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Elevated urinary excretion of aluminium and iron in multiple sclerosis

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Multiple sclerosis (MS) is a chronic, immune-mediated, demyelinating disease of the central nervous system of as yet unknown aetiology. A consensus of opinion has suggested that the disorder is the result of an interplay between environmental factors and susceptibility genes. We have used a battery of analytical techniques to determine if the urinary excretion of i) markers of oxidative damage; ii) iron and iii) the environmental toxin aluminium and its antagonist, silicon, are altered in relapsing–remitting (RRMS) and secondary progressive MS (SPMS). Urinary concentrations of oxidative biomarkers, MDA and TBARS, were not found to be useful indicators of inflammatory disease in MS. However, urinary concentrations of another potential marker for inflammation and oxidative stress, iron, were significantly increased in SPMS ($P < 0.01$) and insignificantly increased in RRMS ($P > 0.05$). Urinary concentrations of aluminium were also significantly increased in RRMS ($P < 0.001$) and SPMS ($P < 0.05$) such that the levels of aluminium excretion in the former were similar to those observed in individuals undergoing metal chelation therapy. The excretion of silicon was lower in MS and significantly so in SPMS ($P < 0.05$). Increased excretion of iron in urine supported a role for iron dysmetabolism in MS. Levels of urinary aluminium excretion similar to those seen in aluminium intoxication suggested that aluminium may be a hitherto unrecognized environmental factor associated with the aetiology of MS. If aluminium is involved in MS then an increased dietary intake of its natural antagonist, silicon, might be a therapeutic option. *Multiple Sclerosis* 2006; **12**: 533–540. www.sagepub.co.uk

Key words: aluminium; environmental factor; iron metabolism; multiple sclerosis; silicon; urinary excretion

Introduction

Multiple sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system. It is characterized pathologically by acute multifocal demyelination as well as axonal loss and death of oligodendrocytes. MS usually presents as a pattern of recurrent attacks followed by partial recovery (relapsing–remitting MS (RRMS)), which in more than half of cases will develop into a chronic progressive form of the disease [1]. The aetiology of MS is not completely understood and is widely believed to involve susceptibility genes, which may or may not be triggered by environmental factors [2]. The inflammatory response and concomitant neuronal damage may be mediated by free radicals [3] and the presence of anomalous iron in MS brain as well as biomarkers of oxidative damage in serum and cerebrospinal fluid would support this view [4]. Disruption in brain iron metabolism could result in iron-driven increases in oxidative damage and increased urinary excretion of iron and urinary biomarkers of, for example, lipid peroxidation. We have looked for these, as well as the pro-oxidant

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aluminium [5], in urine in MS and whilst we did not find increased levels of biomarkers of oxidative damage, we did find evidence of significantly increased excretion of iron and, particularly, aluminium.

Patients and methods

MS patients were recruited from the Neurology Department and Neurology Ward at the University Hospital of North Staffordshire and were diagnosed as clinically definite MS according to Poser's criteria [6]. Ten patients had secondary progressive MS (SPMS) and ten patients had RRMS and were in relapse. Ten healthy individuals recruited by advertisement from within Keele University served as controls. Exclusion criteria included: inflammatory disease other than MS; urinary tract infection; history of receipt of steroid treatment within the past 30 days; use of aluminium-containing medications. All participants provided written informed consent and approval for the study was obtained from the local research ethics committee. Urine samples were collected at the hospital in reagent-free air-tight containers, tested for infection with Multistix 8SG reagent strips and retained on ice before being transported to Keele University where they were sampled for immediate analysis of oxidative biomarkers and the remainder frozen at -20°C for subsequent determination of creatinine, silicon and metals.

Thawed urine samples were thoroughly mixed and acidified to 17% v/v with 14 M HNO_3. Total aluminium and total iron were determined by graphite furnace atomic absorption spectrometry (GFAAS) using programmes developed in our laboratory [7]. The levels of quantitation achieved were 0.04 and 0.09 μmol/L for Al and Fe respectively. Total silicon was determined by inductively coupled plasma emission spectroscopy (ICP-OES) using a modification of a published programme [8]. Reproducibility was consistently within 1% and the validity of calibration was confirmed using the method of standard additions. The coincidence of results from linear calibration and the method of standard additions was always within 5%.

