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Silicon-Rich Mineral Water as a Non-Invasive Test of the 'Aluminum Hypothesis' in Alzheimer's Disease

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Abstract. There has been a plausible link between human exposure to aluminum and Alzheimer's disease for several decades. We contend that the only direct and ethically acceptable experimental test of the 'aluminum hypothesis', which would provide unequivocal data specific to the link, is to test the null hypothesis that a reduction in the body burden of aluminum to its lowest practical limit would have no influence upon the incidence, progression, or severity of Alzheimer's disease. Herein we are testing the hypothesis that silicon-rich mineral waters can be used as non-invasive methods to reduce the body burden of aluminum in individuals with Alzheimer's disease and a control group consisting of their carers and partners. We have shown that drinking up to 1 L of a silicon-rich mineral water each day for 12 weeks facilitated the removal of aluminum via the urine in both patient and control groups without any concomitant affect upon the urinary excretion of the essential metals, iron and copper. We have provided preliminary evidence that over 12 weeks of silicon-rich mineral water therapy the body burden of aluminum fell in individuals with Alzheimer's disease and, concomitantly, cognitive performance showed clinically relevant improvements in at least 3 out of 15 individuals. This is a first step in a much needed rigorous test of the 'aluminum hypothesis of Alzheimer's disease' and a longer term study involving many more individuals is now warranted.

Keywords: Alzheimer's disease, aluminum, body burden, cognitive function, copper, iron, mineral water, silicic acid, silicon, urinary excretion

INTRODUCTION

How might human exposure to aluminum be a contributory factor in the incidence, progression, or severity of Alzheimer's disease (AD)? While we can be confident that aluminum is present in the human brain [1, 2] and that it is a known neurotoxin [3], there has never been a direct experimental test of its contribution to the etiology of AD. There are myriad examples in *in vitro* studies, animal models, and indirectly in humans of aluminum's potential to interact with biochemical systems and markers of AD [4] though none of these have subsequently been translated into an unequivocal contributory role for aluminum in AD etiology. Even in the infamous clinical trial of intramuscular injections of desferrioxamine mesylate (DFO), a trivalent metal ion chelator which was effective in reducing the rate of progression of AD over a two year period by a factor of two, it proved impossible to attribute the clinical

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observations directly to aluminum [5, 6]. It is evidentially clear that unavoidable consequences of living in the Aluminum Age [7] are age-dependent increases in the body burden of aluminum and especially in relation to the brain [1]. However, body and brain burdens of aluminum and their propensities to deliver biologically available aluminum and ultimately toxicity [8] are likely to be specified at the level of individuals as opposed to populations which means that absence as opposed to presence of aluminum should be the most rigorous test of any aluminum hypothesis of AD [9]. The null hypothesis would state that a reduction in the body burden of aluminum toward a lowest practical limit would have no influence upon the onset, incidence, progression, or aggressiveness of AD. A major barrier to testing this hypothesis is the attainment of a lowest practical body burden of aluminum which would require both a reduced systemic intake of aluminum and the removal of aluminum already present in the body. While it is often assumed that absorption across the gut is the major route of entry of aluminum into the body, the lack of quantitative data that describes other possible routes including dermal, olfactory, and respiratory surfaces means that this has not been confirmed experimentally [9]. The enhanced excretion of aluminum in urine is the most accessible approach to a reduction in the aluminum body burden and this should be amenable to chelation therapy [10]. However, there are no clinically-approved drugs which are specific to the chelation and subsequent urinary excretion of systemic aluminum and so the application of known aluminum chelators such as the aforementioned DFO cannot be used in the longer term without affecting the body's status of essential metals such as iron. In addition, an effective chelation strategy should really encompass a drug which can be taken orally and without the need for hands-on medical support. While there are at present no clinically-approved drugs which fulfill these stringent criteria for an effective chelation therapy for aluminum, there is one non-invasive strategy which has shown promise both in facilitating the urinary excretion of aluminum and in limiting its gastrointestinal absorption [11]. Silicon, or more accurately silicic acid, is the natural antagonist to the toxicity of aluminum in biota [12], and in humans it has also been shown to reduce aluminum uptake across the gut [13] and facilitate the excretion of systemic aluminum via the kidney [14]. We have shown previously in a study over 5 days that drinking up to 1.5 L of silicon-rich mineral water each day reduced the body burden of aluminum in individuals with AD [15]. A similar effect was also observed in a single individual suffering from chronic fatigue syndrome [16]. Herein we have tested the null hypothesis that regular drinking of a silicon-rich mineral water over a period of 12 weeks has no influence upon the urinary excretion of aluminum in individuals with AD and their spouse or carer. In addition we have also measured urinary excretion of silicon, iron, and copper as well as performing cognitive testing before and after the period of mineral water therapy. We show that the null hypothesis can be rejected and that silicon-rich mineral water may be a promising therapy to test the aluminum hypothesis of AD.

