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Fatty acid binding protein-4 is associated with disability in multiple sclerosis patients

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Abstract

Background—Adiposity represents a risk factor for multiple sclerosis (MS) and is associated with increased disability scores. Adipokines may mediate the effects of adiposity on MS disease course.

Objective—The objective of this study is to examine the association between the adipokines (leptin and fatty acid binding protein-4, FABP4) and clinical course in individuals with MS.

Methods—Subjects (18–65 years) with relapsing remitting MS or CIS and <10 year disease duration, were selected from a longitudinal clinical study. Cross-sectional and longitudinal models assessed the relationship between two adipokines (leptin and FABP4) and disease severity in women and men, adjusting for age, disease duration and disease type, Vitamin D level, testosterone level, and as well by BMI.

Results—Mean age of subjects (N=163, 56% women) was 39.3 years. Higher FABP4 levels were associated with higher EDSS scores in women in both univariate and multivariate analysis (Odds ratio: 1.30; p=0.005). In men, higher FABP4 level was significantly associated with change

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in EDSS over time (Estimate 0.0062; p=0.035). We found no association of FABP4 levels with time to next relapse or a measure of processing speed.

Conclusion—FABP4 levels may be associated with increased disability in both men and women with MS independent of effects of BMI and other hormones. Future studies should expand these analyses and further explore downstream mechanisms of adiposity-related effects in MS.

Keywords

body mass index; leptin; FABP; gender; multiple sclerosis

INTRODUCTION

Since initial reports of an association between adolescent obesity and multiple sclerosis (MS) risk in the Nurses' Health Study,[1] adiposity in early life has emerged as a risk factor both for adult-onset[2–4] and childhood-onset MS.[5, 6] We have recently found that an increased body mass index is associated with increased EDSS scores in women with MS, but we found an inverse relationship in men[7]. These results suggest that more detailed measures of adiposity are needed to better understand the its effects on an autoimmune disease.

Adiposity-related pro-inflammatory factors, termed adipokines provide a more reliable indicator of adiposity than body mass index (BMI), which can be increased by lean muscle mass, and may shed light on the mechanisms of adiposity-mediated inflammation in MS. Among these, leptin, an appetite signaling hormone that acts as a pro-inflammatory cytokine,[8] has been reported to be elevated in individuals with MS.[9–13] In addition, in a pilot study we have reported adipocyte fatty acid binding protein (a-FABP or FABP4), a cytoplasmic carrier protein in adipocytes and biomarker for metabolic syndrome,[14] is elevated in MS, and more so in progressive forms of MS.[15]

There are several important knowledge gaps. The first is whether adipokines are associated with longitudinal clinical course, and whether these associations are more sensitive than, or independent from, associations with BMI alone. Second, the significance, if any, of known sexual dimorphism in levels is unknown. Additionally, it is not known whether their association with MS is independent from vitamin D and androgen levels, both of which are lower in healthy individuals with obesity, and which we and others have found to been associated with MS disease course.[16, 17]

In the current study, we hypothesized that higher adiposity markers are associated with worse MS cross-sectional and longitudinal outcomes, controlling for BMI as well as other hormonal covariates. We assessed disability, on a cognitive measure of processing speed commonly evaluated in MS. Additionally, we explored the hypothesis that associations between adiposity measures and adverse clinical course were stronger in women than in men.

MATERIALS AND METHODS

Subjects

The subjects included in this study were patients of the Partners MS Center, aged 18–65, enrolled in the Comprehensive Longitudinal Investigation of Multiple Sclerosis at the Brigham and Women's Hospital (CLIMB, www.climbstudy.org)[18]. This study has enrolled over 2,100 patients since 2000, and patients are followed longitudinally with standardized clinical exams every 6 months, and annualized magnetic resonance imaging scans (MRIs) and stored blood samples. Patients were included in this study if they met the diagnostic criteria of relapsing remitting MS (RRMS) by the 2005 McDonald criteria[19] or of clinically isolated syndrome (CIS), had first symptom onset within the 10 years prior to the blood sample, and had at least 2 years of clinical follow up available after a blood sample. We sought an approximately balanced ratio of women to men in this study, in order to increase our power to see male-specific effects. The male subjects represent a subset of a cohort previously described,[16] and were included here to allow analyses of sex differences; importantly, the same demographic and clinical criteria were used to select female and male subjects. Clinical data through 17 October 2013 were included for analysis.

