ABSTRACT

Background There are a lack of studies on the association between obesity and conversion from a clinically isolated syndrome (CIS) to MS.

Objective To determine whether obesity predicts disease activity and prognosis in patients with CIS.

Methods BMI at baseline was available for 464 patients with CIS in BENEFIT. Obesity was defined as BMI ≥30 kg/m2 and normal weight as 18.5≤ BMI< 25. Patients were followed up for 5 years clinically and by magnetic resonance imaging. Hazard of conversion to clinically definite (CDMS) or to 2001 McDonald criteria (MDMS) MS; annual rate of relapse; sustained progression on EDSS, change in brain and lesion volume, and development of new brain lesions were evaluated.

Results Obese individuals were 39% more likely to convert to MDMS (95% CI:1.02-1.91; p = 0.04) and had a 59% (95% CI:1.01-2.31; p = 0.03) higher rate of relapse than individuals with normal weight. No associations were observed between obesity and conversion to CDMS, sustained progression on EDSS or MRI outcomes, except for a larger reduction of brain volume in obese smokers as compared to normal weight smokers (-0.82%; 95% CI: -1.51 to -0.12, p= 0.02).

Conclusions Obesity was associated with faster conversion to MS (MDMS) and a higher relapse rate.

INTRODUCTION

MS is an important cause of neurological disability in young people. The majority of patients present with treatable bouts of inflammatory demyelination followed years after by treatment resistance and brain atrophy.[1,2] The cause of MS is unknown, but is related to genetic and environmental risk factors.[3,5] Obesity in early life has consistently been associated with an increased risk of MS.[6-9] The chronic low-grade inflammatory state linked to obesity and its relationship with endothelial dysfunction, inflammatory, and autoimmune diseases, could in part explain this association[10]. However, few studies have evaluated the relationship between body mass index (BMI) and activity and progression of MS. Therefore, we evaluated whether BMI at the time of a clinically isolated syndrome (CIS) is related to risk of conversion to MS, and MS activity and progression, over 5 years of follow-up, among participants in the Betaferon/Betaseron in Newly Emerging Multiple Sclerosis for Initial Treatment (BENEFIT) clinical trial,[11-13] and whether smoking status modified this association as smoking also has detrimental effects on immune system function and has been associated with MS disease activity and progression.

METHODS

Study population. The BENEFIT trial enrolled 468 participants between 2002 and 2003 who were randomized to receive either interferon beta-1b (INFβ-1b) or placebo within 60 days of experiencing a CIS suggestive of MS. Detailed information on the BENEFIT trial design and participants is provided elsewhere.[11] Briefly, participants were followed for conversion to MS (both clinically definite MS [CDMS][14] and 2001 McDonald MS [MDMS][15]). By current diagnostic criteria,[16] most BENEFIT participants would have been considered to have MS at baseline. However, for consistency with the original trial and subsequent publications, we continue to utilize the definitions of CIS and MS as set forth in the trial. After conversion to CDMS or after the initial 24-month period, placebo patients were placed on INFβ-1b. Participants were then followed through month 60 post-baseline.

A specially trained evaluating physician conducted all standardized neurological evaluations and determined the Expanded Disability Status Scale (EDSS) score. Relapses were assessed and defined using established guidelines.[14]

Serum samples were obtained at baseline (before beginning treatment) and at 6, 12, and 24 months and were received by the central laboratory within 3 days of collection and stored at –20ºC.

Brain MRI was conducted every 3 months in the first year and then at 18, 24, 36, 48, and 60 months. The MRI procedures used in BENEFIT have been previously described.[11-13] Briefly, T2- and T1-weighted images (following administration of 0.1 mmol/kg of gadolinium–diethyl-enetriaminepentaacetic acid) were analyzed centrally at the Image Analysis Centre at the VU University Medical Center in Amsterdam where the number of new lesions (including newly active lesions) and lesion volume were determined. Brain volume was quantified using the SIENAX (structural image evaluation using normalization of atrophy cross-sectional) algorithm. Owing to rigorous criteria with respect to scan quality and brain coverage, approximately 20% of the images were excluded from brain-volume analyses.

Participants in the BENEFIT clinical trial (NCT001185211) provided written informed consent, and this study was approved by Harvard T.H. Chan's School of Public Health's institutional review board. We used deidentified data.