Urine malondialdehyde (MDA) and 2-thiobarbituric acid-reactive substances (TBARS) were measured within 3 hours of collection of urine samples. MDA was determined by reverse-phase high performance liquid chromatography (RP-HPLC) following derivatization with 2,4-dinitrophenylhydrazine using a programme developed in our laboratory [9]. TBARS were also measured using RP-HPLC and a modification of an established method [9,10]. Creatinine in thawed samples of urine was determined by RP-HPLC using a modification of a published method [11].

Two-way analysis of variance was used to compare means of MDA and TBARS as the data were normally distributed. As the data for silicon, aluminium and iron were not normal, Kruskall–Wallis tests were used to compare group medians followed by Dunn's post hoc comparison between pairs of medians [12]. Relationships between variables were determined by Pearson product momentum correlation (r).

Results

Urine aluminium, iron, silicon, MDA and TBARS in MS patients and healthy controls are presented in Tables 1–3. Urine creatinine was measured to correct for differences in glomerular filtration rate.

No significant differences were found between any of the groups for MDA or TBARS. Mean concentrations of MDA (control, 0.025 ± 0.021; RRMS, 0.019 ± 0.010; SPMS, 0.017 ± 0.011 μmol/mmol creatinine) were significantly lower than those for TBARS (control, 0.171 ± 0.052; RRMS, 0.195 ± 0.079; SPMS, 0.213 ± 0.156 μmol/mmol creatinine) across all groups ($F_{1,52} = 109.46$; $P < 0.001$).

Table 1 Urine analyses of MDA, TBARS (corrected for glomerular filtration rate), aluminium, iron and silicon (non-corrected and corrected for glomerular filtration rate) for each individual in control

Sample ID	Sex	Age	MDA (μmol/mmol creatinine)	TBARS (μmol/mmol creatinine)	Si (μM)	Si (μmol/mmol creatinine)	Al (μM)	Al (nmol/mmol creatinine)	Fe (μM)	Fe (nmol/mmol creatinine)
C1	F	50	0.006	0.107	158	18.0	0.35	39.8	0.18	20.5
C2	F	40	0.009	0.101	349	19.5	0.66	36.9	0.33	18.4
C3	F	51	0.028	0.159	268	21.2	0.38	30.1	0.29	23.0
C4	M	42	0.031	0.157	631	36.6	0.39	22.6	0.75	43.5
C5	F	39	0.007	0.243	142	40.5	0.28	79.8	0.09	25.7
C6	F	45	0.077	0.257	173	31.8	0.40	73.6	0.39	71.8
C7	F	50	0.017	0.146	237	37.5	0.29	45.9	0.30	47.5
C8	M	47	0.028	0.156	311	39.6	0.26	33.1	0.29	36.9
C9	F	53	0.030	0.174	254	32.3	0.23	29.2	0.47	59.7
C10	F	55	0.030	0.199	301	44.2	0.26	38.2	0.23	33.8

Table 2 Urine analyses of MDA, TBARS (corrected for glomerular filtration rate), aluminium, iron, and silicon (non-corrected and corrected for glomerular filtration rate) for each individual in RRMS

Sample ID	Sex	Age	MDA (μmol/mmol creatinine)	TBARS (μmol/mmol creatinine)	Si (μM)	Si (μmol/mmol creatinine)	Al (μM)	Al (nmol/mmol creatinine)	Fe (μM)	Fe (nmol/mmol creatinine)
RR1	M	59	0.036	0.333	233	42.1	0.68	122.9	0.25	45.2
RR2	M	30	0.036	0.305	305	39.3	0.31	40.0	0.94	121.3
RR3	F	45	0.010	0.088	156	20.5	11.50	1508.6	1.43	187.6
RR4	F	46	0.018	0.188	232	31.6	10.94	1491.8	0.49	66.8
RR5	F	46	0.011	0.148	221	27.0	39.48	4826.5	1.23	150.4
RR6	F	36	0.011	0.205	180	46.9	8.57	2231.5	0.28	72.9
RR7	F	24	0.021	0.195	68	14.6	0.89	191.4	2.13	458.1
RR8	F	50	0.016	0.102	288	25.2	0.65	56.8	0.79	69.1
RR9	F	53	0.019	0.231	76	23.5	0.78	241.1	0.07	21.6
RR10	F	51	0.014	0.158	119	28.2	1.34	317.6	0.09	21.3

