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Abstract

Background

People with multiple sclerosis (PwMS) have reduced bone mineral density (BMD), but the causes are unclear. Some factors that may cause reduced BMD in PwMS have been understudied, including physical activity, inflammation, cortisol, symptomatic fatigue, and depression. The aim of this study was to investigate factors that may uniquely contribute to reduced BMD in PwMS as compared to people without MS. We hypothesized that physical activity would be the primary determinant of low BMD in PwMS, with additional contributions from inflammation and sympathetic nervous system activation.

Methods

We tested 23 PwMS (16 women; median EDSS: 2) and 22 control participants (16 women). BMD was measured from the femoral neck and lumbar spine with dual x-ray absorptiometry. Disability was measured with the Expanded Disability Status Scale, and functional capacity was measured with the Multiple Sclerosis Functional Composite. Questionnaires measured symptomatic fatigue and depression. A blood draw was used to measure calcium, phosphate, vitamin D, N-terminal telopeptide, osteopontin, and cytokine markers of inflammation. Physical activity was measured with accelerometry. Salivary cortisol and cardiac heart rate variability also were obtained. All outcome variables were compared between groups with independent samples t-tests. Variables that were different between groups and significantly correlated (Pearson product-moment) with femoral neck BMD, were included in a theoretical model to explain femoral neck BMD. The expected direction of relations in the theoretical model were developed based upon the results of previous research. A Bayesian path analysis was used to test the relations of predictive variables with femoral neck BMD and interrelations among predictive variables, as detailed in the theoretical model.

Results

PwMS had lower BMD at the femoral neck than controls (p = =0.04; mean difference: -0.09; 95% CI: -0.2, -0.004; Cohen's d = =0.65), and there was a smaller, statistically non-significant difference in BMD at the lumbar spine (p = =0.07; mean difference: -0.08; 95% CI: -0.17, 0.007; Cohen's d = =0.59). PwMS also had lower functional capacity ($p \leq 0.001$; Cohen's d = =1.50), greater fatigue ($p<0.001$; Cohen's d = =1.88), greater depression ($p<0.001$; d = =1.31), and decreased physical activity (p = =0.03; Cohen's d = =0.62). Using path analysis to test our theoretical model, we found that disability (standardized estimate= -0.17), physical activity (standardized estimate=0.39), symptomatic fatigue (standardized estimate= -0.36), depression (standardized estimate= -0.30), and inflammatory markers (standardized estimate=0.27) explained 51% of the variance in femoral neck BMD. Inflammatory markers were also predictive of disability (standardized estimate=0.44) and physical activity (standardized estimate= -0.40). Symptomatic fatigue and depression were correlated (r = =0.64).

Conclusion

Physical activity, symptomatic fatigue, depression, disability, and inflammation all contributed independently to decreased femoral neck BMD in PWMS. Bone metabolism in PwMS is complex. Efforts to increase physical activity and address symptomatic fatigue and depression may improve bone mineral density in PwMS. Future

research should investigate the mechanisms through which symptomatic fatigue and depression contribute to reduced BMD in PwMS.

Keywords

Bone mineral content, Multiple sclerosis, Physical activity, Symptomatic fatigue, Depression, inflammation,

Abreviation	Definition
25(OH)D	25-hydroxyvitamin D
BDI	beck depression inventory II
BMD	bone mineral density
DIC	deviance information criterion
DXA	dual energy x-ray absorptiometry
EDSS	expanded disability status scale
EPA	expected predictive accuracy
FIS	fatigue impact scale
HRV	heart rate variability
IL-6	interleukin 6
IL-10	interleukin 10
LOO	leave-one-out information criterion
MCID	minimal clinically important difference
MDC	minimal detectable change
MHVH	moderate, hard, and very hard
MS	multiple sclerosis
MSFC	multiple sclerosis functional composite
NTX	N-terminal telopeptide
PASAT	paced auditory serial addition test
ppp	posterior predictive p-values
PSRF	potential scale reduction factor
PWMS	people with multiple sclerosis
SEM	standard error of the mean
sTNF-RII	soluble receptors for tumor necrosis factor type II
WAIC	widely applicable information criterion

1. Introduction

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system. People with MS (PwMS) experience impaired balance, coordination, strength, and vision (Compston and Coles, 2008). PwMS also have reduced bone mineral density (BMD) in multiple regions including the lumbar spine, femoral neck, and hip (Huang et al., 2015). These impairments increase the risk of falls and fractures and threaten independence and quality of life (Dong et al., 2015; Nilsagård et al., 2009). Therefore, it is important to identify factors contributing to reduced BMD in PwMS.

Prior research on BMD in PwMS has primarily focused on the contributions of corticosteroids and 25-hydroxyvitamin D [25(OH)D]. Corticosteroid use is recommended for acute treatment in PwMS (Smets et al., 2017) but has been linked to increased risk of osteoporosis (van Staa et al., 2002). 25(OH)D levels are often low in PwMS and may lead to decreased calcium (Ca^{2+}) absorption, increased bone resorption, and low BMD (Cosman et al., 1998; Nieves et al., 1994; Ozgocmen et al., 2005; Holick, 2007). However, findings related to both factors have been inconclusive. Several studies demonstrated that short-duration or chronic corticosteroid use

does not reduce BMD in PwMS and may even improve BMD secondary to mobility improvements (Cosman et al., 1998; Schwid et al., 1996; Olsson et al., 2015). Other studies found that corticosteroid use is associated with decreased BMD in PwMS (Nieves et al., 1994; Ozgocmen et al., 2005; Triantafyllou et al., 2012). Similarly, there is evidence that 25(OH)D is associated with either higher or lower BMD in PwMS (Cosman et al., 1998; Nieves et al., 1994; Olsson et al., 2015; Triantafyllou et al., 2012).

