



Research Paper

Urinary Excretion of Aluminium and Silicon in Secondary Progressive Multiple Sclerosis



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ABSTRACT

Background: Progressive multiple sclerosis is a chronic autoimmune condition of unknown aetiology and few therapeutic options. Human exposure to aluminium has been linked with multiple sclerosis and affected individuals are known to excrete unusually high amounts of aluminium in their urine. Silicon-rich mineral waters facilitate the removal of aluminium from the body in urine and herein we have tested their efficacy in affecting urinary excretion of aluminium in individuals diagnosed with secondary progressive multiple sclerosis (SPMS). **Methods:** Urinary excretion of aluminium and silicon, measured using transversely-heated graphite furnace atomic absorption spectrometry, was determined in 15 individuals diagnosed with SPMS over 24 weeks, a 12 week baseline period (control) followed by a 12 week treatment period, during which individuals consumed up to 1.5 L of a silicon-rich mineral water every day.

Findings: Individuals with SPMS excreted high amounts of aluminium during the baseline period (135.2 nmol/mmol Cr_t (70.3–222.2, n = 180) and females excreted significantly more aluminium than males. Regular drinking of a silicon-rich mineral water increased the urinary excretion of aluminium significantly (349.0 nmol/mmol Cr_t (231.7–524.7, n = 180; three-way ANOVA, $F_{1,13} = 59.17$, p-value = 0.000003) relative to the baseline period. The majority of individuals, 14 out of 15, excreted more aluminium ($\mu\text{mol}/24\text{ h}$) following drinking of a silicon-rich mineral water (independent-test, $p < 0.05$). Silicon-rich mineral waters may be an effective and non-invasive therapy for the removal of aluminium from the body of individuals with SPMS.

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1. Background

Multiple sclerosis (MS) is a chronic, immune-mediated, demyelinating disease of the central nervous system of as yet unknown aetiology. While there has been progress in understanding the pathogenesis of MS it remains frustratingly slow (Ontaneda et al., 2017). Similarly, effective treatments for MS are few and far between (Thompson, 2017; Montalban et al., 2017). It is widely accepted that MS is likely to involve both genetic and environmental factors acting either in isolation or together in various disease phenotypes. Human exposure to aluminium (Exley, 2013) has been identified as a possible contributor to MS. Individuals with relapsing-remitting (RRMS) and secondary progressive (SPMS) MS were shown to have a higher than expected body burden of aluminium (Exley et al., 2006a). The latter manifested as very high

concentrations of aluminium in urine. A role for aluminium in MS might be expected based upon the known association of the metal with myelin (Verstraeten et al., 1997) and oligodendrocytes in animal models of aluminium intoxication (Golub and Tarrara, 1999). Indeed recent, limited, clinical studies have shown increased urinary excretion of aluminium in MS following chelation with EDTA (Fulgenzi et al., 2012; Zanella and di Sarsina, 2013; Fulgenzi et al., 2015). Aluminium's role in the pathogenesis of MS and the progression of the disease is currently unknown but it may be related to aluminium's activity as a pro-oxidant (Exley, 2004) or as an adjuvant capable of inducing a form of autoimmunity in affected tissues (Exley et al., 2009). Both of these potential mechanisms of aluminium toxicity have implications for myelin breakdown in MS.

We have pioneered silicon-rich mineral waters as non-invasive methods to facilitate the urinary excretion of aluminium in both health and disease (Exley et al., 2006b). Individuals have been shown to excrete significant amounts of aluminium following regular drinking of 1.0–1.5 L of a silicon-rich mineral water and in individuals with Alzheimer's disease

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(AD) this resulted in a lowering of their body burden of aluminium over only 12 weeks (Davenward et al., 2013). Herein we have recruited individuals with SPMS and used urinary excretion of aluminium to establish their body burdens of aluminium both before and after regular drinking of a silicon-rich mineral water for 12 weeks. We show that this is an effective strategy for lowering the body burden of aluminium in SPMS.

2. Methods

2.1. Participants

Participants, 8 females and 7 males, mean age 63 (range 52–73), were recruited from Neurology at the University Hospital of North Staffordshire. Recruits were diagnosed as SPMS according to revised Lublin-Rheingold criteria (Lublin et al., 2014). Exclusion criteria included individuals with urinary infections or impaired renal function and participants using disease-modifying treatments including aluminium-based antacids. None of the participants in the study were current smokers as smoking is known to contribute to the body burden of aluminium (Exley et al., 2006c). Recruits were assessed at the beginning of the trial according to the Expanded Disability Status Scale (EDSS) and all scored between 4 and 7. All participants gave written consent and the trial received NREC approval (14-YH-1115).

2.2. Protocol

The primary objective of the study was to establish if regular drinking of a silicon-rich mineral water could be an effective non-invasive therapy to reduce the body burden of aluminium in individuals with SPMS. Participants were encouraged to live their lives normally during the 24 weeks of the study with the only differences being the collection of their urine samples (see details below) and, during weeks 13 to 24 inclusive, the addition of a silicon-rich (ca 35 mg/L as silicic acid) mineral water to their everyday diet. The latter was provided by the Malaysian mineral water company Spritzer and participants were encouraged to drink up to 1.5 L daily.

The study was conducted over two consecutive 12 week periods. The baseline period (weeks 1–12) was used to estimate normal levels of urinary aluminium and silicon excretion while the treatment period (weeks 13–24) was used to establish how regular drinking of a silicon-rich mineral water affected urinary aluminium and silicon excretion. For Monday to Friday of weeks, 1, 12, 13 and 24 participants collected 24 h urine samples while for weeks 2–11 and 14–23 participants collected their first urine of the day on the Wednesday of that week.

