The role of serum lipid profile and comorbidities in the onset and progression of multiple sclerosis

By

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BSc (Human Biology); MPhil (Medical Microbiology)

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (Medical Studies)

UNIVERSITY OF TASMANIA

Menzies Institute for Medical Research

December, 2015
Declaration of originality

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Statement of Co-authorship

This thesis includes papers for which Prudence Tettey (PT) is not sole author. PT took the lead in this research, developing and implementing the analyses, writing manuscript included herein under the supervision of Ingrid van der Mei (IvM), Bruce V Taylor (BVT), and Steve Simpson, Jr. (SSJ). In this process, however, he was assisted by co-authors to varying extent. Following then, the contribution of each co-author is detailed for each respective project.

The paper reported in Chapter 2:


- Prudence Tettey (PT) undertook the literature review and composed the initial draft with direct assistance from Ingrid van der Mei (IvM). Bruce Vivian Taylor (BVT) and Steve Simpson Jr. (SSJ) provided supervision and were involved in the critical revision of the manuscripts.

The paper reported in Chapter 3:


TETTEY P, VAN DER MEI I. Frequency of autoimmune comorbidities in MS: the role of interferon-beta therapy.

- PT undertook the literature review and composed the initial draft with direct assistance from IvM. BVT and SSJ provided supervision and were involved in the critical revision of the manuscripts.
The paper reported in Chapter 4:


- PT was involved in the development and implementation of statistical analyses. PT composed the drafts of the manuscript and coordinated revision. This was done under the supervision of IvM, BVT and SSJ.

- IvM was involved in the development and acquisition of funding for the MS Longitudinal Study from which the data for this analysis was drawn, along with BVT, Anne-Louise Ponsonby (A-LP), Terence Dwyer (TD). IvM was involved in the data collection for the MS Longitudinal Study. IvM was involved in the initial drafting and critical revision of the manuscript along with BVT and SSJ.

- BVT was involved in the data collection for the MS Longitudinal Study and provided supervision and was involved in the critical revision of the manuscript.

- SSJ provided guidance and supervision for the statistical analyses undertaken in this study, and was involved in the critical revision of the manuscript.

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- A-LP was involved in the development and acquisition of funding for the MS Longitudinal Study and contributed to the critical revision of the manuscript.

- TD was involved in the development and acquisition of funding for the MS Longitudinal Study and contributed to the critical revision of the manuscript.
• Karam Kostner (KK) was involved in the data collection for the MS Longitudinal Study and contributed to the critical revision of the manuscript.

The paper reported in Chapter 5:

• PT was involved in the development and implementation of statistical analyses. PT composed the drafts of the manuscript and coordinated revision. This was done under the supervision of IvM, BVT and SSJ.

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• BVT was involved in the data collection for the MS Longitudinal Study and provided supervision and was involved in the critical revision of the manuscript.

• SSJ provided guidance and supervision for the statistical analyses undertaken in this study, and was involved in the critical revision of the manuscript.

• LB provided guidance and supervision for all statistical analyses undertaken in this study, and was involved in the initial drafting and critical revision of the manuscript.

• A-LP was involved in the development and acquisition of funding for the MS Longitudinal Study and contributed to the critical revision of the manuscript.
• TD was involved in the development and acquisition of funding for the MS Longitudinal Study and contributed to the critical revision of the manuscript.

• KK was involved in the data collection for the MS Longitudinal Study and contributed to the critical revision of the manuscript.

The paper reported in Chapter 6:
TETTEY, P., SIMPSON, S., JR., TAYLOR, B., BLIZZARD, L., PONSONBY, A. L., DWYER, T., LUCAS, RM & VAN DER MEI, I. An adverse lipid profile and increased body mass index significantly predicts clinical course after a first demyelinating event.

• PT was involved in the development and implementation of statistical analyses. PT composed the drafts of the manuscript and coordinated revision. This was done under the supervision of IvM, BVT and SSJ.

• IvM was involved in the development and acquisition of funding for the MS Longitudinal Study from which the data for this analysis was drawn, along with BVT, A-LP and TD. IvM was involved in the data collection for the MS Longitudinal Study. IvM was involved in the initial drafting and critical revision of the manuscript along with BVT and SSJ.

• BVT was involved in the data collection for the MS Longitudinal Study and provided supervision and was involved in the critical revision of the manuscript.

• SSJ provided guidance and supervision for the statistical analyses undertaken in this study, and was involved in the critical revision of the manuscript.

• LB provided guidance and supervision for all statistical analyses undertaken in this study, and was involved in the initial drafting and critical revision of the manuscript.

• A-LP was involved in the development and acquisition of funding for the MS Longitudinal Study and contributed to the critical revision of the manuscript.
• TD was involved in the development and acquisition of funding for the MS Longitudinal Study and contributed to the critical revision of the manuscript.

• KK was involved in the data collection for the MS Longitudinal Study and contributed to the critical revision of the manuscript.

• LMR was involved in the development and acquisition of funding for the MS Longitudinal Study and contributed to the critical revision of the manuscript.

The paper reported in Chapter 7:

• PT was involved in the development and implementation of statistical analyses. PT composed the drafts of the manuscript and coordinated revision. This was done under the supervision of IvM, BVT and SSJ.

• Dylan Siejka (DS) was involved in analyses, initial drafting and critical revision of the manuscript.

• IvM was involved in the development and acquisition of funding for the MS Longitudinal Study from which the data for this analysis was drawn, along with BVT, A-LP and TD. IvM was involved in the data collection for the MS Longitudinal Study. IvM was involved in the initial drafting and critical revision of the manuscript along with BVT and SSJ.

• BVT was involved in the data collection for the MS Longitudinal Study and provided supervision and was involved in the critical revision of the manuscript.
• SSJ provided guidance and supervision for the statistical analyses undertaken in this study, and was involved in the critical revision of the manuscript.

• LB provided guidance and supervision for all statistical analyses undertaken in this study, and was involved in the initial drafting and critical revision of the manuscript.

• A-LP was involved in the development and acquisition of funding for the MS Longitudinal Study and contributed to the critical revision of the manuscript.

• TD was involved in the development and acquisition of funding for the MS Longitudinal Study and contributed to the critical revision of the manuscript.

• KK was involved in the data collection for the MS Longitudinal Study and contributed to the critical revision of the manuscript.

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Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

Signature

8/12/2015

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Date
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Abstract

Multiple sclerosis (MS) is an inflammatory, autoimmune disorder of the central nervous system (CNS) with considerable heterogeneity in clinical outcome and a leading cause of disability in young adults. The disease is caused by interplay of genetic and environmental factors and recent evidence suggests a role for an adverse lipid profile (low HDL, increased LDL and triglycerides) and comorbidity in MS clinical course. This thesis therefore investigates the etiological role of the serum lipid profile and comorbidity in the onset and progression of the disease.

A literature review on the role of vascular comorbidities in the onset and progression of MS showed a potential association of vascular comorbidities with MS risk and disability progression. However, the number of prospective studies was sparse, thus precluding ascription of causality. It was therefore recommended that future studies of the frequency and effects of vascular comorbidities on MS risk and disability should be prospective and objective where relevant. A literature review on the frequency of commonly reported autoimmune comorbidities in MS and the contribution of interferon-beta (IFN-β) to the frequency of thyroid autoimmunity and dysfunction in MS patients identified a consistent increase in the risk of clinical thyroid disorder, inflammatory bowel disease and psoriasis among people with MS compared to a comparator population. It also appears that IFN-β therapy is associated with higher occurrence of thyroid autoimmunity and dysfunction compared to IFN-β naive MS patients. It was recommended that future studies should have a population-based design with large sample size and a concurrent control to minimize heterogeneity due to differences in study design.

The Tasmanian MS Longitudinal Study was used to examine the association between serum lipid profile and disability, progression in disability and time to relapse in a cohort with established disease. A cohort of 178 MS patients was followed for a total of 2.5 years and
serum samples and other data were obtained at each biannual review. Among 141 relapsing–remitting MS patients, neither body mass index (BMI) nor any of the lipid-related measures were associated with the hazard of relapse. In relation to disability, higher levels of total cholesterol (TC), apolipoprotein B (ApoB) and apolipoprotein B to apolipoprotein A-I ratio (ApoB/ApoA-I ratio) were associated with a greater disability. In addition, a significant independent effect of BMI on disability was observed. This suggests that clinicians should monitor and treat adverse lipid profiles and BMI. Importantly, a higher TC/HDL ratio was associated with faster accrual of disability, suggesting that lipid lowering interventions may be of benefit for people with MS.

The Tasmanian MS Longitudinal Study was also used to examine whether the frequency of comorbidities was higher in MS patients compared to the Australian population and whether comorbidities were associated with clinical disability and relapse. It was found that age-standardised prevalence of hypertension, dyslipidaemia, psoriasis, eczema, asthma and anaemia was significantly higher in the MS cohort compared to the general Australian population and that reporting overweight/obesity and dyslipidaemia was associated with significantly higher disability. However, there was no significant association with annual change in disability. In the relapse analysis, rheumatoid arthritis and anaemia were associated with increased risk of relapse.

The final set of analyses was conducted using the Ausimmune Longitudinal Study (AusLong), an internationally unique cohort with a first clinical diagnosis of demyelination, which has been followed annually up to five year review. The association between serum lipids and conversion to clinically definite MS (CDMS), time to relapse and annualised change in disability was investigated. It was found that higher BMI and triglycerides were associated with increased risk of relapse while none of the serum lipid-related variables were
significantly associated with conversion to CDMS. In addition, higher BMI and TC/HDL ratio was associated with a higher annualised change in clinical disability.

In summary, this thesis presents a number of epidemiological studies suggesting that improving the lipid profile, decreasing BMI into the healthy range and increasing physical activity may reduce the risk of comorbidity and modulate the accumulation of disability. Further, long-term monitoring and treatment of lipid profile and comorbidities in MS is recommended in order to prevent increased morbidity as people age with their MS. We examined reverse causality, and this cannot be completely ruled out in these observational studies. Regardless of the direction of some associations, the findings of this thesis are important from a clinical perspective, and the increased awareness of this issue may assist in an improved management of the disease.
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Papers directly arising from the work described in this thesis

Paper published

Chapter two:


Chapter three:


Chapter four:


Chapter five:


Chapter Seven

Yet to be published manuscripts

Chapter Three
TETTEY P. & VAN DER MEI I. Frequency of autoimmune comorbidities in MS: the role of interferon-beta therapy.

Chapter Six

- TETTEY, P., SIEJKA D., SIMPSON, S., JR., TAYLOR, B., BLIZZARD, L., PONSONBY, A. L., DWYER, T., LUCAS, RM & VAN DER MEI, I. An adverse lipid profile and increased body mass index significantly predicts clinical course after a first demyelinating event.

Other publications


- Simpson S, Blizzard L, Taylor B, Tettey P, van der Mei I. Is your association real or just reverse causality?: Some examples from analyses of multiple sclerosis clinical course and tools to assess it. Australasian Epidemiologist 2013;20:34.
Conference presentations arising from work in this thesis

- **European Congress of Epidemiology (2015)**, Maastricht, the Netherlands: “Frequency of comorbidities and their association with clinical disability and relapse in multiple sclerosis”.

- **Multiple Sclerosis Research Association Progress in MS Research Conference (2013)**, Sydney, Australia: “Associations between serum lipids & apolipoproteins and disability in MS” (oral presentation).

- **Student Excellence in Research Conference (2012)**, Hobart, Australia: “The interrelationships between serum vitamin D and serum lipid profile, and their associations with clinical outcomes in multiple sclerosis” (poster presentation).
### List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full term</th>
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<tr>
<td>μg</td>
<td>Micrograms</td>
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<tr>
<td>1,25(OH)$_2$D</td>
<td>1,25-dihydroxyvitamin D</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D</td>
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<tr>
<td>95% CI</td>
<td>95 Percent Confidence Interval</td>
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<tr>
<td>ABS</td>
<td>Australian Bureau of Statistics</td>
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<tr>
<td>Apo-AI</td>
<td>Apolipoprotein AI</td>
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<td>ApoB</td>
<td>Apolipoprotein B</td>
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<td>ApoE</td>
<td>Apolipoprotein E</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<td>CSF</td>
<td>Cerebrospinal Fluid</td>
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<tr>
<td>DMDs</td>
<td>Disease-Modifying Drugs</td>
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<td>EBNA</td>
<td>Epstein-Barr Nuclear Antigen</td>
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<td>EBV</td>
<td>Epstein-Barr Virus</td>
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<tr>
<td>EBV-EA</td>
<td>Epstein-Barr Virus Early Antigen</td>
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<td>EDSS</td>
<td>Expanded Disability Severity Scale</td>
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<tr>
<td>g/L</td>
<td>Grams per Litre</td>
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<td>GWAS</td>
<td>Genome-wide association studies</td>
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<td>HDL</td>
<td>High Density Lipoprotein</td>
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<td>HLA</td>
<td>Human Leukocyte Antigen</td>
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<td>HR</td>
<td>Hazard Ratio</td>
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<tr>
<td>hs-CRP</td>
<td>High Sensitive C-reactive Protein</td>
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<td>IFN-β</td>
<td>Interferon beta</td>
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<td>IgG</td>
<td>Immunoglobulin class G</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>IQR</td>
<td>Interquartile Range</td>
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<td>ISR</td>
<td>Incidence Sex Ratio</td>
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<tr>
<td>Km/m$^2$</td>
<td>Kilometres per Metre Square</td>
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<td>LDL</td>
<td>Low Density Lipoprotein</td>
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<td>LipoA</td>
<td>Lipoprotein A</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>MET</td>
<td>Metabolic Equivalent of Task</td>
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<td>mg/L</td>
<td>Milligram per Litre</td>
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<td>MHC</td>
<td>Major Histocompatibility Complex</td>
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<tr>
<td>mmol/L</td>
<td>Millimoles per Litre</td>
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<tr>
<td>MOB</td>
<td>Month of Birth</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>MS</td>
<td>Multiple Sclerosis</td>
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<tr>
<td>MSL</td>
<td>Multiple Sclerosis Longitudinal Study</td>
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<tr>
<td>MSSS</td>
<td>Multiple Sclerosis Severity Score</td>
</tr>
<tr>
<td>nmol/L</td>
<td>Nanomoles per Litre</td>
</tr>
<tr>
<td>NonHDL</td>
<td>Non High Density Lipoprotein</td>
</tr>
<tr>
<td>NRS</td>
<td>Scripps Neurologic Rating Scale</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PPMS</td>
<td>Primary-Progressive Multiple Sclerosis</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised Controlled Trial</td>
</tr>
<tr>
<td>RRMS</td>
<td>Relapsing-Remitting Multiple Sclerosis</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>TC</td>
<td>Total Cholesterol</td>
</tr>
<tr>
<td>Th1</td>
<td>Helper T-lymphocyte class 1</td>
</tr>
<tr>
<td>Th2</td>
<td>Helper T-lymphocyte class 2</td>
</tr>
<tr>
<td>Th17</td>
<td>Helper T-lymphocyte class 17</td>
</tr>
<tr>
<td>Treg</td>
<td>Regulatory T-lymphocyte</td>
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<tr>
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<td>Micromoles per Litre</td>
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<tr>
<td>UVR</td>
<td>Ultraviolet Radiation</td>
</tr>
<tr>
<td>VCA</td>
<td>Epstein-Barr Virus Viral Capsid Antigen</td>
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Chapter 1  Epidemiology and clinical characteristics of MS

1.1 Preface
This Chapter gives a general background of multiple sclerosis, its geoepidemiology, clinical features, clinical course, risk factors, pathogenesis and immunomodulatory treatment. Information is also provided on serum lipid classification, metabolism and associated diseases. This is to provide succinct literature on MS relevant for understanding of the thesis.

1.2 General overview
Multiple sclerosis (MS) is an autoimmune, inflammatory disorder of the central nervous system (CNS) with considerable heterogeneity in outcome and a leading cause of physical, mental and visual disability in young adults.\(^1\) Although genetic susceptibility explains the clustering of MS within families\(^2\), it cannot fully explain the geoepidemiological variations in MS frequency\(^3\) and the changes in risk factors associated with migration\(^4\) or MS disease.\(^5\)

Further, the observation that concordance rates in monozygotic twins are considerably less than 100\% shows that MS is a complex disease, in which multiple environmental and lifestyle risk factors act together in a genetically susceptible individual to cause the disease.\(^6\)\(^-\)\(^8\)

A number of aetiological factors have been identified to play a role in MS susceptibility and disease progression. Among the aetiological factors associated with MS, low sun exposure or ultraviolet radiation (UVR) exposure,\(^9\) low 25-hydroxyvitamin D (25(OH)D),\(^10\) infection with Epstein-Barr virus,\(^11\) and cigarette smoking\(^12\) stand out because of the strength of supporting evidence. In relation to the genetics of MS, both HLA (Human Leukocyte Antigen) and non-HLA\(^13\) regions have been significantly associated with increased risk and progression of the disease.

Increasing evidence also suggests that serum lipids and comorbidities are associated with the disease course of MS.\(^14\),\(^15\) Serum lipids have been associated with clinical disability,\(^14\),\(^16\)
progression of disability\textsuperscript{14, 16} and disease activity on magnetic resonance imaging\textsuperscript{16}.

Comorbidities have been associated with the clinical course of MS\textsuperscript{17}, disability progression\textsuperscript{15}, health-related quality of life\textsuperscript{18}, and increased mortality\textsuperscript{19}.

MS has a highly variable inter and intra-personal clinical course, both in pattern and rate of deterioration.\textsuperscript{1} While there is a marked female to male sex ratio of the disease\textsuperscript{10}, this seems to be increasing\textsuperscript{20} with generally no difference in the severity of the disease between the male and female.\textsuperscript{21} The typical age of onset of MS is in the third and fourth decades of life but the burden of disease is most marked in the fifth to seventh decades.\textsuperscript{1}

In the study of the geoepidemiology of MS, one of the most striking features observed has been a positive latitudinal gradient in incidence\textsuperscript{22, 23} and prevalence\textsuperscript{24} but recent studies have underlined an attenuation of the gradient in MS incidence.\textsuperscript{25} Conversely, studies continue to report increasing incidence and prevalence of MS\textsuperscript{26, 27} with increase in the sex ratio over time.\textsuperscript{23, 28} This raises the question whether the latitudinal gradient is a real geoepidemiological feature or an artefact and whether the increase in incidence is a real effect or due to alteration in diagnostic criteria and ascertainment. Month of birth (MOB) has previously been described as a risk factor for MS and hypothesised to be related to maternal vitamin D levels during pregnancy\textsuperscript{29, 30} but this effect has not been consistently shown.\textsuperscript{31, 32}

There is currently no cure for MS. Available immunomodulatory therapy may help reduce the number of relapses, severity of symptoms and reduce the progression of disability to improve the quality of life of people with MS. Since the advent of disease-modifying drugs (DMDs) for MS, studies have been carried out to assess their effect on disease outcome.\textsuperscript{33, 34} While they are effective in reducing relapses, their effect on long-term disability is largely unknown.\textsuperscript{34, 35} It is therefore expedient to critically assess whether their extensive use is warranted.
In summary, the scope of this thesis will investigate the role of the serum lipids and comorbidities in the onset and progression of MS. This Chapter is intended to provide a general overview and critical examination of the epidemiology of MS, its clinical course and features, risk factors associated with the disease and those that operate in disability progression, pathophysiology and immunomodulatory treatment of the disease. This is to provide succinct literature on MS relevant for understanding of the thesis.

1.3 The epidemiology of MS

1.3.1 Increasing incidence and prevalence of MS

MS affects more than 2.5 million individuals worldwide, with higher incidence and prevalence in women than men. The prevalence of MS in Australia in 2010 was 21,283 people and at the rate of 95.5 per 100,000 people. The incidence and prevalence of MS have been reported to vary geographically. High prevalence areas of the world (prevalence of ≥30 per 100,000) include New Zealand, Israel, south-eastern Australia, northern parts of Europe, North America and southern Canada. In many of these geographical areas, prevalence is more than 100 per 100,000 with a highest reported estimate of 300 per 100,000 in the Orkney Islands. Medium prevalence areas (5-30 per 100,000) include southern Europe, most of Australia, Ukraine, southern United States, Central and South America. Low prevalence areas (<5 per 100,000) include Africa, Asia and South America. This geographic variation in MS frequency may be explained in part if not all by both differences in environmental factors as well as racial differences.

Accumulating epidemiological studies on MS suggest a trend of increasing incidence over the last few decades which partly explains the observed increase in prevalence. However, it remains to be clarified whether these increased rates reflect a real increase in MS incidence or merely due to temporal changes which can be linked to changes in diagnosis and treatment such as earlier diagnosis, alterations in diagnostic criteria and better case ascertainment.
In Newcastle, Australia, there has been a significant progressive increase in the incidence rate of MS, increasing from 1.2 to 2.4 per 100,000 population between 1950–1959 and 1986–1996 and a significant increase in the age-standardized prevalence from 19.6 and 59.1 per 100,000 population between 1961 and 1996.45 Similarly, in Hobart, Australia, the age-standardized incidence increased from 2.2 per 100,000 to 3.7 per 100,000 between 1951-1961 and 2001-2009 and the crude prevalence increased from 32.5 per 100,000 in 1961 to 125.2 per 100,000 in 2009.46

In Canada47, the incidence rate has increased from 5.2 to 22.3 between 1985–1989 and 1990–2004. In Sassari province of Sardinia, the incidence has increased from 1.1 to 5.8 between 1965–1969 and 1995–1999.40 Increased incidence rates have also been reported in France44, Netherlands48, Japan,39 Finland,49 Norway43,50 and Italy.51

In addition to a meta-analyses of MS prevalence and incidence, Koch-Henriksen and Sorensen23,25 examined cases of incidence studies that had been repeated after some years or decades in the same population. These authors reported that the repeated surveys showed a progressive increase in incidence with time.23,25 However the increase in incidence was not universal. For instance, in the population of Olmsted County, Minn., USA, whose registry depends on the Mayo Clinic, the incidence rates of MS appear to have been stable rather than increasing over the past 20 years.42 Similarly, the trend of incidence in Orkney,52 53 Shetland52,54 and Gothenburg, Sweden55 has been relatively stable or decreasing.

Although improvements in diagnostic accuracy could be responsible for the reported increased incidence, the marked increase over time cannot be explained by alterations in ascertainment or diagnostic criteria alone. For instance in northern Japan, the diagnostic criteria have not been changed since 2000 and therefore the trend of increased incidence observed after this period strongly suggests a possible real increased risk of MS.39 In
Sardinia, where the population is relatively genetically homogeneous and stable, repeated surveys have shown increasing disease incidence. The time scale of these changes tends to suggest changes in environmental factors.\textsuperscript{40}

In summary, evidence from available data shows an increased incidence (leading to increased prevalence) of MS over the last few decades but this increase is not universally found. The increase overrides changes in diagnostic methods, access to care and ascertainment which suggest real increase in the risk of MS. Understanding why these changes occurred would shed light on the causes of MS.

1.3.2 Latitudinal gradient of incidence and prevalence of MS

Early in the study of the epidemiology of MS, one of the most striking hallmarks of the disease was a latitudinal distribution of MS, with increasing latitudinal gradient in incidence and prevalence\textsuperscript{56} in both the northern and southern hemispheres. For instance in Australia, studies have established strong relationship between MS frequency and latitude, with the prevalence of MS reported to be more than six times higher in Hobart, Tasmania, which is in the south compared to tropical Queensland in the north.\textsuperscript{57-59} Also, the incidence and prevalence of MS in Hobart is almost twice that of Newcastle and Perth.\textsuperscript{59, 60} Similarly, in New Zealand, studies have demonstrated a robust association between MS frequency and latitude, with the prevalence of MS increasing three-fold from the Auckland (in the North) to Otago (in the South).\textsuperscript{61}

While latitudinal gradients have also been demonstrated in North America,\textsuperscript{62} Japan and Europe,\textsuperscript{63} this has not been universally found. Other studies have found no significant association between MS prevalence and latitude in Argentine Patagonia,\textsuperscript{64} Canada\textsuperscript{65} and Norway.\textsuperscript{66} Also, studies in Sardinia\textsuperscript{67} have reported higher prevalence than expected for their latitudes, while studies in Norway\textsuperscript{68} have found a lower prevalence than expected for their
latitudes. Evidence also suggests that the gradient of MS incidence may be decreasing.\textsuperscript{5, 69, 70} This has led some authors\textsuperscript{56, 62, 69} to suggest that the gradient is not real.

In an effort to investigate this issue and to provide evidence for or against the existence of a latitudinal gradient of MS frequency, data from individual studies have been combined and analysed using meta-analyses. Zivadinov and colleagues\textsuperscript{71} in the first meta-analysis of MS frequency, combined data from 22 incidence and 69 prevalence estimates published between 1980 and 1998. They reported a highly significant latitudinal gradient for the crude prevalence which was attenuated for the age and sex-standardised prevalence and the effect on incidence disappeared after age-adjustment. Alonso and Hérnan\textsuperscript{22} undertook a meta-analysis of MS incidence, including 38 age-standardised incidence estimates between 1966 and 2007. These authors reported that higher latitude was associated with higher MS incidence, but this latitudinal gradient was attenuated after 1980. They concluded that the gradient which was present in older incidence studies is decreasing.

Recently, Koch-Henriksen and Sørensen published two meta-analyses\textsuperscript{23, 25} of MS prevalence and incidence, reporting a clear trend of latitudinal gradient of incidence in Australia and New Zealand but without a significant latitudinal gradient of prevalence. In Western Europe and North America, the authors reported modest latitudinal gradient of prevalence but no association was found between incidence and latitude even when the search was restricted to surveys published before 1980 or 1970. In 2011, Simpson and colleagues\textsuperscript{24} conducted a more comprehensive meta-analysis of MS prevalence studies, including 650 prevalence estimates from 321 peer-reviewed studies in 59 countries between 1923 and 2009. These authors found a significant positive association between age-standardised prevalence and latitude. Further, these authors noted exceptions to the gradient in the Italian region and northern Scandinavia, which they suggested are explicable by genetic and behavioural-cultural variations. These authors argued that the meta-analyses by Koch-Henriksen and Sørensen\textsuperscript{23} had significant
methodological shortcomings for reporting no association between latitude and prevalence, or incidence after adjusting for study prevalence year in Australasia.

In addition, some migration studies\textsuperscript{72} if not all have reported that persons migrating from lower to higher latitude after the age of puberty are thought to carry their former high risk with them, while those that migrate prior to puberty seem to have the risk associated with the new area to which they migrated.\textsuperscript{69} This finding has been replicated by an Australian study.\textsuperscript{73}

In summary, evidence from the systematic reviews examined above shows that the latitudinal gradient of incidence and prevalence is a real geoepidemiological feature and not a manifestation of poor methodology. However, this gradient is not universally found and the intensity of the gradient may be decreasing, which has been suggested to be due to increasing incidence of MS in lower latitudes or regions closer to the equator.\textsuperscript{22}

1.3.3 Age of onset and sex ratio of MS frequency
The mean age of MS onset ranges from 28 to 31 years. The onset of MS can occur as late as the seventh decade, although rarely. The mean age of onset is a few years earlier for women than for men.\textsuperscript{74} Those who have the type of MS that starts with relapses (relapsing-remitting (RR) MS) tend to have an earlier onset, averaging 25 to 29 years. Those with RRMS may convert to a progressive phase at a mean age of 40 to 49 years.\textsuperscript{75} The mean age of onset for those whose onset is progressive rather than relapsing is substantially later, generally around 40 years of age.\textsuperscript{76} For the definitions of the types of MS clinical course mentioned under this section, see section 1.4.2.

A well-noted feature of MS is the characteristic pattern of higher susceptibility in females compared to males. In relation to this feature, recent studies have suggested that the sex ratio of MS appears to be dynamic, thus varying significantly over time.\textsuperscript{23} While in the past it was shown that there are two women for every man with a diagnosis of MS,\textsuperscript{3} most recent studies
have reported a sex-ratio of about three women for every man, especially in patients diagnosed with RRMS.\(^3\)

In a large population-based study from Canada, the female to male sex ratio increased significantly from 1.9:1.0 to 3.2:1.0 for those born in 1931–1940 and for those in born 1976–1980 respectively.\(^28\) In Denmark\(^77\), the sex ratio increased from 1.3:1.0 in 1950 to 2.0:1.0 in 2005. In Wales\(^27\), the sex ratio increased from 1.8:1.0 in 1985 to 4.3:1.0 in 2007. In Iran\(^78\), the sex ratio increased from 2.0:1.0 in 2002 to 3.1:1.0 in 2008 and in Japan\(^79\), the sex ratio increased from 1.2:1.0 in 1972 to 2.9:1.0 in 2004.

In a meta-analysis by Simpson and colleagues, the global prevalence sex ratio increased from 1.4:1.0 in 1949 to 2.3:1.0 in 2009, but this did not reach statistical significance (p<0.12) in this sample size.\(^24\) However, there was a statistically significant increase in the prevalence sex ratio over time in Australasia (p<0.023) and the UK region (p<0.003).\(^24\) This increasing trend has been noted by another recent meta-analysis showing that the worldwide sex ratio of MS has been substantially increasing over the last century.\(^23\) In another systematic review by Alonso and Hernan, for each five-year period, the male-to-female incidence ratio increased 6% on average, from a predicted mean sex ratio of 1.4 in 1955 to 2.3 in 2000. Recent increase in female incidence of MS has also been reported in France,\(^44\) in Norway,\(^50\) in Australia,\(^45\) and Germany.\(^80\) In conflict with the above finding, Boström and colleagues\(^81\) reported a stable sex ratio trend in Sweden. Simpson and colleagues also did not find any change in the sex ratio in greater Hobart over time.\(^46\) Data from high MS prevalence areas, such as New Zealand, has also shown sex ratio stability over time.\(^61\) This increase in female preponderance mainly affected patients with relapsing onset MS. (RRMS). Interestingly, female sex has also been shown to vary with latitude\(^82\) and that the rate at which MS prevalence increases with decreasing UVB was also higher in females than in males.\(^83\)
In summary, many studies reported an increasing trend of the sex ratio in MS and only few studies documented a stable sex ratio over time. It is therefore apparent that the sex ratio is increasing but this is not universally found. This increasing sex ratio of MS parallels MS incidence and a key driver for the worldwide increasing prevalence of this devastating disease. The rapid increase in the female preponderance occurring over a century is suggestive of environmental exposure to which only females are susceptible rather than genetic cause which requires time. A relative increase in smoking in women has been proposed to partly explain the increasing sex ratio in women. However this was mainly driven by a decline of smoking among men. Other factors such as older age at birth, reduced offspring number and reduced UV radiation in the first trimester of pregnancy have been suggested to contribute to the increasing sex ratio of MS. Further studies are needed to provide evidence as to what environmental factors are influencing the sex ratio.

1.3.4 Month of birth effect in MS
The MOB or season of birth effect describes the relationship between MOB and subsequent or future risk of MS. In the northern hemisphere, people born in spring months (March, April, May) have been reported to have slightly increased risk of MS and those born in winter (November, December, January) have a reduced risk. A recent meta-analysis replicated a significant excess of MS risk in those born in April (1.07, p=0.002) and May (1.11, p=0.0006), and a reduced risk in those born in October (ratio 0.94, p=0.004) and November (0.88, p=0.0002).

Similar to the pattern in the northern hemisphere, two studies conducted in the southern hemisphere reported risk of MS with MOB. In Australia, Staples and colleagues observed an increased risk of MS for those born in early summer (November-December) compared with those born in early winter (May-June) given that the seasons are reversed between the two hemispheres. In Brazil, Becker and colleagues demonstrated that births in the spring
(October, November & December) showed an increased risk of MS, whereas births in the autumn (April, May & June) had lower risk. Conversely, no month of birth effect has been reported in some studies in both hemispheres.89,90

The probability of being born in the spring is positively correlated with latitude, whereas the probability of being born in the winter is negatively correlated. A recent meta-analysis on the subject also showed a latitudinal variation in the month of birth effect.30 This season of birth effect was recently demonstrated to be a manifestation of UV exposure of the mother during early pregnancy.88

In challenge of this finding, Fiddes and colleagues91 reported that the relationship between MOB and risk of MS may be false positive finding because studies have not controlled for year and place of birth. However, a study that showed a significant MOB effect in MS risk by Staples and colleagues88 did adjust for region of birth and year of birth. In another study conducted in Sardinia, where cases were matched with family members as controls, an MOB effect was again observed.92

In summary, evidence from the studies examined suggests that there may be a true relationship between MOB and future MS risk. More studies are however needed that adequately adjusted for confounders including year of birth and region of origin in order to accurately estimate the magnitude of this effect.

1.4 Clinical features and clinical course of MS

1.4.1 Clinical signs and symptoms
MS has no unique clinical signs and symptoms, but some are highly characteristic of the disease. Sign and symptoms of MS may vary depending on the part of the central nervous system affected. The onset is often polysymptomatic where patients usually manifest heterogeneous group of signs and symptoms. The most common symptoms of MS include
sensory symptoms in limbs or face, vision loss (unilateral visual loss, diplopia), ataxia (difficulties with coordination and balance), and acute or subacute muscle weakness and spasms. Additional signs and symptoms that may develop with the disease course include fatigue, pain, gait disturbance and balance problems, Lhermitte’s sign (electric shock-like sensations that run down the back and/or limbs upon flexion of the neck), dysarthria (problems in speech), vertigo, bladder and bowel dysfunction, acute transverse myelopathy, dysphagia (problems in swallowing), tremor and seizures.\textsuperscript{93, 94}

Progression of the disease after onset may eventually lead to accumulation of disability. A number of measures have been created to clinically assess disability progression and symptom severity. Some of these include the Expanded Disability Status Scale (EDSS), Multiple Sclerosis Severity Score (MSSS) and Scripps Neurologic Rating Scale (NRS). The EDSS is the gold standard in assessing physical disability in MS, which utilises a ten-point disease severity scale from 0 (normal) to 10 (death due to MS).\textsuperscript{95} The EDSS is limited, in that it has a limited sensitivity and non-linear scale. MSSS is a useful measure of MS severity, which partially corrects for the limitation of the EDSS. The MSSS is a cross-sectional measure of disability progression, as it uses the EDSS and takes disease duration into account.\textsuperscript{96}

\subsection*{1.4.2 Clinical course of MS}
In 1996, a unified categorisation of MS clinical course was published.\textsuperscript{97} In that publication, the clinical course of MS was characterized as: relapsing-remitting, secondary progressive, primary progressive and progressive-relapsing MS. Since then, an increased understanding of MS and its pathology prompted a re-examination and revision of the disease phenotypes in 2013.\textsuperscript{97-99} The newer characterization of MS phenotypes was dependent on consideration of clinical relapse rate, imaging findings and disease progression. Progressive relapsing MS has been eliminated from the clinical course descriptions and clinically isolated syndrome has
The clinical course of MS has been categorized into four clinical subtypes based on the core phenotype characteristics of relapsing and progressive disease. The clinical subtypes now include clinically isolated syndromes (CIS), relapsing-remitting MS (RRMS), secondary-progressive MS (SPMS) and primary-progressive MS (PPMS).

1.4.2.1 Clinically isolated syndromes (CIS)
CIS is the first clinical presentation of a disease that demonstrates characteristics of inflammatory demyelination isolated in time that could possibly convert to definite MS, but has yet to meet the diagnostic criteria for MS. Common presentations of CIS include optic neuritis, a brainstem and/or cerebellar syndrome and spinal cord syndrome.

The core requirement for the diagnosis of MS is the objective demonstration of dissemination of lesions in the CNS in both space and time, based upon either clinical findings alone or a combination of clinical and MRI findings. Also, for individuals with CIS to become definite MS patients, they may have to develop a second exacerbation or relapse.

For patients with CIS who have MRI lesions at baseline, the chance of developing definite MS is about 60% to 80% by 10 years, and CIS patients who have a normal baseline MRI have a 20% chance of developing MS within 10 years.

1.4.2.2 Relapsing-remitting MS (RRMS)
RRMS is the most predominant form of the disease, accounting for more than 80% of cases. The relapsing-remitting form is characterised by unpredictable relapses followed by remissions. A relapse is defined as the acute or subacute appearance or reappearance of...
a neurological abnormality typical of an acute inflammatory demyelinating event in the central nervous system (lasting at least 24 hours), immediately preceded by a stable, improving, or slowly progressive neurological state for 30 days, in the absence of fever, known infection, concurrent steroid withdrawal, or externally derived increases in body temperature.\textsuperscript{102, 103} Most patients with RRMS will eventually enter a secondary-progressive phase.

1.4.2.3 Secondary progressive MS (SPMS)
A subset of persons with RRMS will after some amount of time convert to a progressive form of disease, featuring a steady accrual or progression to increased disability and worsening of function. Relapses can be seen during the early stages of SPMS, but are uncommon as the disease further progresses. Because there are no established criteria or predictors to determine when RRMS converts to SPMS, the diagnosis of SPMS is made retrospectively.\textsuperscript{99, 104} The conversion from RRMS to SPMS usually takes 10 to 20 years after disease onset. In one study, the median time from CIS to the development of SPMS was 19 years, while the median time from MS diagnosis to SPMS was 12 years.\textsuperscript{105}

1.4.2.4 Primary progressive MS (PPMS)
PPMS is characterized by progressive accumulation of disability from disease onset without any relapses or remission.\textsuperscript{104} The PPMS form of the disease may account for about 10-15\% of MS cases at disease onset.\textsuperscript{104, 106} A common clinical presentation of PPMS is a spinal cord syndrome with spastic paraparesis. PPMS patients have a more even sex distribution than RRMS and tend to have a worse prognosis for ultimate disability in comparison with patients who have RRMS.\textsuperscript{107}

1.5 Environmental, lifestyle, genetic factors in MS onset and progression
1.5.1 Sun exposure and MS
One explanation proposed for the observed association of MS with latitude is that exposure to sunlight may be protective, either because of an effect of ultraviolet radiation (UVR) directly
or via UVR-induced production of vitamin D. The protective effect of UVR-induced immunosuppression, direct or indirect via vitamin D, on MS is a plausible explanation of the gradient as ambient UVR levels decrease with increasing latitude. In line with this hypothesis, a number of studies have found an inverse relationship between sun exposure, serum levels of vitamin D and the risk or disease course of MS.

Sun exposure and risk of MS was investigated in the EnvIMS study. A total of 1660 MS patients and 3050 controls from Italy and Norway were included in the multinational case-control study to estimate the association between MS and measures of sun exposure in specific age periods. They reported significant association between infrequent summer outdoor activity and increased MS risk in Norway and Italy. The association was strongest between the ages of 16 and 18 years in Norway (OR: 1.83, 95% CI: 1.30–2.59) and between birth and age 5 years in Italy (OR 1.56, 95% CI: 1.16–2.10). In Italy, a significant association was also found during winter (OR 1.42, 95% CI: 1.03–1.97).

In one of the pioneering case-control studies conducted by van der Mei and colleagues to investigate whether past high sun exposure is associated with a reduced risk of MS, 136 MS cases and 272 controls were randomly drawn from the community and matched on sex and year of birth. The authors reported a dose-response relationship between MS and decreasing sun exposure when aged 6-15 years. Higher sun exposure when aged 6-15 years was associated with a decreased risk of MS (OR: 0.31, 95% CI: 0.16 - 0.59).

Islam and colleagues also studied the role of childhood sun exposure on the risk of MS in 79 pairs of identical twins where only one of the twins had MS. They found that the twin who developed MS had significantly lower sun exposure during childhood compared to the co-twins without MS. The risk of MS was substantially lower (OR: 0.40, 95% CI: 0.19 - 0.83) for the twin who spent more time in the sun compared with the co-twin.
1.5.2 Vitamin D and MS
There are two major forms of vitamin D. These are ergocalciferol and cholecalciferol. The physiologically active form is 1,25-dihydroxyvitamin D (1,25(OH)₂D) and the circulating metabolite used for assessment of vitamin D status is 25-hydroxyvitmain D (25(OH)D). Vitamin D has been known for its role in calcium homeostasis, brain development and function, cardiovascular and musculoskeletal health, anti-neoplastic properties, regulation of insulin production, anti-inflammatory properties and immunomodulatory effects. A number of prospective studies have shown that low vitamin D intake or serum vitamin D status are associated with increased risk of developing MS. In a prospective, nested case-control study among more than 7 million US military personnel, the risk of MS among the whites significantly decreased with increasing levels of 25(OH)D (Odds Ratio (OR) for a 50 nmol/L increase in 25(OH)D: 0.59; 95% CI: 0.36-0.97).

Consistent with this evidence, a prospective cohort study using the Nurses' Health Study (NHS; 92,253 women followed from 1980 to 2000) and Nurses' Health Study II (NHS II; 95,310 women followed from 1991 to 2001) examined the direct relationship between dietary vitamin D intake and risk of MS and reported that the risk of developing MS was significantly reduced for women in the highest quintile of total vitamin D intake at baseline compared to those in the lowest (Relative Risk (RR): 0.67; 95% CI: 0.40 - 1.12; p-trend 0.03).

A number of studies were also conducted to investigate the relationship between vitamin D and clinical course of MS – relapse and disability. Simpson and colleagues conducted a prospective cohort study of 145 participants with RRMS followed from 2002 to 2005 to investigate whether higher levels of serum 25(OH)D measured every six months were associated with a lower risk of relapses in people with MS. In that study, there was an inverse linear relationship between 25(OH)D levels and the hazard of relapse over the subsequent six
months, with hazard ratio (HR) of 0.91 per 10nmol/L increase in 25(OH)D level (HR: 0.91; 95% CI: 0.85–0.97; p=0.006).

To determine whether serum concentrations of serum vitamin D status predict disease activity and prognosis in patients with a clinically isolated syndrome, 465 patients were followed up for 5 years clinically and by magnetic resonance imaging. From analysis of the results, higher vitamin D levels predicted reduced MS activity and a slower rate of progression. A 50-nmol/L increment in average serum vitamin D levels within the first 12 months predicted a 57% lower rate of new active lesions (p<0.001), 57% lower relapse rate (p=0.03), 25% lower yearly increase in T2 lesion volume (p<0.001), and 0.41% lower yearly loss in brain volume (p=0.07) from 12 to 60 months. In analyses using dichotomous vitamin D levels, values greater than or equal to 50 nmol/L at up to 12 months predicted lower disability (EDSS score: -0.17; p=0.004) during the subsequent four years.

Smolders and colleagues investigated the association between serum levels of 25(OH)D and 1,25-dihydroxyvitamin D (1,25(OH)₂D), and clinical MS severity as expressed by EDSS score and relapse rate. They reported that low 25(OH)D levels were associated with high EDSS scores and high 25(OH)D levels were associated with a high chance of remaining relapse-free. In a study by Thouvenot and colleagues, a retrospective cohort analysis was performed in 181 MS patients without previous vitamin D supplementation. Disability (EDSS) was assessed in relation to vitamin D levels. From the analysis, there was a negative correlation between vitamin D level and EDSS score (r= -0.33, p<0.001).

A longitudinal study by Soilu-Hanninen and colleagues followed MS patients and healthy controls for 12 months and measured their level of serum vitamin D every three months and at the time of MS relapse. They reported that there was a significantly lower serum vitamin D level during MS relapse than in remission. Similarly, a case-control by Soilu-Hanninen confirmed that serum vitamin D levels were significantly lower in MS relapse than in
remission. In the same line, Smolders and colleagues in a cross-sectional study reported that a high serum vitamin D level was associated with a high chance of remaining relapse-free.

However, a case-control study by Nikanfar reported that there was no significant association between serum vitamin D levels and disability in MS patients (r= −0.08, p=0.280). Another study by Hatamian and colleagues showed no significant relationship between EDSS score and serum vitamin D levels in people with MS (p=0.35), after adjusting for covariates. Randomised clinical trials investigating the potential therapeutic benefits of vitamin D in MS patients were conflicting. A systematic review of randomized, double-blind, placebo-controlled trials examining the clinical efficacy of vitamin D in MS was conducted by Pozuelo-Moyano and colleagues. Five clinical trials were included in the review. Out of the five clinical trials included in the review, four of the trials showed no evidence of beneficial effect of vitamin D on any clinical outcome of MS measured and one showed a significant inverse relationship between vitamin D and number of T1 enhancing lesions on brain magnetic resonance imaging. Evidence from these studies was limited by small study sizes, heterogeneity of dosing, form of vitamin D tested and clinical outcome measured.