MATERIALS AND METHODS

Sixteen individuals with a DSM IV diagnosis of AD [17] and their spouses or carers were recruited through memory clinics in Stoke-on-Trent and Birmingham, United Kingdom. National Research Ethics Committee (NREC) approval for the study was obtained prior to its commencement. Both patients and their carers gave informed consent and the latter had to agree to supervise urine collections for the former. Exclusion criteria for both groups included regular use of aluminum-containing medications such as antacids and ongoing medical conditions which might prevent individuals from drinking up to 1 L of mineral water each day for up to 12 weeks. One volunteer pair dropped out of the study following problems with compliance and one female carer had to leave the study due to an unrelated medical condition.

Recruitment occurred over a period of approximately 18 months and once each group was recruited, consisting of one patient and one control, they participated in a study which lasted 13 weeks. In the first week (Wk 0), both patient and control collected their first urine sample of the day over 7 consecutive days. Pristine, acid-washed vessels were provided to collect urine samples. Sample vessels were stored in sealed biohazard bags in home refrigerators before being collected and transported to the laboratory. In the second week (Wk 1), patient and control collected urine samples in the identical manner to Wk 0 and during these 7 days they each drank up to 1 L of a silicon-rich mineral water each day. The silicon-rich mineral water was provided by Spritzer and it was measured by us to contain 35 mg/L total silicon. For the following 10 weeks (Wk 2–Wk 11), both patient and control continued to drink up to 1 L of Spritzer mineral water each day and they collected their first urine sample of the day on the Wednesday of each week. In the final week (Wk

12), patient and carer continued to drink the mineral water and they collected urine samples in the identical manner to Wk 0 and Wk 1.

In addition to the collection of urine samples, the Alzheimer's disease assessment scale-cognitive (ADAS-Cog) [18] was used to measure cognitive performance of both patients and controls at the beginning (Wk 0) and the end (Wk 12) of the trial. These tests were carried out by experienced cognitive test practioners that were blind to the data obtained on urinary excretion. Data were only brought together when completed and as required for statistical analyses and preparation of this document. ADAS-Cog is composed of 11 different components which have been designed to test broad areas of cognition, memory, language, orientation, and praxis. The test is scored out of 70 and a lower score indicates a better performance. Recent research has suggested that a minimal clinically-relevant change (MCRC) is $\geq$3 units on the scale [19]. Following transportation of urine samples to the laboratory, they were mixed thoroughly and sampled for measurement of creatinine (Crt) using the Jaffe method. The remaining urine was acidified using 15.8 M HNO_3 to 20% *v/v* and subjected to microwave digestion to prepare samples for measurement of total silicon, aluminum, iron, and copper by TH GFAAS [2].

