated with BMD at the femoral neck. This test reflects mild ambulatory impairment, related to impaired balance, better than walking distance. Persons with impaired balance may engage in less physical activities with high mechanical loading that are crucial to maintain BMD. BMD was not associated with the present level of physical activity. This is in agreement with a population-based study showing that physical activity during growth, but not current physical activity influenced BMD [Bainbridge *et al.* 2004]. However, high-impact exercise has been shown to significantly increase BMD at the hip in a randomized controlled

trial [Vainionpaa *et al.* 2005]. Age was negatively associated with BMD only at the femoral neck, the site at which bone loss starts first [Vondracek *et al.* 2009].

BMD at the UD radius, a clinically relevant site, has not previously been explored in persons with MS. We found a strong association with strength of grip at this site, an association that also has been demonstrated in healthy individuals [Aydin *et al.* 2006]. Arm dominance has a pronounced effect on bone density, especially if the individual is involved in any type of repetitive unilateral activity. Therefore measurements from the non-dominant arm are used in studies [Bonnick 2004]. Summer outdoor activities age 16–20 were associated with BMD at the UD radius. Interestingly, this measure, a proxy of vitamin production in the skin [van der Mei *et al.* 2006], was associated with MS risk in the same population [Kampman *et al.* 2007]. The difference in variables predicating BMD in UD radius compared to those at the femoral neck and lumbar spine may be a consequence of the UD radius not being weight-bearing, which might result in increased susceptibility to other influences.

Peak bone mass is higher in men than in women [Poole and Compston 2006]. To our surprise, male rather than female sex showed a possible negative association with BMD at the femoral neck and lumbar spine. In addition, at the UD radius, BMD values were only 18% higher in men than in women, compared with the 28% difference reported in a population-based study in the same geographical area [Emaus *et al.* 2005]. Previous studies reporting BMD values for both men and women with MS found results similar to ours, but these findings were not commented on by the authors [Cosman *et al.* 1998;Stepan *et al.* 2004;Tuzun *et al.* 2003]. A recent published case control study report that in men with MS the mean BMD at both lumbar spine and femoral sites were lower by about 8 % than in the healthy controls [Sioka *et al.* 2011].

A causality is established between serum 25(OH)D and skeletal outcomes, including BMD [Ross *et al.* 2011], but in our study population BMD was not associated with serum 25(OH)D. However none of the participants were vitamin D deficient (25(OH)D <25 nmol/L). A correlation of vitamin D status with low BMD in persons with MS was found in patients who had lower serum 25(OH)D levels and vitamin D intake and higher EDSS scores and age than ours [Nieves *et al.* 1994]. Other studies did not find significant associations of vitamin D status with BMD in persons with MS [Cosman *et al.* 1998;Ozgocmen *et al.* 2005;Weinstock-Guttman *et al.* 2004;Triantafyllou *et al.* 2012]. Published studies of persons with MS probably lack the statistical power to confirm the association between serum 25(OH)D and BMD that has been described in healthy individuals [Ross *et al.* 2011;Dawson-Hughes *et al.* 2005].

Treatment with pulsed intravenous methylprednisolone did not affect BMD, which is in agreement with most [Nieves *et al.* 1994;Schwid *et al.* 1996;Tuzun *et al.* 2003;Weinstock-Guttman *et al.* 2004;Zorzon *et al.* 2005;Moen *et al.* 2011b;Sioka *et al.* 2011], but not all [Formica *et al.* 1997;Ozgocmen *et al.* 2005;Triantafyllou *et al.* 2012] previous studies. Interferon-beta treatment induces changes in multiple proteins and mRNAs related to bone homeostasis in MS patients [Weinstock-Guttman *et al.* 2006]. We did not find any association between BMD and Interferon treatment, which is supported by the results from two other recently published studies [Triantafyllou *et al.* 2012;Moen *et al.* 2011b]. Cigarette smoking and alcohol consumption are known risk factors for low BMD [Raisz 2005;Poole and Compston 2006], but in this small study we could not find such association and neither did to other studies [Moen *et al.* 2011b;Sioka *et al.* 2011]. Personal and family fracture history were neither not associated with BMD.

5.1.2 Paper II

Paper II describes the effect of supplementing 20,000 IU vitamin D_3 or placebo on the BMD measurements. There was no difference in change in BMD comparing the to treatment groups. In paper II we presented the change in the BMD measurements between baseline and study end, separately in each group. We found a significant bone loss in the hip in the placebo group and not in the intervention group, and a significant bone increase in the UD radius in the intervention group and not in the placebo group. Such within group analysis is not recommended in RCTs. However in the whole study population mean BMD in the hip decreased significantly by 1% (Paired samples T-test, $P<0.01$). In healthy individuals, studies have reported no significant bone loss at the hip in women before the perimenopausal years [Berger *et al.* 2010;Berger *et al.* 2008;Chapurlat *et al.* 2000;Henry *et al.* 2010;Neer 2010;Recker *et al.* 2000], while men may or may not have a small bone loss before age 50 [Berger *et al.* 2010;Emaus *et al.* 2009;Henry *et al.* 2010]. The general bone loss at the hip, a weight bearing site, may result from decreased physical activity even in fully ambulatory persons with MS [Pugliatti *et al.* 2008b], due to fatigue, visual problems, and impaired balance. We observed no change in BMD at the lumbar spine. This finding is in agreement with two recent studies reporting unchanged BMD at the lumbar spine over a period of respectively 96 weeks and 39 months in interferon beta-treated persons with MS [Ravnborg *et al.* 2010;Sorensen *et al.* 2009]. At the UD radius the mean BMD increased significantly by 1.8% (Paired Samples T-test, $P=0.01$). In healthy individuals, BMD at this site has been

reported to be stable or increasing up to age 40 in both sexes [Chapurlat *et al.* 2000;Emaus *et al.* 2009;Henry *et al.* 2010]. BMD at the UD radius, especially in the non-dominant arm, is not dependent on weight bearing activity and therefore is likely to be less affected by mild to moderate MS-related disability.

There is good evidence that vitamin D supplementation improves bone health [Bischoff-Ferrari *et al.* 2009;Cranney *et al.* 2008]. A meta-analysis concluded that the effect of vitamin D supplementation on bone health was greater in persons with low (<25 nmol/l) serum 25(OH)D than those whose serum level was normal [Tang *et al.* 2007]. Only three individuals in the intervention group of this study were overtly vitamin D deficient at baseline (25(OH)D <25 nmol/L), and participants were allowed to continue vitamin D supplements they used at baseline, both of which could have contributed to the negative findings in this trial. In women, baseline BMD at the hip was significantly higher in the intervention than in the placebo group, which may also have influenced the result of the study.