2.3. Sample Collection, Storage and Measurements

Participants were given instructions, to help reduce issues relating to potential contamination, on collection of urine samples and they were provided with pristine, acid-washed, labelled containers. Urine samples were stored in biohazard bags in participants' domestic refrigerators prior to their collection and transport to Keele University for processing. Upon arrival in the Bioinorganic Chemistry Laboratory the total volumes of the 24 h samples were measured and thereafter all urine samples were thoroughly mixed and sampled for subsequent creatinine analyses using the Jaffe reaction. Further sub-samples were then stored frozen prior to their microwave-assisted acid digestion and analysis of total aluminium and silicon by transversely heated graphite furnace atomic absorption spectrometry (TH GFAAS) (Davenward et al., 2013).

Table 1

Median and IQR of aluminium concentration [nmol/mmol Cr_t] in urine of SPMS patients before (weeks 1–12) and after the treatment (weeks 13–24) with silicon-rich mineral water, N = 12.

Patient ID	Baseline (wks 1–12)	Treatment (wks 13–24)
F001	164.5 (120.9–210.8)	1081.4 (393.2–2270.8)
F003	99.2 (73.6–150.5)	400.4 (131.0–913.1)
F006	175.0 (113.3–239.5)	353.8 (221.1–647.6)
F009	152.1 (84.5–252.6)	435.3 (341.8–684.8)
F012	203.4 (94.1–306.1)	390.2 (312.1–575.1)
F013	240.8 (189.5–366.0)	356.0 (304.2–486.3)
F015	326.1 (201.0–582.7)	399.2 (279.4–682.8)
F016	146.9 (76.8–202.2)	328.3 (279.7–550.7)
M002	89.5 (69.1–133.8)	436.2 (264.0–544.9)
M004	51.80 (39.5–61.8)	452.95 (335.5–511.1)
M005	103.6 (59.8–128.2)	388.5 (156.1–468.3)
M007	102.0 (69.5–131.1)	296.0 (251.3–500.7)
M010	232.0 (88.1–281.0)	270.3 (229.6–387.7)
M011	65.3 (49.9–128.4)	140.5 (124.5–188.7)
M014	152.4 (92.0–225.4)	271.2 (196.2–332.7)

2.4. Statistical Analyses

Data are expressed as medians and interquartile range (IQR), for the baseline (weeks 1–12) and treatment period (weeks 13–24) both for all patients together and separately for each patient. Data are skewed and for further statistical analysis data were $\log(x + 10)$ transformed to fulfil normal distribution. Differences in urinary excretion of aluminium and silicon between females and males were analysed with an independent *t*-test. When we compared first and last week of each baseline and treatment period a paired *t*-test was used.

To detect a possible influence of the silicon-rich mineral water therapy on urinary excretion of aluminium and silicon a nested three-way ANOVA with the factors, *week* nested in *treatment* and the additional factor *gender* was performed. The factor *treatment* encodes for the baseline period and the silicon-rich mineral water therapy, the factor *week* expresses possible changes in urinary excretion over the 12 weeks of each period and the factor *gender* expresses possible differences in urinary excretion of females and males. Additional post-hoc tests were performed using Bonferroni-correction.

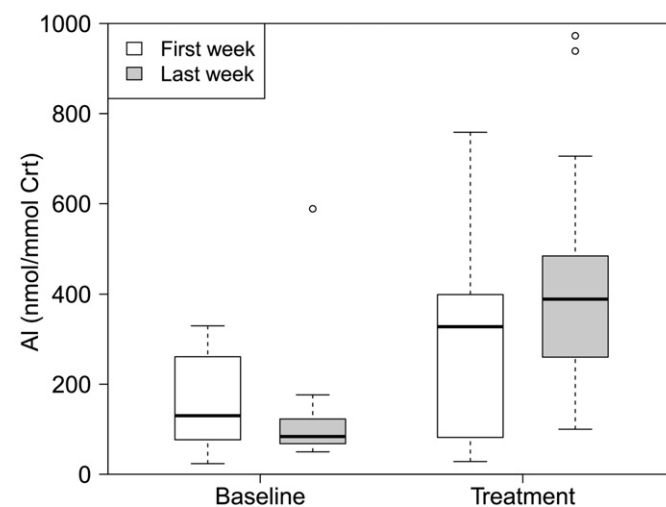


Fig. 1. Comparison of urinary aluminium excretion in control and treatment periods. Boxplot of urinary aluminium excretion (nmol/mmol Cr_t) for the first (n = 15) and last weeks (n = 15) of the baseline (weeks 1–12) and treatment (weeks 13–24) periods respectively. There were no significant differences in aluminium excretion between the first and last weeks of either the baseline or the treatment period, (paired *t*-test, *p* > 0.05, n = 15).

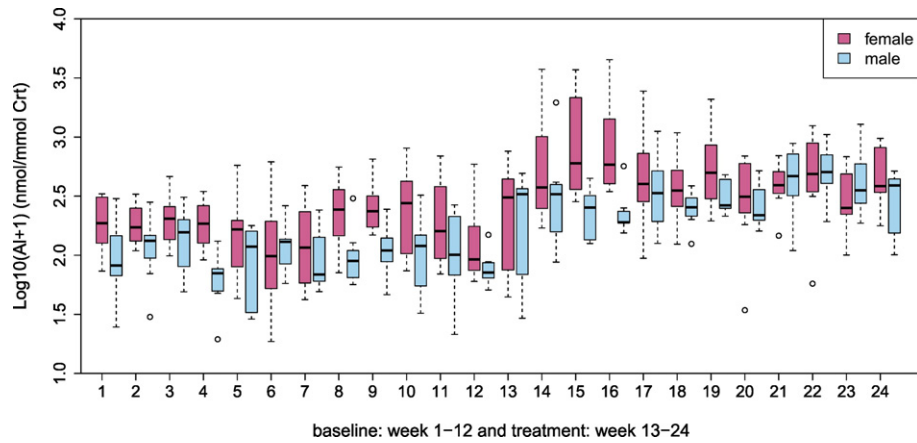


Fig. 2. Urinary aluminium excretion over the full 24 weeks of the study. Boxplots of urinary aluminium excretion (nmol/mmol Cr) from week 1 to week 24 ($n_{\text{females}} = 8$; $n_{\text{males}} = 7$). There was a significant increase in aluminium excretion between the baseline (weeks 1–12) and treatment (weeks 13–24) periods ($p < 0.001$).