Standard Protocol Approvals, Registrations, and Patient Consents

Institutional Review Board approval was granted by the Partners Human Research Committee, and participants provided written informed consent for participation.

Hormonal and metabolic measures

Stored blood samples were selected if they had been drawn between 0830 and 1130 to minimize diurnal variability, and at least 30 days from exposure to exogenous glucocorticoids. In order to minimize the confounding caused by deleterious effects of chronic disease on testosterone levels,[20] only samples drawn within 10 years of first symptom onset were included. If several samples meeting these criteria existed for a given individual, one sample per individual was selected according to the following priorities: shortest disease duration, earliest time of day, and greatest volume of specimen remaining for subsequent CLIMB analyses. According to CLIMB protocol, all samples were stored at −80° C following their collection. All samples were processed in the Harvard Catalyst Clinical laboratory with the exception of FABP4, which was processed in duplicate in the CLIMB laboratory.

Serum leptin levels were measured using Radioimmunoassay (Millipore, St. Charles, MO). The lower limit of detection was 0.1 ng/mL, with an intrassay variation of 5.2–7.5%. FABP4 was measured in serum by ELISA (human FABP4, BioVendor) with an intra-assay coefficient of variant of 1.4% and an inter-assay coefficient of variant of 6.4%. For each measure, the experiments were completed in batches of up to 40 patients per batch. Plasma 25-hydroxyvitamin D (25(OH)VitD) levels were measured using a radioimmunoassay (Diasorin, Inc, Sillwater, MN). The lower limit of detection was 1.5 ng/dL, with an intra-assay variation of 4.4–8.3%. Plasma testosterone levels were measured using Access Chemiluminescent Immunoassay (Beckman Coulter, Fullerton, CA). The lower limit of detection of testosterone was 10 ng/dL, with an intra-assay variation of 1.67–3.93%. BMI

(kg/m^2) was calculated from height and weight measures for 135 of the cohort subjects available in the medical record.

Clinical outcomes

The primary outcome, clinical disability, was measured using the Expanded Disability Status Scale (EDSS), performed every 6 months (mean (SD) follow in this study: 4.20 (2.42) years). Secondary outcomes were: Time to next relapse and Symbol Digit Modalities Test (SDMT) scores which is a measure of cognitive processing speed. Clinical relapse was defined as the appearance of new symptoms lasting more than 24 hours in the absence of fever or intercurrent illness, was determined by the treating physician at the biannual clinical visit, and entered into the study database. All relapses were subsequently validated by an author on the study team by reviewing the research database against the clinical record (RB, CD-C, or NS). The Symbol Digit Modalities Test (SDMT; higher scores indicate better cognitive performance), a test of executive function and processing speed and a sensitive early marker of longitudinal cognitive changes in MS, was administered beginning in 2006 and was available for 106 subjects (mean (SD) follow up: 2.95 (2.02) years).

Statistical Analysis

Analyses

Demographic, disease and hormonal characteristics—We compared demographic, disease and hormonal characteristics in men and women using t-tests for continuous variables (age, hormonal levels, BMI, disease duration), chi-squared for categorical variables (smoking history (yes/no), disease type, DMT treatment category), and Wilcoxon rank sum test for EDSS. We estimated the correlation between the various hormonal markers using Pearson's correlation coefficients between each pair of hormones for the entire cohort.

Associations between hormonal markers and MS outcomes—We assessed the cross-sectional relationship between each hormonal marker and EDSS at the study visit where hormonal levels were drawn (primary outcome) using ordinal logistic regression. In addition to the individual hormone analyses, we fit a multivariate model including raw 25(OH)VitD and testosterone, in addition to leptin or FABP4, to assess the impact of each variable in the presence of the other hormones. In the multivariate model, we included only one adipokine (leptin or FABP4) given the high correlation between leptin and FABP4 noted in Pearson's correlations ($r=0.63$, $p<0.001$; eTable 1, and selected whichever of the two showed the more significant associations in the univariate model). To investigate sex-specific effects of these endocrine markers, we secondarily included an interaction term between each endocrine marker and sex in all analyses. We then assessed the relationship between each baseline hormonal marker and longitudinal change in EDSS using mixed models with random intercept and slope. We then adjusted all analyses for BMI.