BMI

Height and weight at baseline were registered for all participants at enrollment in BENEFIT. BMI was calculated as weight (kilograms) divided by the square of the height (meters), and categorized using the WHO classification of overweight and obesity in adults: underweight (<18.5 kg/m^2), normal weight (18.5-<25 kg/m^2), overweight (25-<30 kg/m^2), and obese (≥30 kg/m^2).

Smoking status

Cotinine levels were measured using an enzyme-linked immunosorbent assay (ELISA) per manufacturer's (DiaMedix Corp) instruction in the baseline, 6-, 12-, and 24-month serum samples. "Smokers" had serum cotinine levels in all measured samples >25 ng/mL—levels of cotinine indicative of regular nicotine use, and "non-smokers" had cotinine levels <10 ng/mL in all measured samples indicative of no nicotine use, as previously described.[19] Individuals with both high and low levels over time were "mixed" and treated as their own category.

Statistical analysis

BMI was modeled as categorical variable as described above. The median BMI within each category was modeled as a continuous variable to assess the linear trend across BMI categories.

There were three broad outcomes of interest based on clinical and MRI assessments: time to a definite diagnosis of MS, MS activity, and MS progression.

The primary outcome of BENEFIT was conversion to CDMS and the secondary outcome was conversion to 2001 MDMS. Cox proportional hazards models were used to estimate the hazard and 95% confidence intervals for the association between BMI and time to MS conversion.

The effect of BMI on MS activity was assessed by rate of relapses and number of new active lesions on brain MRI, defined as new or enlarging T2 lesions or new gadolinium-enhancing lesions from baseline through month 60. Cox proportional hazards models were used to estimate the effect of BMI on relapse rates and negative binomial regression models were used in analyses of number of lesions.

EDSS was assessed every 6 months. Clinical progression on EDSS was defined as an increase of at least 1.0 step from the baseline EDSS that was sustained for at least 6 months (yes/no). Logistic regression models were used to assess the association between BMI and EDSS progression and Cox proportional hazards models were used to assess whether BMI was associated with time to sustained EDSS progression. Progression on MRI was assessed by percentage change in T2 lesion volume,

and percentage change of brain volume. Due to inflammatory processes related to CIS, changes were determined with respect to either the 6-month (EDSS) or 12-month (brain and T2 lesion volume) values.[17,18] Generalized mixed models, treating the participants as a random effect, and including BMI by time interaction, were used to assess associations between BMI and MRI progression outcomes.

All analyses were adjusted for baseline age as a continuous variable, sex, smoking status , region of residence (Central Europe: Belgium, Netherlands, Germany, Austria, Switzerland, Denmark, France, Great Britain, Hungary, Czech Republic, Poland, Slovenia; Southern Europe: Spain, Portugal, Italy, Israel; Scandinavia: Finland, Norway, Sweden; North America: Canada), initial treatment group (INFβ-1b or placebo), number of T2 lesions at baseline, EDSS score at baseline, steroid treatment for CIS (yes/no), and onset type (monofocal or multifocal) for severity of the CIS. Baseline serum levels of 25-hydroxyvitamin D with seasonal correction, and baseline serum Epstein-Barr virus nuclear antigen-1 (EBNA-1) IgG levels were measured as previously described[20,21] and also included in the adjusted analyses.

We also conducted the above analyses stratified by smoking status (non-smoker or smoker) to determine whether smoking modified associations between BMI and MS outcomes.

Data Availability Statement The datasets analyzed in the current study are not publicly available because of restricted access, but further information about the datasets is available from the corresponding author on reasonable request.

RESULTS

Participant Characteristics and BMI

There were 468 participants enrolled in BENEFIT; 292 were randomized to treatment with INFβ-1b and 176 to placebo. Compared with participants with BMI <30 kg/m2, those with a BMI $\geq$ 30 kg/m2 at baseline were older and a higher percentage were smokers. (Table 1) They were more likely to

present with a monofocal event at onset, fewer T2 lesions, and lower T2 lesion volume at baseline than participants in other BMI categories. Only 51% of obese individuals were randomized to treatment with INFβ-1b as compared to over 60% in other BMI groups. EDSS score and steroid use at baseline were similar across BMI categories. Other baseline characteristics of participants are given in table 1.