One-way ANOVA at $\alpha = 0.05$ was carried out on each individual data set to compare the means of Wk 0, Wk 1, and Wk 12 (7 replicates per week per individual). Post-hoc Tukey's paired comparisons were carried out to determine the significance between pairs of means, where tests for normality and equal variance showed a departure from either or both, log transformations were carried out. Two-way ANOVA to compare patients and controls (factor 1) and male and female (factor 2) was not valid because of unequal variance which transformations did not resolve. The non-parametric Mann-Whitney U test was used to compare pairs of medians. Analyses were carried out using Minitab® 15.

RESULTS

Twenty-nine individuals, 15 diagnosed as AD and 14 non-AD, completed the study and informal indices suggested that compliance with study protocols was excellent. Data are summarized according to treatment group (non-AD, Tables 1 and 2; AD, Tables 3 and 4) and gender (males, Tables 1 and 3; females, Tables 2 and 4) and analyzed below as treatment groups and as individuals.

Urinary Al excretion in weeks 0 and 1 in non-AD and AD treatment groups

Mean urinary excretion of Al in non-AD controls and AD patients for Wk 0 were 84.6 (64.3) nmol/mmol Crt and 95.8 (83.7) nmol/mmol Crt, respectively. Mann-Whitney U test of the difference of medians for these groups (68.8 and 64.9 for non-AD control and AD patients, respectively) showed that they were not statistically significant ($p = 0.8440$). However, there were gender differences within these groups as non-AD females (mean 95.4 (52.2) nmol/mmol Crt) excreted significantly (Mann-Whitney U, $p = 0.0147$) more Al than AD females (mean 73.5 (46.7) nmol/mmol Crt) in Wk 0. In males, this trend was reversed as non-AD males (mean 70.1 (75.8) nmol/mmol Crt) excreted significantly (Mann-Whitney U, $p = 0.0291$) less Al than AD males (mean 115.0 (102.0) nmol/mmol Crt). In the non-AD controls, females excreted significantly (Mann-Whitney U, $p < 0.001$) more Al than males in Wk 0 while in AD patients males excreted more Al than females though the difference was not statistically significant (Mann-Whitney U, $p = 0.2437$).

In Wk 1, during which all participants drank up to 1 L of silicon-rich mineral water each day, the mean urinary excretion of Al in non-AD controls was 136.9 (81.4) nmol/mmol Crt and was significantly higher (Mann-Whitney U, $p = 0.0257$) than the same for AD patients (mean 110.6 (62.7) nmol/mmol Crt). Again there were gender differences as non-AD females (mean 152.7 (83.3) nmol/mmol Crt) excreted significantly (Mann-Whitney U, $p = 0.0046$) more Al than AD females (mean 103.5 (42.8) nmol/mmol Crt). However, these differences in Wk 1 were not evident for males with urinary Al excretion of 115.8 (74.7) nmol/mmol Crt and 116.9 (75.9) nmol/mmol Crt for non-AD and AD males, respectively. Non-AD females excreted significantly more Al in Wk 1 than non-AD males (Mann-Whitney U, $p = 0.0293$) while there were no significant differences in Al excretion in Wk 1 for males and females in the AD patients.

Comparisons of individuals within treatment groups

Six of the non-AD controls were male and aged 59–79 (Mean 71 (7.3)). For 5 out of 6 of these, the mean urinary excretion of Al for Wk 0 ranged from 25 to 68 nmol/mmol Crt while in one individual (Id. 23) it was 214 nmol/mmol Crt (Table 1). The mean urinary excretion of Al in Wk 1 was increased for all 6 individuals and in 4, the increases were approximately

Table 1
The urinary excretion of aluminum, silicon, iron, and copper (Mean & SD) in male non-AD controls before (Wk 0) and after drinking silicon-rich mineral water for 1 and 12 weeks. ADAS-Cog scores are given for before (Wk 0) and after drinking silicon-rich mineral water for 12 weeks