The same analyses were used for SDMT except that linear regression was used as opposed to proportional odds logistic regression; and we adjusted for depression using CES-D scores when assessing SDMT as an outcome. For the analysis of time to first relapse from baseline hormonal measurement, a Cox proportional hazards model was used. All models were adjusted for age, disease duration and disease type.

All statistical analyses were completed in the statistical package R version 3.0.2 (www.r-project.org).

RESULTS

Clinical and demographic characteristics

In this cohort of 163 subjects (55.8% women, 87.7% RRMS), at baseline men and women did not significantly differ in age or EDSS, but men had significantly longer disease duration (Table 1). There was no association between any of the hormone levels and either DMT treatment category or smoking history (ever/never). As expected, women had higher levels of leptin, FABP4 and 25(OH)VitD and lower testosterone levels than men. In the 135 individuals with BMI data available, BMI was correlated with both leptin ($r=0.56$, $p<0.0001$) and FABP4 ($r=0.51$, $p<0.0001$); leptin and FABP4 were positively correlated with one another, but not with any other marker (eTable 1). Altogether, 63.0% of individuals had a BMI above 25.0 kg/m^2, the World Health Organization's International Classification categories for overweight or obese.

Hormonal associations with EDSS

Cross-sectional—Higher FABP4 levels in the entire cohort were associated with higher EDSS, our primary outcome measure (Table 2). The significant univariate associations remained significant in the multivariate model, suggesting that FABP4 effects were independent of any effects of 25(OH)VitD or testosterone (Table 2), and also remained significant after adjusting for BMI (eTable 2). This remained significant on sensitivity analysis when including untreated patients only (results not shown). Notably in the multivariate models, lower vitamin D levels were also associated with higher disability. Leptin did not show a significant association with EDSS. When we stratified our analyses by sex, the cross-sectional association between FABP4 and EDSS was significant only in the women; however there was no significant interaction between sex and FABP4 in terms of EDSS as an outcome ($p>0.2$).

Longitudinally, there were no hormonal associations with our primary measure, EDSS, in the entire cohort. However, in men, higher FABP4 levels were associated with worsening EDSS severity, as was lower vitamin D, and these associations remained significant in multivariate analyses (Table 2) after adjusting for BMI (Supplementary eTable 2).

Secondary outcomes measures

Relapse—We did not find an effect of leptin or FABP4 levels on time to next relapse in the entire cohort or when stratified by sex (Table 3).

SDMT—In the whole cohort, there was a significant association of lower cross-sectional Vitamin D level with lower SDMT that did not retain significance in the multivariate model. In women, higher leptin levels were associated with a lesser decline in SDMT in univariate analysis only, but did not remain significant in multivariate analysis (Table 4). There were no associations with FABP4 or leptin and SDMT scores in the men. There was a significant association of lower testosterone levels with longitudinal SDMT decline in the whole cohort

and borderline associations in men in univariate, and multivariate models, consistent with our previously published results.[16]

DISCUSSION

In this study of 163 individuals early in the course of relapsing-onset MS and CIS, 60% of whom were overweight or obese, we performed a comprehensive evaluation of two adipokines (leptin and FABP4). We found that higher levels of the adipokine, FABP4 were associated with higher clinical disability as measured by EDSS. While in women this association was notable at baseline in univariate as well as multivariate analysis. In men, who had lower adiposity markers overall, this became apparent in longitudinal analyses, and remained significant in multivariate analysis adjusting for 25(OH)Vitamin D level and testosterone levels. These results add to our prior cross-sectional analysis which found increased FABP4 levels in patients with secondary progressive MS compared to controls.[15]

It is well established that BMI is not an ideal marker of adiposity, as it can be increased with lean muscle as well as fat.[22] A growing body of research also supports a direct inflammatory role for hormones associated with obesity. Leptin, a hormone release by fat cells, may act as a powerful pro-inflammatory adipokine influencing lymphocyte function, that promotes Th1 responses on one side and inhibits regulatory T cell expansion on the other, leading to increased neuroinflammatory responses *in vitro* and in EAE models; it may trigger inflammatory attacks in humans susceptible to MS.[8]