Furthermore to analyse the relationship of aluminium and silicon a Pearson-correlation and a linear regression analysis of log-transformed data was performed and a p -value smaller than 0.05 was considered as statistically significant. For statistical analysis SPSS Statistics v.22 (IBM Analytics, Armonk, NY, USA) and for additional analysis and figures R Studio (Version 1.0.153 – © 2009–2017 RStudio, Inc.) was used.

3. Results

3.1. Urinary Excretion of Aluminium (Creatinine-Corrected Data)

Median urinary excretion of aluminium during the baseline period (1–12 weeks) ranged from 51.8 (39.5–61.8) to 326.1 (201.0–582.7) nmol/mmol Cr (median + IQR, $n = 15$) (Table 1). The median for all 15 participants over the 12 weeks was 135.2 nmol/mmol Cr (70.3–222.2, $n = 180$). Females, with an overall median concentration of 169.7 nmol/mmol Cr (149.5–222.1, $n = 8$) excreted significantly more aluminium than males (102.0 nmol/mmol Cr, 65.3–152.4, $n = 7$) (independent t -test, $t_{(13)} = 2.52$, $p = 0.026$). There was no significant difference in urinary excretion of aluminium between the start (week 1), 130.1 nmol/mmol Cr (72.4–299.6, $n = 15$) and end (week 12), 83.7 nmol/mmol Cr (67.2–148.0, $n = 15$) of the baseline period (paired t -test, $t_{(14)} = 1.37$, $p = 0.194$) (Fig. 1).

Median urinary excretion of aluminium during the treatment period (13–24 weeks) ranged from 140.5 (124.5–188.7) to 1081.4 (393.2–2270.8) nmol/mmol Cr. (median + IQR, $n = 15$) (Table 1). The median for all 15 participants over the 12 weeks was 349.0 nmol/mmol Cr

(231.7–524.7, $n = 180$). Females excreted more aluminium (394.7 nmol/mmol Cr, 354.9–417.8, $n = 8$) than males, (296.0 nmol/mmol Cr, 270.3–436.3, $n = 7$), but the difference was not statistically significant (independent t -test, $t_{(13)} = 1.75$, $p = 0.103$). There was no significant difference in urinary excretion of aluminium between the start (week 13), 327.7 (47.1–411.5, $n = 15$) and end (week 24), 389.9 (184.6–513.7, $n = 15$) of the treatment period (paired t -test, $t_{(14)} = -1.66$, $p = 0.119$) (Fig. 1).

The urinary excretion of aluminium during the treatment period was higher than during the baseline period (Table 1; Fig. 2) (three-way ANOVA, $F_{1,13} = 59.17$, p -value = 0.000003). In the overall model we also observed significantly different urinary excretion of aluminium between females and males (three-way ANOVA, $F_{1,13} = 15.58$, p -value = 0.002). Post-Hoc tests showed significant differences between females and males in week 4, 8, 9, 15 and 16. The within-factor ‘week’ (nested in treatment) had no significant influence on aluminium concentration in urine (three-way ANOVA, $F_{1,13} = 1.67$, p -value = 0.085).

Table 2

Median and IQR of silicon concentration [$\mu\text{mol}/\text{mmol Cr}$] in urine of SPMS patients before (weeks 1–12) and after the treatment (weeks 13–24) with silicon-rich mineral water $N = 12$.

Patient ID	Baseline (wks 1–12)	Treatment (wks 13–24)
F001	113.7 (66.6–169.0)	349.3 (317.7–448.6)
F003	84.1 (57.5–137.0)	242.9 (134.2–535.0)
F006	100.5 (84.8–118.1)	262.8 (168.8–386.7)
F009	113.4 (81.9–140.6)	313.8 (264.4–473.0)
F012	109.9 (67.7–173.3)	334.4 (219.8–440.1)
F013	89.4 (43.8–114.2)	250.6 (175.3–294.5)
F015	192.7 (129.9–363.2)	277.3 (179.2–382.6)
F016	83.2 (53.7–142.2)	170.9 (129.8–402.6)
M002	44.5 (39.3–73.6)	230.6 (144.2–374.5)
M004	69.2 (57.3–94.3)	140.6 (113.9–285.5)
M005	48.3 (33.8–76.0)	131.6 (112.6–311.3)
M007	77.7 (48.7–108.2)	201.4 (141.5–238.9)
M010	75.6 (42.8–114.0)	195.4 (134.9–250.2)
M011	75.6 (23.5–88.5)	134.7 (73.6–150.5)
M014	45.1 (14.3–69.2)	102.4 (83.7–134.2)

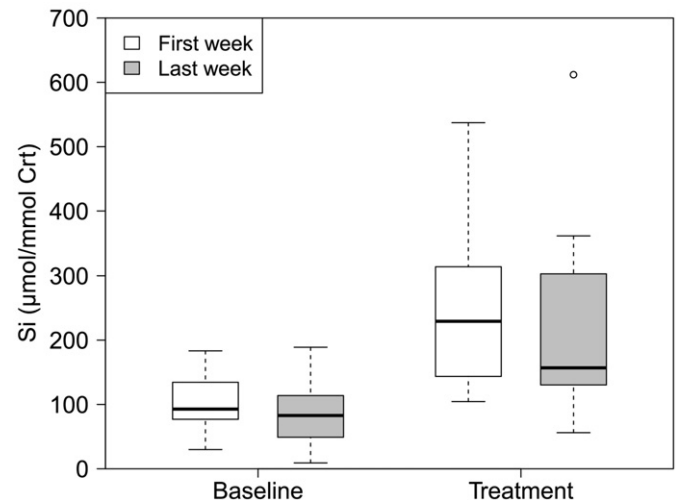


Fig. 3. Comparison of urinary silicon excretion in control and treatment periods. Boxplot of urinary silicon excretion ($\mu\text{mol}/\text{mmol Cr}$) for the first ($n = 15$) and last weeks ($n = 15$) of the baseline (weeks 1–12) and treatment (weeks 13–24) periods respectively. There were no significant differences in silicon excretion between the first and last weeks of either the baseline or the treatment period, (paired t -test, $p > 0.05$, $n = 15$).

