

**An adverse lipid profile and increased levels of adiposity significantly predict clinical course after a first demyelinating event.**

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**Abstract**

**Objective:** To investigate the prospective associations between adiposity and lipid-related variables and conversion to MS, time to subsequent relapse and progression in disability.

**Methods:** A cohort of 279 participants with a first clinical diagnosis of CNS demyelination was prospectively followed to 5-year review. Height, weight, waist and hip circumference were measured, and serum samples taken for measurement of lipids and apolipoproteins. Survival analysis was used for conversion to MS and time to relapse, and linear regression for annualised change in disability (EDSS).

**Results:** Higher BMI (Adjusted hazard ratio (aHR): 1.22(1.04 to 1.44) per 5 kg/m<sup>2</sup> increase, hip circumference (aHR): 1.32(1.12 to 1.56) per 10cm increase and triglyceride levels (aHR:

1.20(1.03 to 1.40) per unit increase were associated with increased risk of subsequent relapse, while adiposity and lipid-related measures were not associated with conversion to MS. In addition, higher BMI ( $\beta$ : 0.04(0.01 to 0.07) per 5 kg/m<sup>2</sup> increase, hip circumference ( $\beta$ : 0.04(0.02 to 0.08) per 10cm increase, waist circumference ( $\beta$ : 0.04(0.02 to 0.07) per 10cm increase, total cholesterol to high density lipoprotein ratio (TC/HDL ratio) ( $\beta$ : 0.05(0.001 to 0.10) and nonHDL ( $\beta$ : 0.04(0.001 to 0.08) at study entry were associated with a higher subsequent annual change in disability.

Conclusions: Higher levels of adiposity, nonHDL and TC/HDL ratio were prospectively associated with a higher rate of disability progression, and higher adiposity and triglycerides were associated with relapse but not with conversion to MS. Improving the lipid profile and losing weight into the healthy range could reduce the accumulation of disability.

## **Introduction**

Multiple sclerosis (MS) is an inflammatory, demyelinating and neurodegenerative disease of the central nervous system (CNS).<sup>1</sup> First demyelinating event (FDE) represents the earliest clinical stage in the development of MS.<sup>2,3</sup> Following FDE, the diagnosis of MS can be made based on the development of a second clinical episode and paraclinical evidence (MRI, oligoclonal bands, evoked potentials) that meet diagnostic criteria.<sup>4</sup> A better understanding of the factors involved in the risk of conversion from FDE to MS could facilitate the identification of predictive biomarkers of conversion, disability progression, quality of life, and potentially also indicate intervention targets.<sup>5,6</sup>

Emerging evidence suggests that dyslipidaemia as a comorbidity in people with MS is linked with a more rapid progression in disability.<sup>7</sup> Similarly, recent evidence suggests a potential adverse 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.<sup>8, 9</sup> Studies have also shown an association between serum lipid variables and disease activity on MRI.<sup>5, 10</sup>

In relation to relapse in MS, Weinstock-Guttman and colleagues evaluated the relationship between serum lipid profile and conversion to MS and subsequent risk of relapse in a prospective cohort of patients with a clinical isolated syndrome (CIS) treated with interferon- $\beta$ . None of the lipid profile variables (total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides) were associated with conversion to MS or subsequent risk of relapse.<sup>5</sup> We verified the relapse outcome of this study, by conducting a study examining whether serum lipids and apolipoproteins predicted subsequent risk of relapse in MS patients with average disease duration of 12 years and on immunomodulatory therapy. Consistent with the earlier study, we found that none of the lipid-related variables were associated with the risk of relapse.<sup>11</sup>

Studies have also been conducted examining how BMI as a measure of adiposity may influence the disease course of MS. The evidence suggests an increased risk of MS onset in people with a high BMI during childhood or adolescence but not adulthood.<sup>12-15</sup> In relation to whether high BMI is associated with disability or progression in disability, studies in this area are scarce and results have been inconsistent.<sup>8, 16, 17</sup> Waist and hip circumference may be a more accurate measure of adiposity in MS compared to BMI<sup>18</sup>, because BMI does not distinguish between fat and lean tissues. Further studies need to incorporate these measures given that they are modifiable and could be a potential therapeutic tool to limit progression of disability.