Identity	Age (y)	Sample Week	Al nmol/ mmol Crt	Si μmol/ mmol Crt	Fe nmol/ mmol Crt	Cu nmol/ mmol Crt	ADAS-Cog/70
01	79	0	41.1 (18.0)	36.5 (12.0)	21.7 (13.4)	32.0 (7.4)	05
		1	81.7 (33.4)	93.9 (41.8)	27.3 (15.7)	37.1 (12.6)	↓
		12	40.3 (14.0)	65.4 (18.7)	24.1 (7.7)	38.0 (8.9)	09
03	71	0	40.6 (14.8)	42.9 (17.6)	25.0 (7.5)	39.1 (19.5)	05
		1	81.1 (16.6)	78.3 (15.2)	28.1 (6.8)	37.5 (18.0)	↓
		12	155.4 (30.3)	151.9 (19.1)	29.4 (10.3)	40.4 (9.9)	07
07	78	0	67.8 (23.7)	38.6 (23.1)	40.6 (6.9)	32.5 (7.5)	07
		1	170.8 (37.7)	109.9 (27.2)	52.4 (24.4)	45.3 (12.5)	↓
		12	82.7 (23.2)	119.1 (18.1)	47.5 (13.8)	46.1 (18.8)	09
11	59	0	25.3 (14.8)	58.6 (17.7)	18.7 (15.1)	5.5 (1.2)	06
		1	38.2 (17.5)	104.2 (31.7)	18.8 (9.2)	7.3 (3.5)	↓
		12	55.8 (28.5)	114.7 (23.8)	20.7 (7.6)	6.7 (2.8)	10
15	71	0	32.3 (8.6)	37.6 (16.4)	21.4 (12.5)	14.1 (1.6)	05
		1	101.6 (43.0)	67.6 (15.8)	36.4 (10.6)	20.4 (10.5)	↓
		12	80.6 (35.3)	71.1 (17.1)	25.3 (16.5)	16.7 (10.5)	02
23	68	0	213.5 (88.7)	32.0 (12.5)	55.2 (26.4)	36.6 (13.2)	00
		1	221.5 (81.1)	63.1 (17.0)	59.2 (11.4)	43.9 (10.2)	↓
		12	156.4 (34.4)	72.6 (17.8)	53.7 (12.3)	43.0 (17.8)	03

100% and statistically significant ($p \leq 0.05$; Post-hoc Tukey's). Four individuals at Wk 12 showed a reduction in mean urinary excretion of Al compared to Wk 1 and for 2 of these individuals, the decreases were statistically significant ($p \leq 0.05$; Post-hoc Tukey's). The mean urinary excretion of Si for Wk 0 for all 6 individuals ranged from 32 to 59 μmol/mmol Crt (Table 1). For all 6, these values were approximately doubled for Wk 1 and all increases were statistically significant ($p \leq 0.05$; Post-hoc Tukey's). Mean urinary excretion of Si remained high for Wk 12 for all individuals with no statistically significant differences with Wk 1. The mean urinary excretion of Fe for Wk 0 for all 6 individuals ranged from 19 to 55 nmol/mmol Crt, and there were neither consistent nor statistically significant differences between mean urinary Fe excretion for all individuals for Wks 0, 1, or 12 (Table 1). The mean urinary excretion of Cu for Wk 0 for all 6 individuals ranged from 6 to 39 nmol/mmol Crt and there were neither consistent nor statistically significant differences between mean urinary Cu excretion for all individuals for Wks 0, 1, or 12 (Table 1).

ADAS-Cog scores ranged from 00 to 07 (Mean 4.7 (2.4)) for Wk 0 and 03 to 10 (Mean 6.7 (3.4)) for Wk 12 (Table 1).