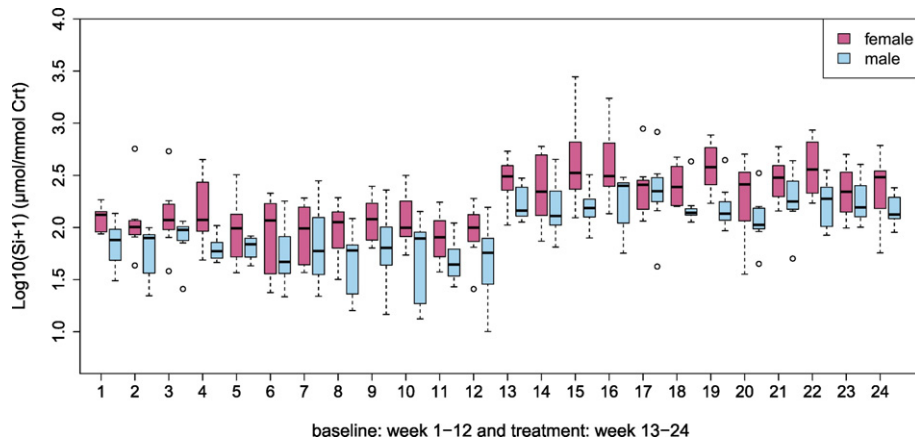


Fig. 4. Urinary silicon excretion over the full 24 weeks of the study. Boxplots of urinary silicon excretion ($\mu\text{mol}/\text{mmol Cr}$) from week 1 to week 24 ($n_{\text{females}} = 8$; $n_{\text{males}} = 7$). There was a significant increase in silicon excretion between the baseline (weeks 1–12) and treatment (weeks 13–24) periods ($p < 0.001$).

3.2. Urinary Excretion of Silicon (Creatinine-Corrected Data)

Median urinary excretion of silicon during the baseline period (1–12 weeks) ranged from 44.5 (39.3–73.6) to 192.7 (129.9–363.2) $\mu\text{mol}/\text{mmol Cr}$. (median + IQR, $n = 15$) (Table 2). The median for all 15 participants over the 12 weeks was 81.3 $\mu\text{mol}/\text{mmol Cr}$. (47.9–118.6, $n = 180$). Females, with an overall median concentration of 105.2 $\mu\text{mol}/\text{mmol Cr}$ (86.8–113.5, $n = 8$) excreted significantly more silicon than males (69.2 $\mu\text{mol}/\text{mmol Cr}$, 45.1–75.6, $n = 7$) (independent t -test, $t_{(13)} = 4.13$, $p = 0.001$). There was no significant difference in urinary excretion of silicon between the start (week 1), 92.8 $\mu\text{mol}/\text{mmol Cr}$ (74.9–135.3, $n = 15$) and end (week 12), 82.7 $\mu\text{mol}/\text{mmol Cr}$ (41.8–114.7, $n = 15$) of the baseline period (paired t -test, $t_{(14)} = 2.04$, $p = 0.06$) (Table 2; Fig. 3).

Median urinary excretion of silicon during the treatment period (13–24 weeks) ranged from 102.4 (83.7–134.2) to 349.3 (317.7–448.6) $\mu\text{mol}/\text{mmol Cr}$. (median + IQR, $n = 15$) (Table 2). The median for all 15 participants over the 12 weeks was 221.5 $\mu\text{mol}/\text{mmol Cr}$ (134.8–332.7, $n = 180$). Females, with an overall median concentration of 270.0 $\mu\text{mol}/\text{mmol Cr}$ (246.8–324.1, $n = 8$) excreted significantly more silicon than males (140.6 $\mu\text{mol}/\text{mmol Cr}$, 131.6–201.4, $n = 7$) (independent t -test, $t_{(13)} = 4.05$, $p = 0.001$). There was no significant difference in urinary excretion of silicon between the start (week 13), 229.2 (142.9–331.7, $n = 15$) and end (week 24), 156.9 (128.5–310.7, $n = 15$) of the treatment period (paired t -test, $t_{(14)} = 1.2$, $p = 0.249$) (Table 2; Fig. 3).

The urinary excretion of silicon during the treatment period was higher than during the baseline period (three-way ANOVA, $F_{1,13} = 220.19$, p -value < 0.001) (Table 2; Fig. 4). The factor ‘gender’ was significant for urinary excretion of silicon during the treatment period (three-way ANOVA, $F_{1,13} = 24.19$, p -value = 0.000281). Post-Hoc tests showed significant differences between females and males in week 1, 2, 4, 8, 15, 16, 18 and 19. The within-factor ‘week’ had also a significant influence on urinary excretion of silicon (three-way ANOVA, $F_{1,13} = 1.89$, p -value = 0.045), though this effect was not proven in a post-hoc test neither for the baseline period ($p = 0.415$) nor the treatment period ($p = 0.495$).

3.3. Correlations Between Urinary Excretion of Silicon and Aluminium (Cr-Corrected Data)

The urinary excretion of aluminium was positively correlated with the urinary excretion of silicon for all participants over the full 24 weeks of the trial (Fig. 5). In females the relationship was more highly correlated in the treatment as compared to the baseline

period (Fig. 6A, B). In males this relationship was very weak in the baseline period becoming stronger during the treatment period (Fig. 6C, D).