Conversion from CIS to MS

During the 5 years of follow-up, 216 patients (46.6 %) converted to CDMS and 377 (81.3 %) converted to MDMS. In unadjusted analyses, obesity did not predict the conversion from CIS to CDMS (HR=0.95, 95% CI: 0.62-1.46) or MDMS (HR=1.18, 95% CI: 0.89-1.58); however, in multivariable analyses, obesity was associated with conversion to MDMS (HR=1.39, 95% CI: 1.02-1.91) through year 5 (Table 2). In multivariable analyses stratified by smoking status, obese non-smokers had a 65% increased hazard of conversion to MDMS as compared to normal weight non-smokers (Table 2). No association was observed in smokers.

MS Activity

New Active MRI Lesions

BMI at baseline was not associated with the number of new active brain lesions on MRI through month 60 (Table 3). There was a suggestion of a 3-fold increased rate of new active lesions associated with being underweight among smokers (Table 3). Among BENEFIT participants randomized to IFNB-1b there was no difference in no new lesions by BMI over the first 24 months (18.5-<25 kg/m2: 33%; ≥25 kg/m2: 31%).

Relapses

On average, patients in BENEFIT experienced 0.2 relapses per year. In unadjusted analyses, there was a non-statistically significant increase in rate of relapse among obese individuals (HR=1.38,

95% CI: 0.88-2.17, p-trend=0.19). In adjusted analyses, the rate of relapse increased to 53% higher in the obese versus the normal weight group (Table 3), with a statistically significant trend across the groups (p-trend=0.03). The overall association between BMI and relapse rate was similar in both smokers and non-smokers (HR for 1 kg/m^2 increase in BMI: smokers: 1.05, 95% CI: 1.01-1.10, p=0.03; non-smokers: 1.04, 95%CI:1.00-1.08 p=0.04). Obese smokers had an about 2-fold increased relapse rate compared to normal weight smokers (Table 3). Obese non-smokers had a non-statistically significant 42% increased rate of relapse as compared to normal weight non-smokers (Table 3).

Progression of MS

Change in T2 Lesion Volume

Obese participants had a lower T2 lesion volume at screening than other BMI groups (Table 1). Overall, there was a positive association between BMI and percent change in T2 lesion volume (% change=3.6, 95% CI: 0.55-6.7, p=0.02), but this association was driven by two extreme outliers with a change in T2 lesion volume > 1,000%. In analyses excluding these individuals, obesity was not associated with percent change of T2 lesion volume from month 12 through month 60 (Table 4). There were also no significant associations between BMI group and percent change in T2 lesion volume when stratifying by smoking status, though the interaction between BMI and time was statistically significant among smokers (p=0.008) (Table 4).

Change in brain volume

BMI was also not associated with percent change in brain volume over months 12 to 60 of the trial (Table 4). However, stratification by smoking status showed a larger reduction of brain volume in overweight and obese smokers as compared to normal weight smokers with a statistically significant trend (p=0.03) and the interaction between BMI and time in smokers was statistically significant

(p=0.03) (Table 4). There was no association between BMI and percent change in brain volume among non-smokers.

Sustained Clinical Progression on EDSS

Over the 60 months of follow-up, 110 participants met the criteria for sustained clinical progression on EDSS. In both unadjusted and adjusted analyses, there were no associations observed between BMI and either having sustained EDSS progression or time to sustained EDSS progression overall or stratified by smoking status. (Table 4) .

DISCUSSION

In this large prospective investigation, obesity was independently associated with an increased hazard of conversion from CIS to MDMS and a higher rate of relapses, but not with other MS related outcomes. Obesity was associated with decreased brain volume only in smokers.

While some of our results may seem paradoxical—for example, obesity was associated with an increased rate of conversion to MDMS and a higher relapse rate but not a higher number of new active lesions, it is important to note that most conversions to MDMS occurred within the first 24 months, but our follow-up goes through 60 months, thus included 3 years or more after MDMS conversion. Additionally, MRIs were only performed at pre-determined study times and not in conjunction with the occurrence of a relapse.