Eight of the non-AD controls were female and aged 63–77 (Mean 70 (6.1)). For Wk 0, the mean urinary excretion of Al across all 8 individuals ranged from 53 to 176 nmol/mmol Crt (Table 2). Mean urinary Al excretion increased in Wk 1 for 7 out of 8 individuals and the increases were approximately two-fold for 4 individuals and statistically significant ($p \leq 0.05$; Post-hoc Tukey's). Five individuals at Wk 12 showed a reduction in mean urinary excretion of Al compared to Wk 1, and for 3 of these 5 individuals, the decreases were statistically significant ($p \leq 0.05$; Post-hoc Tukey's). The mean urinary excretion of Si for Wk 0 for all 8 individuals ranged from 25 to 59 μmol/mmol Crt (Table 2). For all 8, these values increased approximately two-fold for Wk 1 and all increases were statistically significant ($p \leq 0.05$; Post-hoc Tukey's). Mean urinary excretion of Si remained high for Wk 12 for all individuals with no statistically significant differences with Wk 1. The mean urinary excretion of Fe for Wk 0 for all 8 individuals ranged from 24 to 45 nmol/mmol Crt and there were neither consistent nor statistically significant differences between mean urinary Fe excretion for all individuals for Wks 0, 1, or 12 (Table 2). The mean urinary excretion of Cu for Wk 0 for all 8 individuals ranged from 17 to 63 nmol/mmol Crt and there were neither consistent nor statistically significant differences between mean urinary Cu excretion for all individuals for Wks 0, 1, or 12 (Table 2). ADAS-Cog scores ranged from 02 to 07 (Mean 5.4 (21.8)) for Wk 0 and 01 to 09 (Mean 4.5 (2.7)) for Wk 12 (Table 2).

Eight of the AD patients were male and aged 64–81 (Mean 75 (6.3)). The mean urinary excretion of Al for Wk 0 across all individuals ranged from 28 to

Table 2
The urinary excretion of aluminum, silicon, iron, and copper (Mean & SD) in female non-AD controls before (Wk 0) and after drinking silicon-rich mineral water for 1 and 12 weeks. ADAS-Cog scores are given for before (Wk 0) and after drinking silicon-rich mineral water for 12 weeks

Identity	Age (y)	Sample week	Al nmol/ mmol Crt	Si μmol/ mmol Crt	Fe nmol/ mmol Crt	Cu nmol/ mmol Crt	ADAS-Cog/70
05	77	0	57.4 (24.5)	25.5 (12.5)	23.9 (8.6)	25.8 (13.6)	07
		1	85.0 (18.2)	51.7 (12.8)	29.5 (16.1)	29.3 (13.8)	↓
		12	109.1 (49.1)	78.2 (17.7)	29.8 (8.6)	29.4 (11.8)	07
09	75	0	53.4 (26.2)	33.7 (22.0)	26.7 (11.5)	22.8 (11.9)	07
		1	122.2 (49.1)	110.1 (27.4)	41.9 (7.6)	25.4 (12.5)	↓
		12	196.9 (91.6)	99.2 (53.7)	34.2 (18.9)	40.3 (28.9)	06
13	77	0	66.9 (16.2)	58.7 (23.4)	31.1 (12.0)	62.9 (28.3)	05
		1	112.2 (37.1)	137.9 (34.2)	29.8 (4.6)	58.5 (15.1)	↓
		12	63.4 (21.8)	178.5 (54.1)	30.7 (11.7)	67.5 (18.4)	02
17	63	0	72.6 (32.2)	25.3 (4.4)	35.5 (11.7)	22.9 (10.2)	04
		1	189.7 (62.8)	55.7 (8.4)	30.0 (8.6)	14.7 (6.7)	↓
		12	77.1 (18.7)	76.8 (19.2)	22.4 (4.6)	18.5 (8.1)	03
19	71	0	120.5 (54.2)	26.2 (4.2)	44.9 (11.1)	42.1 (12.0)	07
		1	189.1 (86.1)	72.2 (18.3)	52.4 (15.8)	40.0 (5.3)	↓
		12	177.6 (92.0)	106.9 (18.3)	43.2 (10.1)	31.4 (5.7)	09
21	63	0	176.3 (39.3)	26.4 (5.1)	29.7 (13.0)	37.9 (13.4)	06
		1	235.7 (98.2)	61.0 (24.9)	29.3 (8.7)	39.5 (24.4)	↓
		12	138.4 (38.4)	67.3 (17.3)	26.2 (12.3)	37.3 (11.4)	04
25	65	0	115.7 (55.6)	56.6 (16.0)	34.8 (12.2)	18.8 (9.1)	02
		1	224.4 (57.5)	118.5 (25.7)	42.0 (13.5)	23.0 (10.5)	↓
		12	133.2 (59.1)	113.5 (28.8)	40.8 (6.2)	18.1 (9.4)	01
27	66	0	100.6 (26.2)	40.9 (15.0)	33.6 (10.7)	16.8 (6.8)	05
		1	63.0 (13.3)	78.2 (13.2)	36.5 (20.0)	12.2 (2.7)	↓
		12	69.1 (41.9)	103.5 (13.2)	24.5 (12.9)	15.3 (4.5)	04