There was a significant overall difference between the medians of iron concentration ($P < 0.01$). Urine iron concentrations were higher in MS groups compared to controls. The median for SPMS (104.6 nmol/mmol creatinine) was significantly higher than control (35.4 nmol/mmol creatinine) ($P < 0.01$) whereas the median for RRMS (71.0 nmol/mmol creatinine) was not significantly higher than control ($P > 0.05$). The medians for the MS groups were not significantly different ($P > 0.05$) from each other (Figure 1A). Urine iron concentrations were not significantly correlated with urine creatinine concentrations in any of the groups ($P > 0.05$) and normalization of total iron concentrations with creatinine did not alter statistical trends between the groups. There was a significant overall difference between the medians of aluminium concentration ($P < 0.001$). Urine aluminium concentrations were significantly higher in MS groups compared to controls. The median for RRMS (279.4 nmol/mmol creatinine) was more than six times the median for control (37.6 nmol/mmol creatinine) ($P < 0.001$) whereas the median for SPMS (99.2 nmol/mmol creatinine) was approximately twice the control value ($P < 0.05$). Whilst the median for RRMS was approximately three times that for SPMS, the two medians were not statistically different from each other ($P > 0.05$) (Figure 1B). Urine aluminium concentrations were not significantly correlated with urine creatinine concentrations in any of the groups ($P > 0.05$) and normalization of total aluminium concentrations with creatinine did not alter statistical trends between the groups. The median urine silicon concentration in SPMS (20.0 μmol/mmol creatinine) was significantly ($P < 0.05$) lower than control (34.5 μmol/mmol creatinine) and not significantly ($P > 0.05$) lower than RRMS (27.6 μmol/mmol creatinine). The latter was not significantly lower than control ($P > 0.05$) (Figure 1C). Lack of normalization of urine total silicon with creatinine caused a loss of overall significance between medians. Urine silicon concentrations were significantly positively correlated with urine creatinine concentrations in control ($r = 0.756$; $P = 0.011$) and RRMS ($r = 0.752$; $P = 0.012$) but not significantly positively correlated with urine creatinine concentrations in SPMS ($r = 0.624$; $P = 0.134$) (Figure 2A–C). The reduced significance between urine creatinine and silicon

Table 3 Urine analyses of MDA, TBARS (corrected for glomerular filtration rate), aluminium, iron and silicon (non-corrected and corrected for glomerular filtration rate) for each individual in SPMS

Sample ID	Sex	Age	MDA (μmol/mmol creatinine)	TBARS (μmol/mmol creatinine)	Si (μM)	Si (μmol/mmol creatinine)	Al (μM)	Al (nmol/mmol creatinine)	Fe (μM)	Fe (nmol/mmol creatinine)
SP1	F	60	0.024	0.154	92	20.0	0.38	82.7	2.37	515.7
SP2	F	65	0.013	0.173			1.16	98.0	1.21	102.2
SP3	F	42	0.013	0.152			1.07	171.7	1.09	174.9
SP4	M	50	0.015	0.086	324	18.4	0.31	17.6	0.85	48.4
SP5	M	54	0.007	0.151	279	30.9	0.77	85.2	0.80	88.5
SP6	F	59	0.042	0.592	87	23.6	0.37	100.4	1.85	502.0
SP7	F	61	0.019	0.282			0.31	125.5	0.44	178.1
SP8	F	42	0.012	0.239	62	5.0	13.14	1067.0	0.99	80.4
SP9	M	39	0.010	0.088	225	12.1	1.40	75.1	0.52	27.9
SP10	F	39	0.023	0.269	134	23.1	1.52	262.1	0.62	106.9