One consistent finding is that BMD is lower in PwMS with greater disability (Cosman et al., 1998; Nieves et al., 1994; Schwid et al., 1996; Weinstock-Guttman et al., 2004; Pilutti and Motl, 2019). This relation may result from the mediating effects of disuse and decreased physical activity (Olsson et al., 2015; Batista et al., 2012). Physical activity is reduced in PwMS (Motl et al., 2005), which may decrease the mechanical stress imposed upon the skeletal system and lead to decreased BMD (Bielemann et al., 2013). Only one study has investigated the relation between physical activity and BMD in PwMS, and it is still unclear whether disability has an effect independent of physical activity (Mojtahedi et al., 2008).

Other factors that may contribute to reduced BMD in PwMS have received limited investigation. MS is associated with inflammation, which can contribute to bone resorption (Amarasekara et al., 2015). Increased plasma cortisol levels in PwMS (Michelson et al., 1994) could negatively affect bone structure, as seen in hypercortisolism (Fassbender et al., 1998; Mancini et al., 2004). Finally, symptomatic fatigue and depression, affecting over 50% of PwMS, may indirectly lead to decreased BMD by contributing to lower physical activity (Siegert and Abernethy, 2005; Krupp, 2003). Depression also is associated with increased activation of the sympathetic nervous system, which stimulates bone resorption (Yirmiya et al., 2006).

The purpose of this study was to investigate factors that uniquely contribute to reduced BMD in PwMS as compared to people without MS. We used path analysis to test a theoretical model explaining the inter-relations between these factors and BMD. We hypothesized that physical activity would be the primary determinant of low BMD in PwMS, with additional contributions from inflammation and sympathetic nervous system activation.

2. Materials and methods

2.1. Participants

23 PwMS (16 women; 41±7 years; mean±SD) and 21 control participants (16 women; 39±8 years) were recruited through advertisements with the local chapter of the National Multiple Sclerosis Society and from a local neurologic clinic. Participants were 18–55 years old, were non-smokers, had no personal/family history of osteoporosis, did not use proton-pump inhibitors, and had no other major neurologic, metabolic, or cardiovascular diseases. Women were pre-menopausal with regular menstrual cycles. MS participants were ambulatory with an Expanded Disability Status Scale (EDSS) score ≤5, were not concurrently involved in a clinical drug trial, and had not received steroid treatments in the prior 3 months. Control participants were matched for age and sex at the group level. All participants provided written informed consent approved by the Marquette University Institutional Review Board.

2.2. Procedures

Across two visits separated by one week, participants completed: **Visit 1**: a bone mineral density scan, questionnaires of symptomatic fatigue and depression, and a blood draw; **Visit 2**: tests of functional capacity and an assessment of heart rate variability. Between visits, physical activity and late-night salivary cortisol were assessed. All data were collected over a 2-year span (2011–2013) between April and November because of seasonal variations in UVB radiation and vitamin D production (Holick, 1995).

BMD was measured from the femoral neck and lumbar spine (L1-L4) with dual energy x-ray absorptiometry (DXA, Lunar Prodigy software version 11.4, GE Healthcare, Chicago, IL, USA). Z-scores were determined by

normalizing to a sex- and age-matched population (norms from GE Healthcare). Measurements for all participants were performed by a single, trained member of the study team on the calibrated DXA scanner maintained to manufacturer specifications. Scans were reviewed by a rheumatologist, and regions of interest were adjusted if needed.

EDSS was assessed by a neurologist as a measure of MS-related disability (Kurtzke, 1983). Global functional capacity was assessed with the MS Functional Composite (MSFC) comprised of the timed 25 foot walk, 9-hole peg test, and Paced Auditory Serial Addition Test (PASAT) (Fischer et al., 1999). Times/scores for the MSFC were transformed into z-scores based on a representative database of PwMS (Fischer et al., 2001). Symptomatic fatigue and its impact on function and quality of life were assessed with the Fatigue Impact Scale (FIS) (Fisk et al., 1994). Scores were determined for the entire FIS and three subscales: cognitive, physical, and psychosocial. Depressive symptoms were assessed with the Beck Depression Inventory II (BDI) (Beck et al., 1996).

An antecubital venous blood sample was obtained for measurements of plasma or serum levels of Ca^{2+}, phosphate (PO_4), 25(OH)D, N-terminal telopeptide (NTX), osteopontin, and cytokine-related markers of inflammation [interleukin 6 (IL-6), interleukin 10 (IL-10), and soluble receptors for tumor necrosis factor type II (sTNF-RII)]. IL-6 and sTNF-RII are considered pro-inflammatory, while IL-10 is considered an anti-inflammatory marker (Amarasekara et al., 2015). NTX was assayed as a marker of bone resorption (Clemens et al., 1997). Osteopontin is thought to promote osteoclast adhesion to bone matrix, along with other immune functions (Gravallese, 2003) and has been reported to be high in people with relapsing-remitting MS (Vogt et al., 2003).