Whether an adverse lipid profile and increased adiposity drive subsequent clinical course is often hard to disentangle from possible reverse association where those who are more disabled become more sedentary, overweight and develop an adverse lipid profile. A prospective study design where the lipid profile is measured at the start of the disease process is required. Using such a design, we investigated the association between adiposity and lipid-related variables and conversion to MS, time to subsequent relapse, and progression in disability in a prospective cohort of patients with a first clinical diagnosis of CNS demyelination.

## **Methods**

### **Study design**

The Ausimmune Longitudinal (AusLong) Study, is a clinical cohort built upon the original Ausimmune case-control study, that 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 216 who had their initial onset event between 1 Nov 2003 and 31 Dec 2006.<sup>19</sup> 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.

At the 5-year follow-up, 3 case participants were diagnosed with a non-MS disease (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 their onset event just prior to their initial participation in the Ausimmune Study, and who are referred to here as "classic FDE", and 69 who had had an older initial event). Twenty-one participants were now

considered to have primary progressive-onset disease. 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. To satisfy temporality in our investigation, all exposures included in the analysis preceded outcomes evaluated. Therefore, conversion to MS and relapse analyses were restricted to classic FDE (n=169). For the disability analysis, we included all participants with MS who were reviewed at 5 years with an EDSS measurement including participants with primary progressive MS, giving 190 participants in this analysis.

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

### **Measurement of MS and relapse and disability**

For the purposes of the present investigation, a number of clinical outcomes were evaluated: conversion to MS, time to relapse, and annualised disability progression from FDE to 5-year review.

Conversion to MS 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<sup>20</sup> (a minority of cases were diagnosed following MRI based on this latter criterion (n=20)). Conversion to MS was reported at each annual review and cross-checked with neurological records by a neurologist team. A relapse was defined as per the 2001 McDonald Criteria<sup>21</sup> 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 an externally derived increase in body temperature (e.g. heat stroke). Relapses were reported at annual review or derived from medical records, and only relapses diagnosed and verified by a neurologist were included in this analysis. Disability was assessed by the Kurtzke Expanded Disability Status Scale (EDSS)<sup>22</sup> 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.<sup>23</sup>

Clinical history was derived from medical records and a history taken at the 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 participant was diagnosed as having primary progressive MS, 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.

### **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. While triglycerides are traditionally measured in a fasting state, there has been a shift to non-fasting samples as post-prandial non-fasting

values are more representative of the usual metabolic state,<sup>24</sup> providing justification for the use of non-fasting samples for triglycerides in this study. TC and triglycerides were measured using enzymatic colorimetry (Wako Chemicals, Richmond, VA, USA). HDL levels were measured using precipitation and enzymatic assay (Wako Chemicals, Richmond, VA, USA). LDL was measured by direct assay using enzymatic colorimetry (Wako Chemicals, Richmond, VA, USA). Non-HDL-cholesterol levels were computed by subtracting HDL from TC. The apolipoproteins, ApoA-I and ApoB, were also measured as they are the main protein components of HDL and LDL/VLDL (very low density lipoprotein), respectively. Lipoprotein (a) (Lp (a)) is the complex of LDL 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).

BMI was calculated from height and weight, and waist-to-hip ratio was calculated from waist and hip circumference. All were measured at baseline, 2/3 year and 5 year reviews. Smoking (no, yes), statin use (no, yes) and physical activity (expressed as Metabolic Equivalents of Task (METs) using International Physical Activity Questionnaire-short) were measured at each annual review. Age, sex and study site were measured at study entry. Serum 25-hydroxyvitamin D (25(OH)D) levels were measured using liquid chromatography-tandem mass spectrometry standardised to the standard reference method.<sup>25</sup> 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 concentration.<sup>26, 27</sup>

## Data analysis

BMI, waist and hip circumference and lipid-related variables measured at each review were used longitudinally in each analysis, except in the disability analysis. BMI was rendered into categories as normal (18.5 to 25 kg/m<sup>2</sup>), overweight (>25 to 30 kg/m<sup>2</sup>) and obese (>30 kg/m<sup>2</sup>). The effect of serum lipid variables, adiposity indices, and other covariates on conversion to MS (single event) and subsequent relapses (repeated events) were evaluated by Cox regression models. Conversion to MS was evaluated by single-failure Cox regression, while relapse was evaluated using the gap-time model of 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.<sup>28</sup> For Conversion to MS and relapse analyses, time-varying covariates measured at each review were carried forward to the time of Conversion to MS or relapse, such that all parameters were as measured prior to the occurrence of conversion to MS or relapse. The association between continuous serum lipid variables and conversion to MS violated the proportional hazard assumption; the serum lipid variables were thus rendered into quartile categorical variables which satisfied the proportional hazard assumption. All other covariates satisfied the proportional hazard assumption.