There have been a few prospective studies of the association between BMI and MS activity and progression.[22-24] In the AusLong study[23], BMI was measured at four time points over 5-years of follow-up of individuals with a CIS and did not predict conversion to MS, but higher BMI (in 5 kg/m2 increments) was associated with an increased risk of relapse and with an increased risk of annualized worsening in EDSS. Other studies were conducted in individuals with established MS of

187 average duration between 5 and 12 years.[24,25] One study found no association between BMI and
188 change in EDSS.[25] The only other prospective study to evaluate MRI outcomes was conducted
189 among 469 individuals with relapsing-remitting MS in the U.S. and increases in BMI were
190 associated with decreases in normalized gray matter volume and brain parenchymal volume over an
191 average of 4.1 years of follow-up.[24] In our study, there were no associations seen between BMI and
192 MRI outcomes except for a higher percentage brain volume lost with increasing BMI among
193 smokers.

194 A study in Norway conducted among 86 RRMS participants taking INFB-1a in the OFAMS trial
195 found that overweight/obese individuals were less likely to have no MRI activity (20%) over 24
196 months as compared with normal weight individuals (52%),[26] and the authors suggested that doses
197 of INFB-1a may need to be higher among overweight/obese individuals. In our study, among
198 participants in BENEFIT who were randomized to IFNB-1b for the first 24 months there was no
199 difference in the percentage exhibiting no new lesions (overweight/obese: 31% versus normal
200 weight: 33%). IFNB-1b appears to have similar efficacy with respect to MRI activity regardless of
201 BMI.

202 In our previous study on cotinine levels and MS outcomes in BENEFIT[27], we did not find any
203 associations between smoking and clinical or MRI outcomes over 5 years of follow-up. While
204 obesity was associated with an increase in relapse rate in both smokers and non-smokers, obesity
205 was associated with a decrease in brain volume only among the smokers. Similarly, a study among
206 the GEMS and EIMS case-control studies in Sweden reported that obesity at age 20 was associated
207 with risk of conversion to SPMS only among ever smokers.[22] Components of cigarette smoke are
208 known to disrupt immune system function and have neurotoxic effects and the increased adipose
209 tissue in obesity creates a chronic low-grade inflammatory state[10] characterized by an increase of
210 inflammatory and reduction of anti-inflammatory chemokines secreted by adipocytes, increase of
211 type 1 macrophages, increase of Th1 and Th17 lymphocyte proliferation and down-regulation of T

regulatory lymphocytes[10] In a recent study of a small cohort of MS patients, having a BMI >24 kg/m2 appears to modulate monocyte numbers through ceramide-induced DNA methylation of anti-proliferative genes.[28] Obesity is associated with brain volume loss in the general population.[29] While it is possible that we would observe a decrease in brain volume among obese non-smokers if we had a longer follow-up, smoking may accelerate brain volume loss in obese individuals with MS.

Strengths of this study include the longitudinal design, recruitment of all patients at the time of CIS, the large number of participants, standardized treatment (early vs late IFNß-1b), rigorous clinical, including standardized measures of BMI, and MRI assessment of all patients during 5-year period, and information on other predictors of MS activity and progression that we adjusted the analyses for.[11-13] Our study also has limitations to consider. First is that BMI was only measured at baseline. Therefore, we cannot examine whether and how changes in BMI over the course of follow-up are associated with MS disease activity and progression. Second, we did not have a history of smoking status, but rather a biomarker of nicotine exposure. Although stringent criteria were used to define smokers and non-smokers, cotinine does not capture past smoking and any associations on future MS disease activity and progression by past smoking cannot be independently assessed. Third, most participants were eventually treated with IFNß-1b, and although uniform treatment is an important advantage, our results may not apply to patients treated with other disease modifying therapies. Additionally, this was a post-hoc analysis of clinical trial data and multiple comparisons were not corrected for. Nearly all BENEFIT participants were white individuals of European ancestry, thus limiting generalizations to individuals of other races or ethnicities.

CONCLUSIONS

In our study we found that obesity is associated with an increased rate of conversion from CIS to MDMS and with increased MS disease activity (high rate of relapses). Obese smokers may have an

increased rate of brain atrophy. These results suggest that prevention and treatment of obesity may have disease-specific benefits in individuals with MS.