Osteopontin (Human Osteopontin Quantikine HS600B), IL-6 (Human IL-6 Quantikine D0ST00), IL-10 (Human IL-10 Quantikine, D100B), and sTNF-RII (Human sTNF RII/TNFRSF1B Quantikine, DRT200) were measured by enzyme immunoassay per manufacturer instructions (R&D Systems, Minneapolis, MN, USA). Batched serum was sent to the Zablocki VA Medical Center clinical laboratory for Ca^{2+} and PO_4 analysis. 25(OH)D was analyzed by chemiluminescent immunoassay (Diasorin LIAISON 25 OH Vitamin D TOTAL Assay, Saluggia, Italy) (Ersfeld et al., 2004). A serum assay was used to obtain a quantitative measure of cross-linked NTX of type I collagen (Abbott Osteomark NTX Serum ELISA, Abbott Park, IL, USA) (Clemens et al., 1997).

Heart rate variability (HRV) was obtained as an indicator of cardiac autonomic balance. (Anon et al., 2004) A three-lead electrocardiogram was obtained during 10-min of quiet supine rest. HRV was determined as the standard deviation of the R-R interval. Data were sampled at 2000 Hz (CED Power 1401, Cambridge Electronic Design, Milton, Cambridge, UK), and analyzed using Spike2 5.15 (CED).

Cortisol was measured from saliva, in part as a marker of stress. Between experimental visits, participants used a synthetic collection swab (Salimetrics LLC, Carlsbad, CA, USA) to collect a saliva sample prior to sleep (~10:00 PM). Cortisol is at the nadir of its circadian rhythm at night, and a measurement at this time can represent a single measure of average systemic cortisol (Raff et al., 1998). Salivary cortisol was measured with competitive enzyme-linked immunosorbent assay (#1–3002, Salimetrics LLC, Carlsbad, CA, USA) according to manufacturer directions.

Participants wore accelerometers (GT1M, and GT3X set on GT1M mode; Actigraph, Pensacola, FL, USA) around the waist for 6 days during all waking hours except when bathing or swimming. Participants completed a daily activity log (e.g. exercise, transportation) to correlate with accelerometer outputs. Activity was sampled as raw acceleration units (counts) in 1-minute epochs. Using standard cutoffs (Freedson et al., 1998), activity was classified as sedentary (0 counts), light (≤1951 counts), moderate (1952–5724 counts), hard (5725–9498 counts), and very hard (≥9499 counts) based on the counts within each epoch. Average daily counts and minutes within each classification were calculated. Average daily counts and minutes for all activity combined was also

calculated. Moderate, hard, and very hard (MHVH) minutes and counts were combined to represent strenuous activities. For ease of interpretation, average daily count values were divided by 1000. Accelerometers were independently tested for reliability in our laboratory (Wyrick et al., 2008).

2.3. Statistical analysis

Statistical analyses were performed with SPSS 23 (IBM, Armonk, NY, USA) with an alpha=0.05. Data are presented as mean±SD. All variables were compared between groups with independent samples t-tests (MS group as the reference). Proportion of participants with osteopenia or osteoporosis were compared between groups with chi-square analysis. Effect sizes were determined with Cohen's d, calculated as the mean difference divided by the pooled standard deviation.

Variables that were different between groups and significantly correlated (Pearson product-moment) with femoral neck BMD were included in a post-hoc theoretical model to explain femoral neck BMD in PwMS. Variables that were not different between groups or not significantly correlated with femoral neck BMD were excluded because our small sample size limited the number of parameters. Furthermore, we wanted to identify factors that contribute to low BMD, specifically, or to a greater extent in PwMS. We reasoned that limiting factors included in the model to just those that differed between PwMS and controls would identify the factors most likely to account for differences in BMD between groups. A theoretical model and path analysis were only performed for femoral neck BMD because we did not find a statistically significant between group difference for lumbar BMD (post-hoc decision).

We performed a Bayesian path analysis (Kline, 2005) in R (Team, 2008) using the package blavaan (Merkle and Rosseel, 2016), which estimates the model with the general Bayesian software JAGS (Plummer, 2003). Path analysis allowed us to test multiple relations between variables with multiple outcomes while conforming to a hypothesized model and accounting for relations with other variables of interest (Kline, 2005). Specifically, we tested the relations between predictive variables (EDSS, IL-6, physical activity, FIS, and BDI) and absolute (g/cm^2) femoral neck BMD. The path analysis also tested the regressive relation of IL-6 with EDSS and physical activity and the correlation between FIS and BDI. Model priors for each predictive variable identified the expected relations between variables to limit the inferential range. Priors were determined using previously published literature (e.g. see Introduction).

Convergence of the Markov chains was determined using the potential scale reduction factor (PSRF), i.e. univariate R-hat (Gelman and Rubin, 1992). We determined that the model converged when R-hat was lower than 1.10 for every parameter (Brooks and Gelman, 1998). The models were run by burn-in 22,000 iterations and keeping the last 7000 iterations from 3 chains to build the posterior distributions. The priors for the model were weakly informative, indicating that they were not intended to guide the parameters but provide information to delineate the most likely data space for the parameters. Means/intercepts have a prior $\mu \sim N(0,100)$; the standard deviations have a prior $\delta \sim$ half-cauchy(0,2.5); the regressions have a prior $\beta \sim N(0,50)$; and the correlations have a prior $\rho \sim U(-1,1)$. A model without constraints was compared to a model with constraints on whether the β was positive or negative (based on a theoretical model). These constraints were set by specifying the respective β prior as a truncated normal (e.g. $\beta \sim N(0,50)T(0,)$) and not allowing β to be above/below 0. We compared the models with the Bayes factor, leave-one-out information criterion (LOO), and widely applicable information criterion (WAIC) to evaluate which model fit the data better. (Vehtari et al., 2017; , Gelman et al., al. (2019); Kass , 1995; Raftery, 1993)

3. Results

There were no differences between the MS and control group for age, height, mass, or BMI. MS participants were 5 ± 5 years (range: 1–18) post-diagnosis and had a median EDSS score of 2 (range: 0–5). 20 people had a

relapsing-remitting MS phenotype and 3 people had a progressive phenotype. MS participants reported greater depression (p<0.001; Cohen's d = =1.31) and symptomatic fatigue (p<0.001; Cohen's d = =1.88) than controls. Functional capacity (MSFC) was lower in the MS compared to control group ($p \leq 0.001$; Cohen's d = =1.50). See Table 1.