Annualised change in EDSS was calculated by taking the 5-year review EDSS and dividing by the duration between the day before FDE and the 5-year review, this proportion then expressed as an annualised change. The relationships between the lipid-related variables at study entry, 5-year change in lipid-related variables and annualised 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 on the 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 change in EDSS at the mean of model covariates. Log-binomial regression analysis was also used to compare the relationship between the lipid-related variables and annualised change in disability at thresholds of disability accumulation. All analyses were adjusted for age at study entry, sex and study site because age was associated with relapse, while sex and study site were associated with both conversion to MS and relapse. The serum lipid associations were also adjusted for BMI to examine their independent effect. We considered smoking status, physical activity, deseasonalised 25(OH)D level, and statin medication use as potential confounders but they were either not associated with the outcomes or exposures of interest.

All statistical analyses were conducted in Stata/IC 12.1 (StataCorp LP, College Station, Texas, USA).

## **Results**

### **Participant characteristics**

Table 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 (56%) were overweight or obese ( $\geq 25$  kg/m<sup>2</sup>) and 6.1% (n=17) were treated with statins during the study. By the 5-year review, 72.0% (201/279) had converted to MS and had had a total of 446 relapses. The median EDSS at the 5-year review was 1.5 (IQR: 1.0, 2.5). Using the established lipid cut-off points,<sup>29</sup> 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” group (n=169), 78% were female and the mean age at study entry was 37.7 (SD 9.7). By 5-year review, 56.2% (95/169) had converted to MS and

had had 222 relapses. The median EDSS at the 5-year review was 1.5 (IQR: 0.5, 2.0).

**Table 1: Demographic and clinical characteristics of the AusLong cohort**

Characteristics	All persons (279) n/N (%)	Classic FDE (169) n/N (%)
Female sex*	214/279 (77.0)	131/169 (78)
Relapse during study?	170/279 (60.9)	85/169 (50.3)
Immunomodulatory therapy use during study?	146/279 (52.3)	78/169 (46.2)
Any Statin during study?	17/279 (6.1)	11/169 (6.5)
Smoke ever?	174/279 (62.4)	104/169 (61.5)
Body Mass Index (Kg/m <sup>2</sup> )*		
Normal	122/277 (44.0)	76/167 (45.5)
Overweight	80/277 (28.9)	48/167 (28.7)
Obese	75/277 (27.1)	43/167 (25.7)
Mean (SD; Range)		
Age (years)*	38.8 (9.7; 18, 58)	37.7 (9.7; 18, 58)
5-year review		
	Median (IQR)	
MS duration from symptom onset (years)	6.3 (5.7, 7.4)	5.8 (5.3, 6.2)
Annualised change in EDSS	0.3 (0.2, 0.4)	0.3 (0.2, 0.4)
EDSS	1.5 (1.0, 2.5)	1.5 (0.5, 2.0)
MSSS	2.6 (1.3, 4.5)	2.3 (0.3, 3.9)
Physical activity (METs)	8.0 (0.0, 24.0)	8.0 (0.0, 24.0)
Body Mass Index (Kg/m <sup>2</sup> )	26.0 (23.0, 30.4)	25.6 (22.9, 30.0)
Waist circumference (cm)	86.7 (77.0, 97.3)	85.7 (76.3, 95.5)
Hip circumference (cm)	103.5 (97.0, 111.7)	103.3 (97.0, 111.7)
Waist-to-hip ratio	0.83 (0.77, 0.90)	0.83 (0.77, 0.89)
TC (mmol/L)	5.4 (4.7, 6.2)	5.5 (4.7, 6.4)
LDL (mmol/L)	3.2 (2.7, 3.8)	3.2 (2.7, 3.9)
ApoB (g/L)	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)
nonHDL (mmol/L)	4.0 (3.2, 4.7)	4.0 (3.3, 4.9)