5.1.3 Paper III

In paper III we report vitamin D status and its predictors in persons with MS living at high northern latitude and the effect of weekly supplementation with 20,000 IU vitamin D_3 for 96 weeks on 25(OH)D levels. At study start mean serum 25(OH)D was 56 nmol/L and serum 25(OH)D levels were strongly predicted by sun exposure through sun vacation or tanning bed and by vitamin D intake. Half of the participants had insufficient 25(OH)D levels (<50 nmol/L) during winter, even though their mean total daily intake was at 260 IU (95% CI 208;312) which is close to the recommend daily dose (300 IU) by the Norwegian Directorate of Health [Meyer *et al.* 2006]. In the placebo group, the proportion of study participants with sufficient levels of vitamin D increased from 55% during winter to 92% during summer. All participants in the intervention group achieved sufficient winter levels of serum 25(OH)D. During the winter months with insufficient solar UVR to induce cutaneous vitamin D production [Engelsen *et al.* 2005], mean serum 25(OH)D was 56 nmol/L. This is comparable with measurements performed in fair skinned persons with MS during winter at lower latitudes, with reported values in the range of 40 to 80 nmol/L [Amezcua *et al.* 2012;Barnes *et al.* 2007;Burton *et al.* 2010;Cosman *et al.* 1998;Hiremath *et al.* 2009;Holmoy *et al.* 2009;Kimball *et al.* 2007;Knippenberg *et al.* 2011;Loken-Amsrud *et al.* 2012;Lonergan *et al.* 2011;Lucas *et al.* 2011;Nieves *et al.* 1994;Orton *et al.* 2008;Ozgocmen *et al.* 2005;Pierrot-Deseilligny 2009;Runia *et al.* 2012;Simpson *et al.* 2010;Smolders *et al.* 2008b;Soilu-Hanninen *et al.* 2005;Soilu-Hanninen *et al.* 2008;Stein *et al.* 2011;van der Mei *et al.*

2007;Weinstock-Guttman *et al.* 2011;Yildiz *et al.* 2011;Triantafyllou *et al.* 2012;Baarnhielm *et al.* 2012]. A meta-analysis of studies in healthy subjects all over the world during all seasons reported mean serum 25(OH)D levels of 54 nmol/L [Hagenau *et al.* 2009]. Most observational studies comparing 25(OH)D levels in MS patients and controls report on less than 50 individuals in each group and results are inconsistent [Barnes *et al.* 2007;Correale *et al.* 2009;Hiremath *et al.* 2009;Holmoy *et al.* 2009;Ozgocmen *et al* 2005;Soilu-Hanninen *et al.* 2008;van der Mei *et al.* 2007;Triantafyllou *et al.* 2012]. The largest study to date comparing 25(OH)D levels in 1013 Swedish cases of MS and 1194 matched controls found mean values of 62.9 nmol/L in cases and 66.3 nmol/L in controls, a difference that is unlikely to be of clinical significance [Baarnhielm *et al.* 2012].

During winter, the time of the year with no natural sun exposure at 69˚N, we observed that 25(OH)D levels were predicted by UVR exposure through sun vacation or tanning bed use as well as by vitamin D intake. Sufficient vitamin D levels (≥50 nmol/L) were measured in 74% of the participants who at baseline either had had sun exposure during the last three months or who were taking a vitamin D supplement. Only 25% of those who neither had had sun exposure nor were taking a vitamin D supplement were vitamin D sufficient. An association between winter 25(OH)D levels and sun vacation or tanning bed use has previously been reported in Northern Norway [Brustad *et al.* 2004], and an association between serum 25(OH)D levels and sunlight exposure has been described in persons with MS [Simpson *et al.* 2010;van der Mei *et al.* 2007]. Most humans are not dependent upon dietary vitamin D supplies for vitamin D sufficiency [Hollis 2005]. In our study, only 38% of the participants not supplementing vitamin D were vitamin D sufficient during winter, while 69% of those who used supplements containing at least 100 IU vitamin D3 had 25(OH)D values in the sufficient range. This finding is supported by a study in persons with MS from Ireland [Lonergan *et al.* 2011], but not by a study from Australia [Simpson *et al.* 2010;van der Mei *et al.* 2007]. However, supplementation with vitamin D_2 (rather than vitamin D_3) was common in Australia at the time [van der Mei *et al.* 2007], and the contribution of UVR to vitamin D status can be assumed to be larger than in Norway and Ireland. We did not find inverse relationship between serum 25(OH)D level and BMI as has been reported in larger studies [Rosen 2011].

In the placebo group, we analysed seasonal variation in measurements of serum 25(OH)D levels. The mean 25(OH)D level was significantly higher during the summer (87 nmol/L) than during the winter (58 nmol/L), and the proportion of study participants with sufficient levels of vitamin D (≥ 50 nmol/L) increased from 55% during winter to 92% during summer.

Similar seasonal variations have been reported in other studies of MS patients with minimal disability [Soilu-Hanninen *et al.* 2008;van der Mei *et al.* 2007]. In contrast, little or no seasonal fluctuations were observed in more disabled patients [van der Mei *et al.* 2007]. Immobility limits outdoor activities and heat intolerant patients avoid direct sun exposure, both of which reduce the contribution of cutaneous vitamin D production to vitamin D status. In the intervention group, supplementation with 20,000 IU vitamin D_3 per week for 96 weeks raised serum 25(OH)D by 68 nmol/L to 123 nmol/L. This is an increase of 2.4 nmol/L per 100 IU vitamin D_3 daily supplement, which is close to the increase of 2.5 nmol/L per 100 IU vitamin D_3 that has been described earlier [Rosen 2011]. All participants achieved the recommended 25(OH)D levels (≥50 nmol/L) for healthy subjects [Ross *et al.* 2011]. Serum 25(OH)D values of at least 75 nmol/L, as recommended for good bone health [Dawson-Hughes *et al.* 2005], were achieved by 91 % of the participants. Vitamin D_3 supplementation, at a dosage 30% below the tolerable upper intake levels of 28.000 IU per week [Ross *et al.* 2011], was well-tolerated with no clinical or biochemical side effects.

5.2 Methodological aspects of the study

5.2.1 Bias and confounding

Bias is a systematic error, which might result in incorrect estimates. It tends to influence on the results and move the results away from the "truth" and it reduces the validity of the results. In the results of the RCT, selection bias was avoided by having a representative source population and a concealed randomization. Performance bias (systematic difference of care other than the intervention of interest) and detection bias (Systematic difference in outcome assessment) was avoided by the blinding of all personnel in the study. Compliance and attrition bias is systematic difference in compliance and in withdrawals between the groups. In this the study the adherence to medication was > 80% and the withdrawal was only 3 %. To avoid the possible effect of this on the results we analyzed by modified intention to treat. Recall bias is a possible limitation of our study, in particular to the results presented in paper I and III. Assessing vitamin D intake from diet and supplementation and registration of outdoors activity growing up were performed by validated questionnaires [Hjartaker *et al.* 2007;van der Mei *et al.* 2006]. The validation of variables regarding physical activity growing up and at present, sun bed use and sun vacation last three month, personal and family history of fracture and smoking, may be limited by recall bias. Confounding is a hidden variable influencing both the outcome and the exposure, resulting in an observation of a non-causual

association. It is more likely to occur in observational than in experimental studies. In paper II, analyzing the RCT, confounding was not regarded as a problem. In paper I, and partly paper II, however, we report the results of a cross-sectional study at screening, which only gives a moment of time picture and there may be confounding variables which implies that causal relations from association cannot be drawn. To limit the possibility of confounding we performed multivariate regression analysis.