In summary, sun exposure and vitamin D were associated with the risk and disease course of MS. Measurement of sun exposure is subject to measurement error and recall bias since studies rely on subjects to approximate past sun exposure. Further, measurement of 25 (OH) D levels is subject to measurement error and systematic bias and this error increases with increasing 25(OH)D level. Although studies have demonstrated a fairly consistent inverse relationship between sun exposure/vitamin D and MS risk and disease course, randomised controlled trials on the efficacy of vitamin D to modulate the risk and progression of MS are inconsistent. Even though vitamin D supplementation appears to be a promising treatment worthy of further exploration, the paucity of published randomised controlled trials, small
study sizes and use of different doses in studies makes the evidence for vitamin D as a treatment for MS inconclusive. A number of large trials are now being conducted which will hopefully address some of these concerns.

1.5.3 Cigarette smoking
Several epidemiological studies have demonstrated a link between cigarette smoking and subsequent development of MS and rapid progression of the disease. Cigarette smoking may be seen as an important risk factor for MS given that it is relatively easily modified and could provide insights into the pathogenesis of the disease.\textsuperscript{129}

In 2009, a large case–control study by Hedstrom and colleagues demonstrated that cigarette smoking was associated with increased risk of MS. Smokers of both sexes had an increased risk of developing MS compared to never-smokers (OR: 1.4, 95% CI: 1.2–1.7 for women, and OR: 1.8, 95% CI: 1.3–2.5 for men).\textsuperscript{130} This finding was replicated by a larger cohort study of 277,777 male Swedish construction workers that assessed the risk of MS associated with cigarette smoking. Ever-smoking was associated with an increased risk for MS compared with never-smoking (RR: 1.9; 95% CI: 1.4–2.6).\textsuperscript{131}

A meta-analysis was conducted by Hawkes to assess the relationship between smoking and risk of MS. The meta-analysis of six studies showed significantly increased susceptibility to MS in smokers compared to non-smokers, with overall odds ratio ranging from 1.25 to 1.51.\textsuperscript{132} As a follow-up to the earlier meta-analysis, Handel and colleagues conducted a meta-analysis using a total of 14 articles, representing 3,052 cases and 457,619 controls. They reported that smoking was associated with 48% increased risk of MS (RR: 1.48, 95% CI: 1.35–1.63, p<0.001).

In MS disease course, a relationship has been established between cigarette smoking and disability progression. Smoking has also been associated with increased risk of early
progression from clinical isolated syndrome (CIS) to clinically definite MS\textsuperscript{133} and conversion from RRMS to secondary-progressive disease.\textsuperscript{134, 135} In a prospective Tasmanian cohort study, Pittas and colleagues\textsuperscript{136} followed 203 MS patients for a median of 909 days and smoking data were collected at baseline and six-monthly reviews. In a dose–response fashion, smoking was positively associated with the progression of clinical disability measured as MSSS (p=0.001). Similar results were found when EDSS was used as the clinical disability outcome. In addition, smoking was associated with an increased risk of a progressive course at MS onset rather than a relapsing-remitting course. However, smoking during the cohort period was not associated with the hazard of relapse (HR: 0.94; 95% CI: 0.69–1.26).

In a study aimed at investigating the effects of smoking on disability progression and disease severity in a cohort of 895 patients with clinically definite MS, the risk of reaching EDSS score milestones of 4 was 34\% higher in ever-smokers compared to never-smokers (1.34 (95\% CI: 1.12–1.60) and a milestone 6 was 25\% higher in ever-smokers compared to never-smokers (1.25 (95\% CI: 1.02–1.51)).\textsuperscript{137} Similarly, current smokers showed 64\% increased risk of reaching EDSS scores of 4 (1.64 (95\% CI: 1.33–2.02) and 49\% increased risk of reaching EDSS scores of 6 (1.49 (95\% CI: 1.18–1.86) compared with non-smokers. This study demonstrated that regular smoking may be associated with more severe disease and faster disability progression.

To replicate and establish a relationship between cigarette smoking and MS progression, Healy and colleagues\textsuperscript{134} conducted a large cross-sectional survey with 1,465 MS patients and a subsequent longitudinal follow-up for an average of 3.29 years. From the analysis, current smokers had significantly worse disability at baseline than never-smokers as measured by EDSS (p<0.001) and MSSS (p<0.001). In longitudinal analyses, progression from RRMS to secondary-progressive disease occurred faster in smokers than in never smokers (HR: 2.50, 95\% CI: 1.42, 4.41). In addition, compared to never-smokers, smokers had a faster rate of
increase in the T2 lesion volume (p=0.017) and a faster rate of decrease in brain parenchymal fraction (p=0.021).

Not all studies found a significant relationship between smoking and MS progression. For instance, a cross-sectional Dutch survey of 364 patients with MS by Koch and colleagues found no effect of cigarette smoking on disease progression or accumulation of disability.\textsuperscript{138} Also, a meta-analysis of four studies by Handel and colleagues showed a trend towards significant association between smoking and increased risk of secondary-progressive disease from RRMS (RR: 1.88, 95% CI: 0.98–3.61, p=0.06) but with considerable heterogeneity.\textsuperscript{139} In summary, the majority of the current epidemiological data suggest that cigarette smoking is associated with increased MS susceptibility and progression of the disease. A dose–response effect with higher consumption of cigarettes resulting in increased susceptibility and progression of clinical disability was reported in some studies. The overall magnitude of effect of smoking on MS susceptibility or disease course was small but the prevalence is substantial making the population attributable fraction (PAF) relatively high (PAF: 40.8%; 95% CI: 27.3–60.1)\textsuperscript{140} suggesting that smoking prevention efforts are worthwhile. Further studies evaluating the effect of smoking on the risk and course of established MS may shed light on the underlying disease mechanisms which may provide new insights for the better management and prevention of MS.

\subsection*{1.5.4 Epstein-Barr virus (EBV) and MS}
Many infectious agents have been identified to be associated with MS etiology and pathogenesis. The most consistent findings were in relation to past infection with EBV and a history of infectious mononucleosis (IM) and levels of some EBV markers.\textsuperscript{116} Although EBV infection in the general population has been described as being ubiquitous, evidence from
systematic reviews and meta-analyses have consistently demonstrated a positive relationship between EBV and MS risk and disease course.

Infectious mononucleosis (IM), a frequent clinical manifestation of primary EBV infection during adolescence or adulthood, has been consistently associated with the risk of developing MS.\textsuperscript{141} It has been consistently demonstrated that a history of infectious mononucleosis had a positive relationship with the subsequent occurrence of MS and this symptomatic manifestation of EBV infection beyond the immunotolerant phase earlier in life is an independent risk factor contributing to the cause of MS.\textsuperscript{142}

Thacker and colleagues\textsuperscript{143} in 2006 conducted a systematic review and meta-analysis of 11 case–control studies and 3 cohort studies to assess the role of a history of IM as a risk of MS. From the meta-analysis, the combined relative risk of MS after history of IM from the 14 studies was 2.3 (95\% CI: 1.7–3.0; p<0.001). As an extension of this work, Handel and colleagues\textsuperscript{11} identified articles published since the original meta-analysis investigating MS risk following IM. A total of 18 articles were included in this study, representing 19,390 MS cases and 16,007 controls. From the results, history of IM was strongly associated with more than two-fold increased risk of MS (RR: 2.17; 95\% CI: 1.97–2.39; p<0.001).

Several studies have been conducted investigating the association between the risk of MS and serological parameters of EBV. For instance, Ascherio and Munger\textsuperscript{144} investigated the risk of MS in EBV seronegative versus seropositive subjects by reviewing 13 epidemiological studies. They reported an odds ratio of 0.06 (95\% CI: 0.03–0.13, p<0.001) indicating that the risk of MS in the EBV seronegative individuals is many folds lesser than that of the EBV positive individuals. As a follow-up to this work, Pakpoor and colleagues\textsuperscript{145} conducted a meta-analysis of 22 epidemiological studies aiming to investigate the association between MS and EBV. The authors reported that the odd ratio for developing adult MS in EBV
seronegatives was significantly lower compared to the EBV seropositive individuals (OR: 0.18; 95% CI: 0.13–0.26).

A more recent systematic review and meta-analysis of the seroepidemiological association between EBV and risk of MS by Almohmeed and colleagues\textsuperscript{146} included 39 studies in systematic review and meta-analysis. The meta-analysis showed a significant higher seropositivity to anti-EBNA IgG (OR: 4.5; 95% CI: 3.3–6.6, p<0.001) and anti-VCA IgG (OR: 4.5; 95% CI: 2.8–7.2, p<0.001) in cases compared to controls. No significant difference in seropositivity to anti-EA IgG was found (OR: 1.4; 95% CI: 0.9–2.1, p<0.09).

In the disease course of MS, changes in MRI and changes in disability are the main exhibitions of progression, while relapse considered a measure of disease activity. The progression of disability in MS is mainly measured by EDSS or MSSS. In a longitudinal study by Simpson and colleagues\textsuperscript{147} to investigate the relationship between anti-EBV IgG titers and MS clinical course, a prospective cohort of 198 clinically definite MS patients of the Tasmanian MS Longitudinal study was used. They found that neither antibodies to EBNA nor VCA was associated with the hazard of relapse. There was no evidence that higher anti-EBV-EBNA IgG or anti-EBV-VCA IgG levels were associated with level of or change in disability.

Similarly, in a study by Farrell and colleagues\textsuperscript{148} to investigate disease activity, EBNA-1 IgG correlated with change in EDSS (r= 0.3, p=0.035). Further, gadolinium-enhancing lesions on MRI correlated with EBNA-1 IgG (r= 0.33, p<0.001). EBNA-1 IgG also correlated with change in T2 lesion volume (r=0.27, p=0.044).

A study was also conducted to determine the immune responses to EBV candidate viral triggers of MS in patients with CIS, and to evaluate their potential value in predicting conversion to MS.\textsuperscript{149} There was a correlation between immune responses to EBNA1 and EDSS and T2 lesions during follow-up. In a univariable Cox regression model, increased
EBNA1-specific IgG responses predicted conversion to MS (HR: 2.2; 95% CI: 1.2-4.3 p=0.003). In other studies, lesion volume or number of lesions measured by MRI has been associated with antibody levels to EBNA1.\textsuperscript{148-150} Another important viral marker, viral capsid antigen (VCA) has also been associated with a greater atrophy in total brain and increased lesion volume in MRI image.\textsuperscript{151}

Although EBV infection is ubiquitous, epidemiological studies have established a relationship between EBV and MS risk and disease progression. The temporal relationship that exists between infection with EBV in adulthood or development of antibodies against EBV antigens year before disease onset, supports an etiological role of EVB in MS. However, further studies are warranted to investigate whether this relationship is causal.

\textbf{1.5.5 Genetic susceptibility}

There is substantial genetic epidemiological evidence supporting a role of genetic risk factors in the development of MS. Genetic studies of twins, sibling and adoptees have helped to distinguish between environmental and genetic cause of the disease. In these twin studies, the risk of developing MS for identical twins (genetically identical) varies between 15\% and 39\% in different populations. The risk of developing MS for fraternal twins (50\% of genetic component in common) is about 3\% to 5\% which approaches the recurrence rate in other biological siblings.\textsuperscript{6, 7, 152}

Another distinguished feature of the genetic susceptibility of MS is the marked familial aggregation. While MS is not hereditary disease, having a first-degree relative such as a sibling or parent with MS does substantially increase a person’s risk of developing the disease. Nearly 10\% to 20\% of MS patients have another relative with MS.\textsuperscript{153-155} First-degree relatives of an MS patient are 18 to 36 times more likely to acquire MS compared with the general population.\textsuperscript{153, 154}
The frequency of familial MS varies from 3% to 23% in different studies. One well-designed population study of 8,205 Danish patients with MS found that the relative lifetime risk of MS was increased sevenfold (95% CI: 5.8-8.8) among first-degree relatives (n = 19,615). The excess familial lifetime risk for first-degree relatives was 2.5% (95% CI: 2.0-3.2) added to the sporadic absolute risk of MS in Danish women and men of 0.5% and 0.3%. These sporadic rates from the Danish population are among the highest in the world.

Further evidence for genetic susceptibility in MS comes from half-sibling, stepsibling and adoptees studies. Age-adjusted full-sibling (50% of genome in common) risk is reported to be 3.1%, while half-sibling (25% of genome in common) risk in the same families was 1.89%, which is significantly lower and around half of the risk for full siblings. In addition, the risk of MS in stepsiblings who share no genetic component was indistinguishable from that of the general population. From these studies, it could be concluded that the risk of MS increases for the family members of MS patients who have more genes in common.

Data from whole-genome association studies indicate that the risk of developing MS is associated with certain class I and class II alleles of the major histocompatibility complex (MHC) on chromosome 6p21. Variations in the HLA-DRB1 gene are thought to be the strongest genetic risk factors for developing MS. Variations in several HLA genes have been associated with increased MS risk, but one particular variant of the HLA-DRB1 gene, called HLA-DRB1*15:01, is the most strongly linked genetic factor.

Systematic attempts to identify linkage in multiplex families have confirmed that variation within the major histocompatibility complex (MHC) exerts the greatest individual effect on risk. When susceptibility has been fine-mapped to an extended HLA Class II haplotype DQA1*0102 DQB1*0602 DRB1*1501 DRB5*0101, the estimated relative risk ranged between 2 and 4. The three candidate risk genes of this haplotype, HLADRBI*1501
(encoding HLA-DR2b), HLADR5*0101 (encoding HLADR2a) and HLADQB1*0602 (encoding HLA-DQ6), are so tightly linked that they are almost invariably inherited together. The association is strongest in northern Europeans, but is seen in virtually all populations with a notable exception in some Mediterranean populations where MS is associated with DR4.

Apart from HLADR1*15, there are other risk increasing alleles. For instance, HLADR1*17 increases the risk of MS but to a lesser extent when compared to HLADR1*15. When inherited together, HLADR1*14 completely abrogates any risk associated with HLADR1*15. The relative risk of MS for carrying the HLA-DRB1*15 allele is about three. A combination of HLADR1*14 and HLADR1*15 reduces the relative risk to approximately one. Epistasis, (complex gene–gene interactions) has also been indicated to occur in the HLA region. For example, HLA-DRB1*08 which has a modest effect, more than doubles the risk associated with HLA-DRB1*15. In addition, HLADR1*01 and HLADR1*10 protect against MS but only in the presence of HLA-DRB1*15 that operate from the other chromosome—that is in trans, although reports from Sweden suggest that HLADR1*01 may be protective on its own.

The HLADR1 gene belongs to a family of genes called the human leukocyte antigen (HLA) complex. The HLA genomic region encodes the MHC, a key protein complex involved in identification of the body's own proteins from proteins made by foreign invaders (MHC Class I), as well as in antigen presentation (MHC Class II). Each HLA gene has many different normal variations, allowing each person's immune system to react to a wide range of foreign proteins.

Several genome-wide association studies have provided genome-wide significant support for several non-HLA MS risk genes. Among these are IL7R, IL2R, CLEC16A, CD58, CD226, KIF1B, KIF21B, CD6, IRF8, TNFRSF1A, and two loci on
chromosome 12 and 20, which have been suspected to relate to the genes CD40 and CYP27B1\textsuperscript{178}, TYK2 and STAT3.\textsuperscript{179} Strikingly most of these molecules are associated with immune function.

1.6 Serum lipids: Lipoprotein classification, metabolism, and role in MS

1.6.1 Lipoproteins

Lipoproteins are complex aggregate of spheroidal macromolecules of lipids and proteins. Lipoproteins function as transporter molecules for lipids such as cholesterol and triglycerides, for fat and other fat soluble nutrients like vitamins A, K, D and E. Lipoproteins also function in regulating lipid synthesis and catabolism and maintaining cholesterol homeostasis.\textsuperscript{180} Cholesterol like all the other fats and fat-soluble vitamins are not water-soluble and are insoluble in blood, hence the requirement to be carried in “packages” called lipoproteins. They transport lipids from the blood to various tissues (adipose and other cells) and different organs (liver) for energy utilization, lipid deposition, steroid hormone production, and bile acid formation.\textsuperscript{181, 182} Lipoproteins consist of a hydrophobic core containing phospholipid, fat-soluble antioxidants and vitamins, and cholesteryl ester, and a hydrophilic coat that contains free cholesterol, phospholipid and protein molecules referred to as apolipoprotein.\textsuperscript{182} Lipoproteins consist of the following parts:

- A core of fats (triglycerides), cholesterol esters (cholesterol linked to fatty acids), and fat-soluble vitamins.
- A monolayer membrane of phospholipids and small amounts of free cholesterol.
- Proteins called “apoprotein” which may be “integral” apoproteins (apoA or apoB) penetrating as a transmembrane protein through the monolayer membrane, compared to the “peripheral” apoproteins (apoC or apoE) that are on the outer surface of the phospholipid membrane.
Apoproteins determine the overall structures and metabolism of lipoproteins, and the interactions with receptor molecules in liver and peripheral tissues. Lipoproteins are classified or distinguished from each other by size, relative densities of the aggregates on ultracentrifugation, relative mobility on electrophoresis on agarose gels, composition and function. The densities of these lipoproteins are related to the relative amounts of lipids to proteins in the complex. The higher the protein content the higher the density of the lipoprotein. By density, chylomicrons are the lowest in density followed by the chylomicron remnants, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and high density lipoproteins (HDL). According to the nomenclature based on the relative mobility on electrophoresis on agarose gels, HDL, VLDL and LDL corresponds to α, preβ and β lipoproteins respectively.

There are 5 distinct lipoproteins: In increasing order of density, these are

- **Chylomicrons**: They are the largest lipoproteins and transport exogenous lipid (mainly triglycerides) from intestine to all cells.
- **VLDL**: transport endogenous lipid (mainly triglycerides) from liver to cells.
- **IDL**: they are transient and formed during the conversion of VLDL to LDL. They are not normally present in plasma.
- **LDL**: they are formed from VLDL and carry cholesterol to cells
- **HDL**: they transport cholesterol from cells back to liver (reverse cholesterol transport). These lipoprotein can be further divided by density into HDL\(_2\) and HDL\(_3\)

Each lipoprotein contains a specific content of protein, triglyceride, cholesterol and cholesterol ester (table 1.1). From table 1.1 below, the large chylomicron molecules and VLDL are the main carriers of triglyceride in blood compared to the IDL, LDL and HDL
molecules carrying predominantly cholesterol ester. Triglyceride molecules consist of one
glycerol molecule attached by an ester bond to 3 fatty acid molecules. Triglycerides form the
basis of our energy stores of fatty acids and may provide fuel for beta-oxidation to generate
ATP. Cholesterol ester is cholesterol esterified to one fatty acid molecule and is essentially
the mechanism by which cholesterol is transported to various cells for use in cell membranes.
The integrity and stability of cell membranes is dependent on the relative content of these
fatty acids and cholesterol. Cholesterol is also the precursor of many hormones including
Cortisol, Aldosterone, Testosterone and Oestrogen, all vital to optimal health.

| Table 1.1: Physical properties and lipid compositions of lipoprotein classes |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Density (g/ml)                                  | Chylomicron     | VLDL            | IDL             | LDL             | HDL             |
| < 0.94                                         | 0.94-1.006      | 1.006 – 1.063   | 1.006-1.063     | 1.063-1.210     |
| Diameter (Å)                                   | 6000-2000       | 600             | 250             | 250             | 70-120          |
| Protein                                        | 1%              | 10%             | 10%             | 20%             | 50%             |
| Triglyceride                                   | 85%             | 55%             | 10%             | 13%             | 6%              |
| Cholesterol                                    | 2%              | 7%              | 11%             | 10%             | 7%              |
| Cholesterol ester                              | 3%              | 18%             | 50%             | 48%             | 40%             |
| Phospholipids                                  | 8%              | 20%             | 29%             | 24%             | 46              |

Table reproduced from Saba and Oridupa\textsuperscript{183}.

1.6.2 Apoproteins
The protein components of lipoproteins are called apoproteins (apo). They are synthesized in
the liver, intestine, brain and other tissues. The apoproteins have important roles in lipid
transport and metabolism. They have specific structural domains that are recognized by cell
receptors. Apoproteins have amphipathic α-helixes that are thought to be essential for apoprotein-phospholipid interaction.\textsuperscript{180}

The affinities of the apoproteins for the surface components of the lipoprotein change during lipoprotein metabolism. Apoproteins often diffuse from one lipoprotein and bind to another. Only apoprotein B (apoB48 & apoB100) are non-exchangeable and therefore maintains its association with the lipoprotein during the cycle of lipoprotein metabolism.

ApoA is the major apoprotein associated with HDL. ApoA-I and apoA-II are activators of lecithin-cholesterol acyltransferase (LCAT) and hepatic lipase respectively. ApoB100 is the predominant apoprotein found with LDL and it is the ligand for LDL receptor. The ratio of apoA to apoB has been used as a risk of cardiovascular disease risk. Table 1.2 summarizes the main types of apoproteins, lipoproteins associated with and their function. ApoE has three isoforms (E2, E3, and E4). ApoE is a ligand for hepatic chylomicron and VLDL remnant receptor and for LDL receptor. ApoE is also synthesized in the brain and involved in lipid homeostasis. ApoD are mainly associated with HDL and may function as multiple ligand protein.
Table 1.2: Classes of apoproteins, their molecular weight and functions

<table>
<thead>
<tr>
<th>Apoprotein</th>
<th>Molecular weight</th>
<th>Lipoprotein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo A1</td>
<td>28,100</td>
<td>HDL</td>
<td>Lecithin cholesterol acyltransferase (LCAT) activation. Main structural protein. Binds ABCA1 on macrophages</td>
</tr>
<tr>
<td>Apo A2</td>
<td>17,400</td>
<td>HDL</td>
<td>Enhances hepatic lipase activity</td>
</tr>
<tr>
<td>Apo A4</td>
<td>46,000</td>
<td>CM</td>
<td>Enhances triacylglycerol uptake</td>
</tr>
<tr>
<td>Apo AV(5)</td>
<td>39,000</td>
<td>HDL</td>
<td>Derived from Apo B100 – lacks the LDL receptor</td>
</tr>
<tr>
<td>Apo B48</td>
<td>241,000</td>
<td>CM</td>
<td>Depends on Apo B100 – lacks the LDL receptor</td>
</tr>
<tr>
<td>Apo B100</td>
<td>512,000</td>
<td>LDL, VLDL</td>
<td>Binds to LDL receptor</td>
</tr>
<tr>
<td>Apo C1</td>
<td>7,600</td>
<td>VLDL, CM</td>
<td>Activates LCAT</td>
</tr>
<tr>
<td>Apo C2</td>
<td>8,900</td>
<td>VLDL, CM</td>
<td>Activates lipoprotein lipase</td>
</tr>
<tr>
<td>Apo C3</td>
<td>8,700</td>
<td>VLDL, CM</td>
<td>Inhibits lipoprotein lipase</td>
</tr>
<tr>
<td>Apo D</td>
<td>33,000</td>
<td>HDL</td>
<td>Associated with LCAT, progesterone binding</td>
</tr>
<tr>
<td>Apo E</td>
<td>34,000</td>
<td>HDL</td>
<td>At least 3 forms. Binds to LDL receptor</td>
</tr>
<tr>
<td>Apo(a)</td>
<td>300,000-800,000</td>
<td>LDL, Lp(a)</td>
<td>Linked by disulfide bond to apo B100 and similar to plasminogen</td>
</tr>
<tr>
<td>Apo H, J, L</td>
<td></td>
<td>HDL</td>
<td>Poorly defined functions</td>
</tr>
<tr>
<td>Apo M</td>
<td></td>
<td>HDL</td>
<td>Transports sphingosine-1-phosphate</td>
</tr>
</tbody>
</table>

Table reproduced from Saba and Oridupa.\(^{18,9}\)

### 1.6.3 Serum lipid synthesis and metabolism

Lipids are derived exogenously from food or synthesized endogenously in the body. The exogenous pathway starts with the intestinal absorption of dietary cholesterol and fatty acids. The endogenous pathway starts in the liver. The mechanisms regulating the amount of dietary cholesterol and fatty acids that is absorbed are unknown.

#### 1.6.3.1 Chylomicron

Chylomicron is an endogenously synthesized lipoprotein that transports dietary lipids to peripheral tissues and the liver. Within the intestinal cells, free fatty acids released from dietary fats by digestion combine with glycerol to form triglycerides, and cholesterol is esterified by cholesterol acyltransferase (ACAT) to form cholesterol esters. Inside the jejunal enterocytes, triglycerides, cholesterol, phospholipids and ApoB\(_{48}\), are then assembled to form chylomicrons. Chylomicrons enter the circulatory system through the thoracic duct.
The main apoprotein of chylomicron is Apo B48, but Apo CII and Apo E are acquired in the lymph and plasma as the chylomicrons enter the circulation. Lipoprotein lipase (LPL) through the Apo CII cofactor hydrolyses the core triglycerides to free fatty acids and glycerol, a process that yields chylomicron remnant particles. The free fatty acids are then used as an energy source by muscle cells or stored in adipose tissue. The chylomicron remnants enriched in cholesterol, ApoB and ApoE then bind rapidly to hepatic chylomicron remnant receptors (LDL-receptor-related protein), for which apoE is a high affinity ligand. Within the hepatic cells the cholesterol is utilized and the apoprotein catabolized. Thus, chylomicron facilitates the delivery of triglycerides to muscles and adipose tissue and cholesterol to the liver.\textsuperscript{184}

The liver has a central role in the endogenous cholesterol synthesis and metabolism. The endogenous pathway of serum lipid metabolism begins with the synthesis of VLDL by the liver. VLDL is a large particles containing a core of triglycerides (synthesized from fatty acid and glycerol) and cholesterol esters (synthesized locally or derived from chylomicron remnants). VLDL also consists of apoCII which acts as a cofactor for lipoprotein lipase, apoCIII which inhibits this enzyme, and apoB\textsubscript{100} and apoE which serve as ligands for the apoB\textsubscript{100} and ApoE receptor respectively.\textsuperscript{184, 185}

In peripheral tissues, lipoprotein lipase hydrolyses the triglyceride core of nascent VLDL particles to free fatty acids and glycerol. The free fatty acids released by lipolysis can be either used as substrates by skeletal muscle or myocardium, or can be taken up by adipose tissue and then reassimilated into triglyceride for storage.

During lipolysis, the triglyceride core of the VLDL particle is reduced, generating VLDL remnant particles called IDL. VLDL remnants can either be cleared from the circulation by the apoB\textsubscript{100}/apoE receptors or further hydrolysed by the endothelial enzyme hepatic lipase to LDL by losing triglyceride and apoE.\textsuperscript{184, 185}
1.6.3.2 LDL
LDL is produced by VLDL catabolism and not directly secreted from hepatocytes of the liver. LDL particles are concentrated with cholesterol and cholesterol esters and depleted of triglycerides and containing only apoB<sub>100</sub>. They are the major carriers of the total plasma cholesterol.

LDL can be taken up by both hepatic and non-hepatic tissues. Within these tissues or cells, LDL particles are broken down by lysosomes to release cholesterol. This cholesterol internalized by non-hepatic cells is used in steroid hormone production, cell membrane synthesis or stored in the esterified form. In the liver, cholesterol is converted to bile acids and secreted into the intestinal lumen. The internalization of LDL is regulated by cellular cholesterol requirements through negative feedback control of apoB100 and apoE receptor expression. Cells in positive feedback cholesterol balance suppress apoB100 and apoE receptor expression.

Although most of the plasma LDL is removed by LDL receptors, if plasma cholesterol is excessive, LDL particles can infiltrate tissues by passive diffusion and can cause damage as in atheroma formation. Alternatively, LDL is removed through the scavenger cell pathway which only recognises chemically modified LDL as in oxidised LDL.

Decreased activity of HMG CoA reductase, the enzyme that controls the rate of de novo cholesterol synthesis by the cell, leads to a decrease in cell cholesterol, increased expression of apoB100 and apoE receptors, and enhanced uptake of cholesterol from the circulation thereby reducing the plasma cholesterol concentration. Statin drugs inhibit this rate-limiting enzyme to reduce biosynthesis cholesterol.

Chemically modified LDL such as oxidized LDL can also enter macrophages and some other tissues through the unregulated scavenger receptor. This pathway can result in excess accumulation of intracellular cholesterol and the formation of foam cells, which contribute to the formation of atheromatous plaques.
1.6.3.3 HDL

HDL is associated with reverse cholesterol transport, a series of enzymatic reactions that stimulate the transport of intracellular cholesterol from non-hepatic tissues back to the liver for elimination as bile salts or biliary cholesterol.\(^{187}\) HDL is cardio-protective because of the reverse cholesterol transport system, increased atherosclerotic plaque stability, protection of LDL from oxidation and maintaining the integrity of the vascular endothelium.

During chylomicron and VLDL lipolysis, apoproteins in their phospholipid surface coats (apo AI, apo AII, apo CII) are released and can be used to form HDL in serum. HDL particles can also be secreted de novo from hepatocytes and jejunal enterocytes. HDL consists of free cholesterol, phospholipids, apoE with apoA as the predominant apoprotein.

HDL acquires free cholesterol from tissue sites and other lipoproteins as the nascent HDL particles contain relatively little cholesterol. This cholesterol acquisition is stimulated by adenosine triphosphate-binding cassette protein 1 (ABC1). ApoA-I on the surface of HDL plays a central role in this process. It serves as a signal transduction protein to mobilize cholesterol esters from intracellular pools. After diffusion of free cholesterol onto HDL, the cholesterol is esterified to cholesterol esters by lecithin cholesterol acyltransferase (LCAT), a plasma enzyme that is activated primarily by apoA-I. This esterified cholesterol is either directly transported to the liver by HDL or transferred to LDL, VLDL and chylomicron remnants for delivery to the liver for excretion in bile or taken up for steroid hormone synthesis. Cholesterol ester transfer protein (CETP) is involved in these processes. HDL also contains other enzymes including paraoxonase which has antioxidant role.

By a similar mechanism, HDL can act as an acceptor for cholesterol released during lipolysis of triglyceride containing lipoproteins. Cholesterol efflux regulatory protein also appears to play an important role in the uptake of cellular cholesterol by HDL by promoting the transfer of intracellular cholesterol to the cell membrane.\(^{188, 189}\)
1.6.3.4 Lipoprotein (a)
Lipoprotein (a) often shorten as Lp(a) is a unique macromolecular lipoprotein which consists of LDL covalently bound to apoprotein(a) or apo(a) by a disulphide bridge. They are believed to be assembled extracellularly, either in circulation or at the surface of the hepatocytes. The function or metabolic fate of Lp(a) has been elusive probably because of its similarity to LDL. An elevated level of Lp(a) has been shown to be an independent risk factor for cardiovascular disease. This may be due to its high affinity for the arterial wall and athero-thrombogenic properties. Interestingly, lipoprotein-lowering drugs such as statins do not significantly alter or reduce Lp(a) levels. However, nicotinic acid (LDL-apheresis) has been reported to have moderate efficacy.

1.6.4 Disorders of lipid metabolism
1.6.4.1 Familial hypercholesterolemia
This is usually an inherited autosomal dominant disorder. The inheritance of one mutant gene that encodes for the LDL receptor results in impaired LDL catabolism and hypercholesterolemia. Depending on the type of mutation, there may be reduced synthesis of LDL receptor, failure of transport of the synthesized receptor to the Golgi complex within the cell, defective LDL binding or inadequate expression or defective recycling of the LDL receptor at the cell surface.

Clinically, familial hypercholesterolemia is characterized by a high total cholesterol and LDL cholesterol level from birth, a propensity to tendon xanthomata, and early onset coronary heart disease. It affects about 1 in 500 people in most populations. The associated impairment in function of these receptors results in reduced clearance of LDL particles from the circulation and an elevation in plasma LDL cholesterol. There is also increased uptake of modified LDL by the macrophage scavenger receptors, resulting in macrophage lipid accumulation and foam cell formation.
Individuals heterozygous for an LDL receptor mutation express half the normal number of functional receptors on their cell surface. Their cells bind, internalize, and degrade plasma LDL at half the normal rate. This produces a twofold elevation in plasma LDL cholesterol concentration (300 to 500 mg/dl). The excess plasma LDL cholesterol deposits in tendons and arterial walls, forming tendon xanthomas and atherosclerotic plaques (30).

1.6.4.2 Familial combined hyperlipidaemia
Familial combined hyperlipidaemia may be inherited as an autosomal dominant lipid disorder. Others have argued that it may involve multiple susceptibility genes. In affected families, some individuals may experience high triglyceride levels, some high cholesterol levels and some may experience both. In many cases, familial combined hyperlipidaemia may be caused by overproduction of hepatically derived apoB100 associated with VLDL or a decrease in its catabolism. About 0.5 per cent of the European population is affected, and there is an increased incidence of coronary artery disease in family members. Familial combined hyperlipidaemia is thought to cause up to 10% of the coronary heart disease occurring before the age of 60 years.

1.6.4.3 Familial hypoalphalipoproteinemia
Familial hypoalphalipoproteinemia is an autosomal dominant disorder that has been linked to premature coronary heart disease (CHD) and stroke. Affected individuals and their relatives have low levels of high density lipoprotein-cholesterol and apoprotein A. The defect appears to be due to mutations in the apoA-I gene.

1.6.4.4 Familial HDL deficiency and Tangier disease
Familial HDL deficiency is an autosomal dominant disorder associated with very low serum HDL concentrations and premature CHD. Tangier disease is an autosomal co-dominant condition. Homozygotes have elevated plasma triglyceride levels and HDL deficiency and heterozygotes have HDL cholesterol and apoA concentrations about half that of normal individuals. The defect in both familial HDL deficiency and Tangier has been
associated with mutations in the ATP-binding cassette transporter (ABC1), the gene that encodes for the cholesterol efflux regulatory protein.\textsuperscript{205, 208}

HDL mediated cholesterol efflux from macrophages and intracellular lipid trafficking are impaired in this disorder, leading to the presence of foam cells throughout the body and hepatosplenomegaly, peripheral neuropathy, and frequently premature coronary disease.\textsuperscript{207, 209}

\section*{1.6.4.5 Abetalipoproteinaemia (Bassen-Kornzweig Syndrome)}

Abetalipoproteinaemia (ABL) is a rare Mendelian disorder of lipid metabolism caused by a mutation in the gene encoding the microsomal triglyceride transfer (MTTP) protein.\textsuperscript{210} MTTP is an endoplasmic reticulum protein that transfers triglycerides, cholesteryl esters, and phospholipids to apoB100 in the hepatocyte and apolipoprotein apoB48 in the enterocyte. The mutation in MTTP prevents apoB100 and apoB48 from combining with triglycerides to form VLDL and chylomicrons, respectively.\textsuperscript{210} The absence of functioning chylomicrons inhibits effective lipid absorption in the intestines leading to hypolipidaemia, fat malabsorption, and neurologic disorders. The absence of VLDL results in the accumulation of triglycerides within hepatocytes and results in significant steatosis.\textsuperscript{210, 211}

Consequently, patients with ABL exhibit intestinal lipid malabsorption, low plasma lipid levels, hepatic steatosis, and neurological and ophthalmological symptoms.\textsuperscript{210}

Unless treated, abetalipoproteinemic subjects develop gastrointestinal, neurological, ophthalmological, and haematological abnormalities.\textsuperscript{211}

\section*{1.6.5 The role of lipids in the aetiology of MS}

The role of serum lipids in the aetiology of MS will be described in Chapter two.

\section*{1.7 Pathogenesis of MS}

MS is a heterogeneous disorder characterised by variable clinical course. The major pathologic mechanisms that cause the clinical manifestations include inflammation,
demyelination and axonal degeneration. Early in the disease course, MS involves recurrent bouts of CNS inflammation that results in damage to the myelin sheath surrounding axons as well as extensive axonal loss. Histologic examination reveals foci of severe demyelination, decreased axonal and oligodendrocyte numbers, and gliotic scarring. The exact cause of inflammation remains unclear, but an autoimmune response directed against CNS antigens is suspected.

It is believed that early in the disease course, disruption of the blood-brain barrier (BBB) at active lesion points facilitates increased permeability and the migration of inflammatory cells from the periphery into the CNS. T cells are widely considered to be primary effectors of the autoimmune CNS inflammation that characterizes MS. In MS, it has been proposed that autoreactive T cells from the periphery become activated by presentation of antigens, which may include myelin basic protein, proteolipid protein and myelin oligodendrocyte glycoprotein. Upon antigen presentation, naive T cells bearing the CD4-I- surface marker may differentiate into effector subsets including Thl, Th2 and Thl7. T-cell subtypes with particular relevance in MS pathogenesis include T helper-1 (Thl), Th17, superantigen reactive yô and CD8+ cells generally considered to mediate inflammation with resultant neurodegeneration. Functional deficits among regulatory T cells (Tregs) allow proinflammatory cytokine products to tip the balance of intrinsic damage and repair mechanisms in favour of inflammation and subsequent neurodegeneration.

Proinflammatory cytokines stimulate the activation/proliferation of additional T cells, B cells and macrophages, stimulate MHC class II expression on antigen-presenting cells, reduce production of anti-inflammatory cytokines and enhance cytolytic activity of CD8+ cells, macrophages and certain natural killer (NK) cells. The proinflammatory cytokines IL-2, IL-12, IL-17, IL-23, tumour necrosis factor-α and osteopontin are considered pivotal mediators of CNS inflammation in MS.
Activated T-cell migration across the BBB is facilitated by chemokines and activated matrix metalloproteinases (MMPs) such as MMP-9 and MMP-2, which destabilize tight junctions and disrupt the BBB basement membrane. Increased BBB permeability that can often be visualized as gadolinium-enhancing (Gd+) lesions on MRI scans is associated with MS disease initiation and subsequent relapses. Leukocyte migration breaks down the BBB and leaves the CNS vulnerable to cellular, myelin or axonal damage from an array of otherwise restricted substances (e.g. demyelinating antibodies). Macrophages and certain NK cells recruited with the help of proinflammatory molecules become effectors of demyelination, with further axonal damage occurring independently of myelin loss. Microglia within the CNS are normally supportive of neuronal function, but may become activated by proinflammatory molecules to release MMPs, additional proinflammatory mediators and myelinotoxic free radicals such as nitric oxide and glutamate, leading to multiple neurotoxic effects, as well as enhanced phagocytotic activity. Conversely, glial cells, along with endothelial cells, can play a role in promoting recovery from stress and injury through secretion of neurotrophic factors such as brain-derived neurotrophic factor, neurotrophin-3 and nerve growth factor (NGF).

1.8 Diseases modifying therapies (DMTs) in MS

1.8.1 Treatment with DMTs
To date, MS has no cure. Current management of the disease include symptomatic and supportive care and the use of disease-modifying therapies (DMTs). The therapies are used to reduce the occurrence of relapse, severity of symptoms and reduce the progression of disability.

Currently approved DMTs for MS in Australia include intramuscular interferon beta-1a (IFNβ-1a IM; Avonex), subcutaneous interferon beta-1a (IFNβ-1a SC; Rebif), interferon beta-1b (IFNβ-1b; Betaseron; Extavia), glatiramer acetate (GA; Copaxone), natalizumab
Conceptually, MS is considered an autoimmune inflammatory demyelinating disease and therefore DMTs formulated for the disease are anti-inflammatory, immunomodulatory or immunosuppressive in nature. The DMTs are more effective early in the disease course, when there is active inflammation, than in later stages of MS which is typically less inflammatory and more degenerative.

Below we provide an overview of Australian DMTs, focusing on their possible mode of action, the available evidence in terms of efficacy and known adverse effects and their possible treatment implications.

1.8.1.1 Interferon-β
The first-line disease modifying agents approved for use in treating RRMS are those derived from interferon-β, a pro-inflammatory antiviral and anti-tumour cytokine produced by immune cells. The three interferon-β medications available in Australia, Betaseron, Avonex and Rebif, are each recombinant forms of human interferon-β.

Interferon-β balances the expression of pro-inflammatory and anti-inflammatory agents in the brain, probably by increasing expression and concentration of anti-inflammatory agents while down-regulating the expression of proinflammatory cytokines. It reduces the trafficking and number of inflammatory cells that cross the BBB. The precise mechanism by which IFNβ achieves its anti-inflammatory and immunomodulatory effects is yet unclear. However several modes of action have been proposed, including inhibition of T-cell activation and proliferation; apoptosis of autoreactive T cells; induction of regulatory T cells; inhibition of leukocyte migration across the blood-brain barrier; cytokine modulation; and potential antiviral activity.

The efficacy of interferon beta-1b (Betaseron) was investigated in a double-blind, placebo-controlled trial of 372 patients with RRMS. After two years, treatment with high-dose IFNb-
1b (1.17/year) or low-dose IFNb-1b (0.84/year) significantly reduced the annual rate of relapse compared to the compared to placebo group (1.27/year).\textsuperscript{234} However, there was no significant change in disability in this trial. At five-year follow-up, there was about 33 percent reduction in the annual exacerbation rate in the high-dose interferon beta-1b group compared to the placebo group over five years although this was not statistically significant. There was no significant progression of lesion burden in the high-dose interferon beta-1b group (at 4 years, p=0.917) compared to the placebo group (p=0.0001).\textsuperscript{235}

To study the efficacy of interferon beta-1a (Avonex), 301 RRMS patients were randomized into a double-blinded, placebo-controlled, multicentre phase III trial.\textsuperscript{236} Over 2 years, compared to the placebo-group, interferon beta-1a treatment produced a significant delay in time to sustained disability progression (p=0.02). The annual exacerbation rate was significantly (p=0.002) lower in interferon beta-1a-treated patients (0.61 per year) compared to placebo-treated patients (0.90 per year) and a significantly lower number and volume of gadolinium-enhanced brain lesions on MRI was observed in patients treated with interferon beta-la compared to placebo-treated patients (p =0.02).

The randomized, multicentre, double-blind, placebo-controlled PRISMS trial also assessed the benefit of interferon beta-1a (Rebif) in 560 RRMS patients.\textsuperscript{237} After two years there was a significant beneficial effect of treatment (27% 95% CI: 14-39) on relapse rate compared to placebo group (33% 95% CI: 21-44) and MRI outcome measures. Treatment also reduced the MRI lesion burden (number) in both low and high-dose treatment groups (1.2% & and 3%) compared placebo group (10.9%) and 1-point EDSS progression rate (p<0.05) was reduced in the treatment arm compared to placebo arm. When patients initially receiving placebo were randomized to interferon-beta-1a while others continued their original treatment in the two years extension study, outcomes were better for patients treated for 4 years than for patients in crossover groups.\textsuperscript{238}
In a comparative trial, the relative efficacy of Rebif and Avonex were compared in the EVIDENCE trial of 667 RRMS patients randomly assigned to one of these drugs. From the study, the frequency of relapse and the mean number of active MRI lesions was fewer in Rebif compared to Avonex. However Rebif was more frequently associated with adverse effect than Avonex.

The INCOMIN prospective, randomised, multicentre comparative study compared the efficacy of interferon beta-1b and interferon beta-1a in 188 patients with RRMS for two years. Over the period of 2 years, interferon beta-1b was found to be more effective than interferon beta-1a, with 24% of individuals administered interferon beta-1b remaining relapse-free compared with individuals given interferon beta-1a (relative risk of relapse (RR): 0.76; 95% CI: 0.59-0.9; p=0.03). Similarly, 40% of patients who received interferon beta-1b remained free from new T2 lesions on MRI compared to those who received interferon beta-1a (RR 0.6, 95% CI: 0.45-0.8; p<0.0003). Converse to the INCOMIN study, a Danish multicentre, controlled, open-label, randomized trial comparing the efficacy of interferon beta-1a (Rebif) with interferon beta-1b (Betaseron) in 310 patients with RRMS, found that there was no difference in the efficacy between the two treatments. Interferon-β may be associated with adverse effect including fever, flu-like symptoms, Injection site reaction, Myalgia and Abnormal liver function tests (LFT).