Table 1. Anthropometry, depression, symptomatic fatigue, and function.

	MS	**Control**	***p***	**Mean diff.**	**95% CI**	**Cohen's d**
Age (yr)	41.0 (7.5)	39.3 (8.1)	0.46	1.8	-3.0, 6.5	0.23
Height (m)	1.69 (0.10)	1.72 (0.10)	0.23	-0.04	- 0.10, 0.03	0.37
Mass (kg)	73.4 (14.8)	73.1 (15.4)	0.95	0.3	-8.9, 9.5	0.02
BMI (kg/m^2)	25.6 (3.7)	24.5 (4.0)	0.32	1.2	-1.2, 3.5	0.31
BDI	10.6 (7.3)	3.2 (3.4)	<0.001*	7.4	3.9, 10.9	1.31
FIS (total)	60.6 (39.9)	5.9 (10.4)	<0.001*	54.7	36.6, 72.8	1.88
FIS (cognitive)	16.5 (11.3)	2.0 (4.1)	<0.001*	14.6	9.3, 19.9	1.72
FIS (physical)	16.0 (11.0)	1.3 (2.0)	<0.001*	14.8	9.8, 19.7	1.86
FIS (social)	28.0 (18.9)	2.7 (5.0)	<0.001*	25.4	16.8, 33.9	1.84
MSFC (Z-score)	0.2 (0.6)	0.9 (0.3)	<0.001*	-0.7	-1.0, -0.4	1.50
25 ft. walk (s)	5.5 (3.9)	3.7 (0.5)	0.04*	1.8	0.07, 3.6	0.65
9-hole peg test(s)	21.6 (4.9)	17.7 (1.9)	0.002*	3.9	1.6, 6.2	1.05
PASAT (#correct)	44.0 (12.0)	55.1 (4.7)	<0.001*	-11.0	-16.7, -5.3	1.22

MS and control groups differed in depression, symptomatic fatigue, and functional capacity. 9-hole peg test is presented as the mean of dominant and non-dominant hand trials. BDI: Beck Depression Inventory; BMI: body mass index; FIS: Fatigue Impact Scale; MSFC: Multiple Sclerosis Functional Composite; PASAT: Paced Auditory Serial Addition Test. For all comparisons, the MS group was the reference group. Values are mean (SD). Mean difference values and the 95% confidence interval around this measure are presented. Cohen's d was calculated as the mean difference divided by the pooled standard deviation.

* p<0.05.

At the femoral neck, PwMS had lower absolute BMD [0.98 (0.14) vs. 1.06 (0.14) g/cm^2; p = =0.04; Mean difference: -0.09; 95% CI: -0.2, -0.004; Cohen's d = =0.65] and Z-scores [-0.21 (0.97) vs. 0.40 (0.92); p = =0.04; Mean difference: -0.6; 95% CI: -1.2, -0.04; Cohen's d = =0.67] than controls. At the lumbar spine, absolute BMD [1.17 (0.13) vs. 1.25 (0.14) g/cm^2; p = =0.07; Mean difference: -0.08; 95% CI: -0.2, 0.007; Cohen's d = =0.59] and Z-scores [-0.18 (1.00) vs. 0.33 (1.10); p = =0.13; Mean difference: -0.5; 95% CI: -1.2, 0.2; Cohen's d = =0.49] were lower in PwMS, although statistically non-significant. Lumbar spine measurements were excluded from 2 PwMS and 1 control because of metal rods in the lumbar region.

Of the inflammatory or bone metabolism markers, IL-6 and sTNF-RII were greater ($p \leq 0.009$; Cohen's $d \geq 0.85$) and $Ca^{2+}$and PO_4 levels were lower in PwMS compared to controls ($p \leq 0.03$; Cohen's $d \geq 0.74$). However, $Ca^{2+}$and PO_4 were within lab reference ranges limits (8.5–10.1 mg/dL and 2.5–4.9 mg/dL, respectively). There was no difference in salivary cortisol, HRV, or 25(OH)D between groups. Of note, at least 15 (65%) participants in the MS group and 9 (43%) participants in the control group self-reported at least some level of 25(OH)D supplementation. Total physical activity, moderate activity, and MHVH counts were all lower in PwMS compared to controls ($p \leq 0.03$; Cohen's $d \geq 0.62$). Minutes of moderate activity were also lower in PwMS compared to controls (p = =0.01; Cohen's d = =0.81), and there was a trend for minutes of MHVH activity to be lower in PwMS compared to controls (p = =0.07; Cohen's d = =0.75). See Table 2.

Table 2. Potential physiological determinants of bone mineral density.