5.5.2 Random errors

A type I error means reporting a difference which is false. In paper I and III the high number of variables available for analysis in relation to number of participants carried a risk of finding spurious association. Since all analyses were on secondary outcomes, formal correction for multiple measurements was not applied, but results were interpreted with caution when p-values were between 0.05 and 0.005. Where judgments of statistical significance were made, the biological feasibility and consistency of the findings were taken into account. In paper II a type I error affecting the primary outcome of our study would mean that we falsely claimed an effect of vitamin D supplementation on BMD even there was no treatment effect. To avoid this, analyses were performed in a preplanned multiple regression model and a strict statistical criterion was predefined to assess when a finding should be regarded significant and in that paper we set the significance level at 0.05. A type II error means claiming there is no difference between groups or no effect of a treatment, even if there is such effect. This may be avoided by doing pre study power analysis to ensure enough participants in each arm to be able to detect a possible difference between the groups. The major limitation of this study is the small number of participants. Data from BMD measurements in a comparable group of persons with MS were not available when the study was planned.

5.2.3 External validity of the results

The generalisability of the study findings depends on whether the source population is representative also of other populations. In this study all eligible persons with MS from a homogeneous and stable population in a defined geographical area where invited to participate, and the response rate was 65%. Selection bias may be a limitation in our study due to the fact that volunteers in a population based study often are more interested in health issues and may represent a healthier population than those not responding on the invitation. It may also be that the more disabled persons with MS were less likely to participate, but assuming this, the associations of physical function with BMD are rather under- than

overestimated. In addition, persons who probably represent those at highest risk of developing osteoporosis, those with medications or concomitant conditions known to be associated with secondary bone loss, were excluded from this study, thus limiting the external validity of the results. The main outcome, BMD, is a surrogate outcome for fracture risk and the trial protocol supplementing 20,000 IU vitamin D weekly which varies greatly from the routine practice in Norway, both of which reduce the validity of the results. The absence of associations with several known risk factors for low BMD [Bainbridge *et al.* 2004;Lewiecki 2005;Poole and Compston 2006;Vondracek *et al.* 2009] may be ascribed to the small number or the young age of participants in this study. Thus, negative results should not be interpreted in the sense that general risk factors are not relevant in persons with MS.

6. Implications for further research

6.1 Bone loss and vitamin D supplementation

In this phase II trial we did found that a weekly dose of 20,000 IU vitamin D_3 administrated for 96 weeks could not prevent bone loss in fully ambulatory persons with MS age 18-50 year. When the trial was planned power analysis was not performed due to lack of data on BMD change in young ambulatory persons with MS. Even though we invited all eligible persons managed by the Neurological department University Hospital in North-Norway with MS to participate in this study, we were not able to include enough participants in each treatment arm. Based on change in BMD from this study, a trial with approximately 250 persons in each treatment arm will be needed to confirm or reject the hypothesis that a weekly dose of 20,000 vitamin D for 96 weeks could prevent bone loss in persons with MS (two tailed, power 80%, risk of type I error 5% and drop out rate 10%). Adequately powered long-term studies should be performed before drawing a final conclusion.

6.2 Vitamin D levels

Optimising vitamin D status may favorably affect the disease course of MS [Correale *et al.* 2009;Smolders *et al.* 2008b;van der Mei *et al.* 2007], but required vitamin D levels are still unknown. In this study most patients obtain the recommended adequate vitamin D level (≥ 50 nmol/l) with regular sun exposure or vitamin D supplementation. The CHOLINE study (NCT01198132) and the SOLAR study (NCT01285401) and several other clinical trial with vitamin D supplementation in persons with MS are either ongoing or completed and not yet published [http://clinicaltrials.gov]. If future studies find that there is a need for higher serum

vitamin D level, we have shown that supplementation with weekly dose of 20,000 IU Vitamin D_3 brings the majority to serum vitamin D level at least ≥ 75 nmol/L. However, it is important to make regular vitamin D measurements since the individual response of vitamin D supplementation is varying.

7. Concluding remarks

In persons with MS several studies have shown that BMD decreases as disability increasingly limits physical activity. Serum levels of 25(OH)D are associated with higher BMD, and vitamin D and calcium supplementation is the basis in osteoporosis prevention and treatment. In this trial a weekly dose of 20,000 IU vitamin D_3 in 96 weeks did not affect BMD in young fully ambulatory persons with MS. However, almost 25% of the population had lower than expected BMD for sex and age, and over the study period mean BMD at the hip in the whole study population decreased significantly by 1%. This finding may indicate that MS may be a cause of secondary osteoporosis. Progressing physical handicap poses persons with MS at further risk of developing osteoporosis and at the same time increases the risk of falls and fractures. Evidence is accumulating that hypovitaminosis D may play a role in the aetiology and pathogenesis of MS, which by itself might put persons with MS at risk of low peak bone mass. Only half of the participants in this study had at study start sufficient vitamin D levels (≥ 50 nmol/L) during the winter months, but the chance of being vitamin D sufficient increased with regular sunexposure and low dose vitamin D supplementation. Supplementation with high dose vitamin D_3 weekly resulted in all participants in the treatment group being vitamin D sufficient during winter and did not cause adverse effects. Establishing decision rules for selecting individuals for BMD screening has proven difficult [Raisz 2005]. Findings in our limited sample indicate that men as well as women with MS are at increased risk of low BMD. It might be appropriate to perform BMD measurements in all patients, perhaps shortly after diagnosis, but at least when the disease affect the ambulatory function. In order to achieve adequate vitamin D levels, MS patients who have no vitamin D efficient sun exposure and low dietary vitamin D intake during the winter months should be recommended to take vitamin D supplements.

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Paper I and Erratum Paper I

Paper II

Paper III

Appendix A

”Hva spiser du?”
Norwegian version

ID __ __ __ __ __ __

HVA SPISER DU?

Dette spørreskjemaet inneholder nokså detaljerte spørsmål om ditt kosthold. Vi er klar over at kostholdet varierer fra dag til dag og i løpet av året.
Prøv derfor å tenke deg et "gjennomsnitt" av dine spisevaner. Ha det siste året i tankene når du fyller ut. Er du usikker, ber vi deg om å krysse av for det alternativet som passer best, selv om det kanskje ikke passer helt.

Det er viktig at du plasserer kryssene tydelig i boksene. Du må gjerne bruke blyant og viskelær. Bruker du kulepenn og krysser feil, fargelegg hele boksen som er feil og sett kryss i den rette boksen.

Alle opplysningene vil bli behandlet fortrolig.

Kosthold

Påvirker noen av følgende forhold kostholdet ditt? (sett gjerne flere kryss)

- ☐ Er vegetarianer/veganer
- ☐ Spiser ikke norsk kost til daglig
- ☐ Har allergi/intoleranse
- ☐ Kronisk sykdom
- ☐ Har anoreksi
- ☐ Har bulimi
- ☐ Prøver å gå ned i vekt
- ☐ Lav glykemisk mat

Vi er interessert i å få kjennskap til hvordan kostholdet ditt er vanligvis. Kryss av for hvert spørsmål om hvor ofte du i gjennomsnitt siste året har brukt den aktuelle matvaren, og hvor mye du pleier å spise/drikke hver gang.