Trig (mmol/L)	1.5 (1.1, 2.2)	1.5 (1.1, 2.2)
HDL (mmol/L)	1.4 (1.1, 1.6)	1.3 (1.1, 1.7)
ApoA-I (g/L)	1.5 (1.4, 1.8)	1.5 (1.4, 1.8)
Lp(a) (μmol/L)	0.4 (0.2, 1.3)	0.4 (0.2, 1.2)

\*Baseline

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); METs: Metabolic Equivalents

### **Association between adiposity and lipid-related variables and conversion to MS**

Supplementary table 1 summarises the association between adiposity and lipid-related variables and conversion to MS. There was no evidence that serum lipid-related variables, BMI and waist or hip circumference measures were significant predictors of conversion to MS after adjustment for age at study entry, sex, BMI, study site and disease modifying therapy (DMT) use.

### **Association between adiposity and lipid-related variables and hazard of relapse**

Table 2 demonstrates the association between adiposity, lipid-related variables and hazard of relapse. There was a significant positive association between BMI and hazard of relapse with each 5 kg/m<sup>2</sup> increase in BMI associated with a 25% increased risk of relapse after adjustment for sex, age at study entry, study site and DMT use. Compared to normal weight, overweight and obese BMI were significantly associated with increased risk of relapse after adjustment for age at study entry, sex, study site and DMT use ( $p_{\text{trend}}=0.012$ ). 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. Similar associations were found for waist and hip related measures, this being significant for hip

circumference, where an increase of 10 cm was associated with a 29% increased risk of relapse.

There was no evidence of any association between hazard of relapse and total, LDL or HDL cholesterol levels (Table 2). The relationship between serum triglyceride levels and the hazard of relapse showed a significant positive association after adjustment for age at study entry, study site, sex, BMI and DMT use. A 1mmol/L increase in triglyceride level was associated with a 22% increased hazard of relapse. The association was independent of BMI as it was robust to further adjustment for BMI (HR: 1.22; 95% CI: 1.04, 1.42;  $p=0.012$ ). Triglyceride level was then dichotomised into categories of  $<2.30$  mmol/L and  $\geq 2.30$  mmol/L (considered as clinically high risk level for cardiovascular disease)<sup>29</sup> and the association with hazard of relapse re-assessed. Those with  $\geq 2.30$  mmol/L levels of triglycerides had a relapse risk that was 2.22 times ( $p=0.016$ ) higher than those with lower levels after adjustment for age at study entry, sex, BMI, DMT use and study site.

**Table 2: Association between lipid-related variables and hazard of relapse**

	<b>Unadjusted</b> Hazard Ratio (95% CI)	p-value	<b>Adjusted*</b> Hazard Ratio (95% CI)	p-value
BMI (per 5 kg/m <sup>2</sup> )	1.13 (0.96, 1.34)	<i>0.14</i>	1.25 (1.07, 1.47)	<b>0.006</b>
Normal	1.00 [Reference]		1.00 [Reference]	
Overweight	1.08 (0.69, 1.71)	<i>0.73</i>	1.61 (1.06, 2.45)	<b>0.026</b>
Obese	1.34 (0.78, 2.30)	<i>0.29</i>	1.96 (1.13, 3.40)	<b>0.017</b>
Trend		<i>0.30</i>		<b>0.012</b>
Waist circumference (per 10 cm)	1.00 (0.95, 1.04)	<i>0.92</i>	1.04 (1.00, 1.08)	<i>0.07</i>
Hip circumference (per 10cm)	1.25 (1.04, 1.51)	<b>0.015</b>	1.29 (1.09, 1.53)	<b>0.003</b>
Waist-to-hip ratio	0.09 (0.02, 0.56)	<b>0.008</b>	0.88 (0.45, 1.75)	<i>0.73</i>
TC (mmol/L)	1.03 (0.81, 1.31)	<i>0.80</i>	1.19 (0.91, 1.55)	<i>0.20</i>
LDL (mmol/L)	0.93 (0.70, 1.25)	<i>0.64</i>	1.07 (0.77, 1.44)	<i>0.67</i>
ApoB (g/L)	1.49 (0.38, 5.80)	<i>0.57</i>	2.82 (0.68, 11.65)	<i>0.15</i>
nonHDL (mmol/L)	1.02 (0.81, 1.28)	<i>0.88</i>	1.20 (0.92, 1.55)	<i>0.18</i>