	MS	**Control**	***p***	**Mean diff.**	**95% CI**	**Cohen's d**
Ca^{2+} (mg/dl)	9.25 (0.36)	9.52 (0.40)	0.03*	-0.28	-0.52, -0.03	0.74

PO_4 (mg/dl)	3.35 (0.44)	3.72 (0.50)	0.02*	-0.38	-0.68, -0.07	0.82
25(OH)D (ng/ml)	38.7 (13.9)	32.9 (9.8)	0.12	5.8	-1.6, 13.2	0.49
NTX (nM)	13.8 (2.7)	12.5 (4.2)	0.22	1.3	-0.8, 3.4	0.39
Osteopontin (ng/ml)	57.0 (15.9)	56.3 (12.5)	0.88	0.8	-8.9, 10.5	0.05
IL-10 (pg/ml)	1.40 (1.40)	0.90 (0.85)	0.30	0.50	-0.47, 1.46	0.43
IL-6 (pg/ml)	1.60 (0.90)	0.96 (0.59)	0.009*	0.64	0.17, 1.11	0.85
sTNF-RII (pg/ml)	3452 (651)	2880 (428)	0.001*	572	233, 910	1.05
HRV (ms)	0.05 (0.02)	0.05 (0.02)	0.32	-0.006	-0.02, 0.006	0.32
Cortisol (ug/ml)	0.09 (0.07)	0.11 (0.15)	0.56	-0.02	-0.09, 0.05	0.18
Physical activity						
	MS	Control	*p*	Mean diff.	95%% CI	Cohen's d
Total (counts/1000)	263.2 (139.3)	346.6 (135.4)	0.03*	-83.6	-165.8, -0.4	0.62
Sedentary (counts/1000)	6.5 (1.6)	7.0 (1.7)	0.30	-0.5	-1.5, 0.5	0.34
(minutes)	1102.6 (82.8)	1100.8 (82.8)	0.95	1.8	-53.5, 57.1	0.02
Light (counts/1000)	160.7 (55.8)	173.1 (53.3)	0.46	-12.4	-46.1, 21.4	0.09
(minutes)	299.3 (82.1)	288.2 (77.6)	0.65	11.2	-38.2, 60.6	0.14
Moderate (counts/1000)	51.2 (41.9)	106.6 (80.6)	0.006*	-55.4	-94.2, -16.6	0.83
(minutes)	18.9 (13.2)	34.2 (24.7)	0.01*	-15.3	-27.3, -3.3	0.81
Hard (counts/1000)	17.6 (33.6)	31.2 (41.4)	0.24	-13.6	-36.7, 9.5	0.38
(minutes)	3.0 (5.3)	4.3 (5.5)	0.41	-1.4	-4.7, 2.0	0.32
Very hard (counts/1000)	2.9 (10.0)	24.5 (71.7)	0.16	-21.6	-52.1, 8.9	0.20
(minutes)	1.0 (4.4)	1.3 (3.5)	0.80	-0.3	-2.8, 2.1	0.11
MHVH (counts/1000)	71.7 (62.5)	162.3 (147.5)	0.01*	-90.6	-158.7, -22.4	0.64
(minutes)	22.8 (18.1)	39.8 (28.9)	0.07	-16.0	-33.2, 1.2	0.75

Calcium and phosphate were lower and markers of pro-inflammation were higher in PwMS than in controls. Physical activity measures are shown in the bottom half of the table. For physical activity, total, moderate, and MHVH counts were lower in PwMS than in controls. For ease of interpretation, average daily count values for physical activity were divided by 1000. 25(OH)D: 25-hydroxyvitamin D; Ca^{2+}: calcium; HRV: heart rate variability; IL-6: interleukin 6; IL-10: interleukin 10; NTX: N-terminal telopeptide; PO_4: phosphate; sTNF-RII: soluble receptors for tumor necrosis factor type II. For all comparisons, the MS group was the reference group. Values are mean (SD). Mean difference values and the 95% confidence interval around this measure are presented. Cohen's d was calculated as the mean difference divided by the pooled standard deviation.
*$p<0.05$.

3.1. Path analysis

A theoretical model (Fig. 1) was developed to explain femoral neck BMD but not lumbar BMD, because we did not detect statistically significant group differences at this latter site. Variables that rejected the null hypothesis of equality between the MS and control group and correlated with femoral neck BMD were included in the model: disability (EDSS: r=-0.49, p = =0.02), depression (BDI: r=-0.49, p = =0.02), symptomatic fatigue (FIS total: r=-0.65, p = =0.001), and physical activity (total counts: r = =0.48, p = =0.02). The theoretical relationships between these variables and femoral neck BMD were developed based on previously published literature. None of the other variables that were different between the MS and control group were correlated with femoral neck BMD ($p \geq 0.13$). Total physical activity was used in the model because it had a larger bivariate correlation with femoral neck BMD than other physical activity variables. IL-6 was included in the model even though it was not correlated with femoral neck BMD because it was correlated with disability (r = =0.44, p = =0.04), and we had apriori expected it to have an independent impact on BMD and physical activity. Finally, we included a correlation term between depression and symptomatic fatigue (Fassbender et al., 1998).