Drikke

Hvor mange glass melk drikker du vanligvis av hver type? (Sett ett kryss pr. linje)

	aldri/ sjelden	1-4 pr. uke	5-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr.dag
Helmelk (søt, sur)	☐	☐	☐	☐	☐	☐
Lettmelk (søt, sur)	☐	☐	☐	☐	☐	☐
Ekstra lettmelk	☐	☐	☐	☐	☐	☐
Skummet (søt, sur)	☐	☐	☐	☐	☐	☐

Hvor mange kopper kaffe/te drikker du vanligvis av hver sort? (Sett ett kryss for hver linje)

	aldri/ sjelden	1-6 pr. uke	1 pr. dag	2-3 pr. dag	4-5 pr. dag	6-7 pr. dag	8+ pr.dag
Kokekaffe	☐	☐	☐	☐	☐	☐	☐
Traktekaffe	☐	☐	☐	☐	☐	☐	☐
Pulverkaffe	☐	☐	☐	☐	☐	☐	☐
Svart te	☐	☐	☐	☐	☐	☐	☐
Grønn te	☐	☐	☐	☐	☐	☐	☐

Bruker du følgende i kaffe eller te:

	Kaffe		Te	
Sukker (ikke kunstig søtstoff)	☐ Ja	☐ Nei	☐ Ja	☐ Nei
Melk eller fløte	☐ Ja	☐ Nei	☐ Ja	☐ Nei

Hvor mange glass vann drikker du vanligvis?
(Sett ett kryss for hver linje)

	aldri/ sjelden	1-6 pr. uke	1 pr. dag	2-3 pr. dag	4-5 pr. dag	6-7 pr. dag	8+ pr.dag
Springvann/ flaskevann	☐	☐	☐	☐	☐	☐	☐

Hvor mange glass appelsinjuice, saft og brus drikker du vanligvis? (Sett ett kryss for hver linje)

	aldri/ sjelden	1-3 pr. uke	4-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr. dag
Appelsinjuice	☐	☐	☐	☐	☐	☐
Annen juice	☐	☐	☐	☐	☐	☐
Saft/brus med sukker	☐	☐	☐	☐	☐	☐
Saft/brus sukkerfri	☐	☐	☐	☐	☐	☐

Yoghurt/kornblanding

Hvor ofte spiser du yoghurt (1 beger)? (Sett ett kryss)

☐ Aldri/sjelden ☐ 1 pr. uke
☐ 2-3 pr. uke ☐ 4+ pr. uke

Hvor ofte spiser du kornblanding, havregryn eller müsli? (Sett ett kryss)

☐ Aldri/sjelden ☐ 1-3 pr. uke
☐ 4-6 pr. uke ☐ 1 pr. dag

Brødmat

Hvor mange skiver brød/rundstykker og knekkebrød/ skonrokker spiser du vanligvis?
(1/2 rundstykke = 1 brødskive) (Sett ett kryss for hver linje)

	aldri/ sjelden	1-4 pr. uke	5-7 pr. uke	2-3 pr. dag	4-5 pr. dag	6+ pr. dag
Grovt brød	☐	☐	☐	☐	☐	☐
Kneipp/halvfint	☐	☐	☐	☐	☐	☐
Fint brød/baguett	☐	☐	☐	☐	☐	☐
Knekkebrød o.l.	☐	☐	☐	☐	☐	☐

I neste spalte er det spørsmål om bruk av ulike påleggstyper. Vi spør om hvor mange brødskiver med det aktuelle pålegget du pleier å spise. Dersom du også bruker matvarene i andre sammenhenger enn til brød (f. eks. til vafler, frokostblandinger, grøt), ber vi om at du tar med dette når du besvarer spørsmålene.

På hvor mange brødskiver bruker du? (Sett ett kryss pr. linje)

	Aldri/ sjelden	1-3 pr. uke	4-6 pr. uke	1 pr. dag	2-3 pr. dag	4+ pr.dag
Syltetøy	☐	☐	☐	☐	☐	☐
Brunost, helfet	☐	☐	☐	☐	☐	☐
Brunost, halvfet/mager	☐	☐	☐	☐	☐	☐
Hvitost, helfet	☐	☐	☐	☐	☐	☐
Hvitost, halvfet/mager	☐	☐	☐	☐	☐	☐
Kjøttpålegg, Leverpostei	☐	☐	☐	☐	☐	☐
Rekesalat, italiensk o.l.	☐	☐	☐	☐	☐	☐

På hvor mange brødskiver pr. uke har du i gjennomsnitt siste året spist? (Sett ett kryss pr. linje)

	Aldri/ sjelden	1 pr.uke	2-3 pr.uke	4-6 pr.uke	7-9 pr.uke	10+ pr.uke
Makrell i tomat, røkt makrell	☐	☐	☐	☐	☐	☐
Kaviar	☐	☐	☐	☐	☐	☐
Sild/Ansjos	☐	☐	☐	☐	☐	☐
Laks (gravet/røkt)	☐	☐	☐	☐	☐	☐
Annet fiskepålegg	☐	☐	☐	☐	☐	☐

Hva slags fett bruker du vanligvis på brødet?
(Sett gjerne flere kryss)

☐ Bruker ikke fett på brødet
☐ Smør
☐ Hard margarin (f. eks. Per, Melange)
☐ Myk margarin (f. eks. Soft, Vita, Solsikke)
☐ Smørblandet margarin (f.eks. Bremyk)
☐ Brelett
☐ Lettmargarin (f. eks. Soft light, Letta, Vita Lett)
☐ Middels lett margarin (f. eks. Olivero, Omega)

Dersom du bruker fett på brødet, hvor tykt lag pleier 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)

Frukt og grønnsaker

Hvor ofte spiser du frukt? (Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr.mnd.	1 pr.uke	2-4 pr.uke	5-6 pr.uke	1 pr.dag	2+ pr. dag
Epler/pærer	☐	☐	☐	☐	☐	☐	☐
Appelsiner o.l.	☐	☐	☐	☐	☐	☐	☐
Bananer	☐	☐	☐	☐	☐	☐	☐
Annen frukt	☐	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du ulike typer grønnsaker?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr.mnd.	1 pr.uke	2 pr.uke	3 pr.uke	4-5 pr.uke	6-7 pr. uke
Gulrøtter	☐	☐	☐	☐	☐	☐	☐
Kål	☐	☐	☐	☐	☐	☐	☐
Kålrot	☐	☐	☐	☐	☐	☐	☐
Brokkoli/blomkål	☐	☐	☐	☐	☐	☐	☐
Blandet salat	☐	☐	☐	☐	☐	☐	☐
Tomat	☐	☐	☐	☐	☐	☐	☐
Grønnsak-blanding (frossen)	☐	☐	☐	☐	☐	☐	☐
Løk	☐	☐	☐	☐	☐	☐	☐
Andre grønn-saker	☐	☐	☐	☐	☐	☐	☐