Trig (mmol/L)	1.07 (0.94, 1.23)	0.31	<b>1.22 (1.04, 1.42)</b>	<b>0.012</b>
<2.30	1.00 [Reference]		1.00 [Reference]	
≥2.30	1.31 (0.69, 2.47)	0.41	<b>2.22 (1.16, 4.26)</b>	<b>0.016</b>
HDL(mmol/L)	1.12 (0.57, 2.22)	0.75	0.95 (0.44, 2.00)	0.89
ApoA-I (g/L)	1.63 (0.66, 4.05)	0.29	1.44 (0.59, 3.51)	0.42
Lp(a) (μmol/L)	1.02 (0.69, 1.50)	0.94	1.01 (0.73, 1.40)	0.95
TC/HDL ratio	0.99 (0.78, 1.25)	0.92	1.17 (0.89, 1.54)	0.26
LDL/HDL ratio	0.86 (0.61, 1.21)	0.38	1.03 (0.73, 1.47)	0.85
ApoB/ApoA-I ratio	0.68 (0.14, 3.24)	0.63	1.47 (0.24, 8.63)	0.67

\*Adjusted for sex, age at study entry, BMI, disease modifying therapy use & 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

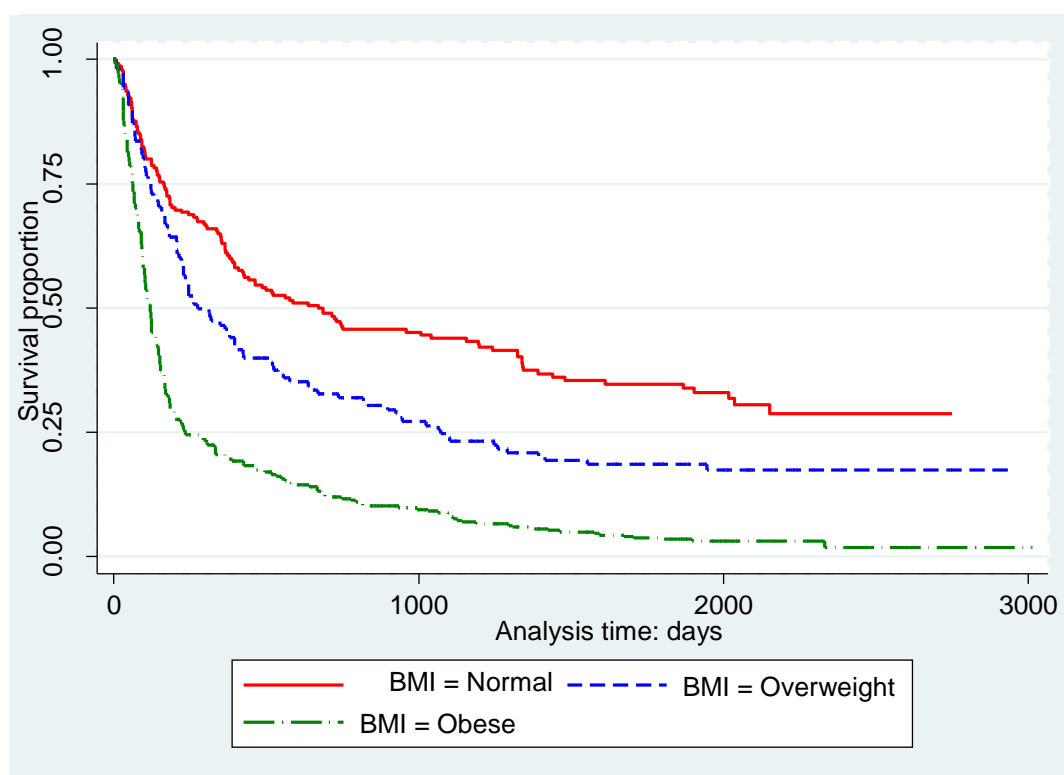


Figure 1: Kaplan-Meier survival plots by body mass index (BMI) category, showing the proportion of subjects that were relapse-free since study entry for those who had a normal BMI, those who were overweight and those who were obese.