298 nmol/mmol Crt (Table 3). The mean urinary excretion of Al in Wk 1 was increased for 5 individuals, and in 3 of these, the increases were approximately 100% and statistically significant ($p \leq 0.05$; Post-hoc Tukey's). Seven individuals at Wk 12 showed a reduction in mean urinary excretion of Al compared to Wk 1 and for 2 of these individuals the decreases were statistically significant ($p \leq 0.05$; Post-hoc Tukey's). The mean urinary excretion of Si for Wk 0 for all 8 individuals ranged from 16 to 65 μmol/mmol Crt (Table 3). For all 8, these values were approximately doubled for Wk 1 and all increases were statistically significant ($p \leq 0.05$; Post-hoc Tukey's). Mean urinary excretion of Si remained high for Wk 12 for 7 out of 8 individuals with no statistically significant differences with Wk 1. In one individual (Id. 10), mean urinary Si excretion increased significantly ($p \leq 0.05$; Post-hoc Tukey's) between Wks 1 and 12 (Table 3). The mean urinary excretion of Fe for Wk 0 for all 8 individuals ranged from 24 to 44 nmol/mmol Crt, and there were neither consistent nor statistically significant differences between mean urinary Fe excretion for all individuals for Wks 0, 1, or 12 (Table 3). The mean urinary excretion of Cu for Wk 0 for all 8 individuals ranged from 11 to 33 nmol/mmol Crt, and there were neither consistent nor statistically significant differences between mean urinary Cu excretion for all individuals for Wks 0, 1, or 12 (Table 3).

ADAS-Cog scores ranged from 15 to 53 (Mean 25.6 (12.3)) for Wk 0 and 11 to 61 (Mean 28.6 (14.9)) for Wk 12 (Table 3).

Seven of the AD patients were female and aged 56–80 (Mean 70 (8.6)). The mean urinary excretion of Al for Wk 0 across all individuals ranged from 39 to 144 nmol/mmol Crt (Table 4). The mean urinary excretion of Al in Wk 1 was increased for 6 individuals and in 1 of these, the increase was statistically significant ($p \leq 0.05$; Post-hoc Tukey's). All seven individuals at Wk 12 showed a reduction in mean urinary excretion of Al compared to Wk 1 and for 2 of these individuals the decreases were statistically significant ($p \leq 0.05$; Post-hoc Tukey's). The mean urinary excretion of Si for Wk 0 for all 7 individuals ranged from 36 to 45 μmol/mmol Crt (Table 4). For 6 individuals, these values were approximately doubled for Wk 1 and were statistically significant ($p \leq 0.05$; Post-hoc Tukey's). Mean urinary excretion of Si remained high for Wk 12 for 6 out of 7 individuals with no statistically significant differences with Wk 1. The mean urinary excretion of Fe for Wk 0 for all 7 individuals ranged from 19 to 47 nmol/mmol Crt, and there were neither consistent nor statistically significant differences between mean urinary Fe

Table 3
The urinary excretion of aluminum, silicon, iron, and copper (Mean & SD) in males diagnosed with AD before (Wk 0) and after drinking silicon-rich mineral water for 1 and 12 weeks. ADAS-Cog scores are given for before (Wk 0) and after drinking silicon-rich mineral water for 12 weeks