1.8.1.2 Glatiramer acetate
Glatiramer acetate (GA), also known as copolymer 1 or Copaxone is a random polypeptide made up of four amino acids. The exact mechanism of action is not known but have been reported to involve binding to major histocompatibility complex molecules and consequent competition with various myelin antigens for their presentation to T cells and induction of specific suppressor cells of the T helper 2 type that migrate to the brain and lead to in situ
bystander suppression. It has also been shown that GA-reactive Th2 cells secrete neurotrophins, important for neuronal survival and for axonal protection.\textsuperscript{243, 244}

The efficacy of glatiramer acetate was reported in a large multicentre, randomized, double-blind, placebo-controlled trial of 251 RRMS patients. At two years, patients treated with glatiramer acetate had a significantly reduced relapse rate compared to placebo group (annualized rates = 0.59 for GA and 0.84 for placebo; p=0.007) and significantly more patients receiving GA reported reduction in 1-point EDSS worsening compared those receiving placebo (p=0.037). There was however no significant change in EDSS progression.\textsuperscript{245} Another double-blind, placebo-controlled, randomized trials with 239 RRMS patients found that glatiramer acetate treatment led to a significant reduction in the total number of enhancing lesions compared with placebo (-10.8, 95% CI: -18.0, -3.7; p=0.003). The relapse rate was also significantly reduced by 33% for GA-treated patients (p=0.012).\textsuperscript{246} Glatiramer acetate may be associated with adverse effect including injection site reaction and post-injection systemic reactions.\textsuperscript{242}

1.8.1.3 Fingolimod

Fingolimod also known as FTY720 (Gilenya) is derived from a metabolite of the fungus \textit{Isaria sinclairii}. The mechanism of action in MS is not completely understood. As a sphingosine analogue, fingolimod modulates the sphingosine-1-phosphate receptor on lymphocytes and alters lymphocyte migration, resulting in sequestration of lymphocytes in lymph nodes.\textsuperscript{247, 248}

The FREEDOMS trial verified the efficacy of fingolimod in a 24-month, double-blind, randomized, placebo-controlled study of 1272 RRMS patients.\textsuperscript{249} They reported a significantly reduced annualized relapse rate in fingolimod-treated group compared to placebo group (p<0.001). Also, treatment with fingolimod significantly reduced the risk of disability progression over the 24-month period (p=0.02). The TRANSFORMS trial, a 12-
month, double-blind, comparative study randomly assigned 1292 RRMS patients to receive either oral fingolimod or interferon beta-1a. At 12 months, the annualized relapse rate was significantly lower in fingolimod-treated groups than in the interferon group (p<0.001). There were no significant differences in progression of disability.\textsuperscript{250} Although there is evidence from the trials that fingolimod is effective for reducing the frequency of relapse, this was associated with adverse events and life-threatening infections.\textsuperscript{249, 250} Fingolimod may also be associated with lymphopenia, macular oedema, bradycardia and Abnormal LFT.\textsuperscript{242}

1.8.1.4 Dimethyl fumarate
Dimethyl fumarate, also called BG-12 is an oral formulation and second-generation fumaric acid.\textsuperscript{251} A study of BG-12 in the CONFIRM placebo-controlled trial (1430 RRMS patients) reported that treatment with BG-12 significantly reduced the relapse rate and improved neuroradiologic outcomes compared to the placebo. There was no significant reduction in disability progression however.\textsuperscript{252} In the DEFINE placebo-controlled trial of BG-12 among 1200 patients with RRMS, treatment with BG-12 was associated with significant reduction in the annualized rate of rate relapse, the rate of disability progression, and the number of lesions on MRI compared to placebo.\textsuperscript{253} Dimethyl fumarate may be associated with adverse effect including lymphopenia, flushing, abdominal pain, and diarrhoea.\textsuperscript{242}

1.8.1.5 Teriflunomide
Teriflunomide is the active metabolite of the pro-drug leflunomide that exhibits anti-inflammatory, anti-proliferative, and immunosuppressive effects.\textsuperscript{254} In a randomized placebo-controlled trial involving 157 RRMS and 29 SPMS patients, oral teriflunomide was effective in reducing MRI lesions compared to placebo.\textsuperscript{255} In the TEMSO placebo-controlled trial of 1088 patients with RRMS, teriflunomide significantly reduced the annualized relapse rate, MRI evidence of disease activity and disability progression (at the higher dose) compared to placebo.\textsuperscript{256} However teriflunomide could be associated with adverse effects.\textsuperscript{255, 256} Adverse
effects of teriflunomide therapy may include lymphopenia, rash, alopecia, Abnormal LFT, diarrhoea, nausea and Peripheral neuropathy.\textsuperscript{242}

\subsection*{1.8.1.6 Alemtuzumab}

Alemtuzumab is a humanised anti-CD52 monoclonal antibody which causes depletion and repopulation of B lymphocytes and T lymphocytes. The CARE-MS I trial was a comparative evaluation of the efficacy of alemtuzumab and interferon beta-1a (Rebif) in 581 RRMS patients.\textsuperscript{257} At two years, alemtuzumab was more effective in reducing the rate of relapse accompanied with a better MRI outcome compared to interferon beta-1a. However, there was no significant difference in sustained accumulation of disability. CARE-MS II comparative trial evaluated 667 RRMS patients with least one relapse while on interferon beta-1a or glatiramer.\textsuperscript{258} At two years, alemtuzumab significantly reduced the relapse rate and sustained accumulation of disability compared to interferon beta-1a but without significant difference in MRI outcomes. Alemtuzumab may be associated with adverse effect of infection and secondary autoimmunity including immune thrombocytopenia.\textsuperscript{257, 258}

\subsection*{1.8.1.7 Mitoxantrone}

Mitoxantrone is an anthracenedione used as an antineoplastic agent in prostate cancer and acute leukaemia and now approved for use in secondary-progressive MS and RRMS.\textsuperscript{259} The MIMS trial of mitoxantrone was a randomised, double-blind, placebo-controlled study of 194 patients with either RRMS or SPMS for 24 months. At 24 months, treatment with mitoxantrone was associated with significantly reduced progression of disability and clinical exacerbations compared to placebo.\textsuperscript{260} However, treatment with mitoxantrone did not reduce MRI outcome measure significantly.\textsuperscript{261} A systematic review and meta-analysis confirmed the positive effect of the drug on disability progression and also reduction in annualised relapse rate.\textsuperscript{262} The use of mitoxantrone may be however associated with serious cardiac toxicity.\textsuperscript{259}
1.8.1.8 Natalizumab
Natalizumab (Tysabri) is a humanized monoclonal antibody that inhibits leukocyte migration across the blood-brain barrier by binding to the α4 subunit of α4β1 and α4β7 integrins which are expressed on the surface of activated T-cells, thus reducing inflammation in central nervous system. To evaluate the efficacy, tolerability and safety of natalizumab in the treatment of RRMS patients, a systematic review and meta-analysis by Pucci and colleagues pooled efficacy data from the AFFIRM (942 RRMS patients) and SENTINEL (1171 RRMS patients) randomized placebo-controlled trials. Over two years period, treatment with natalizumab reduced the risk of sustained progression of disability by 42 percent (HR: 0.58; 95% CI, 0.43 to 0.77; p<0.001) and reduced the risk of relapse by 59% (HR: 0.41, 95% CI: 0.34 - 0.51; p<0.001) compared to placebo. Significant adverse events associated with natalizumab include fatigue and hypersensitivity reactions. Of more serious concern is the association of natalizumab treatment with a risk of developing progressive multifocal leukoencephalopathy.

1.8.2 Is the use of disease-modifying therapies in MS justified?
MS is a complex disorder that results in the accumulation of progressive disability and impaired quality of life in the vast majority of cases. The economic and personal burden of MS increases with level of disability, with the average annual cost of illness increasing from $37,000 p.a. for mild disability to $65,000 p.a. for those with severe disability with overall costs of illness in Australia exceeding $1 billion per year. The principal costs among persons with mild disability are the costs of MS disease-modifying therapies (DMTs).

To date, MS has no cure. Drugs used in the management of the disease are disease-modifying therapies (DMTs). The therapies may help reduce the number of relapse, severity of symptoms and retard the progression of disability.
Since 1993, treatments with DMTs have been available for MS\textsuperscript{269} and as of 2014 there are nine approved DMTs for relapsing-remitting MS in Australia. The crucial questions that remain to be answered is whether the wide use of DMTs over the last 20 years affected the natural history of the disease- that is, whether the DMTs is effectively reducing the duration and number of relapses and long-term disability progression.

DMTs are known to be effective in reducing relapses by 30-80\% and significantly reducing disease activity shown on magnetic resonance imaging.\textsuperscript{270} Studies have shown associations of relapses with disability, quality of life,\textsuperscript{271} cost,\textsuperscript{272} and mortality.\textsuperscript{231} However, the relationship between relapses and subsequent disability progression is a hotly debated topic\textsuperscript{273}, with no clear association found in some longitudinal studies\textsuperscript{273} but with some associations shown in other studies.\textsuperscript{274}

The effect of DMTs on long-term disability progression or quality of life is largely unknown. There are some studies that have attempted to examine the long-term effects of DMTs. Some population-based studies have shown no effects from DMTs on disability progression over a mean follow-up of five years compared to untreated controls.\textsuperscript{275} Other studies have shown that early in follow-up (3-5 years), there are apparent reductions in the risk of converting to definite MS and level of disability progression among those who started therapy early rather than delaying the start of treatment.\textsuperscript{33} Long-term follow-up of the pivotal beta-interferon study demonstrated that, at 16 years there were no differences in disability between those who had been originally randomised to placebo for two years and then offered active treatment and those randomised to active treatment from the start\textsuperscript{34}; other trial extensions have shown associations of treatment persistence and early treatment with long-term disability outcomes.\textsuperscript{276}
Thus, while there is some evidence of both short and long-term benefits of the DMTs, the results are conflicting and the methods ad hoc for disability or wholly absent for other aspects of clinical course. Definitive conclusions are precluded by limitations of these studies, which include large numbers of patients lost to follow-up, uncontrolled open-label treatment with unblinded and retrospective assessment of clinical events. Consequently, a more systematic clinical trials and studies with longer study duration are required to assess the long-term effects of DMTs on MS disability progression. However, this is considered impractical and possibly unethical.

1.9 Structure of thesis
This thesis is partly by publication. Chapter 2 presents a review on the vascular comorbidities in the onset and progression of MS. Data is also reviewed on how vascular comorbidities may influence MS clinical disability and other aspects of the disease course. Chapter 3 presents a review on the frequency of autoimmune comorbidities in MS and how immunomodulatory therapies may influence their occurrence. Chapter 4 presents the results of the analysis on the associations between serum lipids, apolipoproteins and disability in MS in a prevalent cohort. Chapter 5 presents the results of the analysis on the associations between serum lipids, apolipoproteins, body mass index and relapse in MS. Chapter 6 presents the results of the analysis on the relationship between lipid-related variables, conversion to clinically definite MS, time to relapse and progression in disability in a cohort of people with a first clinical diagnosis of demyelination. Chapter 7 presents the results of the analysis of the frequency of comorbidities and their associations with clinical disability and relapse in MS. Chapter 8 presents a summary of the main findings, discussion and future research.
1.10 Postscript
This Chapter has provided some key information about MS, which will help to understand the rest of the thesis. The next Chapter is a review of the literature on the role of vascular comorbidities in the onset and progression of MS.

1.11 References


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Chapter 2  Vascular comorbidities in the onset and progression of multiple sclerosis

2.1 Preface
The manuscript presented in this chapter has been published. The typeset version of the manuscript as it appeared in the journal is in Appendix 2A. The text of this chapter is the same as the published version. In writing this review, relevant articles were retrieved by a variety of methods. Firstly, PubMed, Google Scholar and Web of Knowledge were comprehensively searched using the following terms: “multiple sclerosis” and “obesity”, “multiple sclerosis” and “body mass index”, “multiple sclerosis” and “type 2 diabetes”, “multiple sclerosis” and “hypertension”, “multiple sclerosis” and “coronary heart disease”, “multiple sclerosis” and “myocardial infarction”, “multiple sclerosis” and “stroke”, “multiple sclerosis” and “Atrial fibrillation”, “multiple sclerosis” and “cerebrovascular disease”, “multiple sclerosis” and “lipid, “multiple sclerosis” and “cholesterol.” In addition, a snowballing method was used by retrieving articles back in time by identifying new papers that the key articles cited. Lastly, citation tracking was used (reverse snowballing), where Web of Knowledge was used to retrieve articles forward in time by identifying new papers who cited the key articles.

2.2 Abstract
Vascular comorbidities are common in the general population and are associated with adverse health outcomes. In people with multiple sclerosis (MS), an increasing amount of evidence suggests that vascular comorbidities are also common, but an association with MS risk and disability has not been conclusively established. This review aims to critically examine published data on the relationship between vascular comorbidities (including vascular risk factors) and MS. The evidence suggests an increased risk of MS in people with a high BMI
during childhood or adolescence but not adulthood. People with established MS appear to have a slightly increased risk of cardiovascular disease and a greater proportion of people with MS die from cardiovascular disease, which has important implications for clinicians trying to identify risk factors for cardiovascular disease and reviewing treatment options. In relation to whether vascular comorbidities influence MS clinical disability or other aspects of the disease course, the key finding was that having type-2-diabetes, hypertension, dyslipidaemia or peripheral vascular disease at any point in the disease course may be associated with a greater progression in disability. Additionally, a negative effect of high cholesterol and triglycerides and positive effect of higher HDL (high density lipoprotein) levels on acute inflammatory activity was observed on magnetic resonance imaging. The results of the published clinical trials of statins as an intervention in MS were however conflicting and care need to be taken when treating people with MS with statins. Taken together, the literature seems to indicate a potential association of vascular comorbidities with MS risk and disability, but the number of prospective studies was sparse, thus precluding ascription of causality. We therefore recommend that future studies of the frequency and effects of vascular comorbidities on MS risk and disability should be prospective and objective where relevant.

2.3 Introduction
Multiple sclerosis (MS) is a disorder of the central nervous system (CNS) with autoimmune, inflammatory and neurodegenerative components which may influence each other or alternatively may have independent natural histories. Although the typical age of onset of MS is in the third and fourth decades of life, the burden of disease is most marked in the fifth to seventh decades.\(^1\) MS affects more than 2.5 million individuals worldwide\(^2\), with higher incidence and prevalence in women than men.\(^3\) MS has a highly variable inter and intra-personal clinical course, both in pattern and rate of deterioration.\(^1\)
In relation to aetiology, MS is a complex disease in which multiple environmental and lifestyle risk factors act together in a genetically susceptible individual to cause the disease.\textsuperscript{4} Environmental and lifestyle risk factors include low sunlight exposure and vitamin D, cigarette smoking and exposure to Epstein-Barr virus.\textsuperscript{3-5} However it is not entirely clear whether the same factors also modulate disease progression and whether the putative factors that modulate the inflammatory components of the disease are the same as those that potentially modulate the neurodegenerative components. In the last several years, vascular risk factors and vascular comorbidities such as obesity, dyslipidaemia, type-2 diabetes and cardiovascular disease have been associated with MS onset and disease progression.\textsuperscript{4,6} In line with this background, MS has been proposed to have, in part, a vascular basis due to its shared pathophysiology with these comorbidities, including endothelial dysfunction,\textsuperscript{7} inflammation,\textsuperscript{8} and cardiovascular autonomic dysfunction.\textsuperscript{9}

In this review, we examine the current literature on the relationship between cardiovascular risk factors including obesity, hypertension, dyslipidaemia, type-2 diabetes, and cardiovascular disease with MS risk, disability progression and mortality. Throughout the text, the term vascular comorbidity refers to cardiovascular risk factors and diseases.

2.4 Obesity and MS

2.4.1 Prevalence of obesity in people with MS and comparison with healthy populations
The prevalence of obesity in people with MS has been investigated in several studies. For example, in a large study of persons with MS (n=8,983), 31.3\% of participants were classified as overweight and 25\% as obese.\textsuperscript{10} A study of 123 women with MS from Oregon found 47.5\% of participants to be overweight and 25.8\% were obese.\textsuperscript{11} In a study of by Pilutti and colleagues\textsuperscript{12}, 36.3\% of the 168 MS patients were overweight and 32.7\% were obese. In a 24-month longitudinal study of 269 individuals with relapsing-remitting MS (RRMS), 24.0\%
and 28.3% were classified as overweight and obese respectively. Similarly, a study by Marrie and colleagues reported that nearly half had high BMI at MS onset, with 26.4% being overweight and 23.8% being obese.

A number of studies compared the prevalence of high BMI to a control population. A study by Khurana and colleagues found that 4339 veterans with MS had a slightly higher age and sex-adjusted prevalence of overweight than veterans in general (42.3% vs. 39.6%, respectively) but a lower adjusted prevalence of obesity (20.1% vs. 33.1%). In contrast, other case-control studies were not able to detect any difference in BMI between MS cases and controls, though these studies were relatively small (sample size ranging from 16 to 68) compared to that of Khurana and colleagues. Two other studies even reported a lower BMI in MS cases than controls. Overall, there is currently no evidence that the prevalence of overweight and obesity in MS is higher than that of the general population. The use of self-reported height and weight may have led to underestimation of overweight and obesity in these investigations.

### 2.4.2 Obesity and MS risk

Table 2.1 provides an overview of the studies that have examined the association between BMI and MS risk. It shows that a number of prospective and case-control studies have observed an association between BMI in childhood and adolescence and MS risk; however no associations have been found between BMI in adulthood and MS risk. Interestingly, the two studies that examined childhood BMI both found that the association was only present in females and not in males, both showing a clear dose-dependent relationship between childhood BMI in females and subsequent MS risk. In addition, two studies found a dose-response relationship between BMI and MS when BMI was measured at age 18 and 20 but no association was observed when BMI was measured in adulthood. Certainly having an association with exposure prior to disease onset is potentially
supportive of a causal directionality, but it is interesting and perhaps disruptive to a causal interpretation that the BMI-MS association does not track forward to later adulthood. It may be that by this age other factors that occur among all adults in their later decades of life nullify any appreciable differences in BMI between MS cases and other adults.
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Study information</th>
<th>Number of MS patients/total cohort</th>
<th>Age BMI Measured</th>
<th>Main findings</th>
</tr>
</thead>
</table>
| Munger and colleagues, (2013)<sup>20</sup> | Cohort study (1930-1983) | 774/302,043 | 7 years | 1. Girls: BMI z-score (HR =1.20, (95% CI: 1.10-1.30), p<0.001)  
2. Boys: BMI z-score (HR =1.12, (95% CI: 0.99-1.28), p>0.05) |
| Langer-Gould and colleagues, (2013)<sup>21</sup> | Cohort study (2007-2009) | 75/913,097 | 2-18 years | Girls: Normal weight: OR =1(ref), Overweight: OR =1.58 (95% CI: 0.71-3.50),  
Moderate obesity: OR=1.78 (95% CI: 0.70-4.49), Extreme obesity: OR=3.76 (95% CI: 1.54-9.16), <sup>p-trend</sup>=0.005  
Boys: Normal weight: OR = 1 (ref), Overweight: OR =1.80 (95% CI: 0.83-3.93),  
Moderate obesity: OR=0.89 (95% CI: 0.30-2.64), Extreme obesity: OR=0.82 (95% CI: 0.19-3.56), <sup>p-trend</sup>=0.93 |
| Hedstrom and colleagues, (2012)<sup>21</sup> | Case-control study (2005-2011) | 1571/3,371 | 20 years | Normal weight (BMI): 18.5-21, OR=1(ref), 21-23, OR=1.1 (95% CI: 0.9-1.2), p=0.5,  
23-25, OR=1.2 (95% CI: 1.0-1.5), p=0.03  
Overweight (BMI) : 25-27, OR =1.4 (95% CI: 1.1-1.8), p=0.2, 27-29, OR =2.2 (95% CI: 1.6-3.0), p=1x10<sup>-6</sup>  
Obese(BMI)>30, OR =2.2 (95% CI: 1.5-3.0), p=9x10<sup>-6</sup>, <sup>p-trend</sup>=2x10<sup>-10</sup>  
>20 years | No association between current/adult BMI and MS risk (data not shown) |
| Munger and colleagues, (2009)<sup>22</sup> | Cohort study (1976-2003) | 593/238,371 | 18 years | Normal weight (BMI): 18.5-21, RR=1(ref), 21-23, RR =1.13 (95% CI: 0.91-1.40),  
23-25, RR =0.97 (95% CI: 0.72-1.31)  
Overweight(BMI): 25-27, RR =1.44 (95% CI: 0.87-2.39) 27-29, RR =1.40 (95% CI: 0.92-2.14)  
Obese(BMI): >30, RR =2.25 (95% CI: 1.50-3.37), <sup>p-trend</sup><0.001  
25-55 years | Normal weight(BMI): 18.5-21, RR=1(ref), 21-23, RR =0.87 (95% CI: 0.69-1.10),  
23-25, RR =1.00 (95% CI: 0.78-1.29)  
Overweight(BMI) : 25-27, RR =1.23 (95% CI: 0.93-1.62), 27-29, RR =0.79 (95% CI: 0.30-2.12)  
Obese (BMI): >30, RR =0.91 (95% CI: 0.46-1.79), <sup>p-trend</sup>=0.88 |

Normal: 18.5 to 24.9Kg/m², Overweight:25 to 29.9Kg/m², Obese:≥30Kg/m²
A substantial limitation of these studies was the fact that they were unable to adjust for sun exposure and/or 25-hydroxyvitamin D serum levels. It is well known that individuals with high BMI have less sun exposure and lower vitamin D levels. In part, this may be due to changes in behaviour and the effects of increased adiposity on systemic vitamin D availability. Low sun exposure and vitamin D levels are now established risk factors for MS. It is therefore feasible that the observed associations between childhood/adolescence BMI and MS may be explained by the fact that cases had less sun exposure and lower serum vitamin D levels. That said, there are a number of deleterious effects of obesity that might independently contribute to MS risk, such as increased oxidation and dyslipidaemia, so both BMI and sun/vitamin D are worthy covariates to assess in studies of MS risk and disability progression. Importantly, the absence of association between adult BMI and the risk of MS is interesting and seems to suggest that some aspects of early life or adolescence may be critical in determining the risk of MS.

2.4.3 Obesity and MS disability
Three studies examined the association between BMI and disability. In the first study, 269 individuals with RRMS were prospectively followed for 24 months and participants reported information about their BMI and level of disability as measured by the Patient Determine Disease Steps (PDDS), a self-reported variant of EDSS. Higher BMI at baseline was associated with a higher PDDS at 12 months (β: +0.06, p<0.05), but BMI at 12 months was not significantly associated with PDDS at 24 months (β: -0.04, p>0.05). In the second study by Marrie and colleagues, PDDS was grouped into mild (EDSS≤3; n= 1,318), moderate (EDSS 4.5-5.5; n=350) and severe (EDSS≥6; n=707) disability. No significant association was observed between BMI and disability, because among those with mild, moderate and severe disability, 24.0%, 27.7% and 25.6% were obese, respectively (OR=1.09 (95% CI: 0.90-1.33) for moderate vs. mild disability; OR=0.99 (95% CI: 0.86-1.14) for severe vs. mild
disability; \( p_{\text{trend}}=0.11 \). In our own work,\(^{29}\) BMI was measured objectively and disability assessed by EDSS. We found that BMI was independently associated with higher EDSS (\( p = 0.013 \)). In another study where we investigated the associations between BMI and relapse in MS in cohort of 141 participants with relapsing–remitting MS, BMI was not associated with the hazard of relapse.\(^{30}\)

The fact that two studies used self-reported height and weight to calculate BMI is a potential limitation, because it is well known that those who are more overweight are more likely to under-report their weight.\(^{31}\) This under-reporting could bias an association toward the null, though the fact that the Pilutti study found a significant effect of baseline BMI and disability at 12 months despite a self-reported BMI may just indicate the absence of effect. Regardless, additional studies are needed to assess any association, ideally measuring BMI objectively.

We could not identify any studies that examined disability longitudinally to determine whether obesity could be related to a more rapid change in disability. Certainly such an analysis would greatly improve upon the capacity to assess the veracity of the associations demonstrated with previous retrospective and cross-sectional designs, though the logistics required do complicate implementation. A blended method, prospectively collecting data on early and mid-adulthood BMI and making use of medical records for childhood BMI may allow a more feasible design without being too logistically onerous.

### 2.4.4 Potential biological mechanisms

The mechanisms by which obesity might impact upon MS risk are not yet known. As mentioned, part or all of the association between childhood/adolescence BMI and MS risk could be confounded by other factors such as sun exposure and vitamin D. If there were to be an independent effect of obesity, then the mechanism may be related to the lipid pathway, as obese people are known to have a more adverse lipid profile compared to those of normal weight.\(^{32, 33}\)
It is also possible that there is a shared pathway between vitamin D and lipids.\textsuperscript{34, 35} While serum lipids are prone to oxidation and inflammation in the vascular endothelium, vitamin D is known to exert immunomodulatory and antioxidant effects on the immune system.\textsuperscript{36} The connection between vitamin D and lipids is not unexpected, as vitamin D synthesis starts in the skin from a cholesterol precursor, 7-dehydrocholesterol, and it is thought that higher serum vitamin D levels may improve the plasma lipid profile.\textsuperscript{17} In line with these findings, patients that began using statins in order to improve their cholesterol profile experienced an increase in serum vitamin D levels.\textsuperscript{37}

An alternative hypothesis is that the effects of obesity are mediated by a low-grade chronic inflammatory state. There is evidence that adipose tissue secretes inflammatory factors collectively known as adipokines that influence immune system functioning, including leptin\textsuperscript{38} and interleukin-6,\textsuperscript{39} which has been shown to reduce regulatory T-cell activity\textsuperscript{40, 41} and promote an inflammatory T helper 1 cell response\textsuperscript{42} thought to be responsible for the autoimmune inflammation, development and progression of MS.\textsuperscript{43}

\textbf{2.4.5 Summary: Obesity and MS}
Collectively, the results from the studies on BMI and MS suggest that weight status during early life may be important in determining subsequent MS risk. In relation to the question of whether obesity is associated with disability or a change in disability, studies are required that measure BMI objectively and prospectively.

\textbf{2.5 Type-2 Diabetes and MS}

\textbf{2.5.1 Prevalence of Type-2 Diabetes in people with MS and comparison with healthy populations}
We could not identify any studies that examined T2D and the risk of MS to determine whether T2D is more prevalent prior to the onset of MS. However a number of studies compared T2D frequencies in people with MS and healthy controls. In a study by Hussein
and Reddy\textsuperscript{44}, the prevalence of T2D among a cohort of 1,206 MS patients was significantly higher (prevalence: 6.75\%; 95\% CI: 6.74-6.76, p=0.005) than in the general population. A study by Kang and colleagues reported that the prevalence of T2D in a group of 898 MS patients was 8.6\%, this was 1.5-times higher than that of the 4,490 randomly matched controls without MS (OR: 1.5; 95\% CI: 1.1-1.2; p<0.01).\textsuperscript{45} However, in a study by Marrie and colleagues, the age-adjusted prevalence of T2D in patients with MS was similar to that of the general population (7.62 vs. 8.31\%; prevalence ratio 0.91; 95\% CI: 0.81-1.03).\textsuperscript{46} Fleming and Blake compared T2D frequencies among MS and non-MS cases admitted to hospital, finding the prevalence of T2D was lower in MS cases (3.08 per 100 discharges) compared to controls (6 per 100 discharges).\textsuperscript{47}

A retrospective study by Sternberg and colleague also showed no difference in the prevalence of T2D between MS patients and non-MS patients indicated by the lack of difference in the use of anti-diabetic drugs between the two groups.\textsuperscript{8, 48} Plasma glucose level was however significantly lower in MS patients compared to non-MS patients\textsuperscript{8} and also lower in disease modifying therapies naive (DMTs-naive) MS patients compared to MS patients on disease modifying therapies,\textsuperscript{48} showing that the chronic use of pharmacological agents by MS patients may be associated with increased plasma glucose levels and also associated with the risk of T2D in MS patients.

The heterogeneity of the findings reported could also be due to differences in study design used in these investigations that is, hospital-based versus population-based studies. From these inconsistent results, there is no convincing evidence that the prevalence of T2D is higher among people with MS compared to the general population, and thus no evidence indicative of an association between T2D and MS risk.
2.5.2 Type-2 Diabetes and MS disability

The relationship between T2D and disability in MS was investigated by Marrie and colleagues. In this study, they examined whether T2D was associated with the time to different ambulatory disability endpoints in a large population of 8,983 MS patients enrolled in the North American Research Committee on MS Registry (NARCOMS). Compared with MS patients who did not report T2D, individuals with MS who developed T2D at any point during their disease course had a 29% increased risk of early gait disability (hazard ratio (HR): 1.29; 95% CI: 1.13-1.48), a 28% increased risk of requiring unilateral assistance (HR: 1.28; 95% CI: 1.11-1.49) and a 56% increased risk of requiring bilateral assistance (HR: 1.56; 95% CI: 1.30-1.88).

In another study using the NARCOMS registry, Marrie and Cutter also assessed the effect of T2D on the development of visual disability using the Vision subscale of Performance Scales (PSV) which is scored ordinally as 0 (normal), 1 (minimal), 2 (mild), 3 (moderate), 4 (severe), or 5 (total disability). They reported that developing T2D at any point in the disease course was associated with a 35% (HR: 1.35 (1.15-1.58) increased risk of mild visual disability, a 41% (HR: 1.41 (1.12-1.78) increased risk of moderate visual disability and a 54% increased risk of visual disability (HR: 1.54 (1.04-2.28).

These results are in line with a non-MS systematic review and meta-analysis by Wong et al reporting that diabetes is associated with increased physical disability compared to people without diabetes in the general population which in part, may be due to the complications of diabetic neuropathy which is characterised by muscle weakness and significant autonomic dysfunction. These results are suggesting that T2D comorbidity is associated with a worse progression in disability. The lack of consistently higher prevalence of T2D in people with MS does not preclude any potential association with disability progression, given the distinct pathways for inflammatory and neurodegenerative mechanisms.
2.5.3 Summary: Type-2 Diabetes and MS
Data on the prevalence of T2D in MS patients is limited and the findings are inconsistent. The result on the relationship between T2D and disability suggest that people with MS who also have T2D may have a worse progression in disability compared to people with MS without T2D. Further studies are required to confirm this finding, however.

2.6 Hypertension and MS

2.6.1 Prevalence of hypertension in people with MS and comparison with healthy populations
The frequency of hypertension in MS patients has been investigated by a number of studies. For instance, in a study of 898 MS patients by Kang and colleagues, the prevalence of hypertension was higher in the MS patients compared to the control group (OR: 1.40; 95% CI: 1.10-1.70). In a cross-sectional study of 1142 male veteran MS patients, LaVela and colleagues reported higher prevalence of hypertension in MS patients compared to general veteran population (46.7% versus 41.2%, p<0.001) and the general population (46.7% versus 20.9%, p<0.001). However, in a study using the NACORMS registry, Marrie and colleagues reported that 30.1% of 8983 MS participants reported being hypertensive which was similar to the rate expected for the general population. In another study of 430 MS patients by Marrie and colleagues, the age-adjusted prevalence of hypertension in the MS patients was similar to that of the general population (MS: 20.8% versus general population: 22.5% [PR: 0.9; 95% CI: 0.78-1.06]). Studies reporting actual blood pressure values recorded similar values in MS patients compared to controls. Sternberg and colleagues also reported that there was no significant difference in the use of antihypertensive drugs between MS and non-MS patients. Lower prevalence of hypertension was also reported by some studies. For example, in study of 8281 MS patients by Jadidi and his colleagues, the frequency of hypertension was in MS patients compared to the matched controls (0.42% versus 0.68%). Similarly, Allen and colleagues reported lower prevalence of hypertension
in 9949 hospitalised MS patients compared to non-MS hospitalised controls (18.3% versus 29.3%, p<0.05). Lower prevalence of hypertension in MS patients compared to controls have been reported in other studies.\textsuperscript{8,47}

The data available on the prevalence of hypertension in MS is inconsistent and there is no convincing trend of evidence that the prevalence of hypertension is higher in MS patients compared to the general population or healthy controls. This may be due to differences in demographic and clinical characteristic between studies, including differences in exposure to pharmacological agents in MS.

\subsection*{2.6.2 Hypertension and MS disability}
Marrie and colleague investigated the relationship between hypertension and disability in MS.\textsuperscript{6} They examined whether hypertension was associated with the time to different ambulatory disability endpoints using the NARCOMS registry. Compared with MS patients who did not report hypertension, individuals with MS who developed hypertension at any point during their disease course had a 29% increased risk of early gait disability (HR: 1.29; 95% CI: 1.20-1.39), a 25% increased risk of requiring unilateral assistance (HR: 1.25; 95% CI: 1.15-1.36) and a 17% increased risk of requiring bilateral assistance (HR: 1.56; 95% CI: 1.05-1.31).

Using the NARCOMS registry, Marrie and Cutter\textsuperscript{49} also assessed the effect of hypertension on the development of visual disability using the Vision subscale of Performance Scales (PSV). Developing hypertension at any point in the disease course was associated with 32% (HR: 1.32 (1.20-1.46) increased risk of mild visual disability, 31% (HR: 1.31 (1.13-1.51) increased risk of moderate visual disability and 16% increased risk of visual disability (HR: 1.16 (0.89-1.51)).
These results suggest that hypertension as comorbidity in MS patients may be associated with a worse progression in disability. These findings are consistent with data from non-MS studies which have reported significant association between hypertension and disability in the general population.\textsuperscript{55, 56}

\subsection*{2.6.3 Summary: hypertension and MS}
We found no convincing evidence of higher prevalence of hypertension in MS patients compared to matched controls. However, there is some evidence to suggest that MS patients who develop hypertension at any point in the disease course may experience faster progression in clinical disability.

\section*{2.7 Cardiovascular diseases and MS}
Cardiovascular diseases are common in the general population\textsuperscript{57} and are associated with many adverse health outcomes, including reduced functional status\textsuperscript{58}, severity of the primary disease,\textsuperscript{57, 59} reduced quality of life\textsuperscript{59, 60} and increased mortality\textsuperscript{60}. If cardiovascular diseases were also common in MS and were associated with disability, then this could partly explain the highly variable inter and intra-personal clinical course observed in MS. Despite their potential importance, little information exists about the prevalence of cardiovascular diseases in MS, how the prevalence is changing over time, and how they affect treatment decisions, treatment responses or health outcomes in MS.

\subsection*{2.7.1 Cardiovascular diseases and mortality in people with MS and comparison with healthy populations}
To the best of our knowledge, no study has been conducted that examined the whether the occurrence of cardiovascular disease prior to MS onset was associated with MS risk. However, mortality due to cardiovascular disease in individuals with MS has been investigated by a number of studies. In Denmark, Koch-Henriksen and colleagues\textsuperscript{61} reported that individuals with MS had a 34\% increased risk of death from cardiac and vascular disease compared to the general population (standardised mortality ratio [SMR]: 1.34; 95\% CI: 1.02-
In another Danish study, Bronnum-Hansen and colleagues reported a 32% increased risk for MS patients of dying from cardiovascular disease compared to the general population (SMR: 1.32; 95% CI: 1.22-1.43). Lalmohamed and colleagues reported a stronger effect, with a 2.4-fold increased risk of death from cardiovascular disease compared to gender-matched referent subjects. However Hirst and colleagues reported that MS patients had a 6% increased risk of death from cardiovascular diseases which was not statistically different from the general population (p=0.22).

These studies collectively suggest an association between cardiovascular diseases and mortality in people with MS.

2.7.2 MS and risk of cardiovascular diseases
Table 2.2 provides a summary of the studies that have examined the association between MS and risk of cardiovascular disease. Two studies reported a higher risk of myocardial infarction in MS cases than matched controls. However a third study reported a lower prevalence of myocardial infarction in MS cases than controls. MS cases have been found to have a higher risk of heart failure, stroke or cerebrovascular disease and peripheral vascular disease when compared to matched controls. Coronary heart disease has also been found to be more frequent in MS cases compared to controls. The prevalence of ischemic heart disease was higher in MS cases compared to controls in some studies but lower in others. In a study by Fleming and Blake, the frequencies of acute myocardial infarction, heart failure, atrial fibrillation and cerebrovascular disease were lower in MS cases than matched hospitalised controls.
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Study information</th>
<th>MS patients</th>
<th>Control group</th>
<th>Main findings</th>
</tr>
</thead>
</table>
2. Heart failure: IRR=1.97 (95% CI: 1.52-2.56)  
3. Atrial fibrillation: IRR=0.63 (95% CI: 0.46-0.87)  
4. Stroke: IRR=1.71 (95% CI: 1.46-2.00) |
2. Peripheral vascular disease: OR =6.60 (95% CI: 4.00-11.00)  
3. Cerebrovascular disease: OR =3.70 (95% CI: 2.90-4.10) |
1. Myocardial infarction: IRR=1.84 (95% CI: 1.28-2.65),  
2. Heart failure: IRR=1.92 (95% CI: 1.27-2.90)  
3. Stroke: IRR=1.69 (95% CI: 1.42, 2.71)  
**2-30 year follow-up:**  
1. Myocardial infarction: IRR=1.10 (95% CI: 0.97-1.24)  
2. Heart failure: IRR=1.53 (95% CI: 1.37-1.71)  
3. Stroke: IRR=1.23(95% CI: 1.10-1.38) |
| Allen and colleagues, (2008)     | Case-control (2005-2011) | 9,949       | 19,898 matched controls | 1. Myocardial infarction : OR=0.78 (95% CI: 0.64-0.96)  
2. Ischemic stroke: OR=1.66 (95% CI: 1.33-2.09)  
3. Haemorrhagic stroke: OR=1.14 (95% CI: 0.76-2.32)  
4. Ischemic heart disease: OR=0.58 (95% CI: 0.51-0.66) |
| Fleming & Blake,(1994)            | Case-control (1989) | 7,168       | 7,168 matched controls | 1. Coronary heart disease: 3.81% in MS and 10.69% in control group (p<0.05)  
2. Atrial fibrillation: 2.66% in MS and 6.60% in control group (p<0.05)  
3. Cerebrovascular disease: 1.86% in MS and 2.58% in control group (p<0.05)  
4. Hypertension: 7.42% in MS and 15.95% in control group (p<0.05) |
<table>
<thead>
<tr>
<th>Authors &amp; Study Type</th>
<th>Study Duration</th>
<th>N</th>
<th>Method of Control</th>
<th>Findings: Bilateral Assistance to Walk</th>
</tr>
</thead>
</table>
| Marrie and colleagues, (2010) | Cohort study (2006) | 8,983 | 11,786 matched controls | 1. Hypertension: HR=1.17 (95% CI: 1.05-1.31)  
2. Coronary heart disease: HR=0.99 (95% CI: 0.80-1.23)  
3. Peripheral vascular disease: HR=1.87 (95% CI: 1.35-2.60)  

<table>
<thead>
<tr>
<th>Authors &amp; Study Type</th>
<th>Study Duration</th>
<th>N</th>
<th>Method of Control</th>
<th>Findings: Severe Visual Disability</th>
</tr>
</thead>
</table>
| Marrie and colleagues, (2011) | Cohort study (1984-2005) | 2366 | 11,786 matched controls | 1. Hypertension: HR=1.16 (95% CI: 0.89-1.51)  
2. Coronary heart disease: HR=1.20 (95% CI: 0.80-1.80)  
3. Peripheral vascular disease: HR=1.90 (95% CI: 1.11-3.23)  

<table>
<thead>
<tr>
<th>Authors &amp; Study Type</th>
<th>Study Duration</th>
<th>N</th>
<th>Method of Control</th>
<th>Findings: Ischemic Heart Disease</th>
</tr>
</thead>
</table>
2. Aged 45–59 years: PR=1.21 (95%CI:1.08-1.35)  
3. Age ≥60 years: PR=0.81 (95%CI:0.70-0.92)  

1. Aged 20–44 years: Incidence ratio (IRR)= 2.01 (95%CI:0.94-4.30)  
2. Aged 45-59 years: IRR=1.33 (95%CI:0.95-1.86)  
3. Age ≥60 years: IRR=1.04 (95%CI:0.69-1.56)
Differences in patients’ demographic and clinical characteristics between studies could contribute to the inconsistencies in the results. For instance, in the studies by Jadidi and colleagues\textsuperscript{54} and that of Christiansen et al.,\textsuperscript{64} they used newly diagnosed MS patients and reported higher incidence of cardiovascular disease in MS patients compared to matched controls. One of the factors that may account for this is the activation of the sympathetic autonomic nervous system which is potentiated by heightened inflammation at the initial stages of the disease.\textsuperscript{9,66,67} However, the study by Fleming and Blake\textsuperscript{47} reported contrasting results, since it was conducted in elderly MS patients (≥65 years of age), where inflammation is low, and the sympathetic autonomic function is severely dysfunctional. Secondly, differences in the use or exposure to DMTs of MS and other pharmacological agents between studies may also contribute to the varied results since studies have shown that the use of pharmacological agents in MS may be associated with increased risk of cardiovascular diseases.\textsuperscript{68,69}

In summary, current evidence suggests that people with MS may be at a slightly increased risk of cardiovascular diseases than matched controls. To what extent this is reflective of any causal mechanisms or just a common set of predictors for MS and cardiovascular disease can’t be discerned from these study methods.

2.7.3 Cardiovascular disease and MS disability

Marrie and colleagues evaluated the association between the presence of vascular comorbidities and the time to different ambulatory disability endpoints in 8,983 MS patients from the NARCOMS Registry.\textsuperscript{6} Participants reporting one or more vascular comorbidities at MS diagnosis had an increased risk of ambulatory disability, and the risk increased with the number of vascular comorbidities reported. A single vascular comorbidity at diagnosis was associated with 51\% increased risk of early gait disability (HR: 1.51; 95\% CI: 1.41-1.61) while two vascular comorbidities were associated with a 228\% increased risk of early gait
disability. A single vascular comorbidity at any point in the disease course was also associated with 58% increased risk of early gait disability (HR: 1.58; 95% CI: 1.48-1.68). Similarly, compared with MS patients who did not report peripheral vascular disease, individuals with MS who developed peripheral vascular disease at any point in the disease course had a 29% increased risk of early gait disability (hazard ratio (HR): 1.29; 95% CI: 1.20-1.39), a 25% increased risk of requiring unilateral assistance (HR: 1.25; 95% CI: 1.15-1.36) and a 17% increased risk of requiring bilateral assistance (HR: 1.56; 95% CI: 1.05-1.31). However, compared with MS patients who did not report coronary heart disease, individuals with MS who developed coronary heart disease had similar ambulatory disability.

Using the NARCOMS registry, Marrie and Cutter also assessed the effect of vascular comorbidities on the development of visual disability. They reported that developing peripheral vascular disease at any point in the disease course was associated with 45% (HR: 1.45 (1.14-1.80) increased risk of mild visual disability, 63% (HR: 1.63 (1.17-2.27) increased risk of moderate visual disability and 90% increased risk of visual disability (HR: 1.90 (1.11-3.23). Similarly, coronary heart disease was associated with 27% (HR: 1.27 (1.09-1.46) increased risk of mild visual disability, 45% (HR: 1.45 (1.16-1.80) increased risk of moderate visual disability and 20% increased risk of visual disability (HR: 1.20 (0.80-1.80).

Another study using the NARCOMS Registry assessed the association between pre-existing comorbidity and the severity of disability at diagnosis. Using PDDS, participants were classified as having mild, moderate or severe disability. Among participants enrolled within two years of diagnosis, the adjusted odds ratio of moderate vs. mild disability at diagnosis increased in participants with a vascular comorbidity (OR: 1.51; 95% CI: 1.12-2.05). However, the odds ratio of severe vs. mild disability was not statistically different for participants with a vascular comorbidity (OR: 1.06; 95% CI: 0.77-1.44).
Further, a population-based study by Tinghog and colleague examined whether the presence of cardiovascular comorbidities increases the risk for disability pension in people with MS. From the results, the presence of cardiovascular comorbidity in MS patients (4,519) had no significant influence on the risk for disability pension.\textsuperscript{105}

Taken together, this data suggests that having a cardiovascular disease at diagnosis or at any point in the disease course may be associated with a worse progression in disability compared to those without a cardiovascular comorbidity. As with MS risk, however, whether this reflects a causal mechanism or just shared predictors is unclear.

\textbf{2.7.4 Potential biological mechanisms}

There are some potential hypotheses for the biological mechanisms underlying the relationship between cardiovascular disease and progression in disability. Firstly, vascular comorbidities are associated with increased peripheral inflammation and could act to increase disease progression by activating the inflammatory cascade. Elevated levels of inflammatory markers in MS are associated with an excess activation of cellular and humoral components of the immune system which may increase inflammatory demyelination and neurodegeneration. The neurodegeneration which manifests in disability progression in MS evokes neuronal and axonal loss which leads to brain atrophy and cognitive decline.\textsuperscript{71-73}

Secondly, it has been proposed that the chronic inflammatory process that characterises MS pathogenesis may contribute to the aetiogenesis and dysfunction of the cerebral endothelium.\textsuperscript{7, 72} Serum proinflammatory cytokines such as TNF-\(\alpha\) and IFN-\(\gamma\), which are elevated before clinical exacerbations of MS, can activate the cerebral endothelial cells (CECs) and alter their anatomical distribution and activity leading to the disruption of the blood-brain barrier as evidenced by a decrease expression of endothelial tight junction proteins of the CECs. Dysfunction of the CECs and permeability of the blood-brain barrier
causes adherence and trans-endothelial migration of T-lymphocytes and monocytes to the CNS with destructive and often neurodegenerative consequences.\textsuperscript{7, 74}

2.7.5 Summary: cardiovascular disease and MS
On the whole, the prevalence of cardiovascular disease and the risk of death from cardiovascular disease appear to be higher in people with MS compared to the general population. Whether cardiovascular comorbidities are already frequent prior to the onset of MS is not completely known since only few studies have been published in this area.\textsuperscript{15} More case-control or ideally prospective study designs are needed to assess this. Importantly, whether present at diagnosis or later in the disease course, cardiovascular diseases may be associated with a faster accumulation of disability. Having two or more cardiovascular diseases, which independently cause impairment, could act in concert to increase disability in MS patients.\textsuperscript{70}

While MS in itself may not cause cardiovascular disease, patients with MS may be subject to the risk of cardiovascular diseases because of co-occurring conditions such as dyslipidaemia, depression, neurological impairment or physical disability, which make them less physically active than the general population. Physical inactivity would be expected to increase the risk of cardiovascular diseases. Consequently some MS patients may die from cardiovascular diseases even without a direct relation to the disease.