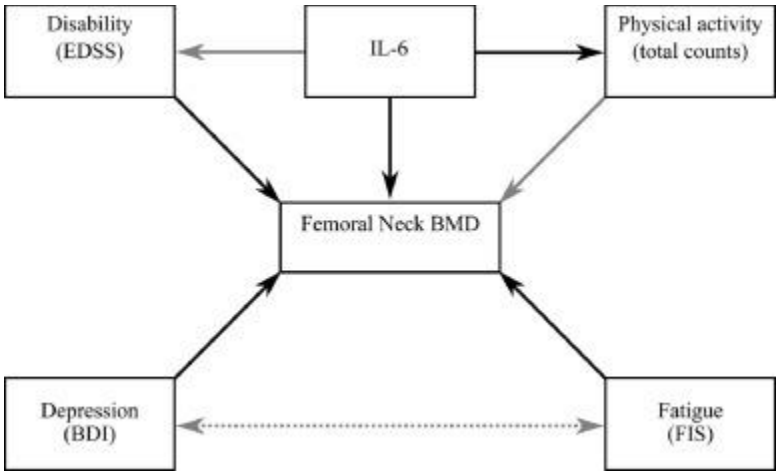

Fig. 1. *Theoretical model*. Solid lines show expected regressions predicting BMD of the femoral neck, EDSS, and physical activity. Dashed lines show expected correlations. Gray lines represent predicted positive relationships, while black line represent predicted negative relationships. BDI: Beck Depression Inventory; BMD: bone mineral density; EDSS: Expanded Disability Status Scale; FIS: Fatigue Impact Scale; IL-6: interleukin 6.

Symptomatic fatigue and physical activity scores had large variances compared to other variables, so they were transformed to z-scores. The unconstrained model (not shown) had an overall good fit (posterior predictive p-values (ppp)=0.11, deviance information criterion (DIC) =382.0, LOO=388.6, WAIC=385.6) and provided a good predictive model for BMD at the femoral neck (R^2=0.47) and good predictions of EDSS (R^2=0.18) and physical activity (R^2=0.15) with IL-6 as a predictor. The constrained model (Table 3) included weakly informative constraints (priors) for negative relationships of depression, symptomatic fatigue, and EDSS and a positive relationship of physical activity with BMD. The relationship between IL-6 and EDSS was constrained to be positive, and the relationship between IL-6 and physical activity was constrained to be negative. The constrained model performed better than the unconstrained model but performed even better when the relationship between IL-6 and BMD was unconstrained. This final, partially constrained model had an overall good fit (ppp=0.14, DIC=378.9, LOO=384.5, WAIC=382.2) and provided a good predictive model for BMD at the femoral neck (R^2=0.51) and good predictions of EDSS (R^2=0.19) and physical activity (R^2=0.16) with IL-6 as a predictor. The similarity between the constrained and unconstrained model suggests that our theoretical model largely matched the actual results. See Fig. 2 and Table 3 for final, partially constrained model with estimate parameters. Table 4 shows the standard error of the mean (SEM) for each predictive variable and how much change in femoral neck BMD would be associated with a 1 SEM change. Generally, a ± 2% change in BMD is considered clinically meaningful. At the femoral neck in PwMS, this translates to 0.0196 g/cm^2.

Table 3. Partially constrained model parameters.

Parameter	**Mean estimate (SD)**	**95% CI**	**Standardized estimate**
Direct effects on femoral neck BMD			
BDI → BMD	-0.006 (0.003)	-0.011, 0	-0.30
FIS → BMD	-0.001 (0.001)	-0.002, 0	-0.36
EDSS → BMD	-0.021 (0.017)	-0.053, 0	-0.17
PA → BMD	0.055 (0.025)	0.005, 0.101	0.39
IL-6 → BMD	0.043 (0.029)	-0.013, 0.100	0.27
Other parameters in the model			
IL-6 → EDSS	0.571 (0.253)	0.054, 1.032	0.44
IL-6 → PA	-0.458 (0.221)	-0.850, -0.013	-0.40
BDI ↔ FIS (covariance)	4.440 (1.939)	1.270, 8.615	0.64

Indirect effects on femoral neck BMD			
IL-6 → EDSS → BMD	-0.119 (0.02)	-0.37, 0.048	-0.07
IL-6 → PA → BMD	-0.028 (0.02)	-0.071, 0.008	-0.16

For each parameter, the predictive variable is before the arrow, and the predicted variable is after the arrow. A one-way arrow represents a regression parameter while a two-way arrow represents a correlation parameter. The mean estimate shows the unstandardized estimate; every 1 unit increase in the predictive variable yields the estimated change in the predicted variable. However, for PA and FIS, every 1 SD increase in the predictive variable yields the estimated change in the predicted variable. Standardized estimates show the SD change in the predicted variable for every 1 SD increase in the predictive variable. BDI: Beck Depression Inventory; BMD: bone mineral density in g/cm^2; CI = Bayesian Credible Interval**;** EDSS: Expanded Disability Status Scale; FIS: Fatigue Impact Scale; IL-6: interleukin 6; PA: physical activity.

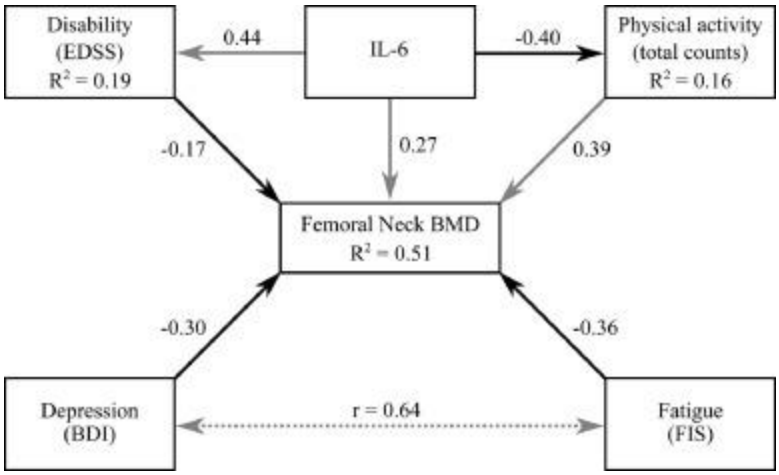

Fig. 2. *Final, partially constrained model with parameter estimates*. Solid lines show regression parameters predicting BMD of the femoral neck, EDSS, and physical activity. Dashed lines show correlation parameters. Gray lines represent positive relationships, while black line represent negative relationships. R^2 values are shown for regression models and represent the variance of the predicted variable (BMD, EDSS, or physical activity) explained by all the predicted variables included in the model. r values are shown for correlations. BDI: Beck Depression Inventory; BMD: bone mineral density; EDSS: Expanded Disability Status Scale; FIS: Fatigue Impact Scale; IL-6: interleukin 6.