For de grønnsakene du spiser, kryss av for hvor mye du spiser hver gang. (Sett ett kryss for hver sort)

Gulrøtter	☐ 1/2 stk	☐ 1 stk	☐ 1 1/2 stk	☐ 2+ stk
Kål	☐ 1/2 dl	☐ 1 dl	☐ 1 1/2 dl	☐ 2+ dl
Kålrot	☐ 1/2 dl	☐ 1 dl	☐ 1 1/2 dl	☐ 2+ dl
Brokkoli/ blomkål	☐ 1-2 buketter	☐ 3-4 buketter	☐ 5+ buketter	
Blandet salat	☐ 1 dl	☐ 2 dl	☐ 3 dl	☐ 4+ dl
Tomat	☐ 1/4 stk	☐ 1/2 stk	☐ 1stk	☐ 2+ stk
Grønnsak-blanding	☐ 1/2 dl	☐ 1 dl	☐ 2 dl	☐ 3+ dl

Hvor mange poteter spiser du vanligvis (kokte, stekte, mos)? (Sett ett kryss)

☐ Spiser ikke/spiser sjelden poteter ☐ 1-4 pr. uke
☐ 5-6 pr. uke ☐ 1 pr. dag ☐ 2 pr. dag
☐ 3 pr. dag ☐ 4+ pr. dag

Ris, spaghetti, grøt, suppe

Hvor ofte bruker du ris og spaghetti/makaroni?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2 pr. uke	3+ pr.uke
Ris	☐	☐	☐	☐	☐
Spagetti, makaroni, nudler	☐	☐	☐	☐	☐

Hvor ofte spiser du grøt ? (Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2-6 pr. uke	1+ pr. dag
Risengrynsgrøt	☐	☐	☐	☐	☐	☐
Annen grøt (havre o.l.)	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du suppe?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2 pr. uke	3+ pr.uke
Som hovedrett	☐	☐	☐	☐	☐
Som forrett, lunsj eller kveldsmat	☐	☐	☐	☐	☐

Fisk

Vi vil gjerne vite hvor ofte du pleier å spise fisk, og ber deg fylle ut spørsmålene om fiskeforbruk så godt du kan. Tilgangen på fisk kan variere gjennom året. Vær vennlig å markere i hvilke årstider du spiser de ulike fiskeslagene.

	aldri/ sjelden	like mye hele året	vinter	vår	sommer	høst
Torsk, sei, hyse, lyr	☐	☐	☐	☐	☐	☐
Steinbit, flyndre, uer	☐	☐	☐	☐	☐	☐
Laks, ørret	☐	☐	☐	☐	☐	☐
Makrell	☐	☐	☐	☐	☐	☐
Sild	☐	☐	☐	☐	☐	☐
Annen fisk	☐	☐	☐	☐	☐	☐

Med tanke på de periodene av året der du spiser fisk, hvor ofte pleier du å spise følgende til <u>middag</u>?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr. uke
<u>Kokt</u> torsk, sei, hyse, lyr	☐	☐	☐	☐	☐
<u>Stekt</u> torsk, sei, hyse, lyr	☐	☐	☐	☐	☐
Steinbit, flyndre, uer	☐	☐	☐	☐	☐
Laks, ørret	☐	☐	☐	☐	☐
Makrell	☐	☐	☐	☐	☐
Sild	☐	☐	☐	☐	☐
Annen fisk	☐	☐	☐	☐	☐

Dersom du spiser fisk, hvor mye spiser du vanligvis pr. gang? (1 skive/stykke = 150 gram)

Kokt fisk (skive)	☐ 1	☐ 1,5	☐ 2	☐ 3+
Stekt fisk (stykke)	☐ 1	☐ 1,5	☐ 2	☐ 3+

Hvor mange ganger pr. år spiser du fiskeinnmat?
(Sett ett kryss pr. linje)

	0	1-3	4-6	7-9	10+
Rogn	☐	☐	☐	☐	☐
Fiskelever	☐	☐	☐	☐	☐

Dersom du spiser fiskelever, hvor mange spiseskjeer pleier du å spise hver gang? (Sett ett kryss)

☐ 1 ☐ 2 ☐ 3-4 ☐ 5-6 ☐ 7+

Hvor ofte bruker du følgende typer fiskemat?
(Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr. uke
Fiskekaker/pudding/boller	☐	☐	☐	☐	☐
Plukkfisk/fiskegrateng	☐	☐	☐	☐	☐
Frityrfisk/fiskepinner	☐	☐	☐	☐	☐

Hvor stor mengde pleier du vanligvis å spise av de ulike rettene? (Sett ett kryss for hver linje)

Fiskekaker/pudding/boller (stk.) (2 fiskeboller=1 fiskekake)	☐ 1	☐ 2	☐ 3	☐ 4+
Plukkfisk, fiskegrateng (dl)	☐ 1-2	☐ 3-4	☐ 5+	
Frityrfisk, fiskepinner (stk.)	☐ 1-2	☐ 3-4	☐ 5-6	☐ 7+

I tillegg til informasjon om fiskeforbruk er det viktig å få kartlagt hvilket tilbehør som blir servert til fisk. Hvor ofte bruker du følgende til fisk?

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr. uke
Smeltet/fast smør	☐	☐	☐	☐	☐
Smeltet/fast margarin/fett	☐	☐	☐	☐	☐
Seterrømme (35%)	☐	☐	☐	☐	☐
Lettrømme (20%)	☐	☐	☐	☐	☐
Saus med fett (hvit/brun)	☐	☐	☐	☐	☐
Saus uten fett (hvit/brun)	☐	☐	☐	☐	☐

For de ulike typene tilbehør du bruker til fisk, vær vennlig å kryss av for hvor mye du vanligvis pleier å spise.

Smeltet/ fast smør (ss)	☐ 1/2	☐ 1	☐ 2	☐ 3	☐ 4+
Smeltet/ fast margarin (ss)	☐ 1/2	☐ 1	☐ 2	☐ 3	☐ 4+
Seterrømme (ss)	☐ 1/2	☐ 1	☐ 2	☐ 3	☐ 4+
Lettrømme (ss)	☐ 1/2	☐ 1	☐ 2	☐ 3	☐ 4+
Saus med fett (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+
Saus uten fett (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+

Hvor ofte spiser du skalldyr (f. eks. reker, krabbe og skjell)? (Sett ett kryss)

☐ Aldri/sjelden ☐ 1 pr. mnd ☐ 2-3 pr. mnd ☐ 1+ pr. uke

Kjøtt

Hvor ofte spiser du reinkjøtt?