2.8 Lipids and MS
Apart from adipose tissue, the brain is the most cholesterol-rich organ in the body,\textsuperscript{75} with cholesterol forming about 80\% of the intact myelin\textsuperscript{76}. Lipids play important roles in the central nervous system and their transport through the blood-brain barrier have been demonstrated in normal physiological conditions\textsuperscript{77, 78} and during breakdown of the blood-brain barrier in MS.\textsuperscript{79} To maintain cholesterol homeostasis in the body\textsuperscript{80} and the CNS\textsuperscript{81, 82} there are antioxidant defence systems and mechanisms to regulate lipid metabolism.
Notwithstanding, the brain is believed to be particularly susceptible to oxidative damage and lipid dysregulation\textsuperscript{83-85} due to its high content of lipids and high oxygen consumption, as well as a less effective antioxidant defence system compared with other tissues.\textsuperscript{85} Dysregulation and oxidation of lipids are closely linked to the development of several neurodegenerative disorders including Alzheimer’s and Parkinson’s disease.\textsuperscript{86, 87}

\subsection*{2.8.1 Lipids in people with MS and comparison with healthy populations}

We could not identify any studies that examined the risk of MS in people with dyslipidaemia (HDL below 1.3mmol/L, LDL above 4.1 mmol/L, triglycerides above 2.3 mmol/L). However, a number of studies were found which compared lipid profiles between MS cases and controls. Table 2.3 provides a summary of the studies that examined the lipid profile in people with MS compared with healthy populations. Generally, a more adverse lipid profile among MS cases was observed in some studies, but this was not consistently found. For example, some studies have shown significantly elevated total cholesterol in people with MS compared to healthy controls,\textsuperscript{8, 88, 89} while other studies found no difference in total cholesterol levels.\textsuperscript{18, 90, 91} Similarly, some studies reported higher LDL levels in people with MS compared with healthy controls\textsuperscript{89} while other studies did not find any significant difference in LDL levels.\textsuperscript{8, 18, 88} Likewise, HDL concentrations have been found to be higher in MS cases compared with controls\textsuperscript{8, 88, 91}, but other studies did not find any significant differences.\textsuperscript{18, 89} Triglycerides levels were also higher in MS cases compared to healthy control in some studies but did not find any significant differences.\textsuperscript{18, 88-91} A study by Sternberg and colleagues which compared the lipid profile difference between MS patients who were on disease modifying therapies (DMTs-users) and DMTs-naïve demonstrated a significantly higher HDL in DMTs-users compared to DMTs-naïve. The level of TC and LDL were higher in DMTs-users but not significantly different.\textsuperscript{48}
Table 2.3: Studies examining the lipid profile in people with multiple sclerosis

<table>
<thead>
<tr>
<th>Author</th>
<th>Study information</th>
<th>Outcome measure of interest</th>
<th>Main findings</th>
</tr>
</thead>
</table>
| Guibilei and colleagues, (2002) | Case-control, 18 MS cases & 18 controls | Lipid profile differences between cases and controls & Correlation between lipid profile and CEL | 1. TC significantly higher in cases than controls  
2. LDL, Trig & ApoE higher in cases than controls but the difference not significant.  
3. TC and LDL correlated significantly with CEL number |
| Salemi and colleagues, (2010) | Case-control, 40 MS cases & 80 controls | Lipid profile differences between cases and controls                                           | 1. HDL significantly higher in cases than controls  
2. TC & Trig higher in cases than controls but differences not significant |
| Jamroz-Wisniewska and colleagues, (2009) | Case-control, 82 RRMS, 55 progressive MS & 40 controls | Lipid profile differences between cases and controls                                           | 1. TC significantly higher in cases than controls  
2. LDL significantly higher in RRMS in remission and progressive MS than controls  
3. HDL higher in cases than controls but difference not significant |
| Sternberg and colleagues, (2013) | Case-control, 206 MS cases & 142 controls | Lipid profile differences between cases and controls                                           | 1. TC and HDL significantly higher in cases than non-MS patients used us controls.  
2. LDL & Trig higher in MS cases than controls but difference not significant |
| Palavra and colleagues, (2013) | Case-control, 30 MS cases & 66 controls | Lipid profile differences between cases and controls & Correlation between lipid profile and EDSS | 1. Trig, Ox-LDL & small HDL particles higher in MS cases than control  
2. LDL significantly lower in MS cases than controls  
3. TC, LDL & Ox-LDL significantly correlated positively with EDSS |
| Çomoğlu and colleagues, (2004) | Case-control, 22 MS cases & 16 healthy controls | Lipid profile differences between cases and controls                                           | 1. Trig & VLDL were significantly higher in MS cases than the healthy controls  
2. TC & LDL higher in cases than controls but difference not significant.  
3. LDL/HDL significantly higher in cases (male ) than the controls (males) |
| Weinstock-Guttman and colleagues, (2011) | Cohort study, 178 CDMS patients | EDSS and MSSS                                                                                   | 1. EDSS associated with TC/HDL ratio  
2. No association between EDSS & MSSS and the other lipid variables |
| Weinstock-Guttman            | Cohort study, 492          | EDSS, MSSS CHEDSS                                                                                | 1. Trend of association between lipid variables and EDSS, MSSS, CHEDSS and |
and colleagues, (2011)\textsuperscript{92} CDMS patients CHMSSS, and T1-LV, T2-LV lesions, CEL, CE-LV and BPF. CHMSSS

Weinstock-Guttman and colleagues, (2013)\textsuperscript{93} Cohort study, 135 CIS patients Relapse, MRI measures

<table>
<thead>
<tr>
<th>Associations between lipids variables and CEL, CE-LV and BPF.</th>
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<tbody>
<tr>
<td>3. Associations between lipids variables and CEL, CE-LV and BPF.</td>
</tr>
<tr>
<td>5. No association between lipid variables, BMI and T1-LV &amp; T2-LV</td>
</tr>
</tbody>
</table>

1. TC & LDL associated with increased cumulative number of new T2 lesions.
2. Trend of association of TC & LDL with lower baseline whole brain volume.
3. Trend of association between TC and CEL. Other lipids were not associated with CEL

**Abbreviations:**

TC: Total cholesterol; LDL: Low density lipoprotein; Ox-LDL: Oxidised LDL; HDL: High density lipoprotein; Trig: Triglycerides; VLDL: Very low density lipoprotein; EDSS: Expanded disability status scale; CHEDSS: Change in EDSS; MSSS: Multiple sclerosis severity score; CHMSSS: Change in MSSS; CDMS: Clinically definite MS; CIS: Clinical isolated syndrome CEL: Contrast enhancing lesions; T1-LV: T1 lesion volume; T2-LV: T2 lesion volume; BPF: Brain parenchyma fraction
Further to this, contrasting results also exist showing that dyslipidaemia is more frequent in people with MS compared with healthy controls. Lavela and colleagues\textsuperscript{52} reported a significantly higher prevalence of dyslipidaemia in 1,142 male veterans with MS (48.5\%) compared to 31,500 non-MS veterans (44.6\%). Similarly Kang and colleagues\textsuperscript{45} reported significantly higher (p< 0.001) dyslipidaemia in 898 MS cases (14\%) compared to 4490 controls (6.9\%). However, significantly (p<0.05) lower prevalence of dyslipidaemia was reported in 9,949 MS cases (3.0\%) compared to 19,898 controls (5.1\%) by Allen and colleagues.\textsuperscript{19}

The disparities in the lipid profile could stem from differences in the\textsuperscript{49} use of DMTs and other unrelated MS pharmacological agents which has been shown to modulate the lipid profiles.\textsuperscript{48} The disparity in dyslipidaemia and lipid profile between the studies examined above could also be due to difference in what constitute a dyslipidaemia (cut off point), difference in population and age of MS patients recruited in the various studies.

\textbf{2.8.2 Lipids and MS disability}

A number of studies have been conducted to determine the impact of serum lipids on disability and disease progression. Using a time-to-event analysis model in the large NARCOMS registry, Marrie and colleagues reported that the presence of hypercholesterolemia (higher LDL and lower HDL) at any time during the disease course was associated with 35\% increased risk of early gait disability (HR: 1.35; 95\% CI: 1.26-1.45), 33\% increased risk of unilateral walking assistance (HR: 1.33; 95\% CI: 1.23-1.44) and 24\% increased risk of bilateral walking assistance (HR: 1.24; 95\% CI: 1.11-1.39).\textsuperscript{6} Similarly, hypercholesterolemia was associated with 83\% (HR: 1.83 (1.67-2.01) increased risk of mild visual disability, 75\% (HR: 1.75 (1.52-2.01) increased risk of moderate visual disability and 59\% increased risk of visual disability (HR: 1.59 (1.23-2.06)).\textsuperscript{49}
In a study by Palavra and colleagues, total cholesterol (r=0.40, p=0.027), LDL (r=0.37, p=0.05) and oxidised-LDL (r=0.46, p=0.01) were positively correlated with EDSS. In a study looking at the association between vitamin D and serum lipid profiles in a population of 178 MS patients, total cholesterol/HDL ratio was associated with higher EDSS (r=0.21, p=0.008). In our own work, TC (p = 0.037), apolipoprotein B (ApoB) (p = 0.003), and the apolipoprotein B to apolipoprotein A-I ratio (ApoB/ApoA-I ratio) (p = 0.018) were independently associated with a higher EDSS.

In terms of measuring progression of disability as an outcome, Weinstock-Guttman and colleagues assessed the associations of baseline lipid profile with subsequent disability progression. A cohort of 492 participants were followed for an average of 2.2 years and data on EDSS and serum lipids were collected at baseline and after a mean period of 2.2 ± 1.0 years follow-up. They found that higher baseline total cholesterol (partial correlation coefficient (rp) =0.15, p=0.001), LDL (rp=0.13, p=0.006), triglycerides (rp=0.10, p=0.025), total cholesterol/HDL ratio (rp=0.09, p=0.005) were significantly associated with a greater increase in EDSS. We also investigated the relationship between the lipid profile and disability progression and reported that TC to HDL ratio (TC/HDL ratio) was prospectively associated with progression in clinical disability (p = 0.029) as measured by annual change in EDSS.

Results from these studies suggest a possible relationship between adverse lipid profile, disability and disease progression. However, data on these relationships, particularly the prospective data, are limited and requires further investigation.

2.8.3 Serum lipids and inflammatory activity in MS
Studies have been conducted to determine whether any relationship exists between serum lipid profile and inflammatory activity as evidenced by MRI. Weinstock-Guttman and
colleagues\textsuperscript{92} found that higher levels of HDL were associated with a reduced likelihood of having contrast-enhancing lesions (p=0.01) and with a reduced lesion volume when they did occur (p<0.001). Higher levels of triglyceride and total cholesterol/HDL ratio were associated with a greater likelihood of having contrast-enhancing lesions (p=0.038) and lesion volume (p=0.023). Giubilei and colleagues\textsuperscript{88} also reported that higher levels of total cholesterol (r=0.59, p=0.01) and LDL (r=0.54, p=0.02) were associated with a greater number of contrast-enhancing lesions. In a cohort of participants with clinically isolated syndromes, Weinstock-Guttman and colleagues\textsuperscript{93} also observed that higher LDL (p=0.006) and total cholesterol (p=0.001) levels were associated with increased cumulative number of new T2 lesions over 2 years. From the MRI studies examined above, individuals with an adverse lipid profile had higher number of inflammatory lesions on MRI in clinically isolated syndromes or clinically definite MS. Since contrast-enhancing lesions represent recent inflammatory activity, their association with serum lipid profile in both early and established MS may be an indication of a causal relationship. However, whether maintaining the lipid profile within a normal range will modulate inflammatory activity is not clear and needs further investigation.

2.8.4 Clinical trials of statins in MS

Statins are a class of lipid-lowering drugs which inhibit the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the main rate-limiting enzyme in cholesterol biosynthesis. In addition to their lipid-lowering effects, statins have been found to have anti-inflammatory and immunomodulatory properties.\textsuperscript{94} A number of small randomised controlled trials using statins have been conducted in MS patients, though with contradictory results. The first single-arm trial (simvastatin 80 mg/day) in 30 RRMS cases found a decrease in gadolinium-enhancing lesion number (44%) and volume (41%) compared to pre-treatment.\textsuperscript{95} A recent double-blind placebo-controlled trial in secondary-progressive MS cases with simvastatin found that those on simvastatin had a 43% reduction in brain atrophy compared
to the placebo group and a 40% reduction in the rate of disability progression between the active and control arms. The ACTIVE study and others reported a protective effect of statin.

However, others found no evidence of a protective effect of statin therapy. A post-hoc analysis of the SENTINEL trial in RRMS patients (natalizumab plus interferon-beta-1a (n=40) vs. placebo plus interferon-beta (n=542)) found no differences in relapse rate, disability progression or number of gadolinium-enhancing lesions between those using statins and those not. The SIMCOMBIN trial, STAyCIS study, and others found no evidence of therapeutic effect of statin.

The inconsistencies in the trial results calls for further research into the role of serum lipids in the pathogenesis of MS and obtaining a mechanistic understanding of cholesterol metabolism and the role of these pathways in MS. Understanding these mechanisms can inform as to the potential benefits of lipid-lowering drugs for MS in the future, which may be critical in designing future randomised controlled trials.

2.8.5 Summary: lipid profile and MS
Results from the case-control studies examined above were largely inconsistent but the best-designed studies showed slightly elevated total cholesterol and HDL in MS cases compared to the controls. In the longitudinal and cross-sectional studies, higher levels of HDL were associated with lower acute inflammatory activity on MRI and lower MS disability, which is consistent with the antioxidant and anti-inflammatory properties of HDL. While MS cases may be expected to have lower levels of HDL than controls, the high concentrations observed may be due to their increased role in the antioxidant and anti-inflammatory activities and reverse cholesterol transfer in MS. This contradiction therefore calls for further investigation to provide a consistent body of knowledge on the relationship between serum lipids and MS in both longitudinal and case-control studies.
On the whole, evidence from the studies examined above suggest a negative impact of high LDL and triglycerides on acute inflammatory activity and disease course in MS patients and a beneficial effect of higher HDL levels on MS. Holistically, the prevalence of dyslipidaemia and its association with disability may be an indication that the serum lipid profile could be a potential target for reducing or modulating the rate of disability progression. However, the results of the statin clinical trials on MS have been contradictory and needs further investigation to explore their potential benefits.

2.9 Conclusions, implications and recommendations
There is a growing body of evidence to indicate that MS is associated with vascular comorbidities such as obesity, type-2 diabetes, cardiovascular diseases and dyslipidaemia in a number of different ways. In relation to MS risk, there is evidence that high childhood/adolescent BMI, but not adult BMI is associated with MS, but it is unclear whether this is due to confounding by other factors like sun and/or vitamin D. The other factors show some indirect evidence of an association with MS, but the directionality of these associations, if any, is unclear. There is no data on whether type-2 diabetes, cardiovascular disease and dyslipidaemia are more common prior to the disease onset in people with MS compared to controls.

Comparing people with established MS to healthy controls, there is insufficient evidence to suggest that the prevalence of obesity, hypertension or T2D is higher in people with MS, and there is inconsistent evidence around the association with lipids. However, people with MS appear to have a slightly increased risk of cardiovascular disease and they also die more often of cardiovascular disease. The question still remains whether this cardiovascular disease risk develops prior to the MS onset or after. Regardless, it is important from a clinical perspective, as the awareness by neurologists of this issue may assist with the prevention of cardiovascular disease or mortality in people with MS. At a patient level, the identification of
personal risk factors of cardiovascular disease, including high BMI, dyslipidaemia, adverse diet, and low physical activity, can inform individual lifestyle modifications or drug treatments.

The presence of vascular comorbidities could increase and complicate the MS disease burden. These comorbidities may be partly responsible for the highly variable inter and intra-personal clinical course of MS. In relation to whether vascular comorbidities influence MS disability and the progression of disability or other aspects of the disease course, the key finding was that having a vascular comorbidity at diagnosis or at any point in the disease course may be associated with a worse progression in disability. In addition, a negative effect of high cholesterol and triglycerides and positive effect of higher HDL levels was observed on acute inflammatory activity, measured by MRI. Little data was available on BMI or T2D and progression. In general, it was clear from our review that prospective studies are sparse.

There are important therapeutic opportunities for lipid lowering drugs. The use of these may be justified when the aim is to reduce a patient’s risk of cardiovascular disease. However, the use of these is not yet justified as an intervention in MS. Importantly, that statin trials have produced inconsistent evidence, including negative effects, is critical and may indicate that previous observational studies demonstrating associations may have been confounded. Adverse effects in people with MS are possibly explained by laboratory evidence showing negative impacts of statins on oligodendrocytes and myelin formation. Thus, care needs to be taken when considering the use of statins in the treatment of MS.

We recommend that future studies of the prevalence and effects of vascular comorbidities on MS risk and disability progression should be prospective where relevant and using sufficiently validated means of assessing outcomes and vascular comorbidities, and where possible objectively assessed (e.g. BMI). Studies must separately investigate vascular comorbidities which occur before the onset of MS and those that occur during the course of
MS in order to determine those that may be important in the onset or progression of MS and those that merely co-vary with age, decreased physical activity and subsequent increased BMI. This research will hopefully lead to potential interventions that may improve outcomes for persons with MS.

2.10 Postscript
This chapter has provided some key information on how vascular comorbidities may influence the onset and disease progression in MS. The next chapter is a review of the literature on the frequency of autoimmune comorbidities in MS and the contribution of immunomodulatory therapy to this frequency.

2.11 References
24. Sioen I, Mouratidou T, Kaufman JM, et al. Determinants of vitamin D status in young children: results from the Belgian arm of the IDEFICS (Identification and Prevention of


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Chapter 3  Autoimmune comorbidities in MS

3.1  Preface
This chapter is made up of two manuscripts on the role of autoimmune comorbidities in MS. The first manuscript is on the frequency of autoimmune comorbidities in MS and the contribution of IFN-β therapy to this frequency. The second manuscript has been published. It describes the co-occurrence of multiple sclerosis and type 1 diabetes and the shared etiologic features and clinical implication for MS aetiology. The typeset version of the manuscript as it appeared in the journal is in Appendix 3A. In writing this review, relevant articles were retrieved by a variety of methods. Firstly, PubMed, Google Scholar and Web of Knowledge were comprehensively searched using the following terms: “multiple sclerosis” and “thyroid”, “multiple sclerosis” and “rheumatoid”, “multiple sclerosis” and “type 1 diabetes”, “multiple sclerosis” and “psoriasis”, “multiple sclerosis” and “systemic lupus erythematosus”, “multiple sclerosis” and “inflammatory bowel disease”, “multiple sclerosis” and “Crohn’s”, “multiple sclerosis” and “ulcerative colitis”. In addition, a snowballing method was used by retrieving articles back in time by identifying new papers that the key articles cited. Lastly, citation tracking was used (reverse snowballing), where Web of Knowledge was used to retrieve articles forward in time by identifying new papers who cited the key articles. These autoimmune comorbidities were particularly examined based on their frequent report in people with MS.

3.2  Frequency of autoimmune comorbidities in MS: the role of interferon-beta therapy

3.2.1  Introduction
Multiple sclerosis (MS) is an autoimmune, inflammatory, demyelinating disease of the central nervous system that is associated with progressive disability in young adults.1 Owing to the chronic and debilitating nature of the disease, MS patients may be at an increased risk of other autoimmune comorbid conditions.2,3
As a presumed autoimmune disease, MS has been associated with increased occurrence or coexistence with other autoimmune diseases and increased frequency of elevated autoantibodies; however, this has not been consistently shown.\textsuperscript{2, 4, 5} Evidence for association of MS with other autoimmune diseases such as type 1 diabetes, psoriasis, autoimmune thyroiditis, inflammatory bowel disease and rheumatoid arthritis\textsuperscript{2, 3} might indicate a shared susceptibility to autoimmunity and may support the hypothesis of an autoimmune pathogenesis for MS.\textsuperscript{6, 7}

Further, recent accumulating evidence indicates that chronic exposure to interferon-beta (IFN-\(\beta\)) therapy in MS patients is associated with the induction or exacerbation of thyroid disease, as either organ dysfunction or autoimmunity.\textsuperscript{8-10} IFN-\(\beta\) has been widely used to treat MS patients because of its capacity to reduce the frequency and severity of exacerbations and retard the progression of disability in secondary progressive MS.\textsuperscript{8-11} Since IFN-\(\beta\) modulates the immunoregulatory system, this cytokine may precipitate autoimmune disorders.\textsuperscript{12, 13} It is therefore important to know whether the frequency of autoimmune thyroid disease is increased in MS patients receiving interferon-beta therapy compared to those not receiving this therapy.

Limited quality data exist on how immunomodulatory therapies may affect the frequency of other autoimmune diseases in MS patients. An increased understanding of factors that affect the frequency and severity of autoimmune comorbidities in MS may provide new insights and enhance MS management. In this review, we aimed to assess the frequency of commonly reported autoimmune comorbidities in MS and investigate the contribution of IFN-\(\beta\) to the frequency of autoimmune thyroid autoimmunity and dysfunction in MS patients.

**3.2.2 Thyroid autoimmunity and dysfunction**

The spectrum of thyroid autoimmunity and dysfunctions reported in MS includes hypothyroidism (Hashimoto’s thyroiditis), hyperthyroidism (Graves’ disease) and positive
anti-thyroid antibodies without clinically significant thyroid disease. Thyroid dysfunction is defined as overt or subclinical hyperthyroidism or hypothyroidism, while thyroid autoimmunity is defined as the presence of anti-thyroid antibodies in serum.

3.2.2.1 Studies examining thyroid autoimmunity and dysfunction in IFN-β naïve MS patients

Three studies compared the incidence of thyroid disease in the MS population to that in the general population. For instance, a 1990 epidemiological study by Wynn and colleagues investigated the incidence of thyroid disease (Graves’ disease or Hashimoto’s thyroiditis) in 137 female MS patients and found no significant difference in the incidence of thyroid disease between the MS patients and that of the general population (RR: 1.33; 95% CI: 0.27-3.87). Similarly, a study of 4,192 MS patients and 20,940 age, sex and geographically matched controls did not find significant difference in the incidence of thyroid disease (Incidence Rate Ratio (IRR): 0.98; 95% CI 0.73–1.30). Furthermore, Nielsen and colleagues reported an incidence of Hashimoto’s thyroiditis of 0% in a study of 10,596 MS patients compared to age, gender, and period-specific national incidence rates from 1977 to 2004. The estimated incidence of thyroid disease from these studies ranges from 0.15% to 0.42%. Marrie and colleagues conducted a meta-analysis of the incidence of thyroid dysfunction in MS patients and reported a summary incidence estimate of 0.17% (95% CI: 0–0.40%). They also reported substantial heterogeneity among the three population-based studies included in the analysis ($I^2=89.9$).

A substantial number of studies have been conducted evaluating the prevalence of thyroid diseases in MS patients, with some comparing it to a control or general population. Edwards and Constantinescu conducted a prospective study of 658 consecutive patients diagnosed with MS between June 2002 and June 2003. Prevalence of autoimmune thyroid disease in these patients was compared with values from population studies. From the study,
the MS population had significantly increased rate of autoimmune thyroid disease compared to the general population (OR: 1.80; 95% CI 1.07–3.02). In a study using the National Health Insurance Research Dataset in Taiwan, Kang and colleagues reported that the prevalence of Hypothyroidism in a group of 898 MS patients was 1.7%, this was 3.2-times higher than that of the 4,490 randomly matched controls without MS (OR: 3.2; 95% CI: 1.7-6.1)\textsuperscript{26}

The association of MS with autoimmune thyroid disease (Graves' disease and Hashimoto's disease) was investigated in 491 MS patients and compared to 532 controls from the general population by Sloka and colleagues.\textsuperscript{27} There was a significantly higher prevalence of Graves' disease in MS (3.1%, \(p = 0.002\)) compared to the general population (0.4%). There was however no difference in the prevalence of Hashimoto’s disease (\(p = 0.097\)).

A number of studies investigated the sex difference in the frequency of autoimmune thyroid disease in MS. For example, Niederwieser and colleagues reported a significantly higher prevalence of autoimmune thyroiditis in male MS patients (9.4 %) than in male controls (1.9 %; \(p = 0.03\)) while prevalence of autoimmune thyroiditis in female MS patients (8.7 %) did not differ from female controls (9.2 %).\textsuperscript{24}

Some studies did not report significant difference in the frequency of thyroid disease in MS compared to a general population. For instance, Marrie and colleagues\textsuperscript{18} reported a similar age-adjusted prevalence of thyroid disease in the MS population (PR: 9.51%; 95CI 8.46–10.6) compared to the general population (PR: 8.56%; 95% CI 8.11–9.02). Likewise, Laroni and colleagues\textsuperscript{23} did not report significant difference in the prevalence of Graves’ disease and Hashimoto’s disease between MS patients (Graves’ disease: 2.04%; Hashimoto’s disease: 1.63%) and general population (Graves’ disease: 1.22%; Hashimoto’s disease: 1.63%).
Overall, the prevalence of thyroid disease in MS patients from these studies ranged from 2.08% to 10%. Hashimoto’s thyroiditis ranged from 0% to 16.1%, and Grave’s disease ranged from 0% to 2.56%.

Two studies conducted a meta-analysis of the prevalence of thyroid disease in MS patients. The meta-analysis by Marrie and colleagues in 2015 reported summary thyroid disease prevalence estimate of 6.44% (95% CI: 0.19–12.7%) with substantial heterogeneity ($I^2=95.4$). The meta-analysis by Dobson and colleagues in 2013 investigated both thyroid dysfunction and thyroid autoimmunity in MS patients. They included 14 studies and reported an overall increased risk of thyroid dysfunction in people with MS (OR: 1.66; 95% CI 1.35–2.05) compared to healthy controls and without between-study heterogeneity (Cochran’s Q $p = 0.16, I^2 = 27\%$). Nine studies were included in the thyroid autoantibodies analysis and reported an overall increased risk of thyroid autoantibodies in patients with MS compared to healthy controls (OR: 2.36, 95% CI 1.32–4.20) but with significant heterogeneity (Cochran’s Q $p = 0.0001, I^2 = 74\%$).

3.2.2.2 Studies investigating IFN-β induced thyroid autoimmunity and dysfunction
There is accumulating evidence showing that MS patients receiving IFN-β therapy are at increased risk of developing thyroid dysfunction and autoimmunity. Few studies on the incidence of IFN-β induced thyroid autoimmunity and dysfunction in MS patients have been conducted. In people with MS, a high incidence of IFN-β induced thyroid dysfunction has been reported by Caraccio and colleagues. In this study, 106 MS patients underwent IFN-β therapy for up to 7 year and thyroid function and autoimmunity were assessed at baseline and every 3–6 months throughout the treatment course. Among these participants, thyroid dysfunction developed in 25 (hypothyroidism in 20 and hyperthyroidism in 5) of the 103 MS patients (24.3%) and thyroid autoimmunity developed in 22 of 97 patients (22.7%). The development of thyroid dysfunction and autoimmunity were mainly within the first year of
treatment. On the whole, both baseline and incident autoimmunity was reported as significant risk factors for subsequent development of IFN-β induced thyroid dysfunction (p=0.001).

The effect of one-year treatment with IFN-β on thyroid function and autoimmunity in 31 MS patients was evaluated by Monzani and colleagues. Systematic thyroid assessment was performed at baseline and every 3 months during treatment. From the analysis, 16% of the patients had autoimmune thyroiditis (Hashimoto’s thyroiditis) before treatment. The overall incidence of thyroid dysfunction was 33% (8/26) over 1 year (10% hyperthyroidism, 23% hypothyroidism) and thyroid autoimmunity developed in 19%. In addition to autoantibody positivity at baseline, female gender and the presence of an ultrasound thyroid pattern suggestive of thyroiditis were identified as additional risk factors for the development of thyroid dysfunction.

To evaluate the frequency of incident thyroid disease during longer term IFN-β therapy, Monzani and colleagues studied 31 MS patients and thyroid assessment was conducted every 3 or 6 months during 3 years of IFN-β treatment. Among six MS patients who developed incident subclinical hypothyroidism (19.4%) during the first year of treatment, thyroid dysfunction persisted in 2 with baseline autoimmune thyroiditis. Three patients developed incident transient hyperthyroidism (9.7%). A positive thyroid autoantibody was continually detected in only two (6.5%) out of five patients without baseline autoimmunity. After the first year of IFN-β treatment, no further cases of thyroid disease were observed.

Quite a substantial number of studies have investigated the prevalence of IFN-β induced thyroid autoimmunity and dysfunction in MS patients. Durelli and colleagues undertook a prospective two-year follow-up of autoimmune events in 40 consecutive IFN-β treated MS patients and in 21 untreated MS controls and monitored thyroid, liver function and serum levels of 12 autoantibodies. In contrast to the controls, thyroid dysfunction
(hyperthyroidism=3, hypothyroidism=1) and anti-thyroid autoantibodies were detected in 4 (10%) IFN-β treated MS patients with persistent autoimmune thyroid dysfunction occurring in 3 IFN-β treated patients with a family history of thyroid disease or anti-thyroid autoantibody positivity at baseline.

Kreisler and colleagues investigated the frequency of clinical thyroid dysfunction in a group of 700 consecutive MS patients treated with IFN-b for at least 3 months, between 1996 and 2000 and without past history or clinical signs of thyroid dysfunction before introduction of IFN-b. A total of eight patients (1.1%) developed clinical thyroid dysfunction. Among these participants, five developed hyperthyroidism (0.71%) and two developed hypothyroidism (0.29%). Out of these five hyperthyroidism cases, it was necessary to stop IFN-b treatment in three cases.

In one of the long-term follow-up studies, a cohort of 787 MS patients was retrospectively followed for 8 years for IFN-β or glatiramer acetate (GA) induced thyroid dysfunction and thyroid autoimmunity at baseline and during treatment every 3–6 months. All patients received at least 2 years of IFN-β or GA treatment. The authors reported higher thyroid dysfunction (7% versus 13.2%; p < 0.02) and thyroid autoimmunity (3.5% versus 13.4%; p =0.01) during IFN-β treatment compared to baseline. On the contrary, there was no difference in thyroid dysfunction and autoimmunity before and after the beginning of GA treatment. The development of thyroid dysfunction and thyroid autoimmunity occurred mainly within the first year of treatment with IFN-β.

Other authors conducted similar studies to investigate the occurrence of thyroid dysfunction and autoimmunity during IFN-β treatment. A number of case reports have also documented the effect of IFN-β on thyroid autoimmunity and dysfunction.
3.2.2.3 Summary and implications: Thyroid autoimmunity and dysfunction

The induction or exacerbation of autoimmune diseases or autoantibodies is a well-known side effect of chronic type I IFN therapy. Long-term experience in chronic viral hepatitis treatment has documented the induction of reversible asymptomatic autoantibodies as well as the development of several autoimmune disorders such as thyroiditis, anaemia, hepatitis, diabetes, systemic lupus erythematosus or psoriasis during IFN-α or IFN-β treatment.16, 32

From the review of current available literature, a substantial number of epidemiological studies have documented the occurrence of thyroid dysfunction (hypothyroidism and hyperthyroidism) and autoimmunity (increased autoantibody levels) in both MS patients receiving IFN-β therapy and MS patients who are IFN-β naive. The frequency of IFN-β induced thyroid dysfunction has been estimated to ranges from 1–13% and that of thyroid autoimmunity ranges from 22–43% (13.4%).29 The frequency of thyroid dysfunction in MS patients not receiving IFN-β have been estimated to ranges from 2–11%, and that of anti-thyroid autoantibodies from 4–22% (26–31).29 It appears from the data available that IFN-β therapy is associated with higher occurrence of thyroid autoimmunity and dysfunction in MS patients than those MS patients that are IFN-β naive. However this needs further investigation including meta-analysis to quantify the magnitude of effect exerted by the IFN-β therapy. This is because only one study actually compared the risk of thyroid autoimmunity and dysfunction in MS patients on IFN-β therapy and those who are IFN-β naïve.

The question remains as to whether there is a true link between IFN-β and the occurrence of thyroid disorder in MS patients. Probably, the short delay between introduction of IFN-β treatment and the onset of clinical symptoms of thyroid dysfunction argue in favour of a possible link. Furthermore, studies have demonstrated the occurrence of IFN-β induced thyroid autoimmunity and dysfunction in MS patients, when baseline analyses were normal in all patients.13, 16 33
The most common form of autoimmune IFN-β-induced thyroid disorder in MS is the presence of thyroid autoantibodies without thyroid dysfunction.\textsuperscript{8, 31, 33} Thyroid dysfunction was generally subclinical and mild in degree and is most often reversible.\textsuperscript{10} The occurrence of thyroid autoimmunity and dysfunction is particularly frequent within the first year of treatment\textsuperscript{34}, although sporadic cases of late onset of dysfunction have been described.\textsuperscript{13, 30}

A number of risk factors have been suggested to be associated with the occurrence of thyroid autoimmunity and dysfunction in MS patients. The female gender has been associated with higher risk or susceptibility to IFN-β induced thyroid dysfunction than men, however in some studies, gender did not emerge as a significant risk factor\textsuperscript{10} or higher risk in the male gender.\textsuperscript{10} Also, the presence of elevated anti-thyroid antibodies prior to treatment with IFN-β or a family history of thyroid disease has been linked to the development of autoimmune dysfunction during treatment.\textsuperscript{34} In addition to pre-existing thyroiditis and female gender, hypoechoic thyroid pattern have been suggested as possible predictor of thyroid abnormalities.\textsuperscript{12}

The evidence provided by contemporary investigations show that IFN-β induced thyroid disorders are becoming a significant clinical problem for MS patients receiving IFN-β therapy. For instance, some cases of IFN-β induced thyroid dysfunction have been reported to result in dose reduction or discontinuation of interferon therapy which may compromise the therapeutic effect of the medication.\textsuperscript{34} There was no consensus on the recommendation for routine thyroid screening test or systematic assessment during IFN-β treatment. Some authors argue that the low frequency of clinical thyroid dysfunction does not warrant systematic thyroid investigation in MS patients receiving IFN-b therapy. However, they recommended that thyroid hormones should be tested as soon as any clinical signs suggestive of thyroid dysfunction are observed, because therapeutic changes may have to be quickly proposed.\textsuperscript{13} When there is severe thyroid dysfunction, periodic withdrawal of treatment and evaluation of
thyroid function while off therapy is recommended to verify the possible recovery of thyroid function.\textsuperscript{32}

Although the mechanisms of IFN-β induced thyroid disorders are not clearly understood, the risk appears to be related to both immune stimulatory effects and direct effects on the thyroid.\textsuperscript{8} IFN-β has been suggested to induce thyroid dysfunction by stimulating the production of thyroid-inhibitory cytokines and/or activation of cytotoxic lymphocytes in the thyroid.\textsuperscript{12} In those patients who develop hypothyroidism without autoantibody production, a direct inhibitory effect on iodine organification may be postulated.\textsuperscript{15}

\textbf{3.2.3 Rheumatoid arthritis}

The incidence of rheumatoid arthritis in MS patients has been investigated by few studies. A 1990 epidemiological study by Wynn and colleagues investigated the incidence of rheumatoid arthritis in 137 female MS patients and found higher incidence of rheumatoid arthritis in people with MS compared to the general population of Rochester but this was not statistically significant, probably due to small sample size (RR: 1.58; 0.43–4.04).\textsuperscript{17} Also, Nielsen and colleagues\textsuperscript{19} investigated the incidence of rheumatoid arthritis in a study of 10,596 MS patients compared to age, gender, and period-specific national incidence rates from 1977 to 2004. They reported a significant decreased risk of rheumatoid arthritis in patients with MS (RR: 0.5; 95% CI: 0.4–0.8). From the systematic review of the literature, Marrie and colleagues reported the incidence of rheumatoid arthritis to range from 0.14–1.28% and a meta-analysis of the incidence of rheumatoid arthritis in individuals with MS was summarized as 0.21% (95% CI: 0.087–0.33%).\textsuperscript{3}

Findings from studies investigating the prevalence of rheumatoid arthritis in MS patients have been inconsistent, with some reporting higher, similar or lower prevalence of the disease in MS patients compared to a comparator population.\textsuperscript{5, 7, 20, 23, 25, 26, 35–37} Kang and colleagues investigated the prevalence of rheumatoid arthritis in a group of 898 MS patients and
compared it to a 4,490 randomly matched controls without MS using the National Health Insurance Research Dataset in Taiwan. From the study, they reported a higher odds of rheumatoid arthritis compared to the matched control group (OR: 4.8; 95% CI: 2.9-8.1)\textsuperscript{26}

Ramagopalan and colleagues\textsuperscript{25} used data from a national, multicentre, population-based sample to investigate the rate of autoimmune disease in 5031 MS patients, 30 259 of their first-degree relatives, and 2707 spousal controls. From the analysis, there was a higher prevalence of rheumatoid arthritis in MS patients (153/5031 = 3.04\%) compared to their spousal controls (Spouses: 66/2,707= 2.44\%) or first degree relatives (529/ 30,259 = 1.75\%).

Edwards and Constantinescu\textsuperscript{5} conducted a prospective study of 658 consecutive patients diagnosed with MS between June 2002 and June 2003. From the analysis, there was no significant difference in the prevalence of rheumatoid arthritis in MS patients compared with the general population (OR: 0.93; 0.23-3.74).

From the systematic review of seventeen studies, Marrie and colleagues reported the prevalence of rheumatoid arthritis to range from 0.30–3.64\%. Meta-analysis of prevalence estimates from two population-based studies was 2.92\% (95\% CI: 1.8–4.0\%).\textsuperscript{3} From the meta-analysis of eleven studies examining the risk of rheumatoid arthritis in MS, there was no significant difference in the prevalence of rheumatoid arthritis in MS patients compared to a comparator group (OR 1.15, 95 \% CI 0.77–1.73, p = 0.49). There was significant heterogeneity between studies.\textsuperscript{2}

3.2.4 Systemic lupus erythematosus

A study on the incidence of systemic lupus erythematosus (SLE) in MS patients was conducted by Nielsen and colleagues. The incidence of SLE in 10,596 MS patients was not statistically different when compared to age, gender, and period-specific national incidence rates from 1977 to 2004 (RR: 0.5; 95\% CI: 0.1-2.0). From a systematic review of the literature, Marrie and colleagues reported the incidence of SLE to range from 0.02–0.35\%.
However, the methods used in the two incidence studies differed substantially.\textsuperscript{19, 38} The study by Nielsen and colleagues with the lower estimate used population-based administrative data\textsuperscript{19}, while the study by Marrie and colleagues with higher estimate used a volunteer sample reporting diagnoses using a validated self-report questionnaire.\textsuperscript{38}

Mixed findings have been reported from studies investigating the prevalence of SLE in MS patients.\textsuperscript{7, 25, 26, 35, 36} For instance, Kang and colleagues using the National Health Insurance Research Dataset in Taiwan, reported significantly higher prevalence of SLE in a group of 898 MS patients when compared to 4,490 randomly matched controls without MS (OR: 26.9; 95% CI: 10.3-70.3)\textsuperscript{26} However, in a population-based case–control study of MS patients enrolled in the Northern California Kaiser Permanente Medical Care Program, the prevalence of SLE in MS patients was not significantly different from that of the matched control (OR: 1.1; 95% CI: 0.8–1.4).\textsuperscript{36}

Marrie and colleagues\textsuperscript{3} conducted a systematic review of nine studies and reported prevalence of SLE to range from 0.14–2.90%. Dobson and colleagues\textsuperscript{2} conducted a meta-analysis of five studies on the prevalence of SLE in MS. There was an increased risk of SLE in MS but this was not significant (OR: 2.80, 95% CI: 0.76–10.25). This may be due to the substantial heterogeneity among the studies included (Cochran’s Q p<0.00001, I\textsuperscript{2} = 88 %).

\textbf{3.2.5 Type 1 Diabetes}

Currently, there are no studies on the incidence of type I diabetes (T1D) in MS patients. However, a study investigated the incidence of MS in T1D patients and reported that the incidence rate for MS in patients with T1D was more than three-fold when compared to the general population (relative risk (RR: 3.26; 95% CI: 1.80-5.88).\textsuperscript{39}

A number of epidemiological studies on the occurrence of T1D in MS patients have reported similar, lower or higher prevalence of T1D in MS patients compared to that in the general population or other comparator population.\textsuperscript{5, 7, 20, 25, 26, 36, 37, 40, 41} These studies have been
assessed in two systematic reviews and meta-analyses.\textsuperscript{2, 3} Marrie and colleagues\textsuperscript{3} reviewed eighteen studies and reported the prevalence of T1D to range from 0–9.4%. Meta-analysis of the prevalence of T1D was estimated as 0.016\% (95\% CI: 0.01–0.21\%). Heterogeneity of the estimates was however substantial ($I^2=95.3$).

In the meta-analysis by Dobson and colleagues\textsuperscript{2}, 16 studies examining prevalence of T1D in MS were included in the analysis. Overall, there was an increased risk of T1D in people with MS compared to a comparator population (OR: 2.02; 95\% CI: 1.22–3.40). However, there was a significant heterogeneity between the studies included in the analysis (Cochran’s Q $p<0.00001$, $I^2 = 91\%$).

Detailed literature review on the co-occurrence of T1D and MS, shared etiological and pathologic mechanisms have been conducted and presented in next section of this chapter.

### 3.2.6 Inflammatory bowel disease

Studies on the incidence of inflammatory bowel disease (IBD: ulcerative colitis & Crohn’s disease) in people with MS are limited. The incidence of IBD in MS patients have been investigated by Nielsen and colleagues.\textsuperscript{19} They reported higher incidence of ulcerative colitis (RR = 2.0; 95\% CI: 1.4–2.8) in a study of 10,596 MS patients compared to age, gender, and period-specific national incidence rates from 1977 to 2004. There was also significant difference in the incidence of Crohn’s disease (RR: 0.7; 95\% CI: 0.3–1.5) when compared to the general population. A systematic review of two studies on the incidence of IBD in MS patients range from 0.33–1.0\%.\textsuperscript{3}

Findings regarding the prevalence of IBD in MS patients were inconsistent, but most studies reported that the prevalence of IBD were higher in the MS population than in the general population.\textsuperscript{5, 7, 19, 25, 36, 42} A prospective study of 658 consecutive MS patients reported increased risk of Ulcerative colitis (OR: 3.15; 95\% CI: 1.30-7.64) and Crohn’s disease (OR:
3.17; 95% CI: 1.01-9.95) in the MS patients compared to the general population. The overall IBD prevalence was higher in MS patients (OR: 3.17; 95% CI: 1.57-6.40). 5

A study was conducted to determine the frequency of IBD prior to the diagnosis of MS. Using the Northern California Kaiser Permanente Medical Care Program, Langer-Gould and colleagues conducted case–control study of 5,296 MS patients and 26,478 matched controls and reported an increased prevalence of IBD prior to the diagnosis of MS compared to the controls (OR: 1.7; 95% CI: 1.2–2.5). 36

Ramagopalan and colleagues 25 used data from a national, multicentre, population-based sample to investigate the rate of autoimmune disease in 5031 MS patients, 30 259 of their first-degree relatives, and 2707 spousal controls. From the analysis, there was no significant difference in the prevalence of ulcerative colitis (0.2%) when compared to the spousal controls (0.2%) or first-degree relatives (0.3%). Similarly there was no significant difference in the prevalence of Crohn’s disease (0.2%) when compared to the spousal controls (0.2%) or first-degree relatives (0.2%). Systematic review of twelve studies estimated the prevalence of IBD to range from 0.36–4.66%. 3

Dobson and colleagues 2 conducted a meta-analysis of the prevalence of IBD in MS patients compared to a comparator population. In the meta-analysis of six studies, they reported an increased risk of ulcerative colitis in people with MS (OR: 2.26: 95% CI: 1.23–4.14), but with significant heterogeneity (Cochran’s Q p = 0.003, I2 = 72 %). Similarly, in a meta-analysis of four studies, they reported increased risk of Crohn’s disease in people with MS (OR: 1.37; 95% CI: 1.12–1.69) and without significant heterogeneity. Overall, there was an increased risk of IBD in MS patients compared to a comparator group when six studies were meta-analysed.
3.2.7 Psoriasis

Fewer studies investigated the incidence of psoriasis in MS patients. Using the Danish Multiple Sclerosis Register, the Danish Hospital Discharge Register, and the Danish Civil Registration System, Nielsen and colleagues reported a higher incidence of psoriasis in the MS population than expected for the Danish general population but this was not statistically significant (RR: 1.5; 95% CI 0.95-2.4). The incidence of psoriasis in two European populations was estimated to range from 0.17–1.63%.