3.4. 24 h Aluminium Data for Weeks 1, 12 (Baseline), 13 and 24 (Treatment)

The amount of aluminium excreted during the baseline period (data for weeks 1 and 12 combined) ranged from 0.85 (0.6–1.3) to 2.98 (2.3–4.2) $\mu\text{mol}/24\text{ h}$ (median + IQR, $n = 10$). This increased from 0.23 (0.2–0.4) to 8.08 (8.0–8.4) for week 13 (Table 3) and 3.01 (3.0–3.3) to 10.77 (10.3–13.1) $\mu\text{mol}/24\text{ h}$ (median + IQR, $n = 5$) for week 24 (Table 4). Increases in the amount of aluminium excreted in week 13 and week 24 relative to baseline were significant for 10 and 14 individuals respectively (independent t -test, $p < 0.05$) (Tables 3 and 4; Figs. 7 and 8). In 6 individuals there were significant differences (independent t -test, $p < 0.05$) in the amount of aluminium excreted between week 13 and week 24 with 1 out of the 6 individuals showing a statistically significant

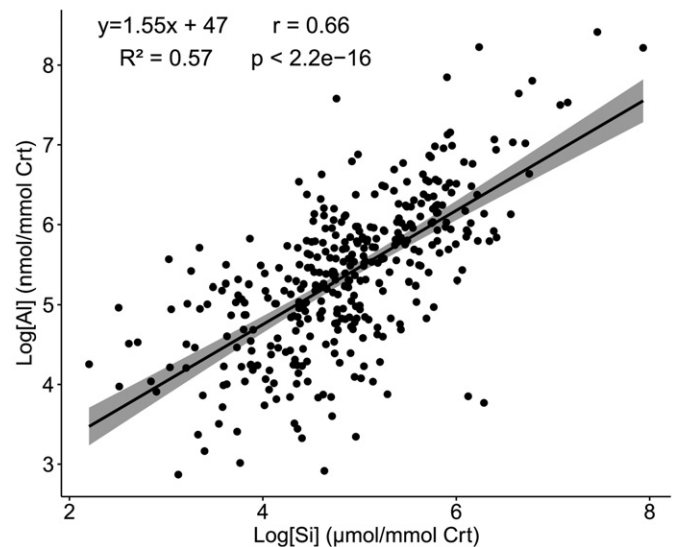


Fig. 5. The relationship between urinary aluminium and silicon excretion for all participants. Scatter-plot of log-transformed aluminium and silicon excretion data (Cr-corrected) for all 15 participants over the full 24 weeks ($n = 180$).

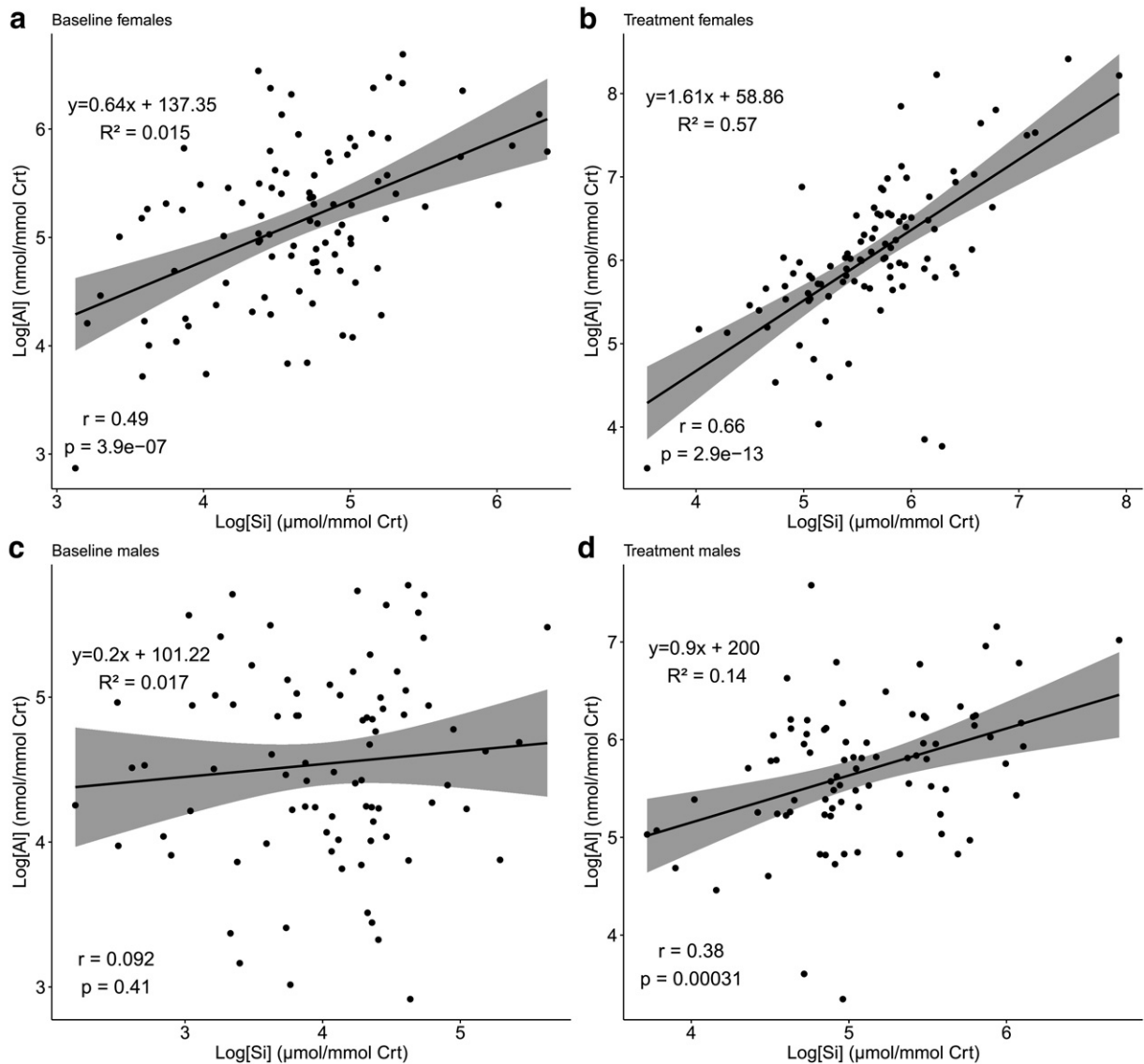


Fig. 6. (A–D) Relationships between urinary excretion of aluminium and silicon based on gender and study period. Scatter-plots of log-transformed aluminium and silicon excretion data (Cr_t-corrected) for females ($n = 96$) and males ($n = 84$) for separate baseline (weeks 1–12) and treatment (weeks 13–24) periods.