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Disclosures

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Table 1 Baseline characteristics by BMI at baseline--BENEFIT

	Body mass index at baseline, kg per m^2			
	<18.5	18.5-<25	25-<30	≥30
	(N = 18)	(N = 271)	(N = 119)	(N = 57)
BMI, kg per m^2	18.0 (17.0-18.0)	22.0 (21.0-23.0)	27.0(25.0-28.0)	32.5 (31.0-35.0)
Age at baseline, years*	29.6 (5.9)	30.1 (7.4)	31.6 (7.6)	32.7 (7.4)
Female, %	91.3	71.9	62.8	69.5
Active smoker[a], %	29.6	32.1	33.7	35.8
Country or region	82.3	69.6	66.2	55.7
Central Europe[b], %				
Canada, %	14.6	4.1	7.1	7.5
Scandinavia, %	0.0	8.9	14.8	16.0
Southern Europe, %	3.1	17.4	11.9	20.8

Table 1 Baseline characteristics by BMI at baseline--BENEFIT

	Body mass index at baseline, kg per m^2			
	<18.5	18.5-<25	25-<30	≥30
	(N = 18)	(N = 271)	(N = 119)	(N = 57)
Time to CDMS, days	415 (141-1,497)	1,176 (351-1,804)	1,064 (342-1,805)	672 (136-1,806)
Time to MDMS[d], days	191 (96.0-372.0)	194 (100-700)	214.0 (96.0-929.0)	100 (86-558)
INFß-1b treatment group, %	62.1	62.6	68.1	51.0
Multifocal onset, %	52.1	47.4	51.6	]29.0
T2 lesion number at screening	33 (9.0-52.0)	19.0 (7.0-40.0)	17.0(8.0-33.0)	13.0 (5.0-35.0)
T2 lesion volume at screening, mm^3	2,044 (1,107-8664)	1,871 (621-4,790)	1,947 (718-4,343)	1,717 (389-2,987)
Brain volume at screening, cm^3	1,073 (1,056-1,097)	1,056 (1,022-1,084)	1,056 (1,019-1,082)	1,048 (1,029-1070)
EDSS at baseline	1.5(1.0-2.0)	1.5(1.0-2.0)	1.5 (1.0-2.0)	1.5(0.0-2.0)
25(OH)D at baseline, nmol/L	45.8 (38.5-61.3)	47.0 (37.6-59.0)	48.0 (37.7- 58.5)	43.9 (34-51.6)

Table 1 Baseline characteristics by BMI at baseline--BENEFIT

	Body mass index at baseline, kg per m^2			
	<18.5	18.5-<25	25-<30	≥30
	(N = 18)	(N = 271)	(N = 119)	(N = 57)
Steroid use at baseline, %	72.9	74.6	62.3	75.6

Values are medians (interquartile ranges) or percentages and are standardized to the age distribution of the study population.

[*] Value is not age adjusted

[a] Active smoker defined as having all cotinine measures—baseline, months 6, 12, and 24— >25 ng/mL

[b] **Central Europe**: Belgium, Netherlands, Germany, Austria, Switzerland, Denmark, France, Great Britain, Hungary, Czech Republic, Poland, Slovenia;

Southern Europe: Spain, Portugal, Italy, Israel; **Scandinavia**: Finland, Norway, Sweden

[d] 2001 McDonald MS

Table 2. Hazard ratios (HR) of conversion to CDMS/MDMS[a] by BMI categories and smoking status

	CDMS[a]			2001 MDMS[b]		
	No. y/n	**HR**	**95% CI**	**No. y/n**	**HR**	**95% CI**
BMI (kg/m^2)						
All						
<18.5	8/10	1.27	0.61-2.63	14/4	1.07	0.65-1.76
18.5-<25	129/142	1	Ref.	224/47	1	Ref.
25-<30	54/65	1.10	0.79-1.54	94/25	1.11	0.88-1.39
>30	25/32	1.00	0.62-1.59	45/12	1.39	1.02-1.91
Non-smokers						
<18.5	5/5	1.47	0.57-3.82	8/2	1.02	0.50-2.08
18.5-<25	70/82	1	Ref.	125/27	1	Ref.
25-<30	27/36	0.98	0.62-1.56	48/15	1.02	0.75-1.40