Studies also compared the prevalence of psoriasis in the MS population with a general population. In a prospective study of 658 consecutive outpatients attending a MS clinic, Edwards and Constantinescu reported increased risk of psoriasis in the MS cohort as compared to published data for the general population (OR: 2.11; 95% CI 1.13-3.94).

Midgard and colleagues conducted a hospital-based case-control study of 155 MS patients and 200 controls to investigate the possible association between MS and autoimmune diseases. They reported increased more than two-fold increased risk of psoriasis in MS patients compared to hospital-based controls without a history of MS (OR: 2.18; 95% CI 0.72-6.70), but this was not statistically significant.

Using the Swedish register, Roshanisefat and colleagues investigated immune-mediated disease risk among 11284 fathers and 12006 mothers of MS patients, compared with 123,158 fathers and 129,409 mothers of index subjects without MS. Similar analyses were conducted among 20,276 index subjects with MS and 203,951 without. From the analysis, index subjects with MS had a consistently increased risk of psoriasis compared with the control population (HR: 2.27; 95% CI 1.81–2.84). There was however no significant increased risk of psoriasis among parents with offspring who have MS, compared with parents of children without MS.
From a systematic review of the literature, the prevalence of psoriasis was estimated to range from 0.39–7.74%. Dobson and colleagues conducted a meta-analysis to assess the risk of psoriasis in MS patients. Eight studies were included in the analysis and they reported a significant increased risk of psoriasis in people with MS compared to a control group (OR: 1.31; 95% CI 1.09–1.57). There was no significant between-study heterogeneity (Cochran’s Q p = 0.16, I² = 34%).

3.2.8 Discussion
The literature was reviewed on the incidence and prevalence of common autoimmune diseases associated with MS including comparison with their respective general population estimates. While there is a substantial amount of data on the prevalence of autoimmune comorbidities in MS, incidence studies are uncommon.

Based on the review of the literature and meta-analyses, the most prevalent comorbid autoimmune diseases were psoriasis and thyroid disease. The studies reviewed demonstrated a consistent increase in the risk of clinical thyroid disorders, IBD and psoriasis among people with MS compared to a general or comparator population. The prevalence of RA and SLE in MS patients was however not significantly different from that of the comparator populations. The interpretation of these results is complicated by the significant heterogeneity between studies included in the meta-analysis.

The prevalence of T1D was significantly higher in MS patients when compared to the general population but this was characterised by substantial between study heterogeneity which limits the interpretation of the result. This heterogeneity may be due to differences in study designs used in the various investigations. The mode or source of data used could also account or explain part of this heterogeneity. For instance some studies used a questionnaire-based studies while other used large administrative or research databases. Questionnaire-based study is subject to high misclassification or mismatching of diseases since these autoimmune
diseases are self-reported by participants. This was evident from a study which compared self-reports to diagnoses verified by general practitioners. They reported that the positive predictive value of a patient-reported condition varied from 32 % for RA to 85 % for thyroid disease.\textsuperscript{43} Moreover, while some studies used population-based samples, others used hospital-based samples.

There was also disparity in the type of comparator populations used in the various studies. While some used population-based controls, others used spousal controls or first-degree relatives, potentially leading to overmatching. Moreover, among the studies that evaluated the frequency of autoimmune disease in MS and a comparator population, fewer studies used concurrent controls that were clearly drawn from the same underlying source population. Additionally, some authors\textsuperscript{2} reported that publication bias clearly affected the results of some of the autoimmune diseases meta-analysed. From their investigation, studies using large databases did not show publication bias or heterogeneity, unlike the smaller-scale studies in which there was evidence of persisting bias.

In summary, current studies available have demonstrated that certain comorbidities may be more prevalent in MS patients than in the general population. Incidence studies were however uncommon. The quality of studies also needs to be improved. A limited number of quality studies using population-based design hamper the ability to calculate good estimates of the incidence or prevalence of autoimmune comorbidities in MS and identify temporal trends in the risk of autoimmune disease in MS patients. We therefore recommend that future studies should have a population-based design with large dataset and a concurrent control to minimize heterogeneity due to differences in study design.
3.3 The co-occurrence of multiple sclerosis and type 1 diabetes: shared etiologic features and clinical implication for MS aetiology

3.3.1 Abstract
We reviewed the evidence for the co-occurrence of type 1 diabetes mellitus (T1D) and multiple sclerosis (MS), and assessed the clinical significance of this association and the shared etiological features of the two diseases. T1D and MS contribute considerably to the burden of autoimmune diseases in young adults. The co-occurrence of MS and T1D has been reported by a number of studies, suggesting the two conditions share one or more etiological components. Both conditions have been associated with distinct human leukocyte antigen (HLA) haplotypes but share a number of similarities in clinical, epidemiological and immunological features, leading to suggestions of possible common mechanisms of development. While underlying genetic factors may be important for the co-occurrence of both conditions, some evidence suggests that environmental factors such as vitamin D deficiency may also modulate an individual’s risk for the development of both conditions. Evidence on whether the co-occurrence of the two autoimmune conditions will affect the disease course and severity of MS is merely absent. Further studies need to be conducted to ascertain whether the neuropathology associated with T1D might influence the disease course and contribute to the severity of MS.

3.3.2 Introduction
Multiple sclerosis (MS) and type 1 diabetes mellitus (T1D) represent significant public health problems worldwide, because of their considerable contribution to medical and social management cost and eventual disability of affected individuals. MS and T1D via diabetic neuropathy and accelerated cerebrovascular disease contribute substantially to the burden of neurologic disability in young adults and significantly affect quality of life.
MS and T1D are considered to be organ-specific autoimmune disorders with an inflammatory component, but with marked differences in their pathogenesis and clinical manifestations. While MS is predominantly associated with neurological and physical disability and loss of function resulting from inflammatory demyelination and neurodegeneration of the central nervous system (CNS), the chronic hyperglycaemic condition characteristic of T1D results from the selective inflammatory autoimmune destruction of the pancreatic Islets of Langerhans responsible for insulin production. Despite the organ specificity of these two disorders, a possible aetiologic and pathologic relationship between the two diseases has been suggested. The framework behind the co-occurrence is unclear. While genetic predisposition appear to be involved in each of these autoimmune conditions, the low concordance among identical twins for MS and T1D and trends of increasing incidence for both diseases over time suggest that environmental factors are also important diseases determinants in the occurrence of the two diseases.

In this review, we assessed the available data on the co-occurrence of MS and T1D, the clinical significance of T1D in patients with MS and the aetiologic similarities between these two autoimmune disorders. Understanding the similarities in aetiology and pathophysiology may help clarify causality and help in the management of both conditions.

3.3.3 Studies investigating the co-occurrence of MS and T1D

3.3.3.1 Incidence studies on the co-occurrence of MS and T1D
Table 3.1 summarises studies that have examined the risk of the co-occurrence of MS and T1D. A Danish population-based cohort study assessed the risk of MS in individuals with T1D and the risk for T1D in first-degree relatives of MS patients. Data from the Danish Hospital Discharge Register, Danish MS Register and Danish Civil Registration System were used to identify patients with T1D (n=6,078), MS (n=11,862) and first-degree relatives of MS patients (n=14,771) respectively. Patients with T1D and first-degree relatives of MS patients
were followed up for the occurrence of MS and T1D respectively. This work found that the expected incidence rate for MS in patients with T1D was more than three-fold higher than what was expected based on available incidence rate data in Denmark (relative risk (RR): 3.26, 95% CI: 1.80-5.88). The observed incidence rate for T1D was also higher in first-degree relatives of MS patients (RR: 1.44, 95% CI: 1.11-1.88).

In summary, this study has demonstrated a higher risk of developing MS in people with T1D compared to the general population. Further to this, data on the risk of T1D in first-degree relatives of MS patients suggest an increased risk of T1D in people at higher risk of MS compared to the general population. Since there is only one study using incidence data, further studies are required to strengthen the evidence of this relationship.
### Table 3.1: Studies investigating the co-occurrence of MS and T1D

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<thead>
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<th>Author</th>
<th>Study information</th>
<th>Outcome measure of interest</th>
<th>Main findings</th>
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<td>Nielsen &amp; colleagues</td>
<td>Cohort study: 11 862 MS cases, 6078 T1D cases &amp; 1&lt;sup&gt;st&lt;/sup&gt;-degree relatives: 4771</td>
<td>1. RR of MS in T1D patients - Observed incidence rate for MS in patients with T1D compared to the expected incidence rate (based on available incidence rate data from Denmark)</td>
<td>1. RR for MS in T1D patients: 3.26 (95%CI: 1.80-5.88). 2. RR for T1D in 1&lt;sup&gt;st&lt;/sup&gt;-degree relatives of MS patients: 1.63 (95% CI: 1.26-2.12).</td>
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<td>Bechtold &amp; colleagues</td>
<td>Cohort study: 19 MS cases &amp; 56,653 T1D cases</td>
<td>1. RR of MS in T1D patients - Observed prevalence rate for MS in a paediatric &amp; adolescent T1D population compared to the expected prevalence rate (based on available incidence rate data from Denmark)</td>
<td>1. RR of MS: 3.35 (95% CI: 1.56 -7.21) to 4.79 (2.01-11.39) for an observed prevalence of 7 to 10 patients per 100,000</td>
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<td>Study</td>
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<td>Marrosu &amp; colleagues (2002)</td>
<td>Cohort study: 1090 MS cases, 2180 parents &amp; 3300 siblings</td>
<td>1. Prevalence of T1D in MS patients compared to healthy siblings of MS patients and the general population; 2. Adjusted odds ratio for risk of T1D in people with MS &amp; in their healthy siblings</td>
<td>1. Prevalence of T1D in MS patients (2.6%) was about 3-fold that in healthy siblings of MS patients (1.0%) (p=0.001) &amp; 5-fold that in the general population (0.4%) (p&lt;0.001). 2. MS patients with relatives with MS versus healthy siblings of MS patients without other relatives with the disease; OR: 6.03 (2.50–14.54). 3. Sibling with other relatives with MS versus sibling without other relatives with MS; OR: 3.41 (1.57-7.37).</td>
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<td>Dorman &amp; colleagues (2003)</td>
<td>Case-control: 143 T1D female cases, 186 sisters &amp; 160 controls</td>
<td>1. Prevalence of MS in women with T1D compared to the general population</td>
<td>1. 20-fold increase in the prevalence of MS in T1D women (2%) compared female general population (~0.1% on average, p=0.003 for difference). 2. 5-fold increase in prevalence of MS in non-diabetic sisters (0.5%) compared with the general population (non-significant).</td>
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<td>Wertman &amp; colleagues (1992)</td>
<td>Cross-sectional: 334 MS cases &amp; T1D in general population in 1950</td>
<td>1. Prevalence of T1D in people with MS under the age of 30 years compared to that of the general population</td>
<td>1. PR of T1D: 94.53; p&lt;0.001</td>
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<td>Hussein &amp; Reddy (2006)\textsuperscript{57}</td>
<td>Cross-sectional: 1,206 MS cases &amp; T1D in general population</td>
<td>1. Prevalence of T1D in people with MS compared to that of the general population</td>
<td>1. Prevalence of T1D: 0.92% (95% CI: 0.38–1.46); p = 0.15 for difference with general population</td>
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<td>2. 36% family history of diabetes among MS patients with T1D</td>
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RR: Relative risk; OR: Odds ratio; PR: Prevalence ratio; T1D: Type 1 diabetes; MS: Multiple sclerosis
3.3.3.2 Prevalence studies on the co-occurrence of MS and T1D

A number of epidemiological studies of MS and T1D co-occurrence have reported either similar or higher prevalence of T1D and MS compared to that in the general population or other comparison group. Table 3.1 summarises studies that have examined the prevalence of MS and T1D.

Bechtold and colleagues investigated the co-occurrence of T1D and MS by estimating the relative risk for MS in a paediatric and adolescent diabetes population. Data on 56,653 patients with T1D in the Diabetes Patienten Verlaufsdocumentation (DPV) database were collected in 248 centres in Germany and Austria and published data on German and Mid-European MS prevalence were used for comparison. The relative risk for MS in the diabetes population was 3.35 (95% CI: 1.56 to 7.21) to 4.79 (95% CI: 2.01 to 11.39) based on the observed prevalence compared to the expected prevalence of 7 to 10 per 100,000 population.

Similarly, a Sardinian cohort study by Marrosu and colleagues assessed the prevalence of T1D in 1,090 individuals with MS and their parents (n=2,180) and siblings (n=3,300) to ascertain the risk of T1D in this cohort of MS patients. The population prevalence of T1D in the province of Oristano was also used as a comparator. The prevalence of T1D in participants with MS was nearly three-fold greater than their healthy siblings (3.0 vs. 1.0%, p=0.001) and five-times greater than that of the general population (3.0 vs. 0.5%); however, there was no difference in the T1D prevalence between MS cases and their parents (2.0 vs. 3.0%, p=0.23). In multivariable analysis, the risk of T1D was six-fold higher in MS patients who had relatives with MS than in healthy siblings of MS patients without other relatives with MS (odds ratio (OR): 6.03, 95% CI: 2.50-14.54, p>0.001). The presence of other relatives with MS also conferred a more than three-fold increased risk of T1D to healthy siblings of individuals with MS (OR: 3.41, 95% CI: 1.57-7.37, p=0.002)
In the Familial Autoimmune and Diabetes Study by Dorman and colleagues\textsuperscript{55}, a significantly increased prevalence of MS in adults with T1D and their first-degree relatives was observed. Data on the clustering of autoimmune thyroid disease, rheumatoid arthritis and T1D were collected in a cohort of adult T1D subjects, as well as self-reported data on other autoimmune disorders. 94 non-diabetic control families were also recruited for comparison; however, no members of these families had any cases of MS. Compared with the average prevalence of MS in the female population of the USA (calculated by the authors as approximately 0.1%), the 2.0% prevalence of MS in this sample constituted a 20-fold increase in the prevalence of MS in the women with T1D (p=0.003). In parallel with this, the 0.5% prevalence of MS in the sisters of T1D women constituted a five-fold higher prevalence of MS compared with the general population, though this did not reach statistical significance. As no MS cases were observed in any male cases or male siblings, these could not be assessed.

In another study by Wertman and colleagues, the prevalence of T1D in 334 MS patients under the age of 30 years was found to be 94.5 times higher than that in the Israeli population of the same age (8.98/1000 vs. 0.095/1000, p<0.001).\textsuperscript{41} This finding of a nearly hundred-fold increase in T1D prevalence may be due to chance as a result of low numbers of T1D cases in the study population. In a larger study of 1,206 MS cases drawn from an MS database by Hussein and Reddy\textsuperscript{57}, the prevalence of T1D in MS patients (0.92% (95% CI: 0.38–1.46)) was similar to that in the general population (p= 0.15). They also reported that, 36% of the patients with the co-occurrence of MS and T1D have a positive family history of diabetes.

In summary, apart from one study which showed a similar prevalence of T1D in people with MS compared to the general population, the prevalence of T1D was higher in people with MS compared to the general population. Studies have also presented data indicating higher prevalence of MS in people with T1D compared to the general population. In addition, the prevalence of T1D in first-degree relatives of MS patients was higher compared to the
general population or the comparison group, and vice versa, the prevalence of MS in first-degree relatives of T1D patients was higher compared to the general population. The individual frequency and familial clustering of MS and T1D may be an indication that both genetic and environmental factors may modulate the risk for the co-occurrence of both diseases.

Furthermore, the occurrence or clustering of autoimmune conditions in MS patients and their families is not limited to MS and T1D. For instance, a recent meta-analysis demonstrated a consistent increase in the risk of autoimmune disorders, such as autoimmune thyroid disease, inflammatory bowel disease as well as T1D amongst people with MS and their first-degree relatives. Studies have also reported that the total combined risk of a number of autoimmune diseases was higher in people with MS and their first-degree relatives than controls.

The co-occurrence of additional autoimmune disease in MS patients suggests the existence of similar genetic, epidemiological and immunological features with a central role in general autoimmunity. Because of the phenomenon of common susceptibility to multiple autoimmune diseases in patients and families, individuals with T1D or MS and their relatives may not only be at increased risk of MS or T1D compared to the general population, but may also be at greater risk of other autoimmune diseases.

### 3.3.4 Studies investigating the effect of T1D on clinical disability in MS

To the best of our knowledge, there is currently no data on the effect of T1D on clinical disability in MS. There is, however, a study available which examined the combined effect of having T1D or T2D. In this study, diabetes was associated with a more rapid progression of ambulatory disability in a population of 8,983 MS patients enrolled in the North American Research Committee on MS (NARCOMS) Registry. While having any vascular condition, including diabetes, hypertension, heart disease, peripheral vascular disease and/or hypercholesterolemia, either at MS diagnosis or developing after MS diagnosis, were
strongly predictive of subsequent disability progression and an earlier occurrence thereof, evaluating these conditions individually found diabetes among the strongest conditions predictive of subsequent disability. Compared with MS patients who did not report diabetes, individuals with MS who developed diabetes at any point during their disease course had a 29% increased risk of early gait disability [EDSS: 4.0] (HR: 1.29; 95% CI: 1.13-1.48), 28% increased risk of requiring unilateral assistance [EDSS: 6.0] (HR: 1.28; 95% CI: 1.11-1.49) and a 56% increased risk of requiring bilateral assistance [EDSS: 6.5] (HR: 1.56; 95% CI: 1.30-1.88), persisting after adjustment for sex, year of symptom onset, age at symptom onset, income, health insurance status, race, and region of residence. It is unclear how the results extrapolate to T1D only given that the prevalence of T1D is roughly ten-fold lower than T2D.

In summary, there is no data specifically on the effect of T1D diabetes on clinical disability in people with MS, but for the combined effect of T1D and T2D there is evidence that this is associated with a worse progression in disability compared to MS patients without T1D or T2D diabetes. Associations were also found for other comorbidities such as dyslipidaemia, heart disease and hypertension. Research specifically on T1D is required to provide high quality evidence on its association with disability.

3.3.5 Shared aetiologic features of MS and T1D

3.3.5.1 Increasing incidence of MS and T1D

Accumulating epidemiological studies on MS and T1D suggest a trend of increasing incidence over the last few decades and the rapid rate of increase in children is of particular concern.\(^61,62\) In the case of MS, the incidence rate in Canada has increased from 5.2 to 22.3 per 100 000 between 1985–1989 and 1990–2004.\(^63\) In the Sassari province of Sardinia, incidence has increased from 1.1 to 5.8 between 1965–1969 and 1995–1999.\(^64\) Increasing incidence rates have also been reported in France\(^65\), Netherlands\(^66\), Australia,\(^67\) Japan,\(^68\) Finland,\(^69\) Norway\(^70,71\) and Italy.\(^72\) Interestingly, an increase in female-to-male ratio of MS
incidence has also been observed, with a systematic review and meta-analysis estimating a ratio increase from 1.4 in 1955 to 2.3 in 2000 in incident cases, when incidence studies were repeated after some years or decades in the same population.\textsuperscript{73}

A rapid increase in the incidence of T1D has also been reported in many countries. For instance, in Europe the pooled annual rate of increase in incidence was 3.4\% (95\% CI: 2.5–4.4\%).\textsuperscript{74} Among T1D patients in Taiwan, the biannual incidence of T1D increased from 2.63 per 100,000 in 1999–2000 to 3.22 per 100,000 in 2009–2010.\textsuperscript{75} A substantial increase in the incidence of T1D among children has been documented as well. For example, in Finland, the incidence of T1D among children diagnosed before the age of 15 years increased from 31.4 per 100,000 per year in 1980 to 64.2 per 100,000 per year in 2005.\textsuperscript{76} In the US state of Colorado, the incidence of T1D among youth (0 to 17 year-old) increased by 2.3\% (95\% CI: 1.6–3.1) per year from 1978 to 2004.\textsuperscript{77} In France, the incidence rate increased from 8.86 per 100,000 per year (95\% CI: 6.27-11.45) in 1988 to 13.47 per 100,000 per year (95\% CI: 10.29-16.65) in 2004, indicating an annual increase in incidence of 3.34\% (95\% CI: 3.33-3.34) in children aged less than 15 years.\textsuperscript{78} In the DIAMOND project, 114 populations in 112 centres in 57 countries were analysed, and an average annual increase in incidence of 2.8\% (95\% CI: 2.4-3.2\%) was calculated among children under 14 years old from 103 centres.\textsuperscript{62} There seem to be no clear sex ratio in the incidence of T1D as observed in MS. A study reported that overall sex ratio is almost equal in children diagnosed under the age of 15, while male preponderance was observed in populations with high incidence. Similarly, approximately 3:2 male: female ratio was reported in populations of European origin aged 15-40 years.\textsuperscript{79} A study in Sweden has reported that there was no sex difference in the incidence rate of T1D in children aged 0-14 years but male preponderance of about twofold was found in subjects aged 15-39 years.\textsuperscript{80}
The observed increase in incidence of MS and T1D cannot be restricted to consequences of changes in diagnostic methods, access to care and ascertainment and enhanced genetic susceptibility alone but may mostly be due to changes in lifestyle and environmental factors because of the marked increase over these few decades. For instance in Northern Japan, the accuracy of diagnostic procedures has not changed since 2000 and therefore the trend of increased incidence observed after this period strongly suggests a real increased risk of MS. In Sardinia, where the population is genetically homogeneous and stable, repeated surveys showed increasing disease incidence. The time scale of these changes tends to suggest changes in environmental factors.

In summary, evidence from available data, documents an increased incidence of MS and T1D over the last few decades. Understanding why these changes occurred could shed light on the causes of MS and T1D.

3.3.5.2 Genetic and immunologic features

The association of one autoimmune disease with another in the same patient is a well-known phenomenon. The pathophysiological mechanism behind the co-occurrence of MS and T1D is not yet clear but may involve both genetic and environmental causes. The observed familial aggregation and higher susceptibility to both diseases in first-degree relatives than the general population supports a genetic predisposition to both diseases. Due to what seems to be mutually exclusive, predisposing HLA haplotypes in patients with T1D and MS, the co-occurrence of MS and T1D has been considered unlikely, given that the HLA haplotype DRB1*1501-DQA1*0102-B1*0602 bestows susceptibility for MS while protecting against T1D. However, with the rapid identification of novel susceptibility loci in both diseases in recent years, it is likely that more overlap will be identified. Indeed, a review from 2009 showed that out of seven single nucleotide polymorphisms (SNPs) shown to be unequivocally associated with T1D, two – rs12708716 from the CLEC16A gene (OR=1.18
(95% CI: 1.08–1.29, p=1.6×10^{-16}) and rs763361 from the CD226 gene (OR= 1.11 (95% CI: 1.02–1.21, p=5.4×10^{-8}) – also demonstrated evidence for associations with MS. There are other non-HLA genes associated with the risk of MS and T1D which include IL2RA and IL7RA. For instance interleukin-2 receptor α gene (IL2RA; SNP: rs2104286) and interleukin-7 receptor α gene (IL7RA; SNP: rs6897932) were associated with 33% (OR=1.33 (95% lower bound: 1.17, p<0.001)) and 15% (OR=1.15 (95% lower bound: 1.01, p=0.035)) increased risk of MS, respectively. Similarly, in T1D, IL2RA (SNP: rs3118470) has been associated with a 28% increased risk of T1D (OR=1.28 (95% CI: 1.10–1.47)).

MS and T1D are thought to share several similarities in immunological and epidemiological features which lend support to possible common mechanisms in the development of both diseases. Immunologically, both diseases are considered T-cell-mediated diseases characterised by auto-antigen-specific T-helper (Th)1 cell responses, decreased T-cell suppressor activity and the presence of various autoantibodies, some which could operate in both diseases. In people with MS, serum pro-inflammatory cytokines such as TNF-α and IFN-γ which are elevated before clinical exacerbations of MS can activate cerebral endothelial cells (CECs) and alter their anatomic structure and function, leading to the disruption of the blood-brain barrier (BBB) as evidenced by a decrease expression of endothelial tight junction proteins of the CECs. Dysfunction of the CECs and permeability of the BBB causes adherence and trans-endothelial migration of T-lymphocytes and monocytes to the CNS, with destructive and often neurodegenerative consequences. T1D is associated with a shift in Th cell differentiation in favour of a pathogenic Th1 pathway and against an immunoregulatory Th2 subset of T-cells. This allows Th1 cytokines, including interleukin-2, interferon-γ and tumour necrosis factor β (TNF-β) -to induce a cascade of immune/inflammatory processes in the islet of Langerhans, culminating in the destruction of the beta-cells of the Islets of Langerhans. Th1 cytokines activate cytotoxic T-cells that...
interact specifically with beta-cells of the Islets of Langerhans and destroy them. They also activate macrophages to produce proinflammatory cytokines and oxygen and nitrogen free radicals that are highly toxic to the beta-cells of the Islets of Langerhans.\textsuperscript{94,95}

Additionally, a study by Winer and colleagues demonstrated that auto-reactive T-cells were capable of targeting both pancreatic Islet of Langerhans and CNS auto-antigens in individuals with MS and people with T1D, including relatives of patients with T1D.\textsuperscript{49} However, a polymorphism in the T-cell receptor did not influence the susceptibility to T1D and MS in a Sardinian population.\textsuperscript{96} IL-2 receptor polymorphisms have been proposed to reflect the existence of a heterogeneous association between T1D and MS, suggesting different immunopathological mechanisms of IL2RA in the two diseases.

In summary, shared genetic and immunological predisposition to both diseases may play a role in the co-occurrence of MS and T1D.

\textbf{3.3.5.3 Latitudinal gradient, ultraviolet radiation (UVR) and vitamin D}

In MS, a latitudinal gradient in MS prevalence and incidence has been reported in North America, Europe and Australasia.\textsuperscript{97} The persistence of a positive gradient in Europe after adjustment for \textit{HLA-DRB1} allele frequencies strongly supports a role for environmental factors which may vary with latitude.\textsuperscript{97} Similarly a positive association of T1D prevalence has been demonstrated, with increasing southern latitude of residence in Australia.\textsuperscript{98} In Europe, incidence of T1D has also been associated with increasing northern latitude.\textsuperscript{74} With respect to MS, persons migrating from lower to higher latitude after the age of puberty are thought to carry their former high risk with them, while those that migrate prior to puberty seem to have the risk associated with the new area to which they migrated.\textsuperscript{99} In the case of T1D, migration studies have reported that the incidence of T1D is similarly increased in population groups who have moved from a low-incidence to a high-incidence region.\textsuperscript{100}
One explanation proposed for the observed association of MS and T1D with latitude is that exposure to sunlight may be protective, either because of an effect of ultraviolet radiation (UVR) directly or via UVR-induced production of vitamin D.\textsuperscript{101} The protective effect of UVR-induced immunosuppression on MS and T1D is a plausible explanation of the gradient as ambient UVR levels decrease with increasing latitude.\textsuperscript{53} In line with this hypothesis, a number of studies have found an inverse relationship between sun exposure, ultraviolet radiation exposure, or serum levels of vitamin D and the risk or prevalence of MS and T1D.\textsuperscript{98, 102-105} For instance, Staples and colleagues\textsuperscript{98} reported that the latitudinal gradient for T1D prevalence in adults reflects a strong negative correlation between ambient UVR and the prevalence of T1D. Similarly, in a study of 51 regions around the world, Mohr and colleagues\textsuperscript{106} found that areas with lower levels of UVR had a higher incidence of T1D. In MS, higher sun exposure during childhood and adolescence was associated with a decreased risk of MS\textsuperscript{102, 107} and regional UVB radiation was predictive of corresponding MS prevalence rates, supporting the hypothesis that sunlight exposure influences MS risk.\textsuperscript{103, 108} Lower UV exposure therefore may predict a higher autoimmune disease susceptibility explaining the co-occurrence of several autoimmune diseases in one person.\textsuperscript{53, 109}

In MS, studies have also shown that low vitamin D, which is predominantly UVR derived, is prospectively associated with the risk and clinical course of MS.\textsuperscript{104, 110, 111} Consistent with this evidence, a prospective cohort study using the Nurses' Health Study (NHS; 92,253 women followed from 1980 to 2000) and Nurses' Health Study II (NHS II; 95,310 women followed from 1991 to 2001) reported that the risk of developing MS was significantly reduced for women taking ≥400 international units/day of vitamin D.\textsuperscript{112} Randomised clinical trials on the efficacy of vitamin D supplementation in MS patients were conflicting, however.\textsuperscript{113-115} In T1D, a retrospective birth-cohort study was conducted by Hypponen and colleagues in Oulu and Lapland, northern Finland which followed 10,821 children up at age
one year and assessed the prevention of T1D by vitamin D supplementation during the first one year of life. Data was retrospectively collected on frequency and dose of vitamin D during the first year follow-up period. This study showed a large decrease in the risk of T1D later in life among the children who regularly received vitamin D supplementation compared to those who did not (RR = 0.12, 95% CI 0.03–0.51). In confirmation, a recent meta-analysis by Zipitis and Akobeng reported that T1D risk was significantly reduced in infants who received supplemental vitamin D as compared with those who did not (pooled odds ratio: 0.71, (95% CI: 0.60–0.84). Since there were no randomised controlled trials, studies included in the meta-analysis were observational in design.

In summary, there is some evidence to suggest a possible influence of latitudinal gradient, UVR and vitamin D on the risk of MS and T1D. Randomised controlled trials on the efficacy of vitamin D to modulate the risk and progression of MS are inconsistent, however. Unlike MS, there were no randomised controlled trials found for the effect of vitamin D on the risk of developing T1D to strengthen the current available evidence. That said, the observational studies that have been done, particularly the birth cohort study by Hypponen and colleagues, are of high methodological rigour and, while not as robust as randomised controlled trials, are potentially supportive of some treatment impact on T1D risk. For this reason, current data suggesting that supplementation with vitamin D in early life might be important in conferring protection against the risk of developing T1D later in life must be interpreted with caution. Even though vitamin D supplementation appears to be a promising treatment worthy of further exploration in both diseases, the paucity of randomised controlled trials and use of different doses in studies is as yet incomplete evidence to recommend supplementation in the prevention of either condition.

Among the other known risk factors for MS, smoking has been associated with the risk of MS and known to increase the progression in disability. In T1D smoking has not been
directly associated with the risk of the disease but with increased risk for diabetic nephropathy, retinopathy, and neuropathy. Other risk factors that have been associated with MS and T1D are viral infection and exposure to cow-milk protein. Evidence for a link between viral infection and the autoimmune process has been repeatedly shown in T1D and MS. The risk of MS has been consistently associated with Epstein Barr virus infection while T1D has been associated with different viruses including enteroviruses, rubella and rotaviruses. Exposure to cow-milk protein, for example, have been tentatively associated with increased risk of development of MS and T1D.

### 3.3.6 Clinical implication of T1D in people with MS

From the studies examined above, T1D was both a consistent risk factor and a significant comorbidity in MS and their first-degree relatives. Consistent with this background, people with T1D have an increased risk of developing MS. Evidence on whether the co-occurrence of the two autoimmune conditions will affect the disease course and progression of disability in MS is absent. However, available data shows that individuals who developed T1D or T2D diabetes or other cardiovascular risk factors or disease at any point during MS disease course may have more rapid progression in ambulatory disability.

The consistent association between MS and T1D calls for a critical assessment of the clinical impact of T1D in patients with MS. The impact of T1D on the risk of MS and possibly disability progression can be hypothesised to be occurring by both direct and indirect mechanisms. Considered an autoimmune disease, T1D may directly worsen the autoimmune dysfunction in MS. Up to 50% of people with T1D are eventually affected by diabetic neuropathy, which is a major cause of morbidity and increased mortality and reduced quality of life. In diabetic neuropathy, damage to the peripheral nervous system is characterised by symptoms such as paraesthesia, sensory loss, neuropathic pain, numbness, tingling, muscle weakness and significant autonomic dysfunction which may mimic and exacerbate MS
symptoms. In T1D, chronic hyperglycaemia and the consequent occurrence of cerebrovascular complications, as well as recurrent episodes of severe hypoglycaemia have been associated with accelerated CNS white matter ischaemic disease leading to cognitive dysfunction, impairment of coordination and other neurological dysfunction that may significantly add to the disease burden of MS.126-128

3.3.7 Conclusions
We have reviewed current data on the concurrence of MS and T1D that has shown that the co-occurrence of the two diseases is not uncommon. The shared similarities in their aetiologic features, including low sun exposure/UVR or vitamin D deficiency, may partly explain the co-occurrence of the two conditions in genetically susceptible individuals. The mechanism by which T1D may impact the clinical course of MS in largely undetermined, as studies in this area are lacking. We have therefore hypothesised that the presence of T1D in patients with MS may potentially increase the rate of disability progression in MS and could worsen cognitive impairment observed in people with MS. Further studies on the co-occurrence of the two diseases are desirable to provide the needed evidence on how T1D might influence progression in clinical disability or determine the clinical course of MS.

3.4 Postscript
This chapter has provided some key information on the frequency of autoimmune comorbidities and contribution of IFN-β to this frequency and their influence on disease progression in MS. The next chapter investigates the role of serum lipids on the disability and progression in disability in people with MS.

3.5 References


These articles have been removed for copyright or proprietary reasons.

Chapter 4 : An adverse lipid profile is associated with disability and progression in disability in people with MS

4.1 Preface
The manuscript presented in this chapter has been published. The typeset version of the manuscript as it appeared in the journal is in Appendix 4A. The text of this chapter is the same as the published version. This investigation describes the relationship between an adverse lipid profile and disability and progression in disability in people with MS.

4.2 Abstract
Background: There is accumulating data suggesting an association between serum lipids, apolipoproteins and disability in MS.

Objective: To investigate the associations between serum lipids, apolipoproteins and disability in MS.

Methods: A cohort of 178 participants with clinically definite MS in southern Tasmania were prospectively followed from 2002-2005 and serum samples were obtained at study entry and at each biannual review and to measure lipid profile and apolipoprotein levels. Associations with disability and annual change in disability were evaluated using linear regression and multilevel mixed-effects linear regression.

Results: In the unadjusted analyses, nearly all lipid-related variables were positively associated with Expanded Disability Status Scale (EDSS). After adjustment for confounders, total cholesterol (TC) (p=0.037), apolipoprotein B (ApoB) (p=0.003), and apolipoprotein B to apolipoprotein A-I ratio (ApoB/ApoA-I ratio) (p=0.018) were independently associated with a higher EDSS. Higher BMI was also independently associated with higher EDSS (p=0.013). With the progression analysis, total cholesterol to HDL ratio (TC/HDL ratio) (p=0.029) was prospectively associated with subsequent change in EDSS.
Conclusion: In this prospective population-based cohort study, an adverse lipid profile was associated with high MS disability and disease progression. Improving serum lipids may be beneficial for MS patients to potentially improve clinical outcomes and vascular comorbidities.

4.3 Introduction
Multiple sclerosis (MS) is an inflammatory, demyelinating condition of the central nervous system (CNS). It has a highly variable inter- and intra-personal clinical course, suggesting multiple contributory factors but there is still little data on factors that modify the disease course.

Lipids play important roles in the CNS and transport through the blood-brain barrier (BBB) has been demonstrated. Lipids are involved in the regulation of neural functions, cell signalling, and in tissue structure and apolipoproteins are key players in the metabolism, transport and delivery of lipids. Oxidative stress and consequent lipid peroxidation may play a role in the inflammatory processes and pathogenesis of MS. Oxidative modifications of low density lipoprotein (LDL), the major carrier of plasma cholesterol, have been established in the parenchyma of MS plaques. Lipid peroxidation and oxidised LDL uptake by activated microglia and infiltrating macrophages in the early stages of MS plaque development, are thought to play crucial roles in demyelination. HDL on the other hand may have protective effects due to its antioxidant properties and role in reverse cholesterol transport.

There is little epidemiological evidence on lipids and MS. High rates of obesity and overweight have been reported in MS, and vascular comorbidities seem to be more common in MS than the general population. A high body mass index (BMI) during adolescence has been associated with an increased MS risk. Strong associations were found between total cholesterol (TC), LDL and the number of gadolinium-enhancing lesions
on magnetic resonance imaging (MRI). Lipoproteins and apolipoproteins were associated with new MRI lesions and grey matter atrophy in clinically isolated syndrome and high cholesterol level was associated with low retinal nerve fibre layer thickness in MS patients with optic neuritis. A prospective study found that higher baseline total cholesterol (TC), LDL, triglycerides and lower high density lipoprotein (HDL) were associated with subsequent worsening clinical disability, but this study did not take confounding factors into account.

In this prospective clinical cohort of people with MS with repeated lipid measures, we examined the association between lipid-related measures (BMI, serum lipid and apolipoprotein levels) and disability and progression in disability.

4.4 Methods

4.4.1 Study design
The Southern Tasmanian Multiple Sclerosis Longitudinal (MSL) Study is a prospective population-based study, which followed a cohort of 203 persons with clinically definite MS living in southern Tasmania, Australia from 2002–2005. An estimated 78% (203/259) of eligible cases were included. The study retention rate was 90% (183/203), with 4% (8/203) withdrawing early and 6% (12/203) lost because they moved interstate or died. One person only participated in a pilot before Wave 1, and four participants were excluded because they did not meet the criteria for definite MS after neurological review at the end of the study (with all available data). For this analysis, the sample was limited to those with BMI data (n=178), excluding 20 people whose weight and height could not be assessed because of their high disability.

The study methodology has been previously described. At each biannual review participants were asked about their lifestyle, including physical activity, smoking, vitamin D supplement use and dosage, statin and other medication use. Height and weight was measured at baseline.
The Expanded Disability Status Scale (EDSS) was assessed each winter by a single physician. The Multiple Sclerosis Severity Score (MSSS) was calculated from the EDSS and disease duration by comparing it to the global MSSS reference dataset. Ethics approval was obtained from the Southern Tasmania Human Research Ethics Committee and all participants provided informed consent.

4.4.2 Biological samples and measurements
Non-fasting serum samples were collected at study entry and at each biannual review and stored at −80°C until use. Traditionally triglycerides were measured in a fasting state; however there has been a shift to non-fasting samples as post-prandial non-fasting values are more representative of the usual metabolic state. Total cholesterol and triglycerides were measured using enzymatic colorimetry (Wako Chemicals USA, Inc., Richmond, VA). HDL-cholesterol levels were measured using precipitation and enzymatic assay (Wako Chemicals USA, Inc., Richmond, VA). LDL-cholesterol was estimated using Friedewald equation except when triglyceride levels were above 5.1mmol/L (n=9), then it was measured by direct assay (Wako). Non-HDL-cholesterol levels were computed by subtracting HDL-cholesterol from total cholesterol.

The apolipoproteins, ApoA-I and ApoB were measured as they are the main proteins components of HDL, and LDL/VLDL, respectively. Lipoprotein(a) (Lp(a)) is the complex of LDL-cholesterol and apolipoprotein (a). ApoB, ApoE and ApoA-1 levels were measured by turbidimetric immunoassay using goat anti-human ApoB or ApoA-I (Wako Chemicals USA, Inc., Richmond, VA) and Lp(a) measured by a sandwich DELFIA (LKB-Pharmacia). Serum levels of highly sensitive C-reactive protein (hs-CRP) were measured with an hs-CRP ELISA Kit (Alpha Diagnostic Int., San Antonio, Texas, USA) according to the manufacturer’s instructions (detection limit 0.35 ng/ml). Samples were diluted 1:100 and studied in duplets and re-measurement with a dilution of 1:200 was performed for samples out of range.
Serum 25-OH-D levels were measured with a commercially available radioimmunoassay (DiaSorin, Stillwater, MN). Inter-batch reproducibility was 4.6% at 32nmol/l and 6.4% at 125nmol/l.

4.4.3 Statistical Analysis
Total cholesterol to HDL ratio (TC/HDL ratio), LDL to HDL ratio (LDL/HDL ratio), ApoB to ApoA-I ratio (ApoB/ApoA-I ratio) were calculated as they are validated predictors of cardiovascular disease risk. We used established cut-points of the American Heart Association for high and normal lipid levels.

Associations with lipid-related variables and disability as outcome variables were assessed by linear regression (cross-sectional analyses). Associations with annual change in disability were assessed by multilevel mixed-effects linear regression to account for intra-individual course over time (prospective analyses).

Associations with disability were adjusted for relevant confounders including relapse at the time of disability assessment (no, yes), age at study entry, sex, smoking (no, yes) and statin use (no, yes), BMI (kg/m²), physical activity (Mets) and 25(OH)D (nmol/L). Associations with annual change in EDSS were also adjusted for a categorical term for baseline EDSS (0-3, 3.5-5.5, 6-7, ≥7.5), because the progression of disability depends on baseline disability, and for change in relapse at the time of disability assessment. Transformation was applied as required to satisfy homoscedasticity; however, all coefficients are reported on the scale of the original disability measure. All analyses were done using STATA/IC for Windows (Version 12.1; StataCorp LP College Station USA).

Our standard analysis evaluated the prospective associations of the lipid-related variables at a winter review with the subsequent annual change in EDSS from that winter to the next winter as the outcome variable. In order to evaluate causality, we used a time lag analysis, where we
shifted the lipid-related variables six and twelve months before or after the outcome variable. If the observed associations were due to reverse causality one would expect the magnitude of effects to become stronger when associations are modelled 6 or 12 months after the outcome variable.

4.5 Results

4.5.1 Participant characteristics
Table 4.1 shows the characteristics of the cohort at study entry. The cohort was followed for an average of 2.2 (SD 0.5) years, 62.9% were overweight or obese (≥25 kg/m²), and only 5.6% (n=11) were treated with statins during the study. Using the established lipid cut-points, 51% of the participants had TC above 5.2 mmol/L, 24% had HDL below 1 mmol/L, 67% had LDL above 2.6 mmol/L and 39% had triglyceride above 1.7 mmol/L.