(independent t -test, $p < 0.05$) fall in the amount of aluminium excreted during the 12 weeks of the treatment period (Table 5; Fig. 8).

3.5. Qualitative Observations

None of the 15 individuals who took part in the study experienced any relapses during the 24 weeks of the trial. Neither did their EDSS scores change during this period.

4. Discussion

Participants were fully compliant with the study and urine samples (spot and 24 h) were obtained as required from all 15 participants for the duration of the 24 week study. We have presented the first comprehensive data set for the urinary excretion of aluminium and silicon in MS over an extended time period.

The data for aluminium demonstrate wide variability across the baseline period (weeks 1–12) with the median + IQR (135.2 nmol/mmol Cr_t + 70.3–222.2, $n = 180$) reflecting significant inter-subject

variability (Table 1; Fig. 2). However, within such variability and for a somewhat limited number of individuals we were still able to discriminate statistically significantly (independent t -test, $t_{(13)} = 2.52$, $p = 0.026$) higher excretion of aluminium in females (169.7 nmol/mmol Cr_t + 149.5–222.1, $n = 8$) than males (102.0 nmol/mmol Cr_t + 65.3–152.4, $n = 7$). This suggests that this relationship, females exhibiting increased urinary excretion of aluminium, would be even stronger for a larger cohort of participants. While there are few comparative data within the scientific literature urinary excretion of aluminium was previously measured using single spot urine samples taken from 10 individuals (7 female and 3 male) diagnosed with SPMS (Exley et al., 2006a) and the computed median of this cohort, 99.2 nmol/mmol Cr_t, was similar to that obtained herein. It is also noteworthy that in the previous study the 3 lowest values, 17.6, 75.1 and 85.2 nmol/mmol Cr_t, were those of the 3 male subjects. In the age and gender-matched control population of the previous study the median urinary excretion of aluminium was 37.6 nmol/mmol Cr_t which was significantly lower than the median for the SPMS group ($P < 0.001$) and adds to the conclusion herein,

Table 3

Median and IQR of urinary Al excretion ($\mu\text{mol}/24\text{ h}$) for all patients for the baseline period (weeks 1 and 12 combined, $n = 10$) compared to the first week of the treatment period (week 13, $n = 5$).

Baseline – week 13		Median	IQR	p-value	Effect
F001	Baseline	0.86	0.4–1.5	0.001	↑
	Treatment	4.54	2.0–4.7		
F003	Baseline	0.92	0.5–1.4	0.466	↑
	Treatment	1.32	1.3–1.9		
F006	Baseline	1.09	0.8–1.5	0.015	↓
	Treatment	0.38	0.3–0.5		
F009	Baseline	0.85	0.6–1.3	0.815	↓
	Treatment	0.23	0.2–0.4		
F012	Baseline	2.98	2.3–4.2	0.002	↑
	Treatment	8.08	8.1–8.4		
F013	Baseline	2.02	1.1–4.1	0.005	↑
	Treatment	6.92	6.8–7.0		
F015	Baseline	1.62	0.3–3.1	0.001	↑
	Treatment	4.96	4.9–5.4		
F016	Baseline	0.86	0.5–1.2	<0.001	↑
	Treatment	2.24	2.2–2.9		
M002	Baseline	1.02	0.8–1.1	0.140	↑
	Treatment	1.15	1.1–1.2		
M004	Baseline	0.87	0.6–1.3	0.001	↑
	Treatment	3.05	2.2–4.6		
M005	Baseline	1.16	0.8–1.7	0.789	↓
	Treatment	0.85	0.8–1.5		
M007	Baseline	1.38	1.2–1.6	<0.001	↑
	Treatment	5.21	3.6–6.8		
M010	Baseline	2.60	1.1–3.9	0.013	↑
	Treatment	5.67	4.5–6.9		
M011	Baseline	2.85	2.2–3.1	<0.001	↑
	Treatment	5.89	4.8–7.0		
M014	Baseline	1.71	1.0–2.0	<0.001	↑
	Treatment	7.26	6.8–7.7		

where the median is 135.2 nmol/mmol Cr_t, that individuals with SPMS, and females in particular, excrete unusually high amounts of aluminium in their urine.

The data for urinary excretion of aluminium during the treatment period (weeks 13–24), when all individuals were drinking up to 1.5 L of a silicon-rich mineral water every day, also showed a high degree of inter-subject variability with the median excretion rising to 349.0 nmol/mmol Cr_t (231.7–524.7, $n = 180$) which was a statistically significant increase relative to the baseline period (three-way ANOVA, $F_{1,13} = 59.17$, $p\text{-value} = 0.000003$) (Table 1; Fig. 2). While females once again excreted more aluminium than males the increase was not statistically significant for the treatment period (independent t -test, $t_{(13)} = 1.75$, $p = 0.103$). Increased urinary excretion of aluminium during the treatment period paralleled the statistically significant increase in urinary silicon excretion between the baseline (81.3 $\mu\text{mol}/\text{mmol Cr}_t + 47.9\text{--}118.6$, $n = 180$) and treatment (221.5 $\mu\text{mol}/\text{mmol Cr}_t + 134.8\text{--}332.7$, $n = 180$) periods (three-way ANOVA, $F_{1,13} = 220.19$, $p\text{-value} < 0.001$) (Table 2; Fig. 4). Urinary excretion of silicon in females was statistically higher than males in both the baseline and treatment periods and so, similar to the overall picture, suggested a role for silicon in the urinary excretion of aluminium. Regression analyses confirmed a positive relationship between urinary excretion of aluminium and silicon and especially so in females (Figs. 5 and 6). In exploring this relationship further we used the 24 h urine data for weeks 1, 12, 13 and 24 to investigate how drinking a silicon-rich mineral water influenced how much aluminium was excreted by each of the 15 participants. Data for weeks 1 and 12 were not significantly different and so were pooled to give a single value for baseline excretion. When baseline data were compared with data for week 13, thus the first week drinking the silicon-rich mineral water, it was found that there were statistically significant increases in the amount of aluminium excreted in 10 of the 15 participants (Table

Table 4

Median and IQR of urinary Al excretion ($\mu\text{mol}/24\text{ h}$) for all patients for the baseline period (weeks 1 and 12 combined, $n = 10$) compared to the last week of the treatment period (week 24, $n = 5$).