>30	16/17	1.07	0.58-1.97	28/57	1.65	1.08-2.50
Active smokers						
<18.5	2/4	0.78	0.17-3.55	5/1	1.27	0.51-3.13
18.5-<25	41/46	1	Ref.	72/15	1	Ref.
25-<30	17/22	1.00	0.54-1.87	32/7	1.06	0.68-1.64
>30	8/12	1.05	0.45-2.48	13/7	0.84	0.47-1.51

*Models adjusted for age, sex, treatment allocation, and baseline: serum 25(OH) vitamin D with seasonal correction, no. T2 lesions, T2 lesion volume, EDSS score, steroid treatment, EBNA-1 IgG levels, smoking status.

[a]CDMS: Clinically Definite Multiple Sclerosis.

[b]2001 McDonald MS

Table 3: Hazard ratios (HR) for new active brain lesions,relapses and time to sustained EDSS progression according to BMI categories--baseline to 60 months								
		<18.5		18.5-<25		25-<30	≥30	
		HR(95% CI)		Ref		HR(95% CI)	HR(95% CI)	P trend
New active brain lesions								
All		1.11 (0.55-2.22)		1		1.18 (0.86-1.61)	1.34 (0.88-2.04)	0.14
Non-smokers		0.47 (0.18-1.23)		1		0.95 (0.62-1.45)	1.28 (0.75-2.18)	0.28
Active smokers		3.13 (1.04-9.39)		1		1.32 (0.79-2.21)	1.40 (0.70-2.79)	0.83
Relapses								
All		0.71 (0.42-1.23)		1		1.12 (0.80-1.57)	1.53 (1.01-2.30)	0.03
Non-smokers		0.60 (0.28-1.28)		1		0.88 (0.56-1.38)	1.43 (0.87-2.34)	0.20
Active smokers		1.02 (0.37-2.90)		1		1.64 (0.93-2.90)	2.04 (0.99-4.19)	0.03

EDSS								
All		1.16 (0.41-3.34)		1		1.09 (0.70-1.71)		0.89 (0.48-1.65)	0.82
Non-smokers		2.20 (0.56-8.6)		1		0.72 (0.36-1.42)		0.88 (0.39-1.99)	0.37
Active smokers		0.84 (0.10-6.81)		1		1.30 (0.61-2.77)		0.51 (0.15-1.71)	0.55

* Adjusted model by age, sex, smoking status, region of residence, baseline serum levels of anti-EBV IgG antibodies, treatment allocation, treatment allocation, baseline serum 25(OH) vitamin D with seasonal correction, no. T2 lesions and brain volume at baseline, EDSS score, steroid treatment at baseline, and CIS onset type.

Table 4: Percentage annual change in cerebral T2 lesion volume and brain volume by BMI

	Body mass index categories				
	<18.5	**18.5-<25**	**25-<30**	**≥30**	**P trend**
T2 lesion volume[*] [a] (% change, 95% CI)					
All	-0.86 (-38.7, 37)		9.8 (-6.8, 26.4)	5.0 (-17.5, 27.6)	0.39
Non-smokers	-10.1 (-55.8, 35.5)	Ref	-3.55 (-23.1, 16)	-0.23 (-25.7, 25.2)	0.97
Active smokers	-2.7 (-91.3, 85.8)		29.9 (-10.4, 70.1)	13.3 (-43.0, 69.6)	0.34
Brain volume, cm^3 [a] (% change, 95% CI)					
All	0.04 (-0.49, 0.56)		-0.09 (-0.34, 0.16)	-0.11 (-0.45, 0.22)	0.39
Non-smokers	0.20 (-0.54, 0.94)	Ref	0.04 (-0.28, 0.36)	-0.02 (-0.44, 0.40)	0.90
Active smokers	0.01 (-0.95, 0.96)		-0.27 (-0.78, 0.23)	-0.78 (-1.47, -0.09)	0.03

Adjusted for age, sex, smoking status, region of residence, treatment allocation, baseline serum 25(OH) vitamin D with seasonal correction, no. T2 lesions at baseline, steroid treatment at baseline, EDSS at baseline (volume analyses only) and CIS onset type.

[a] From 12 to 60 months

[b] From 6 to 60 months

* Two extreme outliers with very high change in T2 lesion volume were excluded.