4.5.2 Determinants of serum lipids and apolipoproteins
Supplementary Table 4.1 shows the correlation between the lipid variables and supplementary Text shows details on the determinants of serum lipids and apolipoproteins. In summary, strong correlations were observed between a number of lipid measures, for example between TC, LDL, nonHDL and ApoB (r>0.87). A higher age was associated with many of the lipid and apolipoprotein measures. Importantly, BMI was a significant independent predictor of lipid and apolipoprotein levels, taking into account factors such as age, sex, smoking, physical activity and statin use, and some associations were also seen for physical activity and smoking.
Table 4.1: Demographic and clinical characteristics of the MS cohort at study entry

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>178/178 (100)</td>
</tr>
<tr>
<td><strong>Female sex</strong></td>
<td>128/178 (72)</td>
</tr>
<tr>
<td><strong>MS Course at study entry</strong></td>
<td></td>
</tr>
<tr>
<td>Relapsing-Remitting MS</td>
<td>149/178 (83)</td>
</tr>
<tr>
<td>Secondary Progressive MS</td>
<td>20/178 (11)</td>
</tr>
<tr>
<td>Primary Progressive MS</td>
<td>9/178 (5)</td>
</tr>
<tr>
<td><strong>Used Immunomodulatory therapy during study?</strong></td>
<td></td>
</tr>
<tr>
<td>Used Immunomodulatory therapy during study?</td>
<td>132/178 (74)</td>
</tr>
<tr>
<td><strong>Smoker during study</strong></td>
<td>48/178 (27)</td>
</tr>
<tr>
<td><strong>Body Mass Index (Kg/m^2)</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>66/178 (37.1)</td>
</tr>
<tr>
<td>Overweight</td>
<td>74/178 (41.6)</td>
</tr>
<tr>
<td>Obese</td>
<td>38/178 (21.3)</td>
</tr>
<tr>
<td><strong>Mean (SD; Range)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>47.4 (11.4; 21-77)</td>
</tr>
</tbody>
</table>

| **MS duration from diagnosis (years)**         | 6.0 (2.0,12.0)  |
| **EDSS at study entry**                        | 3.5 (2.0, 5.0)  |
| **MSSS at study entry**                        | 4.0 (2.3, 6.2)  |
| **Physical activity (Met)**                    | 17.6 (3.3, 40.0)  |
| **Body Mass Index (Kg/m^2)**                   | 26.31 (23.4, 28.8)  |
| **TC (mmol/L)**                                | 5.2 (4.5, 6.1)  |
| **LDL (mmol/L)**                               | 3.4 (2.3, 3.6)  |
| **ApoB (g/L)**                                 | 0.97 (0.82, 1.2)  |
| **nonHDL (mmol/L)**                            | 3.7 (3.0, 4.6)  |
| **Trig (mmol/L)**                              | 1.5 (1.1, 2.2)  |
| **HDL (mmol/L)**                               | 1.4 (1.1, 1.7)  |
| **ApoA-I (g/L)**                               | 1.6 (1.3, 1.8)  |
| **ApoE (mg/L)**                                | 52.0 (41.0, 64.0)  |
| **Lp(a) (µmol/L)**                             | 0.5 (0.2, 1.4)  |
| **hs-CRP (mg/L)**                              | 16.5 (8.0, 36.0)  |

TC: Total cholesterol; LDL: Low density lipoprotein; ApoB: Apoprotein B; NonHDL: Non high density lipoprotein; Trig: Triglycerides; HDL: High density lipoprotein; ApoA-I: Apoprotein A-I; ApoE: Apoprotein E; Lp(a): Lipoprotein a; Hs-CRP: High sensitive C-reactive protein
4.5.3 Association between lipid-related variables and clinical disability

In the basic analyses, nearly all lipid-related variables, including BMI, were associated with clinical disability as measured by EDSS (Table 4.2) and MSSS (data not shown), such that higher total cholesterol, LDL, and triglycerides, as well as ApoE, ApoB, and Lp(a) were associated with higher disability. Age and sex explained part of the associations, as adjustment for age and sex attenuated the associations; additional adjustment for smoking and statin use did not affect the associations. Neither HDL nor its associated apolipoprotein Apo-A-I were associated with disability in any analysis.

In clinical terms, adjusted for age and sex, those who had a 2 mmol/L higher TC, LDL and nonHDL, had on average a 0.61 (p=0.006), 0.54 (p=0.037), and 0.59 (p=0.003) higher current EDSS level, respectively. From the regression lines (adjusted for age and sex), we estimated that those at the cut-point of high TC, HDL, and LDL had a mean EDSS of 3.9, 4.2 and 4.5, respectively. In relation to BMI, those who had a 5 kg/m$^2$ higher BMI score had on average a 0.38 higher EDSS level.

The cross-sectional associations above could reflect reverse causality, where increased disability acts via an increased BMI and reduced physical activity to yield an adverse lipid profile. To account for reverse causality, we further adjusted for BMI and self-reported physical activity in the period prior to lipid measurement. In general, adjustment for BMI and physical activity reduced the magnitude of the coefficients (Table 4.2), with most of it driven by BMI rather than physical activity, even though there was some association between physical activity and EDSS (basic model (-0.012(-0.018, -0.005) p<0.001); adjusted model 2 (-0.005(-0.010, 0.0003) p=0.06). Importantly, total cholesterol, ApoB, ApoB/ApoA-I ratio remained significantly associated, even after taking BMI, physical activity and other confounders into account. In addition, there was an independent effect of BMI on disability. Adjustment for comorbid hypertension status did not affect the magnitude of the associations.
Similar associations were observed with MSSS. In the unadjusted analyses, higher TC, LDL, nonHDL and LDL/HDL ratio were significantly associated with higher MSSS. Again, adjusting for variables such as age, sex, BMI and physical activity explained part of the association, but independent associations were observed for TC (0.51 (0.18, 0.85) p=0.003), LDL (0.68 (0.29, 1.08) p=0.001), nonHDL (0.48 (0.16, 0.79) p=0.003), LDL/HDL ratio (0.43
(0.07, 0.79) P=0.019), ApoB (2.40 (0.85, 3.95) p=0.003) and ApoB/ApoA-I ratio (2.26 (0.40, 4.13) p=0.018). Again, no associations were seen with HDL (p=0.12) and ApoA-I (p=0.12). For BMI, those who had a 5 kg/m² higher BMI score had on average a 0.63 higher MSSS level independent of other confounding factors.

4.5.4 Associations between lipid-related variables and change in clinical disability

We next sought to evaluate the relationship between lipid levels and subsequent change in disability. The prospective model (Table 4.3) is the preferred model where the lipid values are measured at the beginning of the interval of measuring change in disability. Only TC/HDL ratio was significantly associated with annual change in EDSS and this association remained in the fully adjusted model. Examining different lags for this association (Supplementary Table 4.2) did not indicate that the effect was due to reverse causality, as the association was weaker when TC/HDL ratio was measured in the middle, end or after the interval over which change in EDSS was measured.

Stronger associations were observed in the basic model when lipids were measured in the middle of the outcome interval (defined as cross-sectional model), but only HDL was significant in the fully adjusted model. From the findings on HDL using other lags for HDL, we could not determine whether or not this association was due to reverse causality.

BMI (at baseline) was not associated with change in disability (basic model, coefficient +0.003 (-0.011, 0.02) p=0.64; adjusted model 1, coefficient +0.005 (-0.01, 0.02) p=0.53; adjusted model 2, coefficient -0.002 (-0.02, 0.01) p=0.82). No associations were observed for any of the models for triglycerides, Lp(a), Hs-CRP and ApoE, nor for physical activity (data not shown).
Table 4.3: Prospective association between lipid-related variables and annual change in EDSS

<table>
<thead>
<tr>
<th>Basic Model</th>
<th>TC</th>
<th>LDL</th>
<th>ApoB</th>
<th>nonHDL</th>
<th>HDL</th>
<th>ApoA-I</th>
<th>TC/HDL</th>
<th>LDL/HDL</th>
<th>ApoB/ApoA-I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.02 (-0.05, 0.09)</td>
<td>0.04 (-0.04, 0.13)</td>
<td>0.22 (-0.09, 0.52)</td>
<td>0.04 (-0.02, 0.11)</td>
<td>-0.11 (-0.28, 0.06)</td>
<td>-0.05 (-0.24, 0.14)</td>
<td><strong>0.05 (0.00, 0.09)</strong></td>
<td>+0.06 (-0.03, 0.15)</td>
<td>(-0.12, 0.63)</td>
</tr>
<tr>
<td>Basic Model</td>
<td>p=0.50</td>
<td>p=0.32</td>
<td>p=0.16</td>
<td>p=0.20</td>
<td>p=0.19</td>
<td>p=0.62</td>
<td>p=0.045</td>
<td>p=0.17</td>
<td>p=0.18</td>
</tr>
<tr>
<td>Adjusted model</td>
<td>0.04 (-0.05, 0.10)</td>
<td>0.05 (-0.04, 0.13)</td>
<td>0.21 (-0.10, 0.51)</td>
<td>0.04 (-0.03, 0.11)</td>
<td>-0.10 (-0.27, 0.08)</td>
<td>-0.01 (-0.21, 0.19)</td>
<td><strong>0.003 (0.09)</strong></td>
<td>-0.005 (-0.03, 0.14)</td>
<td>(-0.13, 0.61)</td>
</tr>
<tr>
<td>1</td>
<td>p=0.47</td>
<td>p=0.29</td>
<td>p=0.18</td>
<td>p=0.23</td>
<td>p=0.28</td>
<td>p=0.91</td>
<td>p=0.07</td>
<td>p=0.23</td>
<td>p=0.21</td>
</tr>
<tr>
<td>Adjusted model</td>
<td>0.04 (-0.04, 0.11)</td>
<td>0.05 (-0.03, 0.14)</td>
<td>0.26 (-0.05, 0.57)</td>
<td>0.05 (-0.02, 0.11)</td>
<td>-0.10 (-0.28, 0.08)</td>
<td>-0.005 (-0.21, 0.20)</td>
<td><strong>0.01 (0.10)</strong></td>
<td>-0.06 (-0.03, 0.16)</td>
<td>(-0.11, 0.66)</td>
</tr>
<tr>
<td>2</td>
<td>p=0.33</td>
<td>p=0.22</td>
<td>p=0.10</td>
<td>p=0.14</td>
<td>p=0.29</td>
<td>p=0.96</td>
<td>p=0.029</td>
<td>p=0.16</td>
<td>p=0.16</td>
</tr>
</tbody>
</table>
4.6 Discussion
Using a prospective cohort design in people with MS, we have found that higher TC, ApoB and ApoB/ApoA-I ratio were associated with a higher disability and that a higher TC/HDL ratio was associated with a faster accrual of disability, suggesting that lipid lowering interventions may be of benefit for people with MS.

We observed that those with an adverse lipid profile (elevated TC, LDL, nonHDL, triglyceride, ApoB, ApoB/ApoA-I ratio) had higher levels of clinical disability. Part of the effect was explained by age and sex. However as we examined these associations cross-sectionally, this raises the question of reverse causality, i.e. whether an increased BMI and adverse lipid profile results in higher disability, or whether increased disability results in a higher BMI and more adverse lipid profile. We know that a higher BMI results in a more adverse lipid profile. Indeed, adjustment for BMI and physical activity reduced the magnitude of the associations, but independent associations were still observed for TC, ApoB and ApoB/ApoA-I ratio. In addition, we observed an effect of BMI on disability that was independent of the effect of lipids. Our findings agree with studies that have found associations between LDL, TC, TC/HDL ratio, BMI and disability.

We also examined whether lipid-related measures influenced clinical course by examining the association with annual change in disability. It has previously been reported that higher baseline LDL, TC and triglycerides were associated with a worsening in EDSS and MSSS over 2.2 years, but this study did not adjust for potential confounders apart from age and sex. We found in our prospective analysis that the TC/HDL ratio was associated with a higher annual change in EDSS. Examining different lags for this association did not indicate that the effect was due to reverse causality. Our findings are in line with a recent placebo-controlled trial in people with MS, which found that those using lipid lowering statins had a
lower change in EDSS after two years. Baseline BMI was not associated with change in disability.

Although comparable to that of the general population, our MS population had on average high total cholesterol (5.36 mmol/L (SD 1.14)) and LDL levels (3.12 mmol/L (SD 0.97)). The level of dyslipidaemia (TC>5.5 mmol/L) was also high with 35% of participants classified as being dyslipidemic. In analyses adjusted for age and sex, for each 2 mmol/L increase in TC, LDL and nonHDL, EDSS was 0.6 higher, and for each 5 kg/m² higher BMI, EDSS was 0.4 higher. Those with a lipid profile reaching the established cut-points for high lipid levels had on average an EDSS score of 3.6 to 4.8, depending on the lipid type measured. Considering that a 0.03 mmol/L increase in LDL cholesterol confers a >1% increase in cardiovascular disease risk and that vascular comorbidities are common in MS, these levels will be associated with a significantly increased cardiovascular disease burden. Clinicians should therefore monitor lipid levels in people with MS and treat adverse levels as early as possible.

We found stronger associations for ApoB than for LDL in relation to EDSS and change in EDSS, which is in line with data demonstrating that ApoB is a better measure of the relative number of circulating LDL particles and a better indicator of heart disease risk than total cholesterol or LDL. 7-dehydrocholesterol is required as a substrate for the endogenous production of 25(OH)D in the skin, and previously, significant positive associations have been found between 25(OH)D and HDL and negative associations with triglycerides. We observed an independent association between higher 25(OH)D and higher HDL, ApoA-I, ApoE, lower LDL/HDL ratio and lower TC/HDL ratio.
Our study has significant strengths, including being a prospective population-based cohort study with the capability to adjust for relevant confounders and examine mediation pathways. The study had some limitations. A two year change in clinical disability measured is limited, and a longer follow-up is preferable when measuring change in disability. We examined reverse causality, but this cannot be fully ruled out in observational studies. We used non-fasting serum samples, which may have influenced the levels of triglycerides, and this could have contributed to observing non-significant associations with triglycerides. Some associations have been observed with MRI markers, but we could not examine that in this study.

Clinicians should be aware of the associations between lipids, BMI and disability in MS and monitor and treat adverse lipid profiles as early as possible, preferably in a clinical trial setting. Our findings that adverse lipid levels were associated with disability as well as disability progression, suggests that reducing lipids, decreasing BMI into the healthy range and increasing physical activity may significantly reduce disability accumulation. Early interventions in the disease course are likely to be more successful, and our findings provide support for studies of lipid reducing interventions in early MS before sustained disability should occurred.

4.7 Postscript
This chapter has provided some evidence on the relationship between an adverse lipid profile and disability and progression in disability in people with MS. The next chapter investigates the association between serum lipids and the hazard of relapse in people with MS.

4.8 Reference


24. American Heart Association. What your cholesterol levels mean


### Supplementary table 4.1: Correlation of serum lipid-related variables

<table>
<thead>
<tr>
<th></th>
<th>TC</th>
<th>HDL</th>
<th>LDL</th>
<th>Triglycerides</th>
<th>nonHDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p=0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>0.91</td>
<td>-0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p=0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trig</td>
<td>0.43</td>
<td>-0.44</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nonHDL</td>
<td>0.92</td>
<td>-0.32</td>
<td>0.94</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p=0.001</td>
<td>p=0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>ApoE</td>
<td>0.48</td>
<td>0.06</td>
<td>0.30</td>
<td>0.52</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p=0.09</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.87</td>
<td>-0.32</td>
<td>0.88</td>
<td>0.56</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p=0.001</td>
<td>p&gt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>0.18</td>
<td>0.86</td>
<td>-0.10</td>
<td>-0.20</td>
<td>-0.16</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p=0.002</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Lipo(a)</td>
<td>0.23</td>
<td>0.003</td>
<td>0.23</td>
<td>0.09</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p=0.93</td>
<td>p&lt;0.001</td>
<td>p=0.007</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>0.06</td>
<td>-0.10</td>
<td>0.05</td>
<td>0.11</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>p=0.07</td>
<td>p=0.003</td>
<td>p=0.19</td>
<td>p&lt;0.001</td>
<td>p=0.004</td>
</tr>
</tbody>
</table>

TC: Total cholesterol; LDL: Low density lipoprotein; ApoB: Apoprotein B; NonHDL: Non high density lipoprotein; Trig: Triglycerides; HDL: High density lipoprotein; ApoA-I: Apoprotein A-I; ApoE: Apoprotein E; Lipo(a): Lipoprotein a; Hs-CRP: High sensitive C-reactive protein
4.9 Supplementary Text: Determinants of serum lipids and apolipoproteins

Correlation between lipid-related variables – Supplementary Table 4.1 shows the correlations between the serum lipids and apolipoproteins. Strong correlations were observed between TC, LDL, nonHDL and ApoB ($r \geq 0.87$). ApoA-I was only strongly correlated with HDL ($r=0.86$). Triglycerides were moderately correlated with TC, ApoB and ApoE ($0.43 \leq r \leq 0.56$) and inversely with HDL ($r=-0.44$). Correlations with Lp(a) and Hs-CRP were all lower ($r \leq 0.25$).

Age and sex – Age at study entry was strongly associated with higher TC ($p=0.001$), HDL ($p=0.023$), LDL ($p=0.013$), nonHDL ($p=0.015$), ApoE ($p=0.038$), ApoB ($p=0.008$), ApoA-I ($p=0.002$) and Lp(a) ($p=0.003$). Females have significantly higher HDL ($p<0.001$), ApoA-I ($p<0.001$), lower LDL/HDL ratio ($p<0.001$), TC/HDL ratio ($p<0.001$), and ApoB/ApoA-I ratio ($p<0.001$) than males. The other lipids and apolipoproteins variables did not show evidence of variation with age and sex.

BMI – BMI was a significant independent predictor of serum lipid levels and apolipoproteins. After adjustment for age, sex, smoking, physical activity and statin use, higher BMI was significantly associated with lower HDL level ($p<0.001$) and higher TC ($p=0.032$), LDL ($p=0.007$), nonHDL ($p<0.001$), triglycerides ($p=0.003$), TC/HDL ratio ($p<0.001$), LDL/HDL ratio ($p<0.001$). Likewise after adjustment for confounders, higher BMI was associated with lower ApoA-I ($p=0.002$), higher ApoB ($p<0.001$), hs-CRP ($p<0.001$) and ApoB/ApoA-I ratio ($p<0.001$).

25(OH)D – Significant associations were observed between 25(OH)D and serum lipids and apolipoproteins. After adjustment for age at study entry and sex, higher 25(OH)D was associated with higher HDL ($p<0.001$), ApoA-I ($p=0.003$), ApoE ($p=0.16$), lower LDL ($p=0.034$), triglyceride ($p=0.017$), nonHDL ($p=0.012$), TC/HDL ratio ($p<0.001$), LDL/HDL
ratio (p<0.001), ApoB (p=0.027), ApoB/ApoA-I (p=0.002) and hs-CRP (p=0.017). After further adjustment for BMI, physical activity, smoking and statin use, each 10 nmol/L increase in 25(OH)D was associated with higher HDL level (coefficient 0.04 (0.01, 0.07), p=0.004), higher ApoA-I (coefficient 0.03, (0.004, 0.06) p=0.025), higher ApoE (coefficient 1.49, (0.03, 2.94) p=0.045), lower LDL/HDL ratio (coefficient -0.10, (-0.17, -0.03) p=0.004) and lower TC/HDL ratio (coefficient -0.12, (-0.21, -0.03) p=0.01).

Physical activity, smoking, statin use and dietary saturated fat – Physical activity was associated with lower triglycerides (p=0.004), nonHDL (p=0.005), TC/HDL ratio (p=0.012), LDL/HDL ratio (p=0.038), ApoB (p=0.004), and ApoB/ApoA-I ratio (p=0.030). Smoking was associated with lower ApoA-I (p=0.048) and statin use was associated with lower TC (p<0.001), LDL (p=0.001) and nonHDL (p=0.019). Dietary saturated fat was not a significant determinant of serum lipid or apolipoprotein levels.
### Supplementary table 4.2: Association between lipid-related variables and annual change in EDSS

#### Basic Model

<table>
<thead>
<tr>
<th></th>
<th>TC</th>
<th>LDL</th>
<th>ApoB</th>
<th>nonHDL</th>
<th>HDL</th>
<th>ApoA-I</th>
<th>TC/HDL</th>
<th>LDL/HDL</th>
<th>ApoB/ApoA-I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-interval</strong></td>
<td>0.01</td>
<td>-0.01</td>
<td>+0.26</td>
<td>+0.04</td>
<td>-0.21</td>
<td>-0.21</td>
<td>+0.06</td>
<td>+0.05</td>
<td>+0.46</td>
</tr>
<tr>
<td>(Summer values)</td>
<td>(-0.12, 0.13)</td>
<td>(-0.16, 0.13)</td>
<td>(-0.33, 0.86)</td>
<td>(-0.09, 0.17)</td>
<td>(-0.48, 0.06)</td>
<td>(-0.53, 0.10)</td>
<td>(-0.03, 0.15)</td>
<td>(-0.07, 0.16)</td>
<td>(-0.08, 1.01)</td>
</tr>
<tr>
<td>p</td>
<td>0.93</td>
<td>p=0.86</td>
<td>p=0.39</td>
<td>p=0.52</td>
<td>p=0.13</td>
<td>p=0.19</td>
<td>p=0.18</td>
<td>p=0.46</td>
<td>p=0.094</td>
</tr>
<tr>
<td><strong>Prospective</strong></td>
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<td>-0.05,</td>
<td>+0.04</td>
<td>+0.22</td>
<td>+0.04</td>
<td>-0.11</td>
<td>-0.05</td>
<td>+0.05</td>
<td>+0.26</td>
</tr>
<tr>
<td>(Winter values)</td>
<td>0.09</td>
<td>(-0.04, 0.13)</td>
<td>(-0.09, 0.52)</td>
<td>(-0.02, 0.11)</td>
<td>(-0.28, 0.06)</td>
<td>(-0.24, 0.14)</td>
<td>(0.00, 0.09)</td>
<td>(-0.03, 0.15)</td>
<td>(-0.12, 0.63)</td>
</tr>
<tr>
<td>p</td>
<td>0.50</td>
<td>p=0.32</td>
<td>p=0.16</td>
<td>p=0.20</td>
<td>p=0.19</td>
<td>p=0.62</td>
<td>p=0.045</td>
<td>p=0.17</td>
<td>p=0.18</td>
</tr>
<tr>
<td><strong>Cross-sectional</strong></td>
<td>0.04</td>
<td>+0.10</td>
<td>+0.25</td>
<td>+0.07</td>
<td>-0.19</td>
<td>-0.27</td>
<td>+0.05</td>
<td>+0.11</td>
<td>+0.49</td>
</tr>
<tr>
<td>(Summer values)</td>
<td>(-0.02, 0.11)</td>
<td>(0.01, 0.19)</td>
<td>(-0.05, 0.55)</td>
<td>(0.00, 0.14)</td>
<td>(-0.36, -0.02)</td>
<td>(-0.53, -0.02)</td>
<td>(-0.01, 0.11)</td>
<td>(0.01, 0.20)</td>
<td>(0.10, 0.88)</td>
</tr>
<tr>
<td>p</td>
<td>0.21</td>
<td>p=0.034</td>
<td>p=0.098</td>
<td>p=0.041</td>
<td>p=0.025</td>
<td>p=0.034</td>
<td>p=0.075</td>
<td>p=0.024</td>
<td>p=0.013</td>
</tr>
<tr>
<td><strong>Retrospective</strong></td>
<td>0.02</td>
<td>+0.04</td>
<td>+0.12</td>
<td>+0.04</td>
<td>-0.14</td>
<td>-0.27</td>
<td>+0.04</td>
<td>+0.05</td>
<td>+0.21</td>
</tr>
<tr>
<td>(Winter values)</td>
<td>(-0.06, 0.09)</td>
<td>(-0.06, 0.14)</td>
<td>(-0.19, 0.42)</td>
<td>(-0.04, 0.12)</td>
<td>(-0.27, -0.01)</td>
<td>(-0.48, -0.06)</td>
<td>(-0.02, 0.10)</td>
<td>(-0.05, 0.14)</td>
<td>(-0.12, 0.55)</td>
</tr>
<tr>
<td>p</td>
<td>0.69</td>
<td>p=0.46</td>
<td>p=0.45</td>
<td>p=0.34</td>
<td>p=0.041</td>
<td>p=0.013</td>
<td>p=0.16</td>
<td>p=0.33</td>
<td>p=0.21</td>
</tr>
<tr>
<td><strong>Post-interval</strong></td>
<td>-0.002</td>
<td>+0.01</td>
<td>+0.03</td>
<td>+0.02</td>
<td>-0.13</td>
<td>-0.20</td>
<td>+0.03</td>
<td>+0.03</td>
<td>+0.15</td>
</tr>
<tr>
<td>(Summer values)</td>
<td>(-0.06, 0.06)</td>
<td>(-0.08, 0.10)</td>
<td>(-0.21, 0.28)</td>
<td>(-0.05, 0.08)</td>
<td>(-0.27, 0.01)</td>
<td>(-0.39, -0.01)</td>
<td>(-0.01, 0.07)</td>
<td>(-0.04, 0.10)</td>
<td>(-0.15, 0.45)</td>
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<tr>
<td>p</td>
<td>0.95</td>
<td>p=0.88</td>
<td>p=0.80</td>
<td>p=0.61</td>
<td>p=0.078</td>
<td>p=0.042</td>
<td>p=0.18</td>
<td>p=0.39</td>
<td>p=0.32</td>
</tr>
</tbody>
</table>

#### Adjusted model 1

<table>
<thead>
<tr>
<th></th>
<th>TC</th>
<th>LDL</th>
<th>ApoB</th>
<th>nonHDL</th>
<th>HDL</th>
<th>ApoA-I</th>
<th>TC/HDL</th>
<th>LDL/HDL</th>
<th>ApoB/ApoA-I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-interval</strong></td>
<td>-0.01</td>
<td>-0.03</td>
<td>0.23</td>
<td>0.03</td>
<td>-0.23</td>
<td>-0.26</td>
<td>0.07</td>
<td>0.04</td>
<td>0.54</td>
</tr>
<tr>
<td>(Summer values)</td>
<td>(-0.13, 0.11)</td>
<td>(-0.16, 0.11)</td>
<td>(-0.33, 0.80)</td>
<td>(-0.09, 0.16)</td>
<td>(-0.51, 0.04)</td>
<td>(-0.60, 0.08)</td>
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<td>(-0.07, 0.16)</td>
<td>(-0.01, 1.08)</td>
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<tr>
<td>p</td>
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<td>p=0.69</td>
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<td>p=0.58</td>
<td>p=0.10</td>
<td>p=0.13</td>
<td>p=0.13</td>
<td>p=0.45</td>
<td>p=0.06</td>
</tr>
<tr>
<td><strong>Prospective</strong></td>
<td>0.03</td>
<td>0.05</td>
<td>0.21</td>
<td>0.04</td>
<td>-0.10</td>
<td>-0.01</td>
<td>0.04</td>
<td>0.05</td>
<td>0.24</td>
</tr>
<tr>
<td>(Winter values)</td>
<td>(-0.05, 0.10)</td>
<td>(-0.04, 0.13)</td>
<td>(-0.10, 0.51)</td>
<td>(-0.03, 0.11)</td>
<td>(-0.27, 0.08)</td>
<td>(-0.21, 0.19)</td>
<td>(-0.003, 0.09)</td>
<td>(-0.03, 0.14)</td>
<td>(-0.13, 0.61)</td>
</tr>
<tr>
<td>p</td>
<td>0.47</td>
<td>p=0.29</td>
<td>p=0.18</td>
<td>p=0.23</td>
<td>p=0.28</td>
<td>p=0.91</td>
<td>p=0.07</td>
<td>p=0.23</td>
<td>p=0.21</td>
</tr>
<tr>
<td><strong>Cross-sectional</strong></td>
<td>+0.03</td>
<td>0.08 (-0.01,</td>
<td>0.19</td>
<td>0.05</td>
<td>-0.17</td>
<td>-0.24</td>
<td>0.04 (-0.02,</td>
<td>0.08 (-0.01,</td>
<td>0.31</td>
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</table>
### Adjusted model 2

<table>
<thead>
<tr>
<th></th>
<th>TC</th>
<th>LDL</th>
<th>ApoB</th>
<th>nonHDL</th>
<th>HDL</th>
<th>ApoA-I</th>
<th>TC/HDL</th>
<th>LDL/HDL</th>
<th>ApoB/ApoA-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-interval</td>
<td>0.01</td>
<td>-0.01</td>
<td>0.39</td>
<td>0.06</td>
<td>-0.29</td>
<td>-0.31</td>
<td>0.09</td>
<td>0.07</td>
<td>0.74</td>
</tr>
<tr>
<td>(Summer values)</td>
<td>(-0.11, 0.13)</td>
<td>(-0.15, 0.13)</td>
<td>(-0.22, 1.0)</td>
<td>(-0.07, 0.19)</td>
<td>(-0.58, -0.01)</td>
<td>(-0.65, 0.02)</td>
<td>(0.001, 0.19)</td>
<td>P=0.047</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p=0.85</td>
<td>P=0.92</td>
<td>P=0.21</td>
<td>P=0.34</td>
<td>P=0.045</td>
<td>P=0.07</td>
<td>P=0.16</td>
<td>P=0.01</td>
<td></td>
</tr>
<tr>
<td>Prospective</td>
<td>0.04</td>
<td>0.05</td>
<td>0.26</td>
<td>0.05</td>
<td>-0.10</td>
<td>-0.005</td>
<td>0.05</td>
<td>0.06</td>
<td>0.28</td>
</tr>
<tr>
<td>(Winter values)</td>
<td>(-0.04, 0.11)</td>
<td>(-0.03, 0.14)</td>
<td>(-0.05, 0.57)</td>
<td>(-0.02, 0.11)</td>
<td>(-0.28, 0.08)</td>
<td>(-0.21, 0.20)</td>
<td>(0.01, 0.10)</td>
<td>(-0.03, 0.16)</td>
<td>(-0.11, 0.66)</td>
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<tr>
<td></td>
<td>p=0.33</td>
<td>P=0.22</td>
<td>P=0.10</td>
<td>P=0.14</td>
<td>P=0.29</td>
<td>P=0.96</td>
<td>P=0.029</td>
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<td>P=0.16</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>+0.03</td>
<td>0.07</td>
<td>-0.01</td>
<td>0.20</td>
<td>-0.18</td>
<td>-0.24</td>
<td>0.04</td>
<td>0.08</td>
<td>0.42</td>
</tr>
<tr>
<td>(Summer values)</td>
<td>(-0.04, 0.10)</td>
<td>0.16</td>
<td>(-0.09, 0.48)</td>
<td>(-0.01, 0.12)</td>
<td>(-0.35, -0.002)</td>
<td>(-0.52, 0.04)</td>
<td>(-0.02, 0.09)</td>
<td>(-0.01, 0.17)</td>
<td>(0.03, 0.81)</td>
</tr>
<tr>
<td></td>
<td>p=0.38</td>
<td>P=0.10</td>
<td>P=0.18</td>
<td>P=0.10</td>
<td>P=0.047</td>
<td>P=0.09</td>
<td>P=0.19</td>
<td>P=0.08</td>
<td>P=0.036</td>
</tr>
<tr>
<td>Retrospective</td>
<td>-0.01</td>
<td>0.01</td>
<td>0.05</td>
<td>0.02</td>
<td>-0.12</td>
<td>-0.26</td>
<td>0.03</td>
<td>0.03</td>
<td>0.17</td>
</tr>
<tr>
<td>(Winter values)</td>
<td>(-0.08, 0.07)</td>
<td>(-0.09, 0.11)</td>
<td>(-0.24, 0.34)</td>
<td>(-0.06, 0.09)</td>
<td>(-0.26, 0.01)</td>
<td>(-0.48, -0.05)</td>
<td>(-0.03, 0.09)</td>
<td>(-0.03, 0.0)</td>
<td>(-0.18, 0.52)</td>
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<tr>
<td></td>
<td>p=0.89</td>
<td>P=0.85</td>
<td>P=0.74</td>
<td>P=0.68</td>
<td>P=0.08</td>
<td>p=0.015</td>
<td>P=0.39</td>
<td>P=0.34</td>
<td>P=0.35</td>
</tr>
<tr>
<td>Post-interval</td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.03</td>
<td>0.02</td>
<td>-0.12</td>
<td>-0.02</td>
<td>0.02</td>
<td>0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>(Summer values)</td>
<td>(-0.08, 0.05)</td>
<td>(-0.10, 0.06)</td>
<td>(-0.26, 0.21)</td>
<td>(-0.06, 0.09)</td>
<td>(-0.27, 0.04)</td>
<td>(-.41, 0.001)</td>
<td>(-0.02, 0.06)</td>
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<td>(-0.19, 0.36)</td>
</tr>
<tr>
<td></td>
<td>p=0.60</td>
<td>p=0.61</td>
<td>p=0.82</td>
<td>p=0.68</td>
<td>p=0.14</td>
<td>p=0.051</td>
<td>p=0.33</td>
<td>p=0.74</td>
<td>p=0.54</td>
</tr>
</tbody>
</table>

Basic Model: Adjusted for change in relapse, EDSS at baseline

Adjusted model 1: Further adjusted for age at study entry, sex, MS duration, smoking and statin use.

Adjusted model 2: Further adjusted for BMI and physical activity

TC: Total cholesterol; LDL: Low density lipoprotein; ApoB: Apoprotein B; NonHDL: None high density lipoprotein; HDL: High density lipoprotein; ApoA-I: Apoprotein A-I
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  http://dx.doi.org/10.1177/1352458514533162
Chapter 5 : Adverse lipid profile is not associated with relapse risk in MS: Results from an observational cohort study

5.1 Preface
The manuscript presented in this chapter has been published. The typeset version of the manuscript as it appeared in the journal is in Appendix 5A. The text of this chapter is the same as the published version. This investigation describes the association between an adverse lipid profile and the risk of relapse in people with MS.

5.2 Abstract
Background: There is increasing evidence that serum lipids and apolipoproteins may be associated with multiple sclerosis (MS) clinical course.

Objective: To investigate the associations between serum lipids, apolipoproteins, body mass index and relapse in MS.

Methods: A prospective cohort of 141 participants with relapsing-remitting MS was followed from 2002 to 2005. Serum lipid and apolipoprotein levels were measured biannually, and body mass index at baseline. The association with hazard of relapse was assessed using survival analysis.

Results: Neither body mass index nor any of the lipid-related measures were associated with the hazard of relapse.

Conclusion: Serum lipid profile and body mass index are not associated with the hazard of relapse in MS.

5.3 Introduction
The peroxidation of lipoproteins and their associated serum lipids have been implicated as potential agents involved in demyelination and axonal injury in multiple sclerosis (MS) (1).
There is now emerging evidence suggesting that dyslipidaemia as comorbidity in MS is linked with a more rapid progression in disability (2). Recent evidence suggests a role for an adverse lipid profile (low HDL, increased LDL and triglycerides) in MS clinical course with some evidence of an association with both disability and progression in disability (3). Associations have also been found between serum lipids and number of gadolinium-enhancing lesions on magnetic resonance imaging in a small sample of people with first demyelinating events (4). A modification of serum lipids has been reported also in clinically stable MS (5). Only one study has evaluated the association between serum lipid profile and relapse, prospectively following a cohort with a clinically isolated syndrome (CIS) treated with interferon-β and finding no association between lipid profile and relapse risk (6). To date, however, no studies have examined the association between serum lipids and apolipoproteins and the subsequent risk of relapse in people with clinically definite MS.

Here we examine whether an adverse lipid profile is associated with a higher risk of relapse in people with clinically definite relapsing-remitting MS (RRMS) using a prospective cohort study design.

5.4 Methodology

5.4.1 Study design
The Southern Tasmanian Multiple Sclerosis Longitudinal (MSL) Study prospectively followed a cohort of 203 persons with clinically definite MS (using 2001 McDonald criteria) living in southern Tasmania, Australia, between 2002 and 2005. An estimated 78% (203/259) of eligible cases were included and data from 198 participants were obtained. A total of 141 participants had a relapsing-remitting (RRMS) course. When participants discontinued participation (4%) or were lost to follow-up (6%), they were censored as of the date of study exit or last attended review.
The study methodology has been previously described (7). At each biannual review participants were asked about their lifestyle, including physical activity, smoking, and medication use. Clinical disability (Expanded Disability Status Scale (EDSS)) was measured annually by a single physician. Body mass index (BMI) was measured at baseline (weight divided by height squared). Ethics approval was obtained from the Southern Tasmania Human Research Ethics Committee; all participants provided informed consent.

5.4.2 Measurement of relapses
Relapses were reported in real time by phone to the study clinician or subsequently at biannual review, and all relapse reports were validated by the study physician and neurologist. Using a real-time relapse notification system, participants telephoned the study centre if they thought they were experiencing a relapse. Additionally, at each biannual review, participants were queried about the occurrence of a relapse in the preceding 6 months. To ensure each relapse was a true relapse, the study nurse or physician administered a relapse questionnaire detailing relapse symptoms, medical practitioner review, treatment, and co-occurrence of infection or fever. For quality control, the study physician also performed a physical examination for those reporting a relapse by telephone. Throughout the study, each relapse was reviewed rigorously by the study physician and further by the study neurologist.

5.4.3 Biological samples and measurement
Serum samples were collected at study entry and at each biannual review and stored at -80°C until measurement. Triglyceride (trig) was measured using non-fasting samples because post-prandial non-fasting values are more representative of the usual metabolic state (8). Total cholesterol (TC) and trig were measured using enzymatic colorimetry and high density lipoprotein cholesterol (HDL) levels were measured using precipitation and enzymatic assay (Wako Chemicals USA, Inc., Richmond, VA). Low density lipoprotein cholesterol (LDL)
was estimated using the Friedewald equation except for trig levels above 5.1mmol/L (n=5) measured by direct assay (Wako Chemicals USA, Inc., Richmond, VA).

Apolipoprotein A-I (ApoA-I), apolipoprotein E (ApoE) and apolipoprotein B (ApoB) were measured because they are the main proteins components of HDL, and LDL/VLDL (very LDL). Lipoprotein(a) (Lp(a)) is the complex of LDL-cholesterol and apolipoprotein(a). ApoB, ApoE and ApoA-I levels were measured by turbidimetric immunoassay using goat anti-human ApoB or ApoA-I (Wako Chemicals USA, Inc., Richmond, VA) and Lp(a) measured by a sandwich DELFIA (LKB-Pharmacia). Serum levels of highly sensitive C-reactive protein (hs-CRP) were measured with an hs-CRP ELISA Kit (Alpha Diagnostic Int., San Antonio, Texas, USA) according to the manufacturer’s instructions (detection limit 0.35 ng/ml). 25(OH)D levels were measured with a commercially available radioimmunoassay (DiaSorin, Stillwater, MN), which has a detection range of 12.5 to 250nmol/l (inter-batch reproducibility was 4.6% at 32nmol/l and 6.4% at 125nmol/l).

5.4.4 Data analysis
For relapse analyses, covariates measured at each review were carried forward to the time of relapse, such that all parameters were as measured within six months prior to the occurrence of relapse.

The effect of serum lipid variables and other covariates on time-to-relapse was calculated using Cox proportional hazards models for repeated events, whereby multiple relapses by the same persons are treated as independent observations but accounted for at the intra-individual level, and the time until a prior event does not influence the composition of the risk set for a subsequent event.

All covariates satisfied the proportional hazards assumption with the exception of the binary variable for sex and the categorical variable for baseline EDSS (0–<3, 3–<5.5, 5.5–<7.5, 7.5–
9). For this reason, all models were stratified to allow the baseline hazards to differ by sex and baseline EDSS category.

TC to HDL ratio (TC/HDL ratio), LDL to HDL ratio (LDL/HDL ratio), ApoB to ApoA-I ratio (ApoB/ApoA-I ratio) and nonHDL were created because they are predictors of cardiovascular disease risk, and vascular comorbidity is common in MS (9).

All analyses were done using STATA/IC for Windows (Version 12.1; StataCorp LP College Station USA).

5.5 Results

5.5.1 Participant characteristics and correlations between lipid measures
The prospective cohort of 141 participants with RRMS was followed for an average of 2.3 (SD 0.5) years. During the study, a total of 122 confirmed relapses (99 with lipid data) occurred in 70 participants. The cohort was majority female, of overweight BMI (median BMI: 26.1), and of low disability (median EDSS: 3.5) (Table 5.1). Strong correlations were observed between TC, LDL, nonHDL and trig (0.43≤r≤0.94). HDL was negatively correlated with trig, LDL, nonHDL, TC/HDL ratio and LDL/HDL ratio (-0.33≤r≤-0.75). ApoB was strongly correlated with LDL, TC, nonHDL, trig, TC/HDL ratio and LDL/HDL ratio (0.56≤r≤0.94). ApoA-I was only positively correlated with HDL (r=0.83). ApoE was also positively correlated with trig, TC, and nonHDL (0.43≤r≤0.54).
Table 5.1: Demographic and clinical characteristics of the MS cohort at study entry

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>141/141 (100)</td>
</tr>
<tr>
<td>sex (Females)</td>
<td>105/141 (74.5)</td>
</tr>
<tr>
<td>Relapse during study?</td>
<td>70/141 (49.6)</td>
</tr>
<tr>
<td>Any Immunomodulatory therapy during study?</td>
<td>119/141 (84.4)</td>
</tr>
<tr>
<td>Any Statin during study?</td>
<td>9/141 (6.4)</td>
</tr>
<tr>
<td>Smoker during study</td>
<td>39/141 (27.7)</td>
</tr>
<tr>
<td><strong>Body Mass Index (Kg/m²)</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>55/141 (39.0)</td>
</tr>
<tr>
<td>Overweight</td>
<td>55/141 (39.0)</td>
</tr>
<tr>
<td>Obese</td>
<td>31/141 (22.0)</td>
</tr>
<tr>
<td><strong>Mean (SD; Range)</strong></td>
<td></td>
</tr>
<tr>
<td>Age at study entry (years)</td>
<td>44.8 (10.8; 21, 76)</td>
</tr>
</tbody>
</table>

| **Median (IQR)**                             |             |
| MS duration from diagnosis (years)           | 6.0 (2.0,12.0) |
| EDSS at study entry                         | 3.5 (2.0, 5.0) |
| MSSS at study entry                         | 4.0 (2.3, 6.2) |
| Physical activity (Met)                     | 17.6 (3.3, 40.0) |
| TC (mmol/L)                                  | 5.2 (4.5, 6.2) |
| LDL (mmol/L)                                 | 3.4 (2.7, 4.4) |
| ApoB (g/L)                                   | 0.97 (0.82, 1.2) |
| nonHDL (mmol/L)                              | 3.7 (3.1, 4.9) |
| Trig (mmol/L)                                | 1.4 (1.0, 1.9) |
| HDL (mmol/L)                                 | 1.4 (1.1, 1.6) |
| ApoA-I (g/L)                                 | 1.5 (1.4, 1.8) |
| ApoE (mg/L)                                  | 52.0 (40.0, 58.0) |
| Lp(a) (µmol/L)                               | 0.5 (0.2, 1.1) |
| **hs-CRP (mg/L)**                            | 21.0 (10.0, 47.0) |

TC: Total cholesterol; LDL: Low density lipoprotein; ApoB: Apolipoprotein B; NonHDL: non-high density lipoprotein; Trig: Triglycerides; HDL: High density lipoprotein; ApoA-I: Apolipoprotein A-I; ApoE: Apolipoprotein E; Lp(a): Lipoprotein (a); hs-CRP: highly sensitive C-reactive protein
 Associations between lipid-related variables and hazard of relapse

We examined whether lipid-related variables were associated with the hazard of relapse (Table 5.2). We found that none of the lipid-related measures were associated with the hazard of relapse. Adjustment for age at study entry, BMI, 25(OH)D, physical activity, immunomodulatory therapy use during the study, season, smoking, physical activity and statin use during the study did not influence the magnitude of effect. We also found no association between baseline BMI (Table 5.2) or physical activity (data not shown) and hazard of relapse.

### Table 5.2: Association between serum lipid-related variables and hazard of relapse

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted Hazard Ratio (95%CI)</th>
<th>p-value</th>
<th>Adjusted* Hazard Ratio (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>1.02 (0.98, 1.07)</td>
<td>0.29</td>
<td>1.00 (0.95, 1.05)**</td>
<td>0.84</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>0.98 (0.79, 1.20)</td>
<td>0.82</td>
<td>0.99 (0.80, 1.23)</td>
<td>0.94</td>
</tr>
<tr>
<td>LDL (mol/L)</td>
<td>1.02 (0.82, 1.27)</td>
<td>0.87</td>
<td>0.99 (0.79, 1.24)</td>
<td>0.95</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>1.17 (0.48, 2.83)</td>
<td>0.73</td>
<td>1.15 (0.46, 2.92)</td>
<td>0.76</td>
</tr>
<tr>
<td>nonHDL (mmol/L)</td>
<td>1.01 (0.82, 1.23)</td>
<td>0.94</td>
<td>0.99 (0.80, 1.22)</td>
<td>0.90</td>
</tr>
<tr>
<td>Trig (mmol/L)</td>
<td>0.96 (0.76, 1.21)</td>
<td>0.73</td>
<td>0.96 (0.73, 1.26)</td>
<td>0.79</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>0.81 (0.50, 1.31)</td>
<td>0.39</td>
<td>1.05 (0.58, 1.89)</td>
<td>0.87</td>
</tr>
<tr>
<td>ApoA-I (g/L)</td>
<td>0.76 (0.40, 1.44)</td>
<td>0.40</td>
<td>1.08 (0.53, 2.20)</td>
<td>0.84</td>
</tr>
<tr>
<td>ApoE (mg/L)</td>
<td>1.00 (0.99, 1.02)</td>
<td>0.57</td>
<td>1.00 (0.99, 1.02)</td>
<td>0.63</td>
</tr>
<tr>
<td>Lp(a) (µmol/L)</td>
<td>1.06 (0.94, 1.21)</td>
<td>0.35</td>
<td>1.14 (0.99, 1.32)</td>
<td>0.07</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>1.00 (1.00, 1.00)</td>
<td>0.14</td>
<td>1.00 (1.00, 1.01)</td>
<td>0.19</td>
</tr>
<tr>
<td>TC/HDL ratio</td>
<td>1.04 (0.88, 1.23)</td>
<td>0.67</td>
<td>0.98 (0.81, 1.19)</td>
<td>0.86</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>1.05 (0.87, 1.27)</td>
<td>0.59</td>
<td>0.99 (0.80, 1.22)</td>
<td>0.91</td>
</tr>
<tr>
<td>ApoB/ApoA-I ratio</td>
<td>1.59 (0.56, 4.51)</td>
<td>0.38</td>
<td>1.18 (0.38, 3.68)</td>
<td>0.78</td>
</tr>
</tbody>
</table>

*Adjusted for age at study entry, baseline BMI, 25(OH)D (nmol/L), physical activity (METs), smoking during the study (no, yes), statin use during the study (no, yes), season and Immunomodulatory therapy use during the study (no, yes).