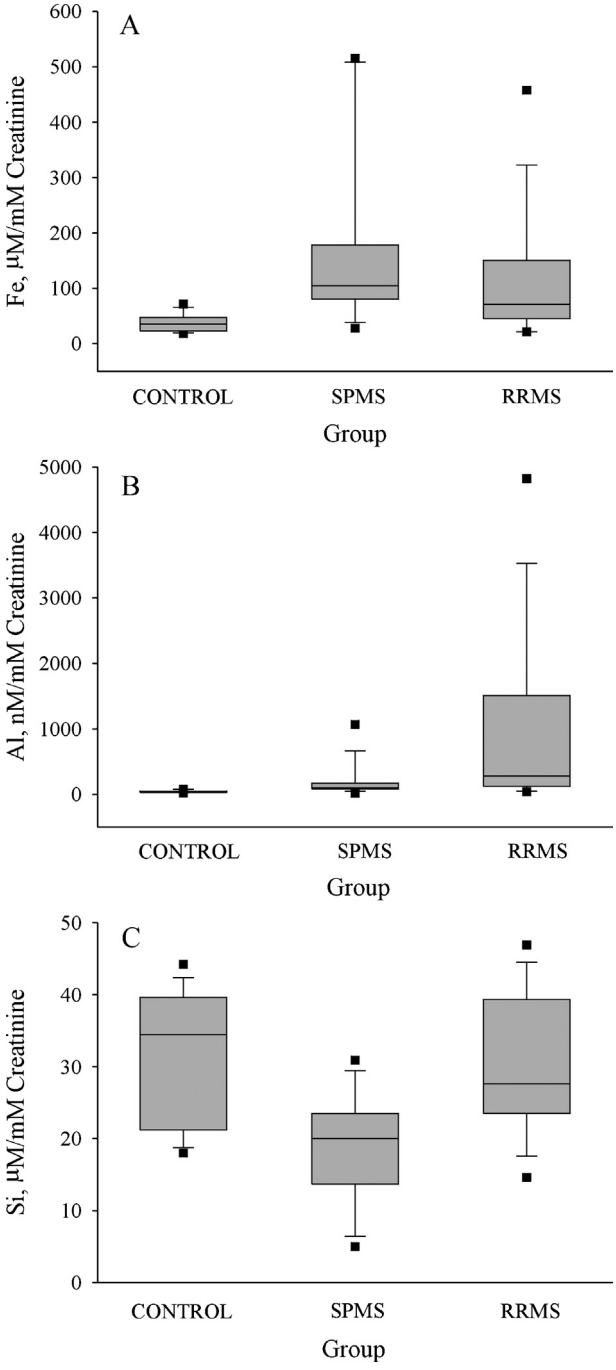

Figure 1 Box and whisker plots of urinary excretion of iron (A), aluminium (B) and silicon (C) in control, SPMS and RRMS groups. The lower boundary of the box indicates the 25th percentile, the line within the box marks the median and the upper boundary of the box shows the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles respectively. Solid squares indicate maximum and minimum values.

in SPMS (Figure 2B) supports the view that renal function may be impaired in progressive MS [13].