### Associations between adiposity and lipid-related variables and disability progression

We next sought to evaluate the relationship between adiposity and lipid-related variables at study entry, 5-year change in adiposity and lipid-related variables and the subsequent change

in disability (Table 3). BMI at study entry was a significant predictor of subsequent annualised change in EDSS. 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 or obese category ( $\text{BMI} \geq 25$ ) had a 60% greater risk of experiencing a change in EDSS greater than 0.5 (after adjustment for age at study entry and sex) compared to those in the normal weight category (RR: 1.60; 95% CI: 1.05, 2.45;  $p=0.029$ ). Significant associations were also observed for the baseline waist and hip circumference measures and change in disability (Table 3). Similar associations were observed when we used MSSS as an outcome after adjusting for age, sex and study site (BMI:  $\beta= 0.09$ ; 95% CI: 0.02, 0.15;  $p=0.008$ ; waist circumference  $\beta= 0.05$ ; 95% CI: -0.01, 0.11;  $p=0.07$ ; hip circumference  $\beta= 0.02$ ; 95% CI: -0.05, 0.10;  $p=0.58$ ; waist-to-hip ratio  $\beta= 3.70$ ; 95% CI: -3.87, 11.28;  $p=0.34$ ).

Upon adjustment for age at study entry, study site and sex, baseline 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;  $p=0.019$ ) were significantly associated with greater five-year change in EDSS. When these associations were further adjusted for BMI, the association with nonHDL (0.049) and TC/HDL ratio ( $p=0.044$ ) remained independently associated.

Using log-binomial regression, participants with TC/HDL ratio level greater than 4.0 had 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 age at study entry, sex and study site (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.<sup>29</sup>

The direction of effect for TC/HDL (adjusted analysis  $\beta$ : 0.49 (-0.07, 1.04)  $p=0.09$ ) and nonHDL ( $\beta$ : 0.45; 95% CI: 0.01, 0.88;  $p=0.045$ ) was similar to EDSS when we examined MSSS as an outcome. With MSSS, trends of associations were also observed 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 age at study entry, sex, study site and BMI.

In the disability analyses, we conducted sensitivity analyses excluding primary progressive MS and FDEs that occurred prior to the study but the findings remained unchanged.

There was no evidence of a significant relationship between the 5-year change in adiposity and lipid-related variables and change in disability after adjustment for age at study entry, sex, BMI and study site.

**Table 3: Association between lipid-related variables at study entry and annualised change in EDSS**

	Basic model# $\beta$ (95% CI)	p-value	Adjusted* $\beta$ (95% CI)	p-value
BMI ( per 5Kg/m <sup>2</sup> )	0.01 (0.003, 0.02)	<b>0.005</b>	0.04 (0.01, 0.07)	<b>0.012</b>
Normal	1.00 [Reference]		1.00[Reference]	
Overweight/obese	0.10 (0.02, 0.18)	<b>0.013</b>	0.09 (0.01, 0.17)	<b>0.021</b>
Waist circumference (per 10 cm)	0.03 (0.01, 0.05)	<b>0.007</b>	0.04 (0.02, 0.07)	<b>0.001</b>
Hip circumference (per 10 cm)	0.04 (0.01, 0.07)	<b>0.008</b>	0.05 (0.02, 0.08)	<b>0.004</b>
Waist-to-hip ratio	0.29 (-0.21, 0.78)	0.25	0.51 (-0.27, 1.29)	0.20
TC (mmol/L)	0.04 (0.01, 0.07)	<b>0.019</b>	0.03 (-0.01, 0.07)	0.09
LDL (mmol/L)	0.02 (-0.02, 0.06)	0.38	0.01 (-0.03, 0.05)	0.68
ApoB (g/L)	0.28 (0.03, 0.52)	<b>0.028</b>	0.22 (-0.03, 0.48)	0.09
nonHDL (mmol/L)	0.04 (0.01, 0.08)	<b>0.009</b>	0.04 (0.001, 0.08)	0.049
Trig (mmol/L)	0.04 (0.01, 0.07)	<b>0.016</b>	0.03 (-0.003, 0.07)	0.07
HDL(mmol/L)	-0.03 (-0.12, 0.07)	0.58	-0.03 (-0.14, 0.07)	0.55
ApoA-I (g/L)	0.003 (-0.12, 0.12)	0.96	-0.01 (-0.26, 0.18)	0.90
Lp(a) ( $\mu$ mol/L)	-0.01 (-0.05, 0.03)	0.64	-0.01 (-0.05, 0.04)	0.78
TC/HDL ratio	0.05 (0.01, 0.09)	<b>0.013</b>	0.05 (0.001, 0.10)	<b>0.044</b>
LDL/HDL ratio	0.04 (-0.01, 0.09)	0.15	0.03 (-0.03, 0.09)	0.32
ApoB/ApoA-I ratio	0.40 (0.01, 0.80)	<b>0.047</b>	0.39 (-0.06, 0.84)	0.09
# Adjusted for relapse at the time of review				