** Further adjusted for TC and Trig

TC: Total cholesterol; LDL: Low density lipoprotein; ApoB: Apolipoprotein B; NonHDL: Non high density lipoprotein; Trig: Triglycerides; HDL: High density lipoprotein; ApoA-I: Apolipoprotein A-I; ApoE: Apolipoprotein E; Lp(a): Lipoprotein a; Hs-CRP: Highly sensitive C-reactive protein
5.6 Discussion
We have evaluated the association between serum lipids and apolipoproteins and the subsequent hazard of relapse in MS patients using a prospective cohort study design, finding none of the lipids, apolipoproteins or baseline BMI were significant predictors of relapse. These findings are in agreement with work by Weinstock-Guttman and colleagues (6) who showed no associations between HDL, LDL and total cholesterol and either time to first relapse or number of relapses in an interferon-β-treated CIS cohort. This is to our knowledge the first study to evaluate the associations between serum lipids and apolipoproteins and relapse in people with established MS.

Dysfunction of the metabolism and oxidation of lipids have been implicated in the pathogenesis of MS (10) and there is evidence of early involvement of serum lipids in the development of MS lesions as oxidized LDL was detected in the parenchyma of MS plaques (11). This is in line with MRI data in CIS cases, where those with higher total cholesterol and LDL had a higher number of gadolinium-enhancing lesions (4) and a higher risk of developing new and newly enlarging T2 lesions over two years (6).

In a study by Palavra and colleagues (12), TC, LDL and oxidised-LDL were positively correlated with MS disability (EDSS). Similarly, Weinstock-Guttman and colleagues found positive associations between MS disability, progression of disability and TC, LDL and trig (3).

The fact that lipid profile may be associated with MS disability, progression of disability and disease activity on MRI, but not with relapses may provide some insights as to how an adverse lipid profile and/or oxidised lipids may exert their effect. Others have found associations with MRI activity among persons with CIS, since the pathologies shown on MRI can correlate with relapse activity (4, 6). However that these results were found in persons with CIS and not clinically definite MS may account for this disparity. Indeed, the differences
between inflammatory attacks and the mechanisms driving disability accumulation and conversion to secondary progressive MS have been a point of considerable interest in the MS literature (13). Indirect evidence from statin trials provides support for this construction since, while a therapeutic effect of simvastatin in reducing brain atrophy by 43% and significantly decreasing progression in EDSS has been recently shown (14), therapeutic effects of statins in relapse reduction have been inconsistent (15-17), as have been studies evaluating the effects of statin treatment on MS disability (15-17). These inconsistencies call for further research into the role of serum lipids in the pathogenesis of MS and the potential therapeutic benefits of statins in MS.

In conclusion, the results of our investigation indicate that serum lipid levels are not associated with the hazard of relapse in established RRMS.

5.7 Postscript
This chapter has provided some evidence on the relationship between an adverse lipid profile and the risk of relapse in people with MS. The next chapter describes the associations between lipid-related variables and conversion to clinically definite MS, time to relapse and progression in disability.

5.8 References


These articles have been removed for copyright or proprietary reasons.

Chapter 6  An adverse lipid profile and increased body mass index significantly predicts clinical course after a first demyelinating event.

6.1 Preface
This investigation examines whether there are relationships between lipid-related variables and conversion to clinically definite MS, time to relapse and progression in disability in a cohort for people with a first clinical diagnosis of CNS demyelination.

6.2 Abstract
Data on whether the accumulation of disability and relapse may be influenced by serum lipid levels and body mass index (BMI) in patients with a first clinical diagnosis of demyelination is limited. We aimed to investigate whether there were any associations between lipid-related variables at study entry and conversion to clinically definite MS (CDMS), time to subsequent relapse and progression in disability.

Methods: A cohort of 279 patients with a first clinical diagnosis of demyelination in the Ausimmune Longitudinal (AusLong) Study was prospectively followed, the present analysis including follow-up to 5-year review. Height and weight were assessed and serum samples obtained to measure serum lipid and apolipoprotein levels. Associations with conversion to CDMS and hazard of relapse were assessed using survival analysis, and associations with annualised change in disability were evaluated using linear regression.

Results: Higher BMI (Adjusted hazard ratio (AHR): 1.04 (95% CI 1.01, 1.08) p=0.014) and triglyceride levels (AHR: 1.20 (95% CI 1.03, 1.40) p=0.021) at study entry were associated with increased risk of subsequent relapse while lipid-related measures were not significantly associated with conversion to CDMS. In addition, higher BMI ($\beta$: 0.01 (95% CI 0.001, 0.13)
p=0.010) and TC/HDL ratio (β: 0.05 (95% CI 0.001, 0.10) p=0.044) at study entry were associated with a higher subsequent annual change in disability.

Conclusions: In this prospective study, we found no significant association with CDMS risk after adjustment. However, we found that higher BMI and triglycerides were associated with relapse, and a higher BMI and TC/HDL ratio were associated with a higher rate in disability progression. These results suggest that improving lipid profile and losing weight into the healthy range may improve the disease course of MS, even early in the course of disease.

6.3 Introduction
Multiple sclerosis (MS) is an inflammatory, demyelinating, neurodegenerative disease of the central nervous system (CNS). Clinical isolated syndrome (CIS) or first demyelinating event (FDE) represents the earliest clinical stage in the development of MS. Following the CIS/FDE, the diagnosis of MS can be made based on the development of a second clinical episode or paraclinical evidence that meet diagnostic criteria. A better understanding of the factors involved in the risk of FDE could help identify biomarkers that predict conversion to clinically definite MS, disability progression, and other elements of clinical course and quality of life, which could lead to interventions targets.

There is now emerging evidence suggesting that dyslipidaemia as a comorbidity in people with MS is linked with a more rapid progression in disability. Similarly, recent evidence suggests a potential role for an adverse lipid profile (low HDL, increased LDL and triglycerides) in MS clinical course with some evidence of an association with both disability and progression in disability. Studies have also shown an association between serum lipid variables and disease activity on magnetic resonance imaging (MRI).

In relation to relapse in MS, Weinstock-Guttman and colleagues evaluated the association between the serum lipid profile and conversion to clinically definite MS (CDMS) and subsequent risk of relapse in a prospective cohort of patients with a CIS treated with
interferon-β in a clinical trial. From the analysis, none of the lipid profile variables (total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides) were associated with conversion to CDMS or subsequent risk of relapse.\textsuperscript{5} We verified the outcome of this study, by conducting a study examining whether serum lipids and apolipoproteins predicted subsequent risk of relapse in clinically definite MS patients with average disease duration of 12 years and on immunomodulatory therapy. Like Weinstock-Guttman, we found that none of the lipid-related variables were associated with the risk of relapse.\textsuperscript{11}

Studies have also been conducted examining how BMI may influence the disease course of MS. Accumulating evidence suggests an increased risk of MS in people with a high BMI during childhood or adolescence but not adulthood.\textsuperscript{12-15} In relation to the question whether high BMI is associated with disability or a change in disability, studies in this area are scarce and results have been inconsistent\textsuperscript{8, 16, 17} and therefore further investigations are needed. Whether an adverse lipid profile and increased BMI drive subsequent clinical course is often hard to disentangle from the reverse association where those who are more disabled are becoming more sedentary, overweight and developing an adverse lipid profile. A prospective study design where the lipid profile is measured right at the start of the disease process, when no disability has accumulated yet, is therefore an ideal observational design to answer this research question. This present research therefore investigates the association between lipid-related variables and conversion to clinically definite MS (CDMS), time to subsequent relapse and progression in disability in a prospective cohort of patients with a first clinical diagnosis of demyelination.
6.4 Methodology

6.4.1 Study design
The Ausimmune Longitudinal (AusLong) Study, which built upon the original Ausimmune case-control study, seeks to elucidate environmental, genetic and lifestyle risk factors for the onset and early progression of MS. The Ausimmune Study recruited 282 participants with a first clinical diagnosis of CNS demyelination, including 169 cases who had their initial onset event just prior to the initial participation in the Ausimmune Study referred to here as “classic FDE” between 1 Nov 2003 and 31 Dec 2006. At the 5-year follow-up, 3 participants had now been diagnosed with a none-MS disease (a case of Susac’s Syndrome, neuromyelitis optica and pineal germinoma). Within the remaining 279 cases, 258 were now considered to have had bout-onset disease (169 of whom had “classic FDE”), and 69 who had had the initial event in the more distant past). Twenty-one participants were now considered to have had progressive-onset disease. Since 2009, the AusLong Study has followed case participants in the Ausimmune Study (retention rate 84.6%); many participants have now been followed for over nine years since their initial participation in the study. The present analysis is for the period from first recorded symptom onset, to the 5-year review, as this is the most recent face-to-face review which all currently enrolled participants have completed.

The Ausimmune and AusLong Study were approved by nine regional Human Research Ethics Committees. All participants provided written informed consent.

6.4.2 Measurement of CDMS and relapse and disability
For the purposes of the present investigation, a number of clinical outcomes were evaluated, including conversion to clinically definite MS (CDMS), occurrence of relapses, and annualised disability progression from FDE to five-year review.

Conversion to CDMS was defined primarily as the occurrence of two or more clinical demyelinating episodes, thus satisfying the diagnostic requirements of dissemination in space and time, or a single episode plus paraclinical evidence, as per the 2005 McDonald criteria.
(a minority of cases were diagnosed following MRI based on this latter criterion (n=20)).

Conversion to CDMS was reported at annual review and cross-checked with neurological records. A relapse was defined according to the 2001 McDonald Criteria\textsuperscript{20} as the acute or subacute appearance or reappearance of a neurological abnormality (lasting at least 24 hours), immediately preceded by a stable, improving, or slowly progressive neurological state for 30 days, in the absence of fever, known infection, concurrent steroid withdrawal, or externally derived increases in body temperature. Relapses were reported at annual review or derived from medical records, and only relapses which were diagnosed and verified by a neurologist were included in this analysis. Disability was assessed by the Kurtzke Expanded Disability Status Scale (EDSS)\textsuperscript{21} at the 5-year review; the EDSS on the day before FDE was assumed to be 0. The Multiple Sclerosis Severity Score (MSSS) was calculated from the EDSS and disease duration, by comparing it to the global MSSS reference dataset.\textsuperscript{22}

Clinical history was derived from medical records at initial presentation, describing the nature of the episode/symptoms which brought the participant into the Ausimmune Study, as well as historical symptoms prior to presentation. In the event that a person had no history preceding their referral symptoms, the referral symptom onset date was taken to be their symptom onset. Where a person had a bout-onset presentation and had symptoms some time previous to the referral episode, this was validated to the extent possible from available clinical notes contemporaneous with the historical episode or taken as valid if judged to be so by the attending neurologist at the referral episode. Finally, where a person was progressive from onset, symptom onset was defined as either the earliest onset of symptoms identified by the attending neurologist at the referral episode, or one year preceding the referral clinic date, whichever was first.

MRI scans were performed as part of the study. The study MRI protocol included a sagittal FLAIR (fluid attenuated inversion recovery) sequence selected for its high contrast sensitivity.
for perivascular and callosal demyelinating lesions. MRI scans ( Corpus callosum area) were measured at baseline and at five-year review.

6.4.3 Biological samples and measurements
Non-fasting serum samples were collected at three time points (cohort entry, 2/3-year and 5-year reviews), and stored at -80°C until use. Traditionally, triglycerides were measured in a fasting state; however, there has been a shift to non-fasting samples, as post-prandial non-fasting values are more representative of the usual metabolic state. Total cholesterol and triglycerides were measured using enzymatic colorimetry (Wako Chemicals, Richmond, VA, USA). HDL-cholesterol levels were measured using precipitation and enzymatic assay (Wako Chemicals, Richmond, VA, USA). LDL-cholesterol was measured by direct assay using enzymatic colorimetry (Wako Chemicals, Richmond, VA, USA). Non-HDL-cholesterol levels were computed by subtracting HDL-cholesterol from total cholesterol.

The apolipoproteins, ApoA-I and ApoB, were measured as they are the main protein components of HDL and LDL/VLDL, respectively. Lipoprotein (a) (Lp (a)) is the complex of LDL-cholesterol and ApoA. ApoB and ApoA-I levels were measured by turbidimetric immunoassay, using goat anti-human ApoB or ApoA-I (Wako Chemicals, Richmond, VA, USA). Lp(a) was measured by a sandwich dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) (LKB-Pharmacia, Stockholm, Sweden).

Serum 25-OH-D levels were measured with liquid chromatography/tandem mass spectrometry and deseasonalised 25(OH)D levels were estimated by sinusoidal regression functions for each of the four study sites, given the widely disparate levels of ambient UV and the strong seasonal variation of 25(OH)D. 25(OH)D values were deseasonalised to reduce the effect of the time of year that the serum samples was taken. This produced values that were more like those that would have obtained if all participants were measured at the same time of year.
6.5 Data analysis

The effect of serum lipid variables and other covariates on conversion to CDMS (single event) and subsequent relapses (repeated events) were evaluated by Cox proportional hazards regression models. CDMS was evaluated by simple single-failure Cox regression, while relapse was evaluated using the gap-time model by Prentice and colleagues, where multiple relapses by the same persons are treated as independent observations, and the time until a prior event does not influence the composition of the risk set for a subsequent event. BMI was also rendered into categories as normal (18.5 to 25 Kg/m2), overweight (>25 to 30 Kg/m2) and obese (>30 Kg/m2) and its relationship with conversion to CDMS and hazard of relapse evaluated. All covariates satisfied the proportional hazard assumption, except where otherwise stated. Because the association between continuous serum lipid variables and conversion to CDMS violates the proportional hazard assumption, the serum lipid variables were rendered into quartile categorical variables which satisfied the proportional hazard assumption.

Annualised change in EDSS was calculated by taking the five-year review EDSS and dividing by the duration between the day before FDE and the five-year review, this proportion then being expressed as an annualised value. The relationships between the lipid-related variables at study entry and annualized change in EDSS were evaluated by linear regression, adjusted for whether persons were having a relapse at the time of 5-year disability measure (n=22). Baseline disability was not included as a covariate as all persons were assumed to be zero EDSS the day before FDE. Because the annualised change in disability was highly skewed, a log-transformation was applied to satisfy linear regression assumptions of minimal heteroskedasticity. All means and coefficients, however, were back-transformed and presented on the original scale of both cross-sectional and change in EDSS at the mean of model covariates. Log binomial regression analysis was also used to compare the relationship the lipid-related variables and annualised change in disability.
All statistical analyses were conducted in Stata/IC 12.1 (StataCorp LP, College Station, Texas, USA).

6.6 Results

6.6.1 Participant characteristics
Table 6.1 shows the characteristics of the study participants. Of the 279 participants, 214 (77%) were female and the mean age at study entry was 38.8 years (SD 9.7), more than half of the cohort (64.6%) were overweight or obese (≥25 kg/m^2), and only 6.1% (n=17) were treated with statins during the study. By the five-year review, 72.0% (201/279) had converted to CDMS and had 446 relapses. The median EDSS at the five-year review was 1.5 (IQR: 1.0, 2.5). Using the established lipid cut-off points, we found that 50.4% of the participants had TC level above 5.2mmol/L, 61% had LDL above 2.6mmol/L and 39% had triglyceride above 1.7mmol/L.

When restricted to the “classic FDE” (n=169), 78% were female and the mean age at study entry was 37.7 (SD 9.7). By five-year review, 56.2% (95/169) had converted to CDMS and had 222 relapses. The median EDSS at the five-year review was 1.0 (IQR: 0.5, 2.0)

6.6.2 Association between lipid-related variables and hazard of relapse
Table 6.2 summarises the association between the serum lipid variables and hazard of relapse. The association between all the continuous serum lipid variables and hazard of relapse satisfied the proportional hazard assumption. The univariable association between BMI and hazard of relapse showed some evidence of positive association but this was not significant (p=0.14). However, upon adjustment for sex and age at study entry, BMI became significantly associated with higher hazard of relapse (HR: 1.04; 95% CI: 1.01, 1.08; p=0.014). Each 5 kg/m^2 increase in BMI, was associated with a 22% increased risk of relapse (HR: 1.22; 95% CI: 1.04, 1.44; p=0.014). Compared to normal weight, overweight and obese BMI were significantly associated with increased risk of relapse after adjustment for age at
study entry, sex and study site (p-trend=0.021). Figure 1 shows the Kaplan-Meier survival plots by category of BMI demonstrating that those with higher BMI levels experienced more relapses compared to those with lower BMI. The results were similar when we included BMI levels at 2/3-year review.

The relationship between triglycerides and the hazard of relapse showed some evidence of positive association but this was not significant in the univariable analysis (p=0.31). However, upon adjustment for age at study entry and sex, the association was enhanced and triglycerides became significantly associated with a 23% increased hazard of relapse (HR: 1.23; 95% CI: 1.06, 1.42; p=0.006). The association was independent of BMI as it was robust to further adjustment for BMI (HR: 1.20; 95% CI: 1.03, 1.40; p=0.022). Triglyceride levels was then dichotomised into categories of <2.30 mmol/L and ≥2.30 mmol/L (considered as clinically high risk for cardiovascular disease) and it association with hazard of relapse assessed. Those with ≥2.30 mmol/L levels of triglycerides had a relapse risk that was 2.18 times (p=0.022) higher than those with lower levels after adjustment for age at study entry, sex, BMI and study site. The results were similar when we included the lipid levels at 2/3-year review.

6.6.2.1 Other predictors of hazard of relapse
Age at study entry was significantly associated with hazard of relapse (HR: 0.97; 95% CI: 0.95, 0.98; p<0.001). Female sex was also a significant predictor of relapse (HR: 2.25; 95% CI: 1.27, 3.97; p=0.005). Compared to New South Wales, Tasmanian participants (HR: 0.57; 95% CI: 0.35, 0.93; p=0.025) had a decreased hazard of relapse, but no difference was seen for Queensland (p=0.67) or Victoria (p=0.52). In addition, neither smoking status (p=0.26), physical activity (p=0.24), 25(OH)D (p=0.34) nor statin use (p=0.30) were significant predictors of relapse.
Table 6.1: Demographic and clinical characteristics of the MS cohort at study entry

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All persons (279) n/N (%)</th>
<th>Classic FDE (169) n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex (Females)</td>
<td>214/279 (77.0)</td>
<td>131/169 (78)</td>
</tr>
<tr>
<td>Relapse during study?</td>
<td>170/279 (60.9)</td>
<td>85/169 (50.3)</td>
</tr>
<tr>
<td>Immunomodulatory therapy use during study?</td>
<td>146/279 (52.3)</td>
<td>78/169 (46.2)</td>
</tr>
<tr>
<td>Any Statin during study?</td>
<td>17/279 (6.1)</td>
<td>11/169 (6.5)</td>
</tr>
<tr>
<td>Smoke ever?</td>
<td>174/279 (62.4)</td>
<td>104/169 (61.5)</td>
</tr>
<tr>
<td>Body Mass Index (Kg/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>122/277 (44.0)</td>
<td>76/167 (45.5)</td>
</tr>
<tr>
<td>Overweight</td>
<td>80/277 (28.9)</td>
<td>48/167 (28.7)</td>
</tr>
<tr>
<td>Obese</td>
<td>75/277 (27.1)</td>
<td>43/167 (25.7)</td>
</tr>
</tbody>
</table>

Mean (SD; Range)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All persons (279) n/N (%)</th>
<th>Classic FDE (169) n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at study entry (years)</td>
<td>38.8 (9.7; 18, 58)</td>
<td>37.7 (9.7; 18, 58)</td>
</tr>
</tbody>
</table>

Median (IQR)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All persons (279) n/N (%)</th>
<th>Classic FDE (169) n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS duration from symptom onset (years)</td>
<td>6.3 (5.7, 7.4)</td>
<td>5.8 (5.3, 6.2)</td>
</tr>
<tr>
<td>Annualised change in EDSS</td>
<td>0.3 (0.2, 0.4)</td>
<td>0.3 (0.2, 0.4)</td>
</tr>
<tr>
<td>EDSS score</td>
<td>1.5 (1.0, 2.5)</td>
<td>1.5 (0.5, 2.0)</td>
</tr>
<tr>
<td>MSSS score</td>
<td>2.6 (1.3, 4.5)</td>
<td>2.3 (0.3, 3.9)</td>
</tr>
<tr>
<td>Physical activity (Met)</td>
<td>8.0 (0.0, 24.0)</td>
<td>8.0 (0.0, 24.0)</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>5.4 (4.7, 6.2)</td>
<td>5.5 (4.7, 6.4)</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>3.2 (2.7, 3.8)</td>
<td>3.2 (2.7, 3.9)</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>0.8 (0.7, 1.0)</td>
<td>0.8 (0.7, 1.0)</td>
</tr>
<tr>
<td>nonHDL (mmol/L)</td>
<td>4.0 (3.2, 4.7)</td>
<td>40 (3.3, 4.9)</td>
</tr>
<tr>
<td>Trig (mmol/L)</td>
<td>1.5 (1.1, 2.2)</td>
<td>1.5 (1.1, 2.2)</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.4 (1.1, 1.6)</td>
<td>1.3 (1.1, 1.7)</td>
</tr>
<tr>
<td>ApoA-I (g/L)</td>
<td>1.5 (1.4, 1.8)</td>
<td>1.5 (1.4, 1.8)</td>
</tr>
<tr>
<td>Lp(a) (µmol/L)</td>
<td>0.4 (0.2, 1.3)</td>
<td>0.4 (0.2, 1.2)</td>
</tr>
</tbody>
</table>

TC: Total cholesterol; LDL: Low density lipoprotein; ApoB: Apolipoprotein B; NonHDL: non-high density lipoprotein; Trig: Triglycerides; HDL: High density lipoprotein; ApoA-I: Apolipoprotein A-I; Lp(a): Lipoprotein (a)
Table 6.2: Association between lipid-related variables at study entry and hazard of relapse

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th></th>
<th>Adjusted*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard Ratio (95%CI)</td>
<td>p-value</td>
<td>Hazard Ratio (95%CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>BMI</td>
<td>1.03 (0.99, 1.06)</td>
<td>0.14</td>
<td>1.04 (1.01, 1.08)</td>
<td>0.014</td>
</tr>
<tr>
<td>Normal</td>
<td>1.00 [Reference]</td>
<td>1.00 [Reference]</td>
<td>1.00 [Reference]</td>
<td>1.00 [Reference]</td>
</tr>
<tr>
<td>Overweight</td>
<td>1.08 (0.69, 1.71)</td>
<td>0.73</td>
<td>1.44 (0.96, 2.16)</td>
<td>0.08</td>
</tr>
<tr>
<td>Obese</td>
<td>1.34 (0.78, 2.30)</td>
<td>0.29</td>
<td>1.78 (1.07, 2.96)</td>
<td>0.027</td>
</tr>
<tr>
<td>Trend</td>
<td>0.30</td>
<td>0.30</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>1.03 (0.81, 1.31)</td>
<td>0.80</td>
<td>1.16 (0.89, 1.53)</td>
<td>0.27</td>
</tr>
<tr>
<td>LDL (mol/L)</td>
<td>0.93 (0.70, 1.25)</td>
<td>0.64</td>
<td>1.05 (0.77, 1.44)</td>
<td>0.74</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>1.49 (0.38, 5.80)</td>
<td>0.57</td>
<td>2.75 (0.61, 12.39)</td>
<td>0.19</td>
</tr>
<tr>
<td>nonHDL (mmol/L)</td>
<td>1.02 (0.81, 1.28)</td>
<td>0.88</td>
<td>1.17 (0.90, 1.53)</td>
<td>0.23</td>
</tr>
<tr>
<td>Trig (mmol/L)</td>
<td>1.07 (0.94, 1.23)</td>
<td>0.31</td>
<td>1.20 (1.03, 1.40)</td>
<td>0.021</td>
</tr>
<tr>
<td>&lt;2.30</td>
<td>1.00 [Reference]</td>
<td>1.00 [Reference]</td>
<td>1.00 [Reference]</td>
<td>1.00 [Reference]</td>
</tr>
<tr>
<td>&gt;=2.30</td>
<td>1.31 (0.69, 2.47)</td>
<td>0.41</td>
<td>2.18 (1.12, 4.25)</td>
<td>0.022</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.12 (0.57, 2.22)</td>
<td>0.75</td>
<td>0.93 (0.44, 1.94)</td>
<td>0.84</td>
</tr>
<tr>
<td>ApoA-I (g/L)</td>
<td>1.63 (0.66, 4.05)</td>
<td>0.29</td>
<td>1.36 (0.56, 3.31)</td>
<td>0.49</td>
</tr>
<tr>
<td>Lp(a) (µmol/L)</td>
<td>1.02 (0.69, 1.50)</td>
<td>0.94</td>
<td>0.94 (0.563 1.42)</td>
<td>0.78</td>
</tr>
<tr>
<td>TC/HDL ratio</td>
<td>0.99 (0.78, 1.25)</td>
<td>0.92</td>
<td>1.16 (0.88, 1.52)</td>
<td>0.29</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>0.86 (0.61, 1.21)</td>
<td>0.38</td>
<td>1.03 (0.73, 1.46)</td>
<td>0.87</td>
</tr>
<tr>
<td>ApoB/ApoA-I ratio</td>
<td>0.68 (0.14, 3.24)</td>
<td>0.63</td>
<td>1.49 (0.24, 9.38)</td>
<td>0.67</td>
</tr>
</tbody>
</table>

*Adjusted for sex, age at study entry, BMI & study site

BMI: Body mass index; TC: Total cholesterol; LDL: Low density lipoprotein; ApoB: Apolipoprotein B; NonHDL: Non high density lipoprotein; Trig: Triglycerides; HDL: High density lipoprotein; ApoA-I: Apolipoprotein A-I; Lp(a): Lipoprotein a
6.6.3 Association between lipid-related variables and conversion to CDMS
Table 6.3 summarises the association between the serum lipid-related variables at study entry and conversion to CDMS. In the univariable analysis, HDL was significantly associated with conversion to CDMS (p-trend=0.042). Although the magnitude of effect remained robust, this became non-significant on adjustment for age at study entry, sex and study site (p-trend=0.14). Similarly, LDL/HDL ratio showed an inverse association with CDMS, but the association became non-significant upon adjustment (p-trend=0.15). Similar associations were observed for the corresponding ApoA-I and ApoB/ApoA-I ratio. From the analysis, TC, LDL, triglycerides, ApoB, BMI or any other serum lipid-related variable was not a significant predictor of conversion to clinical definite MS. The results were similar when we included the lipid levels at 2/3-year review to allow for possible changes over time.

6.6.3.1 Other predictors of conversion to clinically definite MS
Age at study entry was significantly associated with conversion to CDMS (HR: 0.97; 95% CI: 0.95, 0.99; p=0.008). Similarly, sex was a significant predictor of conversion to CDMS (HR: 1.94; 95% CI: 1.27, 3.97; p=0.012). Compared to New South Wales, Tasmania (HR: 0.51; 95% CI: 0.28, 0.95; p=0.033) as a study site was associated with a lower risk of conversion to CDMS, though the other sites did not materially differ. Neither smoking status (p=0.58), physical activity (p=0.25), 25(OH)D (p=0.94) nor statin medication use (p=0.45) was a significant predictor of conversion to CDMS.
<table>
<thead>
<tr>
<th>Table 6.3: Association between lipid-related variables at study entry and conversion to CDMS</th>
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<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td>BMI (Kg/m²)</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Overweight</td>
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<tr>
<td>Obese</td>
</tr>
<tr>
<td>Trend</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
</tr>
<tr>
<td>3.0-4.7</td>
</tr>
<tr>
<td>&gt;4.7-5.3</td>
</tr>
<tr>
<td>&gt;5.3-6.1</td>
</tr>
<tr>
<td>&gt;6.1-10.4</td>
</tr>
<tr>
<td>Trend</td>
</tr>
<tr>
<td>LDL (mol/L)</td>
</tr>
<tr>
<td>1.3-2.5</td>
</tr>
<tr>
<td>&gt;2.5-2.9</td>
</tr>
<tr>
<td>&gt;2.9-3.5</td>
</tr>
<tr>
<td>&gt;3.5-7.4</td>
</tr>
<tr>
<td>Trend</td>
</tr>
<tr>
<td>nonHDL (mmol/L)</td>
</tr>
<tr>
<td>1.9-3.1</td>
</tr>
<tr>
<td>&gt;3.1-3.7</td>
</tr>
<tr>
<td>&gt;3.7-4.6</td>
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<td></td>
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<tr>
<td>-------</td>
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<tr>
<td>&gt;4.6-9.2</td>
</tr>
<tr>
<td><strong>Trend</strong></td>
</tr>
<tr>
<td><strong>Trig (mmol/L)</strong></td>
</tr>
<tr>
<td>0.5-1.1</td>
</tr>
<tr>
<td>&gt;1.1-1.5</td>
</tr>
<tr>
<td>&gt;1.5-2.2</td>
</tr>
<tr>
<td>&gt;2.2-10.9</td>
</tr>
<tr>
<td><strong>Trend</strong></td>
</tr>
<tr>
<td><strong>HDL (mmol/L)</strong></td>
</tr>
<tr>
<td>0.8-1.3</td>
</tr>
<tr>
<td>&gt;1.3-1.5</td>
</tr>
<tr>
<td>&gt;1.5-1.8</td>
</tr>
<tr>
<td>&gt;1.8-2.8</td>
</tr>
<tr>
<td><strong>Trend</strong></td>
</tr>
<tr>
<td><strong>ApoA-I (g/L)</strong></td>
</tr>
<tr>
<td>0.9-1.3</td>
</tr>
<tr>
<td>&gt;1.3-1.5</td>
</tr>
<tr>
<td>&gt;1.5-1.7</td>
</tr>
<tr>
<td>&gt;1.7-2.6</td>
</tr>
<tr>
<td><strong>Trend</strong></td>
</tr>
<tr>
<td><strong>Lp(a) (µmol/L)</strong></td>
</tr>
<tr>
<td>0.05-0.2</td>
</tr>
<tr>
<td>&gt;0.2-0.4</td>
</tr>
<tr>
<td>&gt;0.4-1.1</td>
</tr>
<tr>
<td>&gt;1.1-3.3</td>
</tr>
<tr>
<td><strong>Trend</strong></td>
</tr>
<tr>
<td><strong>TC/HDL ratio</strong></td>
</tr>
<tr>
<td>2.0-2.9</td>
</tr>
<tr>
<td>&gt;2.9-3.5</td>
</tr>
<tr>
<td>&gt;3.5-4.3</td>
</tr>
<tr>
<td>&gt;4.3-8.9</td>
</tr>
<tr>
<td>Trend</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>0.8-1.5</td>
</tr>
<tr>
<td>&gt;1.5-2.0</td>
</tr>
<tr>
<td>2.0-2.5</td>
</tr>
<tr>
<td>2.5-6.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trend</th>
<th>ApoB/ApoA-I ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2-0.4</td>
<td>9/46.38 (0.19)</td>
</tr>
<tr>
<td>&gt;0.4-0.5</td>
<td>12/38.70 (0.31)</td>
</tr>
<tr>
<td>&gt;0.5-0.7</td>
<td>7/49.17 (0.14)</td>
</tr>
<tr>
<td>&gt;0.7-1.22</td>
<td>7/72.42 (0.10)</td>
</tr>
</tbody>
</table>

*Trend: 0.09 0.54

*Adjusted for sex, age at study entry, & study site

BMI: Body mass index; TC: Total cholesterol; LDL: Low density lipoprotein; ApoB: Apolipoprotein B; NonHDL: Non high density lipoprotein;
Trig: Triglycerides; HDL: High density lipoprotein; ApoA-I: Apolipoprotein A-I; Lp(a): Lipoprotein a
6.6.4 Associations between lipid-related variables and disability progression

We next sought to evaluate the relationship between lipid-related variables at study entry and the subsequent change in disability (Table 6.4). BMI at study entry was a significant predictor of subsequent annualised change in EDSS ($\beta$: 0.01; 95% CI: 0.003, 0.02; $p$=0.005). This association was robust to adjustment for age at study entry and sex ($\beta$: 0.01; 95% CI: 0.001, 0.013; $p$=0.01). Using log binomial regression, participants with BMI level in the overweight and obese category (BMI $\geq$25) had 60% increased risk of experiencing a change in EDSS greater than 0.5 after adjustment for confounders (RR: 1.60; 95% CI: 1.05, 2.45; $p$=0.029).

When we examined a related outcome variable, MSSS, we also observed a significant association between MSSS and BMI after adjusting for confounders ($\beta$: 0.09; 95% CI: 0.02, 0.15; $p$=0.008).

In relation to the lipids, in the basic model, TC ($p$=0.019), ApoB ($p$=0.028), nonHDL ($p$=0.009), triglycerides (0.016), TC/HDL ratio (0.013) and ApoB/ApoA-I ratio (0.047) were significantly associated with greater five-year change in EDSS. Upon further adjustment for age at study entry and sex, the association with TC ($\beta$: 0.04; 95% CI: 0.03, 0.07; $p$=0.032), ApoB ($\beta$: 0.26; 95% CI: 0.01, 0.52; $p$=0.042) nonHDL ($\beta$: 0.04; 95% CI: 0.01, 0.08; $p$=0.018), triglycerides ($\beta$: 0.04; 95% CI: 0.005, 0.07; $p$=0.026) and TC/HDL ratio ($\beta$: 0.06; 95% CI: 0.01, 0.10; $p$=0.019) remained significant. When these associations were further adjusted for BMI, only the association with TC/HDL ratio remained independently associated ($p$=0.044). There was no significant interaction between sex and TC/HDL ratio ($p$=0.40) and between TC/HDL ratio and age at study entry and between BMI and TC/HDL ratio ($p$=0.11).

Using log binomial regression, participants with TC/HDL ratio level greater than 4.0 have a more than doubled risk of experiencing an annualised change in EDSS greater than 0.5 points compared to participants with TC/HDL ratio level of $\leq$4.0 after adjustment for confounders.
(RR: 2.07; 95% CI: 1.40, 3.06; p<0.001). A TC/HDL ratio of 4 is considered a clinically high level for the risk of cardiovascular disease.

The direction of effect for TC/HDL was similar when we examined MSSS as an outcome (adjusted analysis β: 0.49 (-0.07, 1.04) p=0.09). With MSSS, significant associations were observed for nonHDL (β: 0.45; 95% CI: 0.01, 0.88; p=0.045) and a trend with TC (0.36 (-0.03, 0.76), p = 0.07), ApoB (2.68 (-0.01, 5.37), p = 0.051) and ApoB/ApoA-I ratio (3.36 (-0.56, 7.29), p = 0.09) after adjustment for confounding factors.

6.6.4.1 Other predictors of change in EDSS

Neither age at study entry (p=0.19), sex (p=0.74), smoking status (p=0.38), physical activity (p=0.90), 25(OH)D levels (p=0.26) nor statin use (p=0.07) were significantly associated with change in EDSS. Compared to New South Wales, there was no difference in the change in EDSS among participants from Tasmanian (p=0.92), Queensland (p=0.33) or Victoria (p=0.30).

<table>
<thead>
<tr>
<th>Table 6.4: Association between lipid-related variables at study entry and annualised change in EDSS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basic model</strong></td>
</tr>
<tr>
<td><strong>β (95%CI)</strong></td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
</tr>
<tr>
<td>LDL (mol/L)</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
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<tr>
<td>nonHDL (mmol/L)</td>
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<td>Trig (mmol/L)</td>
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<td>HDL(mmol/L)</td>
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<td>ApoA-I (g/L)</td>
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<td>Lp(a) (µmol/L)</td>
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<tr>
<td>TC/HDL ratio</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
</tr>
<tr>
<td>ApoB/ApoA-I ratio</td>
</tr>
</tbody>
</table>

# Adjusted for relapse at the time of review
*Adjusted for sex, age at study entry, study site and BMI
Figure 6.1: Kaplan-Meier survival plots by category of BMI at study entry. The plots show the proportion of subjects relapse-free each day since study entry.

6.7 Discussion
Using a prospective cohort design in people with a first clinical diagnosis of CNS demyelinating disease, we found that a higher BMI and TC/HDL ratio at study entry was associated with a higher annual change in clinical disability, and higher BMI and triglycerides were associated with increased risk of subsequent relapse but not conversion to CDMS, suggesting that improving lipid profile and losing weight into the healthy range may improve the disease course of MS.

We found that higher BMI and TC/HDL ratio were independently associated with a higher annual change in EDSS. Similar findings were seen with MSSS. Overweight and obese people had a 60% increased risk of experiencing an annualised change in EDSS greater than 0.5 and those with a TC/HDL ratio level greater than 4.0 had a doubling in risk of
experiencing an annualised change in EDSS greater than 0.5 points. The same was found in our previous study in people with established MS, with the same magnitude of effect. It is also in agreement with previous work by Weinstock-Guttman and colleagues who showed that higher baseline LDL, TC and triglycerides were associated with a worsening in EDSS and MSSS over 2.2 years after adjustment for age and sex. The association between BMI and annual change in EDSS was not observed in our previous study.

In the relapse analysis, we found that being obese was associated with a 1.78 fold higher risk of relapse compared to those of normal weight (HR: 1.78; 95% CI: 1.07, 2.96; p=0.027). In addition, an independent effect was observed for those with high triglycerides, where those with clinically high levels (≥2.30 mmol/L) having a 2.18 fold risk of relapse compared to those with lower levels (HR: 2.18; 95% CI: 1.12, 4.25; p=0.022). These findings do not align with two earlier studies from our group and Weinstock-Guttman and colleagues. The reason for this disparity may be due to the fact that participants of the current study were at a much earlier disease state (FDE/CIS) compared to the participants of the Tasmanian MS Longitudinal study (MSL study) who were prevalent cases of CDMS with longer disease duration (median: 12 years) and largely on treatment, thus significantly reducing the relapse rate in that group. In the current study treatment was not as widely applied and not instituted after CIS in the vast majority of cases (slow uptake of immunomodulatory therapy after conversion of CDMS with 52% of participants on therapy at the five year review). Further, the study by Weinstock-Guttman and colleagues was an RCT while ours was a population-based cohort study.

The lack of significant association between serum lipid-related variables and conversion to clinically definite MS in our study is in line with the findings of from Weinstock-Guttman and colleagues. The study by these authors prospectively followed a cohort of participants with a clinically isolated syndrome (CIS) treated with interferon-β and reported finding no
significant association between serum lipid-related variables and conversion to CDMS. While this finding needs further investigation, it is suggesting that serum lipid-related variables may not be as strongly associated with the pathophysiological mechanisms that are involved in susceptibility to MS but rather with the clinical course of the disease.

The findings that BMI and adverse lipid levels are associated with disease course raises the crucial question whether interventions aimed at lowering adverse serum lipid levels into healthy ranges may reduce the accumulation of disability in people with MS. This question was partly answered by a recent phase II placebo-controlled trial conducted by Chataway and colleagues. In that trial, MS patients using high dose simvastatin had a lower change in EDSS after two years compared to the placebo arm (difference -0.254; 95% CI: -0.464 to -0.069). Similarly, a trial by Togha and colleagues demonstrated a lower EDSS score in MS patient on both simvastatin and interferon beta-1a compared to those on only interferon beta-1a. However, a trial conducted to determine the efficacy of a combined therapy of low-dose atorvastatin plus high-dose interferon beta-1a showed that, adding low-dose atorvastatin was not beneficial in terms of reducing the progression of disability. This disparity in outcome may stem from the type of statin used, dosing of statin and whether the statin is combined with immunomodulatory therapy or not. On the other hand, due to the pleomorphic therapeutic effect of statins, the benefit of reducing the rate of relapse and disability progression in MS may not be directly related to its effect on lipid levels but rather it may be due to the anti-inflammatory and immunomodulatory properties of statin. Further studies unravelling these properties will open-up therapeutic opportunities for people with MS.

The mechanisms by which BMI and serum lipid-related variables may influence MS disability and risk of relapse are not yet established. However, serum lipid-related variables may act through the lipid pathway where adverse serum lipid levels may directly influence disability levels or lead to increased weight and reduced physical activity, which may also
lead to increased disability in MS patients. Alternatively, there may be shared pathway between vitamin D and lipids where adverse lipid levels may lead to increased weight and reduced physical activity, which may also lead to reduced vitamin D to yield increased disability.

Our study has significant strengths, including being a prospective population-based cohort study with the capability to adjust for relevant confounders. This study has a longer follow-up period (5 years) which improves upon our previous study with only 2.3-year average follow-up period. This is important because a longer follow-up is preferable when measuring change in disability. Also, lipids and BMI at study entry were measured prior to the accrual of disability and associated with subsequent change over five years, therefore limiting the potential for reverse causality. The study had some limitations. Some associations have been observed with MRI markers, but we could not examine that in this study. We used non-fasting serum samples, which may have influenced the levels of triglycerides but this is becoming more accepted as a better measure of overall lipid and metabolic status.

In conclusion, we have demonstrated that higher BMI and TC/HDL ratio were associated with a higher rate of disability progression, and higher BMI and triglycerides were associated with relapse but not conclusively with conversion to CDMS, suggesting that improving lipid profile and losing weight into the healthy range may reduce the accumulation of disability. It is now important to test this in trials.

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6.8 Postscript
This chapter has provided some evidence on how lipid-related variables may influence MS relapse and disability progression. The next chapter describes the frequency of comorbidities and their association with clinical disability and relapse in people with MS.

6.9 References


15. Hedstrom AK, Olsson T, Alfredsson L. High body mass index before age 20 is associated with increased risk for multiple sclerosis in both men and women. Mult Scler 2012;18:1334-1336.


Chapter 7  Frequency of comorbidities and their association with clinical disability and relapse in multiple sclerosis

7.1 Preface
The manuscript presented in this chapter has been published. The typeset version of the manuscript as it appeared in the journal is in Appendix 7A. The text of this chapter is the same as the published version. This investigation describes frequency of comorbidities and their association with clinical disability and relapse in people with MS.

7.2 Abstract
Background: MS patients may be at an increased risk of comorbidities due to the debilitating and chronic nature of the disease. An increased understanding of comorbidities and disease course in MS may provide new insights and enhance MS management. We aimed to investigate the frequency of comorbidities and their associations with clinical disability and relapse in MS.

Methods: A prospective cohort of 198 MS patients, followed 2002–2005, and queried about specific doctor-diagnosed comorbidities. Prevalences of comorbidities in the MS cohort were compared to the 2007 general population in Australia. Multilevel mixed-effects linear regression was used to assess the difference in subsequent disability between those who reported comorbidities and those who did not. The association with hazard of relapse was assessed using survival analysis.

Results: The age-standardised prevalences of hypertension, dyslipidaemia, asthma, psoriasis, eczema and anaemia were significantly higher in the MS cohort compared to the general Australian population. The level of disability (MSSS) in those who reported overweight/obesity (β: 0.76 (95%CI: 0.04, 1.48), p=0.037), or dyslipidaemia (β: 1.05 (95%CI: 0.07, 2.02), p=0.036) was significantly higher compared to those who did not report
these comorbidities, even after adjustment for potential confounders. There were no significant associations between comorbidities and change in disability. For relapse analyses, rheumatoid arthritis and anaemia were associated with more than three-fold (HR: 3.70 (95% CI: 1.80, 7.58), p<0.001) and two-fold (HR: 2.04 (95% CI: 1.11, 3.74), p=0.022) increased risk of subsequent relapse respectively.

**Conclusions:** The prevalences of some comorbidities were higher in MS patients and associated with greater disability and relapse risk. Treatment of these comorbidities in patients with MS has the potential to improve disease course and understanding of the prognosis and outcomes of MS.