Identity	Age (y)	Sample week	Al nmol/ mmol Crt	Si μmol/ mmol Crt	Fe nmol/ mmol Crt	Cu nmol/ mmol Crt	ADAS-Cog/70
06	81	0	28.3 (10.6)	28.2 (5.1)	25.6 (11.9)	30.0 (7.7)	20
		1	68.4 (39.7)	52.4 (22.3)	39.6 (16.6)	37.6 (16.2)	↓
		12	65.0 (16.3)	80.1 (22.6)	40.9 (16.2)	44.5 (9.1)	26
10	75	0	188.4 (34.6)	33.7 (9.3)	38.7 (20.0)	24.8 (9.4)	29
		1	199.5 (78.1)	54.3 (15.4)	35.8 (14.7)	22.2 (11.6)	↓
		12	88.6 (21.2)	146.3 (25.3)	29.9 (10.4)	29.5 (10.4)	36
14	80	0	37.7 (16.0)	65.0 (18.4)	26.9 (4.8)	33.4 (15.0)	30
		1	73.3 (20.0)	132.1 (17.6)	30.9 (5.8)	46.6 (13.5)	↓
		12	56.2 (33.9)	125.0 (31.6)	29.3 (8.8)	48.9 (19.1)	29
18	69	0	298.3 (107.9)	25.6 (10.4)	27.4 (15.0)	33.2 (9.0)	15
		1	189.7 (80.5)	80.6 (32.0)	20.4 (7.5)	32.6 (31.6)	↓
		12	141.3 (75.8)	79.2 (15.6)	24.7 (7.7)	27.2 (7.0)	22
20	71	0	48.8 (32.7)	16.4 (5.3)	23.5 (9.7)	10.9 (3.8)	21
		1	47.2 (19.1)	46.2 (24.5)	21.8 (10.6)	21.6 (9.2)	↓
		12	79.3 (28.5)	69.8 (18.6)	29.0 (11.0)	21.2 (11.6)	23
22	81	0	83.7 (52.8)	46.6 (15.2)	43.7 (24.0)	33.1 (14.6)	53
		1	189.4 (37.8)	125.7 (23.8)	41.4 (16.6)	52.1 (16.9)	↓
		12	61.6 (25.8)	111.6 (14.4)	40.0 (15.1)	30.4 (16.2)	61
26	64	0	179.5 (30.4)	28.2 (10.7)	31.2 (4.5)	27.7 (9.0)	17
		1	70.3 (33.9)	82.3 (35.4)	21.9 (8.3)	22.1 (8.7)	↓
		12	65.8 (23.0)	140.0 (34.8)	30.5 (13.3)	25.3 (18.6)	21
30	77	0	58.6 (47.3)	28.7 (14.0)	35.5 (14.7)	30.3 (11.2)	20
		1	97.1 (37.2)	64.8 (16.5)	41.6 (9.9)	28.6 (9.9)	↓
		12	85.6 (40.8)	60.2 (11.4)	43.6 (13.4)	36.8 (7.7)	11

excretion for all individuals for Wks 0, 1, or 12 (Table 4). The mean urinary excretion of Cu for Wk 0 for all 7 individuals ranged from 13 to 52 nmol/mmol Crt and there were neither consistent nor statistically significant differences between mean urinary Cu excretion for all individuals for Wks 0, 1 or 12 (Table 4).

ADAS-Cog scores ranged from 16 to 48 (Mean 30.1 (11.6)) for Wk 0 and 18 to 50 (Mean 30.4 (12.3)) for Wk 12 (Table 4).