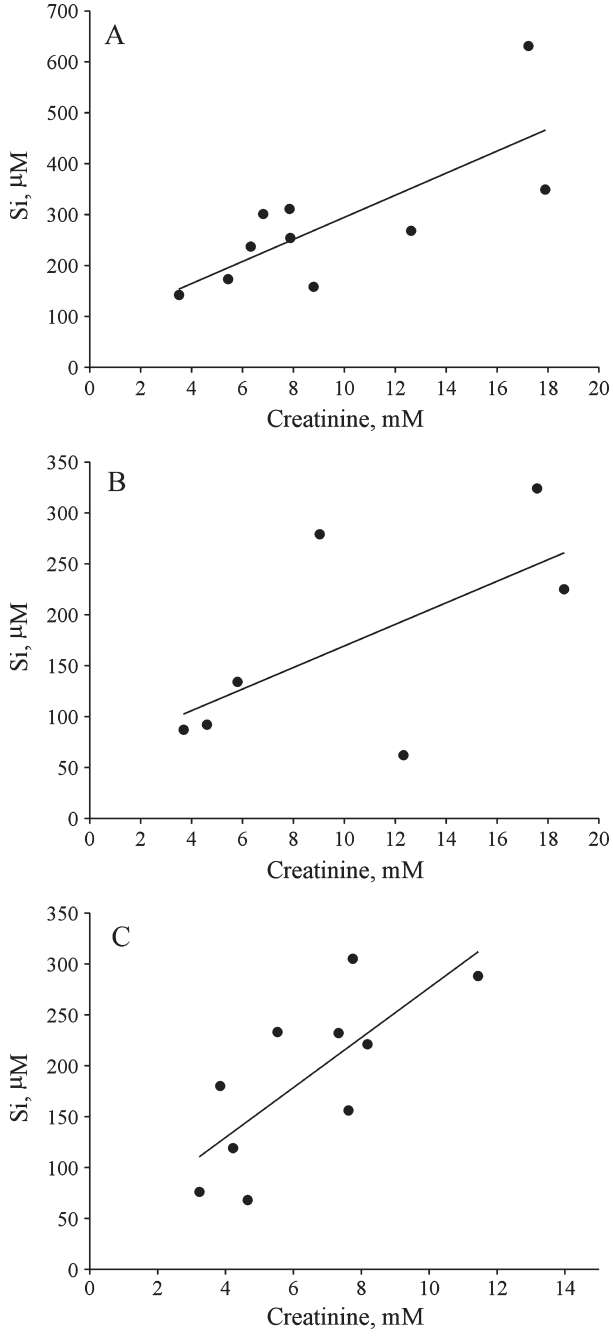

Figure 2 Pearson product momentum correlation analyses of urine silicon with urine creatinine for control (A) ($r=0.756$; $P=0.011$), SPMS (B) ($r=0.624$; $P=0.134$) and RRMS (C) ($r=0.752$; $P=0.012$) groups.

Discussion

The urine of individuals diagnosed as suffering from MS had higher concentrations of iron and aluminium than the urine of age- and gender-matched healthy controls. There were no statistical differences ($P>0.05$) of mean age between any of

the groups (mean ± SD, years; C, 47.2 ± 5.5; RRMS, 44.0 ± 10.9; SPMS, 51.1 ± 10.0). Neither were there any significant correlations between age and metal excretion ($P > 0.05$). The higher concentrations of metals were unrelated to urine creatinine and were statistically significant when expressed as either total or normalized data. In particular, MS urine showed a wide range of concentrations of these metals and the medians of the ranges segregated according to the diagnosis of MS.

The most significant differences were found for urine aluminium concentrations in RRMS. All patients in this group had urine aluminium concentrations above the median of the control group and in four individuals the urine aluminium concentrations were more than 40 times the median of the control group. Urine aluminium concentrations were also higher in SPMS where nine out of ten individuals showed concentrations higher than the median of the control group. In the majority of individuals the increases above the control median were of the order of three to five times though it was as much as 30 times for one individual. Urine aluminium concentrations in RRMS were clearly higher than SPMS though the lack of statistical significance between the medians of these groups probably reflected the wide range of concentrations in the former. For example, two individuals with RRMS had urine aluminium concentrations below the median in SPMS and only marginally above the median for the control group. Overall MS urine had a higher concentration of aluminium than control urine and 19 out of a possible 20 individuals showed concentrations above the median for the control group.

Urine iron concentrations were highest in SPMS where all but one individual showed concentrations above the median for the control group and several individuals showed concentrations between five and fifteen times that of the control group. A similar range of urine iron concentrations were found in RRMS such that the median for this group was neither statistically higher than the control group nor statistically lower than in SPMS. Similar to aluminium, MS urine had a higher concentration of iron than control urine and 17 out of a possible 20 individuals showed concentrations above the median for the control group.

Silicon in urine was significantly positively correlated with urine creatinine in the control group and in RRMS but not significantly positively correlated with urine creatinine in SPMS. The latter showed the lowest median silicon concentration; all individual concentrations were below the median for the control group and all but one below the median in RRMS. The statistical significance of the lower median in SPMS was weak ($P < 0.05$) though 80% of individuals with MS showed urine silicon concentrations below the median for the control group.

There were no statistically significant differences for either MDA or TBARS between MS and controls. We confirmed the previous finding in urine that MDA constituted only a small proportion of the TBARS fraction [9] and our results concurred with the previous suggestion that urinary aldehydes were not reliable indicators of oxidative stress *in vivo* [14].