Table 4. Change in BMD based upon β values.

Parameter	**SEM**	**Δ in BMD**	**MCID or MDC**	**Δ in BMD**
BDI → BMD	1.5 points	-0.009 g/cm^2	1.9 points	-0.011
FIS → BMD	8.3 points	-0.008 g/cm^2	11.7 points	-0.012
EDSS → BMD	0.25 points	-0.005 g/cm^2	0.5 points	-0.010
PA → BMD	29,000 counts	0.011 g/cm^2		
IL-6 → BMD	0.19 pg/mL	0.008 g/cm^2		

Change in femoral neck BMD is shown for a 1 standard error of the mean (SEM) change in each predictive variable. For example, a 1.5-point increase in BDI is associated with a 0.009 g/cm^2 decrease in femoral neck BMD. The change in femoral neck BMD is also shown for a change in each predictive variable (BDI, FIS, and EDSS) that meets the minimal clinically important difference (MCID) or minimal detectable change (MDC) levels. For example, the MCID for FIS is 11.7 points (19.3% of baseline), and a change of this magnitude is associated with a

0.012 g/cm^2 decrease in femoral neck BMD. Note that for PA and FIS, β values (Table 3) represented a 1 SD change in the predictive variable. Generally, *a* ± 2% change in BMD is considered clinically meaningful. At the femoral neck in PwMS, this translates to 0.0196 g/cm^2. BDI: Beck Depression Inventory; BMD: bone mineral density; EDSS: Expanded Disability Status Scale; FIS: Fatigue Impact Scale; IL-6: interleukin 6; PA: physical activity.

We compared the unconstrained and partially constrained models with Bayes Factor. The resulting value of 11.5 indicated that the partially constrained model fit the data 11 times better than the unconstrained model. We also compared the models with LOO and WAIC, which yielded the expected predictive accuracy (EPA). The difference in EPA for LOO was 2.04 (SE=0.829), which represents a difference of 2.46 standard errors. The difference in EPA for WAIC was 1.67 (SE=0.727), which represents a difference of 2.29 standard errors. These EPA differences also indicated that the partially constrained model fit the data better than the unconstrained model.

4. Discussion

In this study, we found that BMD at the femoral neck was decreased in PwMS compared to healthy controls. BMD at the lumbar spine was also decreased in PwMS, but statistically non-significant. Path analysis showed that physical activity, depression, symptomatic fatigue, disability, and IL-6 all contributed independently to BMD in PwMS within a theoretical framework. Together, these factors explained 51% of the variance in BMD, with the strongest contributions from physical activity, depression, and symptomatic fatigue. Below, we discuss the implications of these findings.

Physical activity was decreased in PwMS compared to controls, as found previously (Motl et al., 2005), with most of the difference in moderate or higher physical activity. This is of interest because current physical activity recommendations for PwMS stress the importance of moderate intensity activity (American College of Sports Medicine. 2014). Our results suggest that lower physical activity affects BMD in this population; decreased physical activity was associated with decreased BMD at the femoral neck. The only other study that has evaluated the relation between physical activity and BMD in PwMS found an association between lower physical activity and lower femoral BMD in women, but intensity was not quantified (Mojtahedi et al., 2008). An association between physical activity and BMD in PwMS is consistent with a similar relation found in adults without MS (Bielemann et al., 2013). For our study, we used standard intensity cutoffs for physical activity (Freedson et al., 1998). Activity cutoffs for PwMS may not be the same as for those without MS and may underestimate activity intensity (Agiovlasitis and Motl, 2014). Nevertheless, our results suggest that an increase in physical activity of 29,000 accelerometer counts per day (~15 min of moderate physical activity) is associated with an increased femoral neck BMD of 0.01 g/cm^2. Thus, increased physical activity may improve BMD in PwMS. Interestingly, BMD in the lumbar spine of PwMS was not statistically different from controls. This finding suggests that sitting may provide enough lumbar postural loading to partially maintain BMD, as seen in people with spinal cord injury (Leslie and Nance, 1993).

We found that depression and symptomatic fatigue independently contributed to decreased BMD. In addition, greater symptomatic fatigue was correlated with depression which is consistent with symptomatic fatigue and depression being interrelated but independent constructs (Krupp, 2003; Greeke et al., 2017). Both depression and symptomatic fatigue contributed to decreased BMD independently of physical activity. Prior work suggests that depression is associated with decreased BMD in non-MS populations and that this relation can also be independent of physical activity (Bab and Yirmiya, 2010). Mechanistically, depression may increase activation of the sympathetic nervous system, or vice versa, stimulating bone resorption (Yirmiya et al., 2006; Cherruau et al., 1999). However, our measure of cardiac sympathetic balance (HRV) was not different between groups nor was it associated with depression or BMD. Depression may contribute to decreased BMD through other mechanisms,

or cardiac HRV may be an insufficient measure of overall sympathetic activation or autonomic balance. Alternatively, altered sympathetic activity may be better revealed during tests of reactivity instead of at rest. Furthermore, an informal assessment suggests that a large portion of our sample used medications (such as antidepressants) that may have skewed sympathetic balance or otherwise affected BMD. Lack of group difference in HRV is somewhat consistent with lack of group difference in late-night salivary cortisol whereas both could indicate basal "stress" levels. Immune dysregulation may be associated with depression and bone density (Cizza et al., 2009), but IL-6 was not related to depression in our study. Altered endocrine function has also been hypothesized to mediate the relationship between depression and bone density (Cizza et al., 2009), but our data provide no insight into this other than that our subjects seemed to be 25(OH)D replete.