FABP4, also known as adipocyte FABP (A-FABP) is present in adipose tissue and mature adipocytes and macrophages. This protein has also been termed adipocyte P2 (aP2) since there is high sequence similarity (67%) with the myelin P2 protein (M-FABP/FABP8).[23] FABP4 is highly expressed in adipocytes and consists of about 1% of all soluble proteins in adipose tissue[24]. FABPs function to actively facilitate the transport of FAs to specific organelles in the cell for lipid oxidation in the mitochondrion or peroxisome; lipid-mediated transcriptional regulation in the nucleus; signaling, trafficking, and membrane synthesis in the endoplasmic reticulum (ER); and regulation of enzyme activity and storage as lipid droplets in the cytoplasm[25]. FABP4 is highly upregulated during foam cell formation in response to lipid loading[26]. Higher FABP4 levels are associated with increased risk for metabolic syndrome,[27] and has been associated with increased risk and mortality from ischemic stroke controlling for conventional risk factors including cholesterol levels.[28] FABP4 is induced through TLR-4 signaling and modulates inflammatory responses in macrophages through a positive feedback loop involving c-Jun NH2-terminal kinases and activator protein-1.[29] Rao et al. demonstrated that A/E-FABP (FABP4 and 5) deficient mice have a milder course of EAE[30]. In this study, FABP4 exhibited both sexual dimorphism and independent associations with MS disability scores.

There is a growing literature demonstrating an association with lipids and MS. Total cholesterol levels, low density lipoprotein and triglyceride levels have been associated with EDSS worsening in MS patients.[31, 32] Another study found higher levels of adiposity and the total cholesterol/high density lipoprotein (HDL) ratio were prospectively associated with a higher rate of disability progression, while higher adiposity and triglycerides were

associated with relapse in early MS patients,[33] providing a further link with adipose markers and MS disease course. Lipids can activate the immune system through CD1a-, CD1b-, CD1c-, or CD1d-restricted T cells, which in the case of CD1d, are termed natural killer T (NKT) cells. These data combined with ours suggest that lipid metabolism may influence MS disease course, and that these markers may be used to monitor MS disease course, and be a target of both lifestyle as well as therapeutic intervention.

The strengths of this study include a well-characterized cohort with large numbers of males and females and adjustment for other hormones known to be associated with MS course. Because adiposity is associated with low vitamin D,[34] which is the best studied hormonal modulator of MS risk,[35] an important contribution of this study is to show an association of adipokine signaling hormones with disease course that is independent of vitamin D. These associations were also independent of testosterone, which has been associated with MS risk and course, including in a larger cohort that included the men presented in this sample.[16]

Another strength of the study is the exploration of potential sex differences in the association between obesity and MS risk, as well as sex differences in adiposity markers noted in the current study. One interpretation of our results could be that in men, who have lower levels of adipokines and vitamin D, the magnitude of the effect of these hormones on disease severity is similar to women, but a larger cohort is required to achieve significance. Overall, FABP4 appeared to show more significant associations with MS course. In fact, both FABP4 and 25(OH)VitD were significant in the longitudinal analyses of EDSS in men. Important for further studies, the sex ratio of a clinical cohort may determine the magnitude and significance of any hormonal effect noted. The prioritization of which hormones to target in terms of therapeutic interventions might also differ by sex.

Limitations of the study include the lack of standardization of treatment, however over half our sample was untreated at the time of blood sampling, and the rest were on first line treatment (B-IFN or glatiramer acetate). In the current study, in addition to our primary outcome (EDSS), we also explored adiposity associations with SDMT, increasing our number of exploratory analyses and the likelihood of false discoveries. An additional limitation is that, while samples were selected to identify individuals early in MS course, this naturally limited the range of EDSS values (mean and median: 1) so that impact of hormones on patients with more severe course could not be identified in this sample. Further studies should include progressive MS patients and those with more advanced disease, as well as MRI analysis.