## **Discussion**

Using a prospective cohort design in people with a first clinical diagnosis of CNS demyelinating disease, we found that higher levels of adiposity, nonHDL and TC/HDL ratio at study entry were associated with a higher annual change in clinical disability, and higher levels of adiposity and triglycerides were associated with an increased risk of subsequent relapse but not conversion to MS.

We found that higher BMI, waist circumference and hip circumference all were associated with a higher annual change in EDSS. Similar findings were seen with MSSS but not for hip circumference. 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 latter was also found in our previous study in people with established MS, with a similar magnitude of effect.<sup>8</sup> 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.<sup>9</sup>

In the relapse analysis, we found that being obese was associated with a near doubling in the risk of relapse compared to those of normal BMI (HR: 1.78; 95% CI: 1.07, 2.96; p=0.027), and higher hip circumference was also associated with relapse. In addition, an independent effect was observed for those with high triglycerides, where those with clinically high levels



( $\geq 2.30$  mmol/L) had a doubling in the 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<sup>8,11</sup>, nor those of Weinstock-Guttman and colleagues.<sup>5</sup> The reason for this disparity may be due to the fact that participants of this current study were at a much earlier disease state (FDE/CIS) compared to the participants of the Tasmanian MS Longitudinal study (MSL study)<sup>11</sup> who were prevalent cases of MS 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 FDE/CIS for most participants (52% of participants on therapy at the five-year review) compared to the study by Weinstock-Guttman and colleagues where all participant were on treatment.

The lack of a significant association between serum lipid-related variables and conversion to MS in our study is in line with the findings of from Weinstock-Guttman and colleagues.<sup>5</sup> The study by these authors prospectively followed a cohort of participants with CIS treated with interferon- $\beta$  and reported no significant associations between serum lipid-related variables and conversion to MS.<sup>5</sup> While this finding needs further investigation, it is suggestive 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 higher levels of adiposity and an adverse lipid profile are associated with a more adverse 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.<sup>30</sup> In that trial, MS patients ( $n=70$ )

using high-dose simvastatin had a lower EDSS accumulation after two years compared to the placebo arm (difference -0.254; 95% CI: -0.464 to -0.069).<sup>30</sup> Similarly, a trial by Togha and colleagues (n=42) demonstrated a lower EDSS score in MS patient on both simvastatin and interferon beta-1a compared to those on only interferon beta-1a.<sup>31</sup> 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.<sup>32</sup> 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 may yield therapeutic opportunities for people with MS.

The mechanisms by which adiposity 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 indirectly lead to increased weight and reduced physical activity, which may also lead to increased disability in MS patients. Further, adiposity may also be related to the lipid-vitamin D pathway as obese individuals are known to have a more adverse lipid profile and decreased level of serum vitamin D compared to individuals with normal weight.<sup>33</sup> Alternatively, the effect of adiposity may be mediated by low grade inflammatory state where increased levels of adipokines such as leptin and visfatin may promote inflammatory response and subsequent progression in MS.<sup>34, 35</sup>

Our study has significant strengths, including being a prospective population-based cohort study with the capability to adjust for multiple confounders. It also has a longer follow-up period (5 years). This is important because a longer follow-up is preferable when measuring change in disability. As an observational study prone to reverse causality, this was limited by ensuring that exposures of interest precede outcomes. Also, lipids and adiposity indices at study entry were measured prior to a significant accrual of disability and yet predicted 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,<sup>5 10 36</sup> 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.<sup>37</sup>

In conclusion, we have demonstrated that higher levels of adiposity, nonHDL and TC/HDL ratio were associated with a higher rate of disability progression, and higher adiposity and triglycerides were associated with relapse but not with conversion to MS. Improving the lipid profile and losing weight into the healthy range could reduce the accumulation of disability.

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