Baseline – week 24		Median	IQR	p-value	Effect
F001	Baseline	0.86	0.4–1.5	<0.001	↑
	Treatment	3.99	3.4–4.5		
F003	Baseline	0.92	0.5–1.4	0.046	↑
	Treatment	3.60	1.5–4.5		
F006	Baseline	1.09	0.8–1.5	<0.001	↑
	Treatment	7.11	5.4–8.2		
F009	Baseline	0.85	0.6–1.3	<0.001	↑
	Treatment	10.77	10.3–13.1		
F012	Baseline	2.98	2.3–4.2	0.942	↓
	Treatment	3.07	2.3–3.2		
F013	Baseline	2.02	1.1–4.1	0.030	↑
	Treatment	5.06	4.7–7.0		
F015	Baseline	1.62	0.3–3.1	0.016	↑
	Treatment	4.13	4.0–4.7		
F016	Baseline	0.86	0.5–1.2	<0.001	↑
	Treatment	3.86	2.8–4.5		
M002	Baseline	1.02	0.8–1.1	<0.001	↑
	Treatment	5.32	5.0–5.8		
M004	Baseline	0.87	0.6–1.3	<0.001	↑
	Treatment	8.50	6.4–9.9		
M005	Baseline	1.16	0.8–1.7	0.004	↑
	Treatment	3.01	3.0–3.3		
M007	Baseline	1.38	1.2–1.6	<0.001	↑
	Treatment	4.90	3.7–5.1		
M010	Baseline	2.60	1.1–3.9	0.039	↑
	Treatment	3.42	3.4–4.8		
M011	Baseline	2.85	2.2–3.1	0.017	↑
	Treatment	4.97	3.0–5.4		
M014	Baseline	1.71	1.0–2.0	<0.001	↑
	Treatment	4.75	4.5–5.5		

3; Fig. 7). Similarly for week 24, when participants had been drinking the silicon-rich mineral water for 12 weeks, 14 out of 15 participants showed statistically significant higher amounts of aluminium in their urine relative to the baseline period (Table 4; Fig. 8). While all data, both creatinine-corrected and 24 h, showed that the excretion of aluminium during the baseline period was homogenous, unaffected by week, we were interested to know if such was also true between the beginning (week 13) and end (week 24) of the treatment period. When urinary excretion in week 13 was compared with week 24 for each individual we found a fall in urinary aluminium excretion in 3 individuals though such was only statistically significant in 1 (female) patient (Table 5; Fig. 9). In the only other published long term study of the excretion of aluminium following drinking silicon-rich mineral waters individuals with a diagnosis of Alzheimer's disease showed a statistically significant fall in urinary aluminium excretion over a 12 week period. This was interpreted as evidence that longer term drinking of a silicon-rich mineral water would begin to reduce the body burden of aluminium as estimated using urinary aluminium excretion. Herein in SPMS we observed preliminary evidence that this was happening in some individuals. A longer study over months and years would help to test the validity of this hypothesis. It is of note that the median urinary excretion of aluminium in week 12 of the previous AD study (Davenward et al., 2013) was 64.8 nmol/mmol Cr_t compared to 389.9 nmol/mmol Cr_t in SPMS. This may be further evidence that the body burden of aluminium in MS may be significantly higher than it is in AD and as such it may take considerably longer to lower the body burden of aluminium in MS through regular drinking of a silicon-rich mineral water. Only, as suggested previously, a longer-term study will help to resolve this question.

We are able to conclude that individuals with SPMS excrete an unusually high amount of aluminium in their urine and this confirms the results of a previous much smaller study. Regular drinking of a

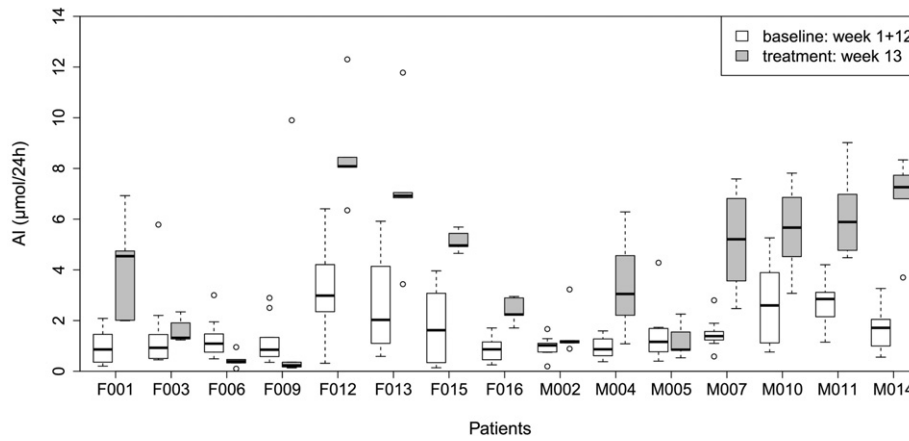


Fig. 7. Comparison of amount of aluminium excreted in control and the first week of the treatment period. Boxplots of urinary aluminium excretion ($\mu\text{mol}/24\text{ h}$) for all patients for the baseline period (week 1 and 12 combined, $n = 10$) compared to the first week of the treatment period (week 13, $n = 5$).