☐ Aldri/sjelden ☐ 1 pr. mnd. ☐ 2-3 pr. mnd. ☐ 1 pr. uke
☐ 2-3 pr. uke ☐ 4+ pr. uke

Hvor ofte spiser du følgende kjøtt- og fjærkreretter? (Sett ett kryss for hver rett)

	aldri/ sjelden	1 pr.mnd.	2-3 pr.mnd.	1 pr.uke	2+ pr.uke
Steik (okse, svin, får)	☐	☐	☐	☐	☐
Koteletter	☐	☐	☐	☐	☐
Biff	☐	☐	☐	☐	☐
Kjøttkaker, karbonader	☐	☐	☐	☐	☐
Pølser	☐	☐	☐	☐	☐
Gryterett, lapskaus	☐	☐	☐	☐	☐
Pizza med kjøtt	☐	☐	☐	☐	☐
Kylling	☐	☐	☐	☐	☐
Bacon, flesk	☐	☐	☐	☐	☐
Andre kjøttretter	☐	☐	☐	☐	☐

Dersom du spiser følgende retter, oppgi mengden du vanligvis spiser: (Sett ett kryss for hver linje)

Steik (skiver)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5+
Koteletter (stk.)	☐ 1/2	☐ 1	☐ 1,5	☐ 2+	
Kjøttkaker, karbonader (stk.)	☐ 1	☐ 2	☐ 3	☐ 4+	
Pølser (stk. à 150g)	☐ 1/2	☐ 1	☐ 1,5	☐ 2+	
Gryterett, lapskaus (dl)	☐ 1-2	☐ 3	☐ 4	☐ 5+	
Pizza m/kjøtt (stykke à 100 g)	☐ 1	☐ 2	☐ 3	☐ 4+	

Hvilke sauser bruker du til kjøttretter og pastaretter? (Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr. uke
Brun saus	☐	☐	☐	☐	☐
Sjysaus	☐	☐	☐	☐	☐
Tomatsaus	☐	☐	☐	☐	☐
Saus med fløte/rømme	☐	☐	☐	☐	☐

Hvor mye bruker du vanligvis av disse sausene?

Brun saus (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+
Sjysaus (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+
Tomatsaus (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+
Saus med fløte/ rømme (dl)	☐ 1/4	☐ 1/2	☐ 3/4	☐ 1	☐ 2+

Andre matvarer

Hvor mange egg spiser du vanligvis i løpet av en uke? (stekte, kokte, eggerøre, omelett) (Sett ett kryss)

☐ 0 ☐ 1 ☐ 2 ☐ 3-4
☐ 5-6 ☐ 7+

Hvor ofte spiser du iskrem? (til dessert, Krone-is osv.) Sett ett kryss for hvor ofte du spiser iskrem om sommeren, og ett kryss for resten av året

	aldri/ sjelden	1 pr. mnd.	2-3 pr. mnd.	1 pr. uke	2+ pr.uke
Om sommeren	☐	☐	☐	☐	☐
Resten av året	☐	☐	☐	☐	☐

Hvor mye is spiser du vanligvis pr. gang? (Sett ett kryss)

☐ 1dl ☐ 2 dl ☐ 3 dl ☐ 4+ dl

Hvor ofte spiser du bakevarer som boller, kaker, wienerbrød eller småkaker (Sett ett kryss pr. linje)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-3 pr uke	4-6 pr. uke	1+ pr.dag
Gjærbakst (boller o.l.)	☐	☐	☐	☐	☐	☐
Wienerbrød, kringle	☐	☐	☐	☐	☐	☐
Kaker	☐	☐	☐	☐	☐	☐
Pannekaker	☐	☐	☐	☐	☐	☐
Vafler	☐	☐	☐	☐	☐	☐
Småkaker, kjeks	☐	☐	☐	☐	☐	☐
Lefser, lomper	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du dessert? (Sett ett kryss pr. linje)

	aldri/ sjelden	1 pr. mnd.	2-3 pr mnd.	1 pr uke	2-3 pr. uke	4+ pr. uke
Pudding sjokolade/karamell	☐	☐	☐	☐	☐	☐
Riskrem, fromasj	☐	☐	☐	☐	☐	☐
Kompott, fruktgrøt, hermetisk frukt	☐	☐	☐	☐	☐	☐
Jordbær (friske, frosne)	☐	☐	☐	☐	☐	☐
Andre bær (friske, frosne)	☐	☐	☐	☐	☐	☐

Hvor ofte spiser du sjokolade? (Sett ett kryss)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-3 pr uke	4-6 pr. uke	1+ pr.dag
Mørk sjokolade	☐	☐	☐	☐	☐	☐
Lys sjokolade	☐	☐	☐	☐	☐	☐

Dersom du spiser sjokolade, hvor mye pleier du vanligvis å spise hver gang? Tenk deg størrelsen på en Kvikk-Lunsj sjokolade, og oppgi hvor mye du spiser i forhold til den.

☐ 1/4 ☐ 1/2 ☐ 3/4 ☐ 1 ☐ 1,5 ☐ 2+

Hvor ofte spiser du snacks? (Sett ett kryss)

	aldri/ sjelden	1-3 pr. mnd.	1 pr. uke	2-3 pr. uke	4-6 pr. uke	1+ pr. dag
Potetchips	☐	☐	☐	☐	☐	☐
Peanøtter	☐	☐	☐	☐	☐	☐
Andre nøtter	☐	☐	☐	☐	☐	☐
Annen snacks	☐	☐	☐	☐	☐	☐

Mølje en gang til:

Dette siste spørsmålet gjelder denne "møljesesongen"

Har du spist skreimølje denne vinteren? ☐ ja ☐ nei

Hvis ja, hvor mange ganger?

	Desember	Januar	Februar
Antall måltider mølje			

Dersom du spiser fiskelever, hvor mange spiseskjeer pleier du å spise hver gang?
☐ 1 ☐ 2 ☐ 3-4 ☐ 5-6 ☐ 7-10 ☐ 11 eller flere

Bruker du da kraften/fettet som leveren er kokt i?
☐ ja ☐ nei

Hvis ja, hvor mye bruker du hver gang?
☐ 1-2 ss ☐ 0,5 dl ☐ 1 dl ☐ mer enn 1 dl

Appendix B

Spørreskjema start
Norwegian version

Røyking & Alkoholbruk

Røyker du?

❑ ja ❑ nei

Hvis ja, hvor mange sigaretter? _____/dag

Har du røykt mer eller mindre over lengre perioder tidligere?

fra (alder) _____ til _____ år,

gjennomsnittlig _____ sigaretter/dag

fra (alder) _____ til _____ år,

gjennomsnittlig _____ sigaretter/dag

Hvor mange år har du røykt daglig? ____ år

Bruker du alkohol?

❑ ja ❑ nei

Hvis ja, hvor mange enheter i uka? _____
Skriv "0" hvis det er mindre enn én enhet i uka i gjennomsnitt. En enhet er: øl (1/2 l), vin (glass), brennevin (drink), likør/hetvin (glass)

Har du hatt høyere eller lavere forbruk over lengre perioder tidligere?

fra (alder) _____ til _____ år,

gjennomsnittlig _____ enheter/uke

fra _____ til _____ år,

gjennomsnittlig _____ enheter/uke

Familie og språkbakgrunn

I Nord-Norge bor det folk med ulik etnisk bakgrunn. Det vil si at de snakker ulike sprak og har forskjellige kulturer. Eksempler på etnisk bakgrunn eller etnisk gruppe er norsk, samisk og kvensk.