In summary, adiposity biomarkers appeared to show important associations with disease severity in individuals with early MS, potentially providing a mechanistic understanding of the role of adiposity in inflammatory diseases. FABP4 appears to be an important factor in disability accrual in MS patients. Future studies should expand these analyses to subjects with progressive onset disease and thus potentially more aggressive disease, as well as mechanistic studies to shed further light on the impact of a growing obesity epidemic, and potential pro-inflammatory milieu, on MS course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Demographic, disease and hormonal characteristics of study participants.

	Women	Men	p-value
N	91	72	
Demographic characteristics			
Age (years, mean (SD))	38.7 (8.5)	39.9 (9.0)	0.40
% White[^]	97.8	94.4	0.41
Disease characteristics			
Disease duration (years, mean (SD))	3.0 (1.4)	4.4 (2.5)	**<0.001**
% Relapsing-remitting	83.5	93.1	0.11
EDSS (median (range))	1 (0, 3.5)	1 (0, 3.5)	0.097
SDMT (mean (SD))[&]	56.9 (10.4)	55.3 (11.9)	**0.55**
CES-D (mean (SD))[#]	10.8 (9.0)	7.7 (6.8)	**0.043**
Disease modifying therapy at blood draw (N)			0.24
None	57	34	
Interferon	18	22	
Glatiramer acetate	13	12	
Other	3	4	
Endocrine Variables			
Leptin (ng/mL)	22.6 (16.9)	8.8 (5.9)	**<0.001**
a-FABP (ng/mL)	27.7 (20.7)	20.7 (11.0)	**0.007**
BMI, kg/m^2 (mean (SD))[*]	26.6 (6.5)	28.0 (4.3)	0.16
25(OH)VitD(ng/mL)	30.2 (12.2)	26.5 (9.6)	**0.033**
Testosterone (ng/dL)	17.8 (17.2)	335.7 (124.7)	**<0.001**

BMI: body mass index. CES-D: for Epidemiologic Studies Depression scale. EDSS: Expanded Disability Status Scale. 25(OH)VitD: 25-hydroxy-vitamin D; a-FABP: adipocyte-fatty acid binding protein. SD: standard deviation. SDMT: Symbol Digit Modalities Test.

[^]Two women's race was listed as unknown or not reported.

[&]35 women and 30 men had SDMT scores to contribute to this analysis

[#]62 women and 48 men had CES-D scores to contribute to this analysis

[*]73 women and 62 men contributed a BMI measurement.

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Table 2

Cross-sectional and longitudinal adjusted associations between adiposity markers and MS disability (EDSS).

	UNIVARIATE ASSOCIATIONS			MULTIVARIATE ASSOCIATIONS		
	All subjects	**Women**	**Men**	**All subjects**	**Women**	**Men**
Cross-sectional EDSS (N=163) – Odds ratio greater than 1 indicates worse function						
Adiposity Markers						
Leptin	1.20 (0.060)	1.25 (0.072)	2.08 (0.079)	-	-	-
a-FABP	**1.26 (0.004)**	**1.27 (0.012)**	1.18 (0.44)	**1.27 (0.003)**	**1.30 (0.005)**	1.05 (0.81)
Additional markers						
25(OH)VitD	**0.71 (0.012**)	**0.69 (0.036)**	0.76 (0.24)	**0.69 (0.007)**	**0.64 (0.015)**	0.77 (0.29)
Testosterone	0.99 (0.55)	1.00 (0.97)	**0.95 (0.008)**	1.00 (0.72)	1.04 (0.74)	**0.95 (0.009)**
Longitudinal EDSS (N=163) – a positive value indicates worsening function						
Adiposity Markers						
Leptin	0.00029 (0.85)	−0.00016 (0.93)	−0.0052 (0.39)			
a-FABP	0.0018 (0.16)	0.0004 (0.78)	**0.0067 (0.025)**	0.0017 (0.19)	0.0002 (0.91)	**0.0062 (0.035)**
Additional markers						
25(OH)VitD	−0.00188 (0.36)	0.0001 (0.96)	**−0.011 (0.009)**	−0.0019 (0.37)	−0.0006 (0.81)	**−0.010 (0.017)**
Testosterone	−0.00004 (0.72)	0.0035 (0.17)	0.00023 (0.41)	−0.00003 (0.80)	0.0035 (0.18)	0.0004 (0.11)

Cross-sectional EDSS analyses: Odds ratio for a ten-unit increase in each of the hormonal markers (p-value). Adjusted for age, disease duration and type.