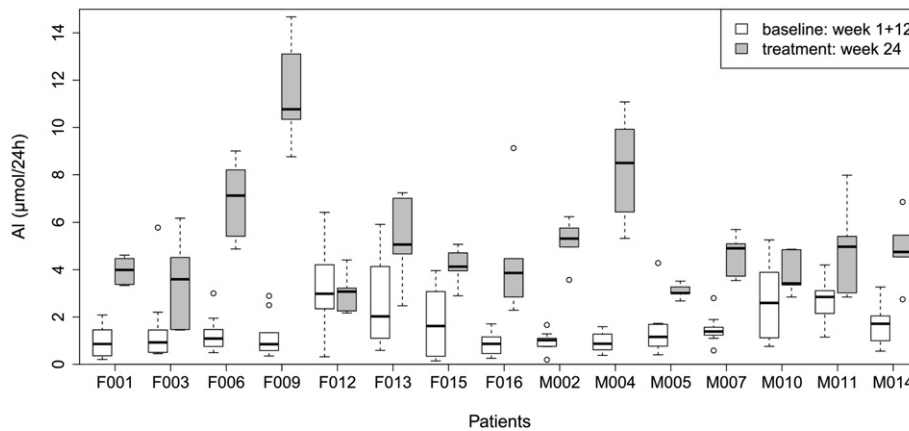


Fig. 8. Comparison of amount of aluminium excreted in control and the last week of the treatment period. Boxplots of urinary aluminium excretion ($\mu\text{mol}/24\text{ h}$) for all patients for the baseline period (week 1 and 12 combined, $n = 10$) compared to the last week of the treatment period (week 24, $n = 5$).

silicon-rich mineral water increased their urinary excretion of aluminium which suggested that individuals with SPMS have a high body burden of aluminium. Drinking a silicon-rich mineral

water for 12 consecutive weeks provided limited evidence that such could help individuals in reducing their body burden of aluminium if this was continued for months and years thereafter. Females had a higher content of aluminium in their urine than males which suggested a higher body burden of aluminium in females. The incidence of MS is also higher in females which may begin to suggest that the body burden of aluminium predisposes females to the disease. Metabolomic profiling may in the future reveal further gender differences in MS (Villoslada et al., 2017) some of which may also shed some light on how aluminium is handled by the body in MS. If human exposure to aluminium has a role to play in the aetiology of MS then regular drinking of a silicon-rich mineral water may act as a simple, non-invasive therapy for the removal of aluminium.

Table 5

Median and IQR of urinary Al excretion ($\mu\text{mol}/24\text{ h}$) for all patients and comparing the first week (week 13, $n = 5$) and the last week (week 24, $n = 5$) of the treatment period.

	Week 1		Week 2		P-value	
	Median	IQR	Median	IQR		
F001	4.5	2.0–4.7	4.0	3.4–4.5	0.816	↓
F003	1.3	1.3–1.9	3.6	1.5–4.5	0.106	↑
F006	0.4	0.3–0.5	7.1	5.4–8.2	<0.001	↑
F009	0.2	0.2–0.4	10.8	10.3–13.1	0.003	↑
F012	8.1	8.1–8.4	3.1	2.3–3.2	<0.001	↓
F013	6.9	6.8–7.0	5.1	4.7–7.0	0.295	↓
F015	5.0	4.9–5.4	4.1	4.0–4.7	0.057	↓
F016	2.2	2.2–2.9	3.9	2.8–4.5	0.080	↑
M002	1.2	1.1–1.2	5.3	5.0–5.8	<0.001	↑
M004	3.0	2.2–4.6	8.5	6.4–9.9	0.012	↑
M005	0.9	0.8–1.5	3.0	3.0–3.3	0.007	↑
M007	5.2	3.6–6.8	4.9	3.7–5.1	0.800	↓
M010	5.7	4.5–6.9	3.4	3.4–4.8	0.117	↓
M011	5.9	4.8–7.0	5.0	3.0–5.4	0.252	↓
M014	7.3	6.8–7.7	4.8	4.5–5.5	0.142	↓

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Conflict of Interests

None of the authors report any conflict of interests.

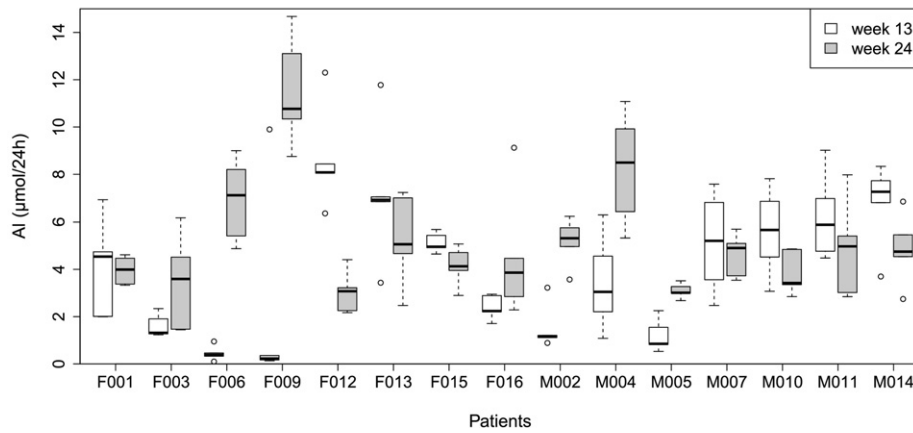


Fig. 9. Comparison of amount of aluminium excreted in the first and last weeks of the treatment period. Boxplots of urinary aluminium excretion ($\mu\text{mol}/24\text{h}$) for all patients and comparing the first week (week 13, $n = 5$) and the last week (week 24, $n = 5$) of the treatment period.

Author Contributions

CE designed the study, supported KJ and wrote the manuscript. KJ carried out the majority of the study as part of her PhD. CH provided all clinical support for the project. CL performed all statistical analyses.

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