We have not found previous literature on the urinary excretion of iron, aluminium or silicon in MS. The median urine iron concentration in the control group was 35.4 nmol/mmol creatinine which, for an expected urine creatinine excretion of ca. 12.5 mM/24 h [15], equates to a daily excretion of 0.44 μmoles of iron. Literature data of urinary iron excretion in healthy individuals are scarce though recent research suggested 1.0 μmole/24 h as an upper limit [15]. Seven SPMS and four RRMS excreted more than 1.0 μmole Fe/24 h and three of these eleven excreted more than five times this amount. The latter are similar to rates of iron excretion in individuals undergoing iron-chelation therapy with, for example, desferrioxamine (DFO) [16]. Urinary iron excretion was reported to be increased in Parkinson's disease [17] though literature data of urine iron in neurodegenerative diseases are scarce. The median urine aluminium concentration in the control group was 37.6 nmol/mmol creatinine and equates to a daily excretion of 0.47 μmoles of aluminium. This compares well with the literature range for healthy adults of ca. 0.20–0.70 μmoles/24 h [18–20]. The median urine aluminium concentration in SPMS equated to a daily excretion of 1.24 μmoles of aluminium and was identical to urinary aluminium excretion in normal individuals following ingestion of 300 mg of aluminium hydroxide antacid [21]. In RRMS the median urinary aluminium excretion was 3.49 μmoles/24 h and was well in excess of what has been measured in individuals suffering aluminium intoxication (1.71 μmoles/24 h) [22] and in individuals with Alzheimer's disease (2.89 μmoles/24 h) [18]. Four individuals with RRMS presented with urinary aluminium excretion in the range 18.86–60.33 μmoles/24 h, which is a level of aluminium excretion normally only associated with aluminium-chelation therapy with DFO [23]. The median urine silicon concentration in the control group was 34.5 μmol/mmol creatinine and equates to a daily excretion of 431.3 μmoles. This is within the normal range for silicon excretion [20] with the proviso that the absorption and excretion of silicon would be expected to reflect diet [24] and geographical variations in its availability in potable water [25]. All individuals in this study were

drawn from the same geographical region and so the lower levels of silicon excretion in SPMS (250.0 μmoles/24 h) and RRMS (345.0 μmoles/24 h) were unexpected. Urinary silicon excretion has not hitherto been linked to neurodegenerative disease though lower concentrations of silicon in potable waters are linked with an increased incidence of Alzheimer's disease [26,27].

The significance of either higher levels of urinary iron and aluminium excretion or lower levels of silicon excretion in MS is unknown. Urinary excretion of these elements might be influenced by any one of a number of characteristics of MS including, for example, reduced mobility, and future research should include control groups which take account of such differences in MS. The deposition of iron in MS brain has been known for some time [28,29] though, as yet, there is no consensus as to whether anomalous iron contributes to the aetiology of the disease or is simply an epiphenomenon linked to brain atrophy [4]. Recent research suggested that iron metabolism was altered in MS [30] and results herein support this conclusion. There are no previous data on aluminium in MS though a high incidence of MS was reported in macrophagic myofascitis [31], which is an inflammatory myopathy that has been linked to intramuscular injections of aluminium-containing vaccines [32]. Animal studies have shown that myelin bound and accumulated aluminium [33] and was the preferred target of aluminium neurotoxicity [34]. Oligodendrocytes were particularly susceptible to the uptake and accumulation of aluminium with concomitant disruption in cellular iron metabolism [35]. Several reports of aluminium intoxication have shown dramatic thinning of myelin sheath in both the spinal cord and hippocampus [36–38]. In addition aluminium was shown to increase the activity of a kinase that phosphorylates myelin basic protein [39] and decrease the activity of the myelin-specific enzyme, 2′3′-cyclic nucleotide phosphohydrolase [40]. There are clearly precedents, in animal research if not yet in humans, for the involvement of aluminium in MS. Individuals with a higher body burden of aluminium may accumulate the metal in oligodendrocytes and myelin where it could disrupt iron metabolism and facilitate iron-mediated oxidative damage through its known pro-oxidant activity [41]. The extremely high urinary excretion of aluminium in RRMS might be consistent with a prior association with myelin and, concomitant with demyelination, its excretion with myelin basic protein-like material [42]. The vulnerability of oligodendrocytes and myelin to neurotoxic insults in MS is mirrored in Alzheimer's disease [43] and might explain the putative role of aluminium in the latter [44] and suggest a role for aluminium in the former.

There are myriad ways by which we are exposed to aluminium in our everyday lives [45]. Higher levels of silicon in the diet are expected to lower the body burden of aluminium by reducing its gastrointestinal absorption [25] and facilitating its excretion in urine [46]. Urinary silicon excretion was lower in MS, which might suggest that this natural protection against the retention of aluminium in the body was compromised in MS. Both active and passive smoking of tobacco and cannabis are likely contributors to the body burden of aluminium [47] and this might begin to explain why smoking is a risk factor both for MS [48] and for the progression of MS [49].

MS has invariably been described as a disorder resulting from an interplay between as yet unidentified environmental factors and susceptibility genes [1]. Could aluminium, perhaps in concert with iron dysmetabolism [50], be the unidentified environmental factor? If aluminium is involved with disease progression in MS, could an increased dietary intake of silicon be a therapeutic option?

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