Longitudinal EDSS analyses: Estimate and p-value of the association between hormonal markers and change in EDSS over time are provided. Adjusted for age, disease duration and type.

25(OH)VitD: 25-hydroxy-vitamin D; a-FABP: adipocyte-fatty acid binding protein. EDSS: Expanded Disability Status Scale.

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Table 3

Associations between adiposity markers and secondary outcome: time to next relapse

	UNIVARIATE ASSOCIATIONS			MULTIVARIATE ASSOCIATIONS		
	All subjects	**Women**	**Men**	**All subjects**	**Women**	**Men**
Time to next relapse (N=163) – a positive value indicates worsening function						
Adiposity Markers						
Leptin	0.97 (0.66)	0.99 (0.88)	0.93 (0.82)			
a-FABP	0.95 (0.47)	0.93 (0.42)	1.10 (0.54)	0.96 (0.51)	0.93 (0.42)	1.12 (0.47)
Additional markers						
25(OH)VitD	1.12 (0.26)	1.15 (0.24)	0.96 (0.86)	1.11 (0.29)	1.13 (0.32)	0.96 (0.86)
Testosterone	1.00 (0.98)	1.10 (0.28)	1.01 (0.69)	1.00 (0.96)	1.10 (0.32)	1.01 (0.54)

25(OH)VitD: 25-hydroxy-vitamin D; a-FABP: adipocyte-fatty acid binding protein

Time to next relapse: cox proportional hazards ratio increase in time to next relapse for one unit increase in each of the hormone measures (p-value). Adjusted for age, disease duration and type.

Table 4

Cross-sectional and longitudinal adjusted associations between adiposity markers and secondary outcomes SDMT

	UNIVARIATE ASSOCIATIONS			MULTIVARIATE ASSOCIATIONS		
	All subjects	**Women**	**Men**	**All subjects**	**Women**	**Men**
Cross-sectional SDMT (N=65) – positive score indicates better function						
Adiposity Markers						
Leptin	−0.01 (0.90)	−0.04 (0.70)	0.15 (0.76)	-	-	-
a-FABP	−0.13 (0.092)	−0.12 (0.16)	−0.15 (0.58)	−0.11 (0.15)	−0.11 (0.16)	−0.11 (0.68)
Additional markers						
25(OH)VitD	0.25 (0.058)	0.27 (0.087)	0.029 (0.93)	0.24 (0.076)	0.28 (0.087)	−0.015 (0.96)
Testosterone	0.002 (0.82)	0.005 (0.97)	0.019 (0.23)	0.002 (0.73)	0.056 (0.66)	0.018 (0.27)
Longitudinal SDMT (N=96) * - a positive value indicates less worsening in function						
Adiposity Markers						
Leptin	−0.007 (0.57)	**0.031 (0.042)**	−0.0075 (0.90)	0.019 (0.20)	0.031 (0.057)	−0.0042 (0.94)
a-FABP	0.007 (0.58)	0.030 (0.055)	0.0073 (0.79)	-	-	-
Additional markers						
25(OH)VitD	−0.0009 (0.96)	0.006 (0.74)	−0.015 (0.79)	0.0078 (0.64)	−0.004 (0.83)	−0.022 (0.70)
Testosterone	**0.0039 (<0.001)**	0.011 (0.64)	0.0040 (0.056)	**0.0048 (<0.001)**	−0.004 (0.87)	0.0040 (0.060)

*: Random intercept only model was used for the SDMT because of problems fitting random intercept and slope model for some of the predictors.

Cross-sectional analyses: Increase in mean score for one unit increase in each of the hormone measures (p-value). Adjusted for age, disease duration and type. SDMT analyses also adjusted for CES-D.

Longitudinal analyses: Estimate and p-value of the association between hormonal markers and change in score over time are provided. Adjusted for age, disease duration and type. SDMT analyses also adjusted for CES-D.