### 7.3 Introduction

Comorbidities, referring to the presence of one or more additional diseases co-occurring with a primary disease, are relatively common in the general population\(^1, 2\) and their frequency increases with age.\(^3\) These comorbidities are associated with adverse health outcomes\(^1\), a negative impact on quality of life, increased health care use and concomitant increased medical costs.\(^4\)

In people with MS, increasing evidence suggests that cardiovascular and immune comorbidities are prevalent\(^5, 6\) and may be associated with the clinical course of the disease,\(^7\) disability progression\(^5\) and health-related quality of life,\(^8\) and with increased mortality.\(^9\) The association of comorbidities with MS has prompted some authors to suggest that comorbidities may be partly responsible for the highly variable inter and intra-personal clinical course of MS.\(^5\)

Despite their potential importance in understanding the aetiology and heterogeneity of MS, little and inconsistent information exists about the prevalence of cardiovascular and immune
comorbidities in MS patients and how they may affect the progression of the disease. From the existing literature it is also unclear whether people with MS are at increased or reduced risk of cardiovascular and immune comorbidities compared with the general population.\textsuperscript{10}

This analysis aimed to determine whether MS patients were at differential risk of comorbidities compared with that of the general Australian population. We also sought to determine whether reporting comorbidity was prospectively associated with subsequent level of disability and risk of relapse in participants with MS.

\subsection*{7.4 Methodology}

\subsubsection*{7.4.1 Study design}

The Southern Tasmanian Multiple Sclerosis Longitudinal (MSL) Study was a prospective population-based cohort study, which followed a population of 203 persons with clinically definite MS\textsuperscript{11} living in southern Tasmania, Australia from 2002–2005. An estimated 78\% (203/259) of eligible cases were included and the study retention rate was 90\%. Ethics approval was obtained from the Southern Tasmania Human Research Ethics Committee and all participants provided informed consent.

The study methodology has been previously described.\textsuperscript{12} Briefly, at each biannual review participants were asked about their lifestyle, including physical activity, smoking, vitamin D supplement use and dosage, and medication use. Using a questionnaire modelled on that used by the Australian Bureau of Statistics, participants were also asked whether a doctor had diagnosed them with a list of specific conditions and at what age the onset of the condition was. The comorbidities included in this study were based on those frequently reported in people with MS.\textsuperscript{6} Height and weight was measured at baseline. Expanded Disability Status Scale (EDSS)\textsuperscript{13} were assessed annually by a single physician and Multiple Sclerosis Severity Score (MSSS) was calculated from the EDSS and disease duration by comparing it to the global MSSS reference dataset.\textsuperscript{14}
7.4.2 Measurement of relapses
Using a real-time relapse notification system, participants telephoned the study centre if they thought they were experiencing a relapse. Additionally, at each biannual review, participants were queried about the occurrence of a relapse in the preceding 6 months. To ensure each relapse was a true relapse, the study nurse or physician administered a relapse questionnaire detailing relapse symptoms, medical practitioner review, treatment, and co-occurrence of infection or fever. For quality control, the study physician also performed a physical examination for those reporting a relapse by telephone. Throughout the study, each relapse was reviewed rigorously by the study physician and further by the study neurologist.

7.4.3 Statistical analysis
Prevalence of comorbidities in the MSL study were estimated and compared to that of the general Australian population estimates in the 2007 National Health Survey by the Australian Bureau of Statistics.\textsuperscript{15}

Information about the National Health Survey design and methodology, the quality and interpretation of results has been described.\textsuperscript{15} Briefly, approximately 22,000 people from all States and Territories and across all age groups were included in the survey. Estimates from the survey were obtained using a complex estimation procedure which ensures that survey estimates compares with independent population estimates by state, part of state, age and sex. The survey was designed to acquire national benchmarks on a range of health issues, and to allow changes in health to be surveyed over time. Information was also obtained about the health status of the population, health-related aspects of lifestyle and other health risk factors and the use of health services and other actions people had recently taken for their health.

Log-binomial regression was used to estimate the prevalence ratio of comorbidities in people with MS at study entry compared with that of the general Australian population. Age
standardisation was calculated by the direct method. Associations between comorbidities and measured disability (EDSS, MSSS) or disability progression (annual change in EDSS) were assessed by multilevel mixed-effects linear regression to take into account the repeated measures. EDSS/MSSS was also dichotomised and its relationship comorbidities assessed using log-binomial regression. Associations with disability were adjusted for relevant confounders, including having a relapse at the time of disability assessment, age at study entry, sex, BMI (kg/m²), physical activity (Mets) and smoking status (no, yes). Log-binomial regression was used to assess the relationship between comorbidities and sustained disability progression. Sustained disability progression is defined as having a deterioration of at least 0.5 in consecutive measures of EDSS compared to baseline EDSS.

For relapse analyses, the effect of comorbidity on time-to-relapse was calculated using Cox proportional hazard models for repeated events, whereby multiple relapses by the same persons are treated as independent observations but accounted for at the intra-individual level, and the time until a prior event does not influence the composition of the risk set for a subsequent event. All covariates satisfied the proportional hazard assumption with the exception of the binary variable for sex and the categorical variable for baseline EDSS (0–<3, 3–<5.5, 5.5–<7.5, 7.5–9). For this reason, all models were stratified to allow the baseline hazards to differ by sex and baseline EDSS category. Associations with relapse were adjusted for relevant confounders, including age at study entry, BMI (kg/m²), physical activity (Mets), vitamin D, smoking status (no, yes), statin use (yes/no) and immunomodulatory therapy use (yes/no). All analyses were done using STATA/IC for Windows (Version 12.1; StataCorp LP College Station USA). We did not conduct adjustment for multiple comparisons because although this reduces the likelihood of type I error, it increases the likelihood of type II error. We have used epidemiologic analysis to assist the assessment of whether an
association has causal features and to exclude non-causal explanations, such as confounding. Significance level was set at \( p < 0.05 \).

7.5 Results

7.5.1 Participant characteristics
Table 7.1 summarises the clinical and demographic characteristics of the MS cohort at study entry. The cohort was 72% female, and 75.3% of participants were of relapsing-remitting MS type, 62.9% were overweight or obese \((\geq 25 \text{ kg/m}^2)\), and 72.5% were on immunomodulatory therapy during the study, largely interferon-β-based medications. The median EDSS at study entry was 3.0 and median MS duration from diagnosis was six years.

Table 7.1: Demographic and clinical characteristics of the MS cohort at study entry

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex</td>
<td>128/198 (72)</td>
</tr>
<tr>
<td>MS course at study entry</td>
<td></td>
</tr>
<tr>
<td>Relapsing-remitting MS</td>
<td>149/198 (75.3)</td>
</tr>
<tr>
<td>Secondary Progressive MS</td>
<td>40/198 (20.2)</td>
</tr>
<tr>
<td>Primary Progressive MS</td>
<td>9/198 (4.5)</td>
</tr>
<tr>
<td>Used Immunomodulatory therapy during study?</td>
<td>132/198 (66.7)</td>
</tr>
<tr>
<td>Smoker during study</td>
<td>53/198 (26.8)</td>
</tr>
<tr>
<td>Body Mass Index ( \text{Kg/m}^2 )</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>66/178 (37.1)</td>
</tr>
<tr>
<td>Overweight</td>
<td>75/178 (42.1)</td>
</tr>
<tr>
<td>Obese</td>
<td>37/178 (20.8)</td>
</tr>
<tr>
<td>Age at study entry</td>
<td>47.4 (11.21; 21-77)</td>
</tr>
<tr>
<td>MS duration from diagnosis (years)</td>
<td>6.0 (3.0, 12.0)</td>
</tr>
<tr>
<td>EDSS at study entry</td>
<td>3.0 (2.0, 4.5)</td>
</tr>
<tr>
<td>MSSS at study entry</td>
<td>3.8 (2.2, 5.7)</td>
</tr>
<tr>
<td>Physical activity (Mets)</td>
<td>20.1 (6.0, 43.0)</td>
</tr>
</tbody>
</table>
Table 7.2: Prevalence of comorbidities in MS patients compared to the general population at study entry

<table>
<thead>
<tr>
<th>Comorbidities</th>
<th>Total number (percentage)</th>
<th>Prevalence ratio (PR): (95% CI)</th>
<th>Prevalence ratio (PR): (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS: (198) n (%)</td>
<td>Crude</td>
<td>Age standardised</td>
</tr>
<tr>
<td></td>
<td>GP: (20,643,100) n '000 (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight/obesity</td>
<td>112 (62.92)*</td>
<td>1.03 (0.92-1.15)</td>
<td>0.96 (0.79, 1.16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.66</td>
<td>p=0.49</td>
</tr>
<tr>
<td>Hypertension</td>
<td>42 (21.21)</td>
<td>2.25 (1.72, 2.94)</td>
<td>1.73 (1.25, 2.34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Type 1 diabetes</td>
<td>2 (1.01)</td>
<td>2.55 (0.64, 10.12)</td>
<td>2.01 (0.23, 7.27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.18</td>
<td>p=0.31</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>4 (2.02)</td>
<td>0.58 (0.22, 1.53)</td>
<td>0.71 (0.19, 1.83)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.27</td>
<td>p=0.49</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>29 (14.65)</td>
<td>2.56 (1.83, 3.59)</td>
<td>1.83 (1.23, 2.63)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>15 (7.58)</td>
<td>3.31 (2.04, 5.39)</td>
<td>2.65 (1.48, 4.38)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>4 (2.02)</td>
<td>0.97 (0.37, 2.57)</td>
<td>0.69 (0.19, 1.76)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.96</td>
<td>p=0.44</td>
</tr>
<tr>
<td>Thyroid disease</td>
<td>11 (5.56)</td>
<td>2.36 (1.33, 4.19)</td>
<td>1.67 (0.83, 3.00)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.003</td>
<td>p=0.08</td>
</tr>
<tr>
<td>Asthma</td>
<td>37 (18.69)</td>
<td>1.88 (1.41, 2.52)</td>
<td>1.85 (1.28, 2.58)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Hay fever</td>
<td>30 (15.15)</td>
<td>1.01 (0.72, 1.40)</td>
<td>0.85 (0.57, 1.23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.97</td>
<td>p=0.35</td>
</tr>
<tr>
<td>Eczema</td>
<td>26 (13.13)</td>
<td>14.31 (10.0, 20.47)</td>
<td>26.20 (17.11, 38.38)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Anaemia</td>
<td>25 (12.63)</td>
<td>6.90 (4.78, 9.95)</td>
<td>5.68 (3.64, 8.45)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>1 (0.51)</td>
<td>3.60 (0.51, 25.40)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Condition</td>
<td>GP (p)</td>
<td>BMI (SD)</td>
<td>Obs (p)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>1 (0.51)</td>
<td>35.1 (0.17)</td>
<td>2.97 (0.42, 20.98)</td>
</tr>
<tr>
<td>Coeliac disease</td>
<td>2 (1.01)</td>
<td>41.286 (0.20)</td>
<td>5.05 (1.27, 20.05)</td>
</tr>
</tbody>
</table>

*178 of participants with BMI data, GP= General population, NA= Not applicable, NC= No case, ND= No data
7.5.2 Prevalences of comorbidities
Table 7.2 presents the prevalence, prevalence ratio and standardisation of comorbidities by sex and age. In the age-standardised prevalence analysis, patients with MS were more likely than the general Australian population to have hypertension and dyslipidaemia. A significantly higher prevalence in the MS cohort than the general population was also observed for psoriasis, asthma, eczema and anaemia, with a particularly strong magnitude of association for eczema (PR: 26.20 (95% CI: 17.11, 38.38)).

Prevalences of comorbidities were also stratified by sex. In these analyses, both male and female MS patients had higher prevalences of vascular comorbidities like hypertension and dyslipidaemia than males and females in the general population and the increased prevalence in the MS cohort was generally higher among females than males, but not statistically significantly. Similarly, the prevalence of psoriasis, eczema and asthma in both males and females were significantly higher in the MS cohort than males and females in the general population.

7.5.3 Association between comorbidities and disability and progression in disability
We next examined whether having particular comorbidities was associated with a higher disability and disability progression (Table 7.3). From Model 1 (adjusted for relapse at the time of disability measurement), MS patients reporting overweight/obesity (β: 0.65 (95% CI: 0.09, 1.21), p=0.024), hypertension (β: 1.25 (95% CI: 0.43, 2.06), p=0.003) and dyslipidaemia (β: 0.99 (95% CI: 0.05, 1.92) p=0.038), had higher EDSS compared to those who did not report these comorbidities. Further adjustment for sex, BMI, physical activity, MS duration and smoking status did not substantially reduce the association of disability with reporting hypertension (β: 1.15 (95% CI: 0.49, 1.81), p=0.001) and dyslipidaemia (β: 0.97 (95% CI: 0.24, 1.69), p=0.009). However, further adjustment for age at study entry (Model 2)
attenuated the associations with overweight/obesity, hypertension and dyslipidaemia, suggesting that these associations were at least partly driven by older age.

The results were similar when EDSS was dichotomized into EDSS<4.0 and EDSS>=4.0. From this adjusted analysis, dyslipidaemia (Adjusted Prevalence ratio (PR): 1.23 (95% CI: 1.01, 1.50), p=0.041), hypertension (Adjusted PR: 1.24 (95% CI: 1.02, 1.51), p=0.034) and hay fever (Adjusted PR: 1.35 (95% CI: 1.09, 1.69), p=0.007) were associated with increased risk of higher disability compared to those who did not report these comorbidities.

For associations with MSSS, similar results were found to those for EDSS. However, even after adjustment for confounders, MS patients reporting overweight/obesity (β: 0.76 (95% CI: 0.04, 1.48), p=0.037) and dyslipidaemia (β: 1.05 (95% CI: 0.07, 2.02), p=0.036) had significantly higher MSSS compared to those who did not report these comorbidities. Additionally, the combined effect of reporting hypertension, diabetes and dyslipidaemia (vascular disease group) was associated with significantly higher EDSS (β: 0.64 (95% CI: 0.14, 1.14), p=0.012) and MSSS (β: 0.90 (95% CI: 0.10, 1.69), p=0.027) compared to those who did not report these comorbidities, persisting on adjustment for confounders.

When MSSS was dichotomized into MSSS<4.0 and MSSS>=4.0. From this adjusted analysis, dyslipidaemia (Adjusted Prevalence ratio (PR): 1.32 (95% CI: 1.11, 1.58), p=0.002), overweight/obesity (Adjusted PR: 1.31 (95% CI: 1.08, 1.59), p=0.006 and thyroid disease (Adjusted PR: 1.34 (95% CI: 1.09, 1.66), p=0.005) were associated with increased risk of higher disability compared to those who did not report these comorbidities.

For other comorbidities, such as psoriasis, rheumatoid arthritis, thyroid dysfunction, asthma, hay fever, eczema, anaemia, the disability level of those who reported these comorbidities was not significantly different from those who did not.
We found no significant associations between comorbidities and annual change in EDSS (data not shown).
<table>
<thead>
<tr>
<th>Comorbidity</th>
<th>EDSS: Model 1</th>
<th>p-value</th>
<th>EDSS: Model 2</th>
<th>p-value</th>
<th>MSSS: Model 1</th>
<th>p-value</th>
<th>MSSS: Model 2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overweight/obesity</td>
<td>0.65 (0.09, 1.21)</td>
<td>p=0.024</td>
<td>0.35 (-0.09, 0.80)**</td>
<td>p=0.12</td>
<td>0.82 (0.09, 1.55)</td>
<td>p=0.027</td>
<td>0.76 (0.04, 1.48)**</td>
<td>p=0.037</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.25 (0.43, 2.06)</td>
<td>p=0.003</td>
<td>0.46 (-0.12, 1.04)</td>
<td>p=0.12</td>
<td>0.83 (-0.12, 1.78)</td>
<td>p=0.09</td>
<td>0.07 (-0.81, 0.96)</td>
<td>p=0.87</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>0.99 (0.05, 1.92)</td>
<td>p=0.038</td>
<td>0.44 (-0.16, 1.05)</td>
<td>p=0.15</td>
<td>1.03 (-0.08, 2.14)</td>
<td>p=0.07</td>
<td>1.05 (0.07, 2.02)</td>
<td>p=0.036</td>
</tr>
<tr>
<td>Vascular disease group*</td>
<td>1.52 (0.81, 2.23)</td>
<td>p&lt;0.001</td>
<td>0.64 (0.14, 1.14)</td>
<td>p=0.012</td>
<td>1.87 (0.61, 2.30)</td>
<td>p=0.001</td>
<td>0.90 (0.10, 1.69)</td>
<td>p=0.027</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>0.64 (-0.59, 1.88)</td>
<td>p=0.31</td>
<td>-0.03 (-0.85, 0.79)</td>
<td>p=0.94</td>
<td>0.89 (-0.58, 2.37)</td>
<td>p=0.24</td>
<td>0.19 (-1.11, 1.49)</td>
<td>p=0.78</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>0.46 (-1.83, 2.75)</td>
<td>p=0.69</td>
<td>0.71 (-0.78, 2.19)</td>
<td>p=0.35</td>
<td>0.67 (-2.09, 3.43)</td>
<td>p=0.63</td>
<td>0.69 (-1.63, 3.00)</td>
<td>p=0.56</td>
</tr>
<tr>
<td>Thyroid dysfunction</td>
<td>0.83 (-0.48, 2.14)</td>
<td>p=0.22</td>
<td>0.47 (-0.35, 1.30)</td>
<td>p=0.26</td>
<td>0.82 (-0.72, 2.36)</td>
<td>p=0.30</td>
<td>1.00 (-0.33, 2.33)</td>
<td>p=0.14</td>
</tr>
<tr>
<td>Asthma</td>
<td>-0.65 (-1.38, 0.08)</td>
<td>p=0.08</td>
<td>0.02 (-0.51, 0.56)</td>
<td>p=0.93</td>
<td>-0.29 (-1.20, 0.63)</td>
<td>p=0.54</td>
<td>0.04 (-0.76, 0.87)</td>
<td>p=0.92</td>
</tr>
<tr>
<td>Hay fever</td>
<td>-0.40 (-1.22, 0.41)</td>
<td>p=0.34</td>
<td>0.21 (-0.36, 0.78)</td>
<td>p=0.48</td>
<td>-0.12 (-1.13, 0.89)</td>
<td>p=0.82</td>
<td>0.36 (-0.53, 1.26)</td>
<td>p=0.43</td>
</tr>
<tr>
<td>Eczema</td>
<td>-0.42 (-1.29, 0.44)</td>
<td>p=0.34</td>
<td>0.16 (-0.47, 0.78)</td>
<td>p=0.62</td>
<td>0.21 (-0.89, 1.31)</td>
<td>p=0.71</td>
<td>0.38 (-0.61, 1.37)</td>
<td>p=0.45</td>
</tr>
<tr>
<td>Anaemia</td>
<td>0.08 (-0.85, 1.01)</td>
<td>p=0.87</td>
<td>0.34 (-0.30, 0.98)</td>
<td>p=0.30</td>
<td>0.46 (-0.68, 1.60)</td>
<td>p=0.43</td>
<td>0.79 (-0.24, 1.81)</td>
<td>p=0.13</td>
</tr>
</tbody>
</table>

*Vascular disease group: Hypertension, Types I & II Diabetes, Dyslipidaemia

Model 1: Adjusted for relapse at the time disability measurement

Model 2: Adjusted for relapse, age, sex, BMI, physical activity, smoking status (yes/no) and MS duration (MSSS analysis not adjusted for MS duration)

**Adjusted for relapse, age, sex, physical activity, smoking status and MS duration
7.5.3.1 Sensitivity analysis

We conducted a sensitivity analysis excluding those people whose disability was measured during a relapse. The findings were unchanged. The level of disability (MSSS) in those who reported overweight/obesity (β: 0.76 (95%CI: 0.03, 1.47), p=0.042), or dyslipidaemia (β: 1.04 (95%CI: 0.05, 2.03), p=0.039) compared to those who did not report these comorbidities were not significantly affected. When the relationship between comorbidities and sustained disability progression was examined, there was a significant positive association with hay fever (Adjusted PR: 1.74 (95%CI: 1.45, 2.11), p<0.001) and a trend with overweight/obesity (Adjusted PR: 1.21 (95%CI: 0.97, 1.49), p=0.09).

7.5.4 Association between comorbidities and risk of subsequent relapse

During the study, a total of 122 confirmed relapses occurred in 70 out of the 149 patients with relapsing-remitting MS (RRMS). The mean age was 45.6 years, 75% were female, the average BMI was 26.8 kg/m² and 82% was on immunomodulatory therapy. We found significant positive associations between rheumatoid arthritis, and anaemia and the risk of subsequent relapse. The association with rheumatoid arthritis was more than three-fold (HR: 3.70 (95% CI: 1.80, 7.58), p<0.001) and that with anaemia was two-fold (HR: 2.04 (95% CI: 1.11, 3.74), p=0.022). These associations were robust to the adjustment for potential confounders, including age at study entry, BMI, physical activity, smoking during study, vitamin D, statin use and use of immunomodulatory therapy during the study (Table 7.4).
Table 7.4: Association between comorbidity and hazard of relapse

<table>
<thead>
<tr>
<th>Condition</th>
<th>Unadjusted Hazard Ratio (95%CI)</th>
<th>p-value</th>
<th>Adjusted* Hazard Ratio (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overweight/obesity</td>
<td>1.20 (0.78, 1.87)</td>
<td>0.41</td>
<td>1.16 (0.61, 2.22)</td>
<td>0.65</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.09 (0.57, 2.09)</td>
<td>0.78</td>
<td>1.08 (0.53, 2.20)</td>
<td>0.84</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>0.97 (0.50, 1.89)</td>
<td>0.93</td>
<td>0.79 (0.31, 2.01)</td>
<td>0.62</td>
</tr>
<tr>
<td>Vascular disease group</td>
<td>1.12 (0.66, 1.91)</td>
<td>0.67</td>
<td>1.06 (0.57, 1.94)</td>
<td>0.86</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>0.62 (0.27, 1.44)</td>
<td>0.27</td>
<td>0.50 (0.18, 1.42)</td>
<td>0.20</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>3.02 (1.94, 4.69)</td>
<td><strong>0.001</strong></td>
<td>3.70 (1.80, 7.58)</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Thyroid dysfunction</td>
<td>1.00 (0.46, 2.15)</td>
<td>1.00</td>
<td>1.12 (0.50, 2.49)</td>
<td>0.79</td>
</tr>
<tr>
<td>Asthma</td>
<td>1.04 (0.63, 1.72)</td>
<td>0.88</td>
<td>1.09 (0.27, 1.44)</td>
<td>0.78</td>
</tr>
<tr>
<td>Hay fever</td>
<td>1.22 (0.72, 2.07)</td>
<td>0.46</td>
<td>1.11 (0.62, 2.00)</td>
<td>0.72</td>
</tr>
<tr>
<td>Eczema</td>
<td>1.31 (0.79, 2.17)</td>
<td>0.30</td>
<td>1.26 (0.74, 2.16)</td>
<td>0.40</td>
</tr>
<tr>
<td>Anaemia</td>
<td>1.81 (1.05, 3.16)</td>
<td><strong>0.034</strong></td>
<td>2.04 (1.11, 3.74)</td>
<td><strong>0.022</strong></td>
</tr>
</tbody>
</table>

*Adjusted for age, BMI, physical activity, smoking during study (yes/no), vitamin D, statin use (yes/no) & Immunomodulatory therapy use (yes/no)

7.6 Discussion

Using a cohort of clinically definite MS patients, with the aim to determine whether they had different frequencies of comorbidities than that of the general population, we found that the age-standardised prevalence of hypertension, dyslipidaemia, psoriasis, eczema, asthma and anaemia was significantly higher in our MS cohort compared to the general Australian population. In the disability analysis, reporting overweight/obesity and dyslipidaemia was most consistently associated with a higher disability as measured by EDSS or MSSS measures. In the relapse analysis, rheumatoid arthritis and anaemia were associated with an increased risk of subsequent relapse.
In the regression analysis, the unadjusted analysis showed a significantly higher level of disability as measured by EDSS in MS patients who reported overweight/obesity, hypertension and dyslipidaemia compared to those who did not report these comorbidities. Adjustment for age at study entry substantially attenuated the associations, suggesting that advancing age of participants may explain a large part of the relationship between EDSS and these comorbidities. Despite this, age-independent associations were observed between overweight/obesity and dyslipidaemia and MSSS, which was also observed when we dichotomised MSSS. We also found a significant positive association between hay fever and sustained disability progression. Also, the combined effect of these vascular comorbidities was associated with significantly higher disability after adjusting for confounders including age at study entry and BMI. We found no association with annual change in disability. The findings on the level of disability were consistent with our previous work using this cohort where we reported significant positive associations between BMI (β: 0.06 (95%CI; 0.01, 0.10) p=0.013), total cholesterol (β: 0.23 (95%CI; (0.01, 0.44)) p=0.037) and disability.19 Our findings are consistent with work by Weinstock-Guttman20 and colleagues who reported that dyslipidaemia is associated with higher disability in MS. Similarly, Marrie and colleagues5 reported that people who reported comorbidities of diabetes, hypertension and dyslipidaemia have increased risk of ambulatory disability compared to those who did not. With reference to other comorbidities such as, psoriasis, rheumatoid arthritis, thyroid dysfunction, asthma, hay fever, eczema, and anaemia, the level of disability in those who reported developing these comorbidities was not significantly different from those who did not report these comorbidities although numbers were small.

In the relapse analysis, rheumatoid arthritis was associated with a more than three-fold increased hazard of subsequent relapse and anaemia was associated with a two-fold increased hazard of relapse. To the best of our knowledge, we are the first to report a relationship
between these comorbidities and hazard of relapse. Similar to our study, Marrie and colleagues investigated the relationship between comorbidities and relapsing-remitting course at MS onset.\(^7\) In that study, both men and women who reported mental comorbidities had an increased odds of a relapsing-remitting course at MS onset compared to participants who did not report these comorbidities (OR: 1.48; 1.08–2.01) and also gastrointestinal comorbidities (OR 1.78; 1.25–2.52) and obesity (OR 2.08 1.53–2.82) at MS onset were associated with a relapsing-remitting course at onset in women but not in men. In the wake of the increasing prevalence of relapsing-remitting MS, finding a relationship between comorbidity and relapse raises the possibility that comorbid conditions could be a significant factor in this phenomenon. The association between relapse and rheumatoid arthritis however need to be interpreted with caution. This is because the small number of participants reporting rheumatoid arthritis limits the interpretation of this association.

Our finding of higher prevalence of comorbidities in MS patients is generally consistent with the literature. For example, Kang and colleagues\(^{21}\) reported a higher prevalence of hypertension and dyslipidaemia in MS cases compared to matched controls. However, their finding of a higher prevalence of type 2 diabetes was not found in our study. For patients with MS compared to control groups, the prevalences of dyslipidaemia\(^{21, 22}\), diabetes\(^{21, 22}\) and hypertension\(^{21, 22}\) have been found to be higher in some studies, while other studies found similar prevalences\(^{23}\) or lower prevalence\(^{24, 25}\) of these comorbidities among persons with MS compared to control groups. The prevalence of obesity has also been found to be higher\(^{26}\), similar\(^{27}\) or lower\(^{24, 26}\) in MS cases compared to a control group in different studies. However, evaluation of the whole body of evidence together suggests a slightly elevated prevalence of these cardiovascular comorbidities in MS than the general population.

In relation to other comorbidities, our findings are in line with Kang and colleagues who reported a higher prevalence of asthma in MS cases compared to matched controls,\(^{21}\) and
Edwards and colleagues who found higher prevalences of asthma, eczema, and anaemia in MS patients compared to matched controls. In relation to autoimmune diseases, which are generally less common in the general population than more general comorbidities, our magnitudes of effect are in line with the findings of a recent meta-analysis, although our findings did not reach statistical significance. For example, in the meta-analysis, the pooled odds ratio for type 1 diabetes was 2.02 (95% CI: 1.22, 3.40) and the pooled odds ratio for thyroid dysfunction was 1.66 (95% CI: 1.35, 2.05), while in our study the prevalence ratio for type 1 diabetes was 2.01 (95% CI: 0.23, 7.27), and for thyroid dysfunction was 1.67 (95% CI: 0.83, 3.00). Our estimate of effect for psoriasis was stronger and in the same direction (prevalence ratio 2.65 (95% CI: 1.48, 4.38) as the pooled estimate of 1.31 (95% CI: 1.09, 1.57) in the meta-analysis.

Our study had significant strengths and limitations. The significant strengths include a rigorous ascertainment of MS diagnosis and relapses by neurologist. One limitation of our study is the small sample size which provided limited power for rare comorbidities and for interaction effects by sex and for effect on disability or relapse. As a longitudinal study, however, its sample size is balanced with the logistics inherent in prospective and comprehensive follow-up such as those here. Furthermore, the use of self-reported, doctor-diagnosed comorbidity information, while valid for conditions which are readily diagnosed such as diabetes and hypertension (kappa >0.82), this is not the case for conditions such as arthritis and anaemia (kappa <0.40) which are not readily diagnosed. This has the potential of introducing misclassification bias which may have also reduced the power to identify an impact of comorbidities on the outcomes of interest. The probability that the diagnosis of these conditions may also be due to surveillance bias resulting from frequent health care utilizations cannot be completely ruled out.
The mechanisms by which comorbidities might influence MS disability and risk of relapse are not yet established. We are therefore hypothesizing that, the association between obesity, dyslipidaemia and clinical disability may act via the vitamin D pathway where obesity and dyslipidaemia may lead to reduced physical activity, which may also lead to reduced vitamin D to yield increased disability. The fact that obesity and dyslipidaemia may be associated with MS disability but not with relapses and likewise rheumatoid arthritis and anaemia may be associated with increased risk of relapse but not disability provides some insights as to how these comorbidities exert their effect. Proposed potential mechanisms assume that comorbidities may influence MS at the pathophysiologic level. Alternatively, independent etiologic effects of comorbidities on MS could additively or synergistically increase the risk of relapse and disability.

MS is a chronic, debilitating, lifelong disease and therefore the risk of developing comorbidities in the course of the disease is high. While MS in itself may not cause cardiovascular or other comorbidities, people with MS may be subject to an increased risk of comorbidities due to a diminished ability to engage in physical activity due to symptoms or conditions associated with MS. Studies have confirmed that people with MS have reduced physical activity compared to healthy control group.\(^31\) Less physical activity is associated with an increased risk of cardiovascular comorbidities.\(^33\) Consequently, MS patients may have increased morbidity and mortality from comorbidities despite the underlying pathophysiology potentially being unrelated.

In conclusion, we found the age-standardised prevalence of hypertension, dyslipidaemia, psoriasis, eczema, asthma and anaemia to be significantly higher in our MS cohort compared to the general Australian population. Participants who reported rheumatoid arthritis and anaemia had a higher risk of relapse. Reports of being overweight/obese and having dyslipidaemia were associated with a higher level of disability when compared to those who
did not report these comorbidities. These associations were in part determined by the older age of participants; however, MSSS associations with comorbidities were robust to these adjustments, indicating a potential age-independent mechanism for these associations. We recommend long-term monitoring and treatment of comorbidities in MS in order to prevent increased morbidity as people age with their MS. Many of the comorbidities discussed here are treatable or preventable and therefore this is an area of MS management that can be emphasised, particularly in those with progressive disease where there are no effective treatment strategies to slow progression. Studies of comorbidity treatment and prevention in patients with MS have the potential to improve our understanding of the prognosis and outcomes of MS.

7.7 Postscript
This chapter has provided some data on the frequency of comorbidities in MS patients and their association with clinical disability and relapse. The next chapter describes the conclusions drawn from the various studies undertaken and the future direction of MS research I will be carrying out.

7.8 References


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Chapter 8 Conclusions
MS has so far proven to be a complex disease of the central nervous system. Even though the aetiology and the pathophysiology of the disease have been substantially studied, no reliable biomarker, diagnostic test, biological mechanism, or specific therapy has been identified for all MS patients.¹ One of the reasons accounting for this failure is the considerable heterogeneity in clinical course and pathophysiological outcomes. The clinical course of MS is highly variable, including four different clinical phenotypes with various subcategories. Moreover, the pathophysiology of the disease is highly diverse and may be marked by various combination and degrees of inflammation, demyelination, axonal damage and loss, neurodegeneration and gliosis.

MS is thought to be a multifactorial disease that results from the intricate, yet not well-understood aetiology. From all the identified etiological factors of MS, not a single factor is in itself complete to initiate the onset or cause a substantial progression of the disease. Therefore, the identification of factors that can wholly predict the onset or progression by acting independently and in concert with each other would be a major success in MS research. Greater understanding of the aetiology of the disease will lead to early diagnosis and identification of individuals at risk of developing MS, prediction of disease course and monitoring of disease progression.²

The search for these etiological factors has led to research into how serum lipids may influence the onset and progression of MS. Earlier laboratory investigations have identified oxidatively modified serum LDL cholesterol in the parenchyma of MS plaques.³ This finding has led to the question whether there is any relationship between presence of serum lipids in the brain parenchyma and MS onset, disability and disease progression. Further, the role of comorbidities in the onset and progression of MS was also investigated because of the
relationship between serum lipids and vascular comorbidities. For instance, it is a well-established knowledge that elevated levels of serum lipids (dyslipidaemia) are associated with vascular diseases such as coronary heart disease, stroke, hypertension and diabetes.

In this regard, findings from the various investigations on how lipid-related variables and comorbidities may influence the onset, severity of clinical disability and disease progression has been summarised below.

8.1.1 Lipid-related variables and relapse
To investigate whether there is a relationship between lipid-related variables and conversion or susceptibility to MS and time to next relapse, two datasets - the Tasmanian MS Longitudinal Study (MSL) and the Ausimmune Longitudinal Study (AusLong) were used. The AusLong study is an internationally unique cohort of participants with incident first clinical diagnosis of demyelination, which has been followed annually up to five-year review. The MSL study is made up of a cohort of prevalent MS patients living in southern Tasmania which was followed for a total of 2.5 years.

The AusLong study was used to investigate the association between lipid-related variables and conversion to CDMS and time to subsequent relapse. In that investigation, we found that none of the serum lipid-related variables at study entry were significantly associated with conversion to CDMS. However, higher BMI and triglycerides were associated with increased risk of subsequent relapse. This disparity may be due to the fact that the association between lipid-related variables and risk of subsequent relapse has multiple outcomes per person, resulting in more power than the association between serum lipid-related variables and conversion to CDMS which has only one outcome per person. The MSL study was also used to examine the association between the lipid-related variables and time to subsequent relapse. From the analysis of the associations between lipid-related variables and the subsequent
hazard of relapse in MS patients using the MSL study, none of the lipid-related variables were associated with the hazard of relapse.

While this finding needs further investigation, the lack of association between lipid-related variables and CDMS is suggesting that serum lipid-related variables may not be associated with the pathophysiological mechanisms that are involved in conversion or susceptibility to CDMS. The study for the factors which may significantly influence conversion to CDMS was motivated by the fact that significant minority of patients who develop first demyelinating event never progress to CDMS. Therefore identifying modifiable factors that can influence the progression of first demyelinating event to CDMS could provide both potential therapeutic and management target to either prevent conversion to CDMS or delay progression to CDMS. This therefore makes the window between first demyelinating event and conversion to CDMS an important research focus.

The finding that higher BMI and triglycerides were associated with increased risk of subsequent relapse in the AusLong study do not align with the finding from the MSL study which did not report any significant finding with the lipid-related variables. The reason for this disparity may be due to the fact that participants of the AusLong study were incident first demyelinating event cases who are at a much earlier disease state compared to the participants of the MSL study who were prevalent cases of CDMS with longer disease duration and largely treated hence significantly reducing the relapse rate. In addition, the AusLong study has a larger sample size and more power than the MSL study.

8.2 Lipid-related variables and disability and disability progression
The MSL study and AusLong study datasets were used to examine the association between the lipid-related variables and disability and progression in disability. From the investigation whether an adverse lipid profile in people with MS is associated with disability and progression in disability in the MSL study, it was demonstrated that higher TC, ApoB and
ApoB/ApoA-I ratio were associated with higher disability and that higher TC/HDL ratio was associated with significant progression of disability. Moreover, we observed an effect of BMI on disability that was independent of the effect of lipids. In the disability progression analysis using the AusLong study dataset, higher BMI and TC/HDL ratio was associated with a higher annual change in clinical disability.

There was a consistency in the finding of a significant association between TC/HDL ratio and a higher change in clinical disability in both AusLong and MSL study. However, the finding of a significant association between baseline BMI and change in disability in the AusLong study could not be replicated in the MSL study. The disparity may be due to the fact that the AusLong study used cases of first demyelinating event where disability was measured over a 5 years period compared to the MSL study which used cases of established or prevalent, well-treated MS patients where disability was measured over a shorter period of 2.5 years on average. The two-year change in disability is limited and a longer follow-up is preferable when measuring disability progression. The AusLong study would be followed up to 10 and 15 years on average, making it a better study for the investigation of the progression of MS in relation to adverse lipid profile. It also makes it a better study to investigate whether the observed associations would persist further as the duration of the disease advances.

The findings that BMI and adverse lipid levels were associated with disability progression raises the crucial question whether lipid-lowering interventions aimed at lowering adverse serum lipid levels into healthy ranges may reduce the accumulation of disability in people with MS. This research adds to the argument to test whether interventions aimed at increasing physical activity and improving diet is beneficial. It also opens up potential therapeutic opportunities for the use of lipid-lowering agents such as statins to lower serum lipid levels, but further investigation through clinical trial is needed to ascertain the benefit. From the data available, the use of statin in MS may be justified when the aim is to reduce a patient’s risk of
cardiovascular disease since the benefit of reducing the risk of cardiovascular disease has been established. However, the use of statins is not yet justified as an intervention to reduce the accumulation of disability in MS patients because clinical trials of statins as an intervention in MS have produced inconsistent evidence, including negative effects, which may be an indication that previous observational studies demonstrating associations may have been confounded. Adverse effects in people with MS are possibly explained by laboratory evidence showing negative impacts of statins on oligodendrocytes and myelin formation. Thus, care needs to be taken when considering the use of statins in the treatment of MS.

8.3 Comorbidities and MS
The MSL study was also used to examine whether the frequency of comorbidities was higher in MS patients compared to the Australian population and whether comorbidities were associated with clinical disability and relapse. From the investigation, it was found that the age-standardised prevalence of hypertension, dyslipidaemia, psoriasis, eczema, asthma and anaemia was significantly higher in the MS cohort compared to the general Australian population. When disability data was analysed, it was found that reporting overweight/obesity and dyslipidaemia was associated with significantly higher disability after adjustment for potential confounders. These associations were in part determined by the advancing age of participants. We found, however, no significant association with annual change in disability. In the relapse analysis, rheumatoid arthritis and anaemia were associated with an increased risk of subsequent relapse. The association between relapse and rheumatoid arthritis, however, need to be interpreted with caution. This is because the small number of participants reporting rheumatoid arthritis limits the interpretation of this association.

Although our study is a prospective population-based study, it is limited by the small sample size which provided limited power for rare comorbidities and for interaction effects by sex.
and for effect on disability or relapse to be detected. The number of studies in this area of research is limited and therefore further investigation is warranted. The only sufficiently large study in this area with sufficient power is the NARCOMS study by Marrie and colleagues which found that vascular comorbidity in MS patients was associated with more rapid progression in ambulatory disability.\textsuperscript{4}

Studies of comorbidity treatment and prevention in patients with MS have the potential to improve our understanding of the prognosis and outcomes of MS. Many of the comorbidities examined here are treatable or preventable and therefore this is an area of MS management that can be emphasised, particularly in those with progressive disease where there are no effective treatment strategies to slow progression.

8.4 Final conclusion of PhD

Studies included in this thesis assessed the role of serum lipids and comorbidities in the onset and progression (clinical disability and relapse) of the disease. From these studies, the following conclusions regarding MS have been drawn:

- None of the serum lipid-related variables were significantly associated with conversion to CDMS.

- It was demonstrated that higher BMI, TC, ApoB and ApoB/ApoA-I ratio were associated with higher disability; and that a higher BMI and TC/HDL ratio was associated with progression of disability.

- There was significant association between BMI, triglycerides and the hazard of relapse in the AusLong study but not in the MSL study.

- The age-standardised prevalence of hypertension, dyslipidaemia, psoriasis, eczema, asthma and anaemia was significantly higher in the MSL study compared to the general Australian population.
• Overweight/obesity and dyslipidaemia as comorbidity in MS patients was associated with significantly higher disability.

• Rheumatoid arthritis and anaemia as comorbidity in MS patients were associated with increased risk of subsequent relapse.

8.5 Future directions
A number of research questions that warrant further investigation have arisen from this work. Australian datasets such as the Australian Multiple Sclerosis Longitudinal Study (AMSLS) and Ausimmune Longitudinal Study (AusLong) datasets can be used to contribute to the body of evidence. I have secured a Post-Doctoral Fellowship which will enable me conduct large part of the research proposed below.

8.5.1 Is a history of particular comorbidity associated with a higher risk of MS?
In the general population, the occurrence of comorbid kidney disease, diabetes and hypertension has been associated with increased risk of cardiovascular disease. There is currently limited data as to whether history of particular comorbidity may be associated with a higher risk of MS. In a Danish population-based cohort study assessing the risk of MS in individuals with type 1 diabetes (T1D), the expected incidence rate for MS in patients with T1D was more than three-fold higher than what was expected based on available incidence rate data in Denmark. Further studies using these large datasets should be conducted to verify this finding.

8.5.2 Are people with clinically definite MS at increased risk of developing comorbidities compared to the general population?
In people with MS, increasing evidence suggests that comorbidities are prevalent and may be associated with the clinical course of the disease. From the existing literature it is however unclear whether people with MS are at increased or reduced risk of comorbidities compared with the general population. In a study by Kang and colleagues, MS patients were
more likely to have rheumatoid arthritis, hypertension, hyperlipidaemia, diabetes, systemic lupus erythematosus, and peripheral vascular disorders. However in other studies, the prevalence of comorbid rheumatoid arthritis\textsuperscript{11}, hypertension\textsuperscript{12}, hyperlipidaemia and\textsuperscript{13} diabetes\textsuperscript{14} were lower in MS patients compared to control group. Also, studies on the comorbidities in MS patients from the Australian region are lacking.

8.5.3 Are people with MS worse off in their relapse and disability if they have comorbidities?
While there is enough evidence in the general population that comorbidities are associated with increased disability,\textsuperscript{15} there is only one high quality study that investigated the relationship between comorbidities and level of disability in people with MS. In this particular study, Marrie and colleague reported that individuals with MS who developed vascular comorbidities at any point during their disease course had significantly increased risk of ambulatory disability compared with MS patients who did not report comorbidities.\textsuperscript{4} Our study group has shown that anaemia and rheumatoid arthritis may be associated with increased risk of relapse. We will have the opportunity to investigate this further using larger datasets.

8.5.4 Is there a relationship between comorbidities and reduced health-related quality of life in people with MS?
While comorbidities are associated with reduced health-related quality of life (HRQOL) in the general population,\textsuperscript{16, 17} there is little information on the relationship between comorbidities and HRQOL in people with MS. In a study restricted to persons with relapsing-remitting MS, patients with musculoskeletal and respiratory comorbidities were reported to have reduced physical HRQOL\textsuperscript{18} and another study reported that physical comorbidity is associated with substantially lower physical HRQOL in MS.\textsuperscript{19} Identification to what extent
comorbidities affect HRQOL could prioritise prevention efforts and improve the quality of lives of MS patients.

8.5.5 Is the use of disease modifying therapies (DMTs) associated with increased risk of comorbidities?
As demonstrated in the general population, there is accumulating evidence to suggest that DMTs may be associated with increased risk of comorbidity in MS. Treatment of MS patients with interferon beta has been associated with the risk of developing thyroid disorder\textsuperscript{20}, psoriasis\textsuperscript{21} ulcerative colitis\textsuperscript{22} and systemic lupus erythematosus.\textsuperscript{23} Unpublished data from our Tasmanian Multiple Sclerosis Longitudinal Study showed that use of DMTs may be associated with significantly increased risk of developing thyroid dysfunction and eczema. The period of investigation in these studies is often too short to determine the long term effect of these agents. Large longitudinal datasets with data on DMTs and comorbidities should investigate any relationship between long term use of DMTs and risk of comorbidity.

8.5.6 Are comorbidities associated with high health care costs?
Comorbidities are known to have strong negative effects on health status and health services. In the general population, comorbidity have been associated with greater health care utilisation, including more hospital admissions, longer stays in hospital.\textsuperscript{17} In a recent study by Marrie and colleagues, MS patients with one or more comorbidities had a two-fold higher all-cause hospitalization rate compared to MS patients without any comorbidity.\textsuperscript{24} Higher health care usage and treatments will lead to higher health care costs. A study in the general population attributed 36.4\% of health care expenditure for people with diabetes to comorbidities.\textsuperscript{25} There is currently no study on the relationship between comorbidities and health care cost in people with MS. Using the economic survey data from the AMSLS, we will be able to investigate the health care cost due to comorbidities.
8.6 References