Hvilket hjemmespråk hadde du, dine foreldre og besteforeldre?

Sett ett eller flere kryss

	Norsk	Samisk	Kvensk	Usikkert	Annet, beskriv
Morfar	❑	❑	❑	❑	❑.....
Mormor	❑	❑	❑	❑	❑.....
Farfar	❑	❑	❑	❑	❑.....
Farmor	❑	❑	❑	❑	❑.....
Far	❑	❑	❑	❑	❑.....
Mor	❑	❑	❑	❑	❑.....
Jeg selv	❑	❑	❑	❑	❑.....

Hva er din, din fars og din mors etniske bakgrunn?

Sett ett eller flere kryss

	Norsk	Samisk	Kvensk	Usikkert	Annet, beskriv
Min etniske bakgrunn er	❑	❑	❑	❑	❑.....
Fars etniske bakgrunn er	❑	❑	❑	❑	❑.....
Mors etniske bakgrunn er	❑	❑	❑	❑	❑.....

Hva regner du deg selv som?

Sett ett eller flere kryss

Norsk	Samisk	Kvensk	Annet, beskriv
❑	❑	❑	❑.....

ID __ __ __ __ __ __

MS og beinskjørhet - Spørreskjema

Beinskjørhet

Kjenner du til at beinskjørhet/osteoporose forekommer blant dine foreldre eller søsken?

❑ ja ❑ nei

Hvis ja, hvem har beinskjørhet?_____

For kvinner

Hvor gammel var du da du fikk menstruasjonen for første gang? _____ **år**

Har du hatt regelmessig menstruasjon (d.v.s. med 3-5 ukers mellomrom) det siste året?

❑ ja ❑ nei

Hvis du bruker p-piller eller har brukt p-piller tidligere: i hvor mange år tilsammen? _____ **år**

Kosthold

Har du endret kostholdet ditt etter at du fikk diagnosen MS? ❑ ja ❑ nei

Hvis ja, hvordan? _____

Når (årstall)? _____

Har du endret kostholdet ditt av andre grunner? ❑ ja ❑ nei

Hvis ja, hvordan? _____

Når (årstall)? _____

Kosthold det siste året

I de følgende spørsmålene ønsker vi svar på hvilke matvarer du har brukt det siste året.
Forsøk å tenke deg et gjennomsnitt for hele året.

Hva spiser du til middag?

	aldri	0-1 ganger i uka	2-3 ganger i uka	4-7 ganger i uka
Fisk eller fiskemat	❑	❑	❑	❑
Kjøtt, kjøttkaker, pølser	❑	❑	❑	❑
Grønnsakretter	❑	❑	❑	❑
Grøt, pannekaker, pizza, pasta?	❑	❑	❑	❑

Har du gjort noen større endringer i kostholdet det siste året? ❑ ja ❑ nei

Hvis ja, hvilke? _____

Har du i løpet av det siste året slanket deg mer enn 5 kg eller forsøkt å slanke deg i lengre perioder? ❑ ja ❑ nei

Uteaktiviteter og soling det siste året

Har du vært på solferie i utlandet etter mai 2007?

Land	Måned	antall uker

Var du i Troms eller Finnmark **hele** sommeren 2007 (juni – august)?

❑ ja ❑ nei

Hvis nei, oppgi sted og varighet på reiser over en uke.

Sted	Antall uker

Har du vært i **solarium** det siste året?

❑ nei

❑ ja, _____ (antall) ganger det siste året, hvorav _____ ganger de siste tre måneder og _____ ganger den siste måneden. Jeg soler meg i gjennomsnitt i _____ minutter hver gang.

Fysisk aktivitet det siste året

Er du i lønnet eller ulønnet arbeid? ❑ ja ❑ nei

Hvis ja, hvor mange timer i uken?_____

Hvis du er i arbeid: Hvor stor er den fysiske belastningen? (sett **ett** kryss)

- ❑ For det meste stillesittende arbeid (for eksempel skrivebordsarbeid, montering)
- ❑ Arbeid som krever at du går mye (for eksempel ekspeditørarbeid, husmor, undervisning)
- ❑ Arbeid hvor du går og løfter mye (f.eks. postbud, pleier, bygningsarbeid)
- ❑ Tungt kroppsarbeid (for eksempel skogsarbeid, tungt jordbruksarbeid, tungt bygningsarbeid)

Hvordan har din fysiske aktivitet *i fritiden* vært det siste året?
Tenk deg et ukentlig gjennomsnitt for året. **Arbeidsvei** regnes som fritid. Besvar begge spørsmålene

	Timer per uke:			
	Ingen	under 1	1-2	3 og mer
Lett aktivitet, ikke svett/andpusten	❑	❑	❑	❑
Hard fysisk aktivitet, svett/andpusten	❑	❑	❑	❑

Angi bevegelse og kroppslig anstrengelse i *din fritid*. Hvis aktiviteten varierer meget, så ta et gjennomsnitt. Spørsmålet gjelder bare det siste året. Sett kryss i den ruta som passer best

- ❑ Leser, ser på fjernsyn eller annen stillesittende beskjeftigelse.
- ❑ Spaserer, sykler eller beveger meg på annen måte *minst 4 timer i uka.*
  Regn også med gang eller sykling til arbeidsstedet, søndagsturer m.m.
- ❑ Driver mosjonsidrett, tyngre hagearbeid eller lignende.
- ❑ Trener hardt eller driver konkurranseidrett.

Hvor har du bodd til du fylte 20 år?

Hvor mange år har du bodd i Troms eller Finnmark? _____ år

Hvor har du bodd resten av tiden?

Kosthold i oppveksten (tiden før du flyttet hjemmefra for godt)

Hva spiste du til middag i oppveksten?

	aldri	0-1 ganger i uka	2-3 ganger i uka	4-7 ganger i uka
Fisk (kokt, stekt) eller fiskemat	❑	❑	❑	❑
Kjøtt, kjøttkaker, pølser	❑	❑	❑	❑
Grønnsakretter	❑	❑	❑	❑
Grøt, pannekaker, pizza, pasta	❑	❑	❑	❑

Hvor mye melk og yoghurt brukte du i oppveksten?
(med melk menes alle melkeslag inkludert kefir, kulturmelk etc.1 beger yoghurt tilsvarer ett glass melk)

Gjennomsnittlig _____ glass per dag (skriv ”0” hvis du ikke brukte melk eller yoghurt hver dag)

Fikk du tran i oppveksten?

- ❑ ja, hele året (minst 4 dager i uka)
- ❑ ja, om vinteren (minst 4 dager i uka)
- ❑ ikke eller uregelmessig (mindre enn 4 dager i uka)

Har du hatt spiseforstyrrelser? ❑ ja ❑ nei
Har du forsøkt å slanke deg over lengre perioder? ❑ ja ❑ nei

Uteaktiviteter i oppveksten

Sett kun **ett** kryss for hver aldersgruppe.