The mechanism by which symptomatic fatigue is related to decreased BMD in PwMS is also unclear, but cardiovascular autonomic impairment has been suggested to result in decreased bone density and symptomatic fatigue in PwMS (Sternberg, 2018). Further, alterations in sympathetic nervous system activation could be a shared mechanism in the associations between symptomatic fatigue, bone density, and depression, although the caveats discussed above must still be considered.

Corresponding with numerous other studies, we found that PwMS with greater disability (i.e., EDSS) had lower BMD (Cosman et al., 1998; Nieves et al., 1994; Olsson et al., 2015; Weinstock-Guttman et al., 2004; Pilutti and Motl, 2019). Many investigators have suggested that the relationship between disability and BMD is mediated by physical activity (Olsson et al., 2015; Batista et al., 2012). However, in our study, EDSS had an independent contribution to BMD and was not associated with physical activity. This finding suggests that some aspect of the disease process decreases BMD independent of its effect on physical activity or other variables in the theoretical model. Like in MS, decreased BMD has been reported in rheumatoid arthritis, autoimmune thyroid disease, and other autoimmune disorders, pointing toward a shared autoimmune mechanism not elucidated by our findings (Iseme et al., 2017).

Inflammation, as indicated by IL-6, was the only factor related to BMD that differed from our theoretical model. Previous work has suggested that increased inflammation is associated with decreased BMD (Amarasekara et al., 2015). However, we found that increased inflammation was associated with greater BMD at the femoral neck. This could be related to the effects of corticosteroid use whereby individuals using corticosteroids had lower inflammation, but also lower BMD. Participants in this study were excluded if they had used corticosteroids in the previous 3 months, but we did not assess historical use of corticosteroids. While IL-6 was associated independently with greater BMD in our model, it also was associated with lower physical activity and greater disability. As a result, the total or net effect of IL-6 to BMD was close to zero (standardized estimate of the total effect=0.04), which is consistent with our finding that there was no significant bivariate correlation between IL-6 and femoral BMD (r=-0.04, p = =0.86). IL-6 is a pleotropic cytokine with both direct signal pathways and trans pathways with soluble receptors (Mihara et al., 2012). The actions of IL-6 are complex and can be contradictory; while IL-6 is usually thought to be osteoclastic, its actions can both stimulate or inhibit bone resorption (Mihara et al., 2012; Yoshitake et al., 2008). For example, IL-6 deficiency has been associated with bone resorption in some animal models (Balto et al., 2001). Furthermore, IL-6 is just one of several markers of inflammation, and our results found both greater pro-inflammatory markers (IL-6 and sTNF-RII) and greater anti-inflammatory markers (IL-10). However, these other factors were not correlated with femoral neck BMD.

Lack of difference between PwMS and controls does not negate the importance of factors known to influence BMD. Rather, our study highlights factors that may specifically explain differences in BMD between PwMS and controls. For example, 25(OH)D is important for bone metabolism (Holick, 2007), but was not different between the MS and control groups. Contrary to reports of low 25(OH)D in PwMS (Nieves et al., 1994), 25(OH)D was replete in both the MS and control group in this study (Holick and Vitamin, 2009). At least 15 participants (65%) in the MS group reported regularly taking some form of 25(OH)D supplementation. That 25(OH)D was replete

and so many PwMS were supplementing may speak to the beneficial widespread attention to 25(OH)D in PwMS and their medical team. However, some recommendations suggest that >40 ng/mL is safe and optimal, meaning that additional supplementation may be beneficial for both people with and without MS (Bischoff-Ferrari et al., 2006).

One limitation of this study is that we restricted our theoretical model to include only variables that were different between groups and correlated with femoral neck BMD. We constrained the number of relationships reflected in our theoretical model because of limited sample size. Furthermore, we reasoned that the criteria imposed were likely to yield the most relevant factors for decreased BMD in PwMS as compared to people without MS. Other factors related to BMD might not have been captured because of this limitation, and their contribution to BMD in PwMS and/or people without MS should not be minimized. The role of vitamin D supplementation, chronic corticosteroid use, and different disease-modifying treatments were not explored and likely impact BMD, inflammation, and other factors in PwMS. In the future, larger sample sizes may allow a more complete evaluation of the factors contributing to decreased BMD and interrelations among these factors. Another limitation is that our study was cross-sectional. Any interpretations from our findings must be tempered by the limitation that it takes time (months to years) to build BMD, and our findings may not be reflective of prior lifestyle. Finally, this study was performed in PwMS with mild disability (median EDSS of 2), and the results may not extend to individuals with greater disability (i.e. EDSS).

5. Conclusions

Our study illustrates the complexity of bone metabolism in PwMS while stressing the importance of physical activity to maintain BMD. This importance cannot be overstated. Physical activity is safe and efficacious for persons with MS (Latimer-Cheung et al., 2013) and an inexpensive, non-pharmacological intervention to improve BMD and potentially decrease fracture risk.

Declaration of Competing Interest

None.

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