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

	Lite	Middels	Ganske mye	Ute stort sett hele tiden
6-10 år	❑	❑	❑	❑
11-15 år	❑	❑	❑	❑
16-20 år	❑	❑	❑	❑

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

	Lite	Middels	Ganske mye	Ute stort sett hele tiden
6-10 år	❑	❑	❑	❑
11-15 år	❑	❑	❑	❑
16-20 år	❑	❑	❑	❑

Fysisk aktivitet i oppveksten

Angi bevegelse og kroppslig anstrengelse i *din fritid*. Her skal hele oppveksten ses under ett.

Hvilket av de fire aktivitetsnivåene beskriver ditt aktivitetsnivå best?

- ❑ Leste, så på fjernsyn eller hadde annen stillesittende beskjeftigelse.
- ❑ Spaserte, syklet eller beveget meg på annen måte *minst 4 timer i uka.*
  Regn også med gang eller sykling til skolen, søndagsturer m.m.
- ❑ Drev med mosjonsidrett eller tilsvarende fysisk aktivitet.
- ❑ Drev med hard trening eller konkurranseidrett.

Appendix C

Spørreskjema hver 12. uke
Norwegian version

ID __ __ __ __ __ __ __ __

MS og beinskjørhet

Kosthold

Har du gjort noen større endringer i kostholdet de siste tre månedene?

❑ ja ❑ nei

Hvis ja, hvilke? _____

Har du i løpet av de siste tre månedene slanket deg mer enn 5 kg eller forsøkt å slanke deg i lengre perioder?

❑ ja ❑ nei

Kosthold den siste måneden

I de følgende spørsmålene ønsker vi svar på hvilke matvarer du har brukt den siste måneden. Forsøk å tenke deg et gjennomsnitt.

Hvor ofte har du spist fet fisk til middag den siste måneden?

Sett kun et kryss på hver linje.

	ikke	1 gang	2-3 ganger	1 pr. uke	2 pr. uke	3+ pr. uke
Laks, ørret						
Makrell						
Sild						

Dersom du spiser fisk, hvor mye spiser du vanligvis per gang?
(1 skive/stykke = 150 gram)

❑ 1 skive ❑ 1,5 skive ❑ 2 skiver ❑ 3 skiver ❑ 4 eller flere skiver

Fiskepålegg

Har du brukt fiskepålegg den siste måneden?
❑ ja ❑ nei

Hvis ja, hvor mange skiver med fiskepålegg spiser du i uka?

	0 pr. uke	1 pr. uke	2-3 pr. uke	4-6 pr. uke	7-9 pr. uke	10+ pr. uke
Laks						
Makrell i tomat, røkt makrell						
Sild						
Kaviar						
Annet fiskepålegg						

Melk

Har du drukket TINE ekstra lett melk (vitamin D beriket, grønn kartong) den siste måneden?

❑ ja ❑ nei (eller mindre enn 1 glass per uke)

Hvis ja, hvor mange glass drikker du vanligvis?

❑1-4 per uke ❑5-6 per uke ❑1 per dag ❑2-3 per dag ❑4+ per dag

Smør og margarin

Har du brukt smør eller margarin på brødskiva den siste måneden?

❑ ja ❑ nei

Hvis ja, hvor mange skiver spiser du per dag ____ (antall)

Hvor tykt lag pleier du å smøre på?

Sett ett kryss (en kuvertpakke med smør/margarin veier 12 gram)

❑ skrapet (3g) ❑ tynt lag (5g) ❑ godt dekket (8g) ❑ tykt lag (12g)

Tran og fiskeolje

Har du brukt medisinsk tran eller fiskeoljekapsler den siste måneden?

❑ ja, hver dag

❑ ja, minst 4 dager i uka

❑ mindre enn 4 dager i uka

❑ nei

Hvis ja, hva er navnet på tranen/fiskeoljekapslene? _____

Mølje

Har du spist skreimølje denne vinteren? ☐ ja ☐ nei

Hvis ja, hvor mange ganger?

	Desember	Januar	Februar
Antall måltider mølje			

Dersom du spiser fiskelever, hvor mange spiseskjeer pleier du å spise hver gang?

☐ 1 ☐ 2 ☐ 3-4 ☐ 5-6 ☐ 7-10 ☐ 11 eller flere

Bruker du da kraften/fettet som leveren er kokt i?

☐ ja ☐ nei

Hvis ja, hvor mye bruker du hver gang?

☐ 1-2 ss ☐ 0,5 dl ☐ 1 dl ☐ mer enn 1 dl

Soling

Har du vært på solferie i utlandet de siste tre månedene?

❑ ja ❑ nei

Hvis ja, vennligst svar på følgende

Sted (Land)	fra – til (dato)	Omtrent hvor mange **timer pr uke** solte du deg?

Har du vært i **solarium** de siste tre måneder?

❑ nei

❑ ja, _____ (antall) ganger de siste tre måneder, hvorav _____ ganger den siste måneden. Jeg soler meg i gjennomsnitt i _____ minutter hver gang.

Fysisk aktivitet de siste tre månedene

Angi bevegelse og kroppslig anstrengelse i *din fritid*. Hvis aktiviteten varierer meget, så ta et gjennomsnitt. Spørsmålet gjelder bare de siste tre månedene.
Sett kryss i den ruta som passer best

- ❑ Leser, ser på fjernsyn eller annen stillesittende beskjeftigelse.
- ❑ Spaserer, sykler eller beveger meg på annen måte *minst 4 timer i uka.*
  Regn også med gang eller sykling til arbeidsstedet, søndagsturer m.m.
- ❑ Driver mosjonsidrett, tyngre hagearbeid eller lignende *minst 4 timer i uka.*
- ❑ Trener hardt eller driver konkurranseidrett.

Røyking

Røyker du?

❑ ja ❑ nei

Hvis ja, hvor mange sigaretter? _____/dag

Medikamenter og kosttilskudd

Har du **begynt eller sluttet med medisiner de siste 3 månedene**?
❑ ja ❑ nei

Angi navnet på nye medisiner du bruker (også **naturmedisin**, men **ikke kosttilskudd**) og hvilken grunn det er til at du tar disse, samt navnet på medisiner du har sluttet med.

Navn på medisinen	Grunn til bruk (sykdom eller symptom)	Fast	Ved behov	Begynt (dato for nye medisiner)	Sluttet (dato)

Dersom det ikke er nok plass her, kan du fortsette på eget ark som du legger ved.

Har du **begynt eller sluttet med kosttilskudd de siste 3 måneder**?

❑ ja ❑ nei

Hvis et eller flere av kosttilskuddene inneholder vitamin D, hvor mye?
Noter kun navnet på kosttilskudd du har brukt daglig eller flere ganger i uka.

Navn på kosttilskuddet	Grunn til bruk	Vitamin D innhold	begynt (dato)	sluttet (dato)

Dersom det ikke er nok plass her, kan du fortsette på eget ark som du legger ved.

For kvinner

Har du hatt regelmessig menstruasjon (d.v.s. med 3-5 ukers mellomrom) de siste tre månedene?

❑ ja ❑ nei ❑ bruker hormonspiral eller p-sprøyte

Kommentarer

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