DISCUSSION

Spot urine samples collected on consecutive days during Wks 0, 1, and 12 and corrected for differences in glomerular filtration rate are reliable estimates of the urinary excretion of Si, Al, Fe, and Cu during these periods. Previous research has demonstrated statistically significant linear relationships between the amount of element excreted in 24 h and the concentration of element expressed per mmol Crt [21]. There were no statistically significant differences between the non-AD control group and the AD patient group in the urinary excretion of each of these elements during Wk 0. Thus there were no significant differences between the urinary excretion of Al in individuals with AD and a similar (age and gender) group of individuals who did not have AD. The range of values for urinary Al excretion were similar to our previous study though there was no evidence herein that individuals with AD excreted more Al [15]. However, closer scrutiny of the data showed significant gender-related effects for Al in that while non-AD females excreted significantly more Al than females with AD, the opposite was true for males as AD males excreted significantly more Al than non-AD males. The only clear difference between the two female groups was the diagnosis of AD, while in the male groups, the AD patients were older and age is a known risk factor for a higher body burden of Al [1] and consequently higher urinary excretion of Al. Overall in this study, females, in which there is a known higher incidence of AD [20], excreted more Al than males and this difference was also noted in a previous study [15] and may point towards gender-specific characteristics which relate to the absorption and retention of Al.

In Wk 1, during which all recruits drank up to 1 L of silicon-rich mineral water each day, the urinary excretion of Si was significantly higher than in Wk 0 for both non-AD and AD groups, though there were no statistically significant differences between these groups. Thus there was no evidence that individuals with AD

Table 4
The urinary excretion of aluminum, silicon, iron, and copper (Mean & SD) in females diagnosed with AD before (Wk 0) and after drinking silicon-rich mineral water for 1 and 12 weeks. ADAS-Cog scores are given for before (Wk 0) and after drinking silicon-rich mineral water for 12 weeks

Identity	Age (y)	Sample week	Al nmol/ mmol Crt	Si μmol/ mmol Crt	Fe nmol/ mmol Crt	Cu nmol/ mmol Crt	ADAS-Cog/70
02	79	0	55.2 (22.8)	37.8 (9.8)	25.0 (10.0)	27.5 (13.6)	42
		1	77.0 (30.8)	82.4 (28.9)	22.8 (13.3)	38.3 (16.0)	↓
		12	48.1 (16.5)	51.4 (15.3)	26.1 (20.5)	31.8 (6.9)	42
04	74	0	74.6 (28.4)	39.8 (8.3)	37.7 (30.0)	38.0 (19.3)	16
		1	99.9 (28.4)	102.2 (53.4)	28.9 (9.2)	40.4 (14.5)	↓
		12	62.0 (32.1)	74.2 (26.8)	32.1 (10.0)	46.0 (15.7)	23
08	80	0	144.2 (46.3)	37.7 (17.7)	47.0 (20.4)	52.0 (16.4)	26
		1	126.1 (63.6)	85.2 (37.3)	56.7 (24.5)	51.6 (23.2)	↓
		12	85.6 (45.0)	149.7 (35.2)	44.8 (18.9)	49.3 (21.9)	21
12	56	0	84.0 (52.4)	44.6 (20.8)	21.0 (10.5)	23.6 (12.8)	48
		1	119.2 (19.9)	57.6 (22.1)	16.5 (8.1)	23.2 (8.7)	↓
		12	100.6 (56.9)	58.7 (30.2)	19.2 (10.0)	25.5 (21.3)	50
16	67	0	38.8 (14.8)	35.8 (11.3)	20.5 (11.5)	31.9 (21.0)	29
		1	123.2 (45.6)	84.0 (33.5)	27.1 (6.0)	32.3 (8.6)	↓
		12	44.5 (22.4)	108.4 (15.8)	17.0 (4.7)	35.1 (14.7)	36
24	68	0	76.1 (25.7)	36.1 (16.7)	19.1 (8.6)	13.7 (5.2)	31
		1	107.1 (47.7)	110.6 (25.4)	23.8 (9.7)	9.3 (3.7)	↓
		12	56.4 (10.4)	92.0 (12.3)	29.4 (11.5)	14.1 (5.1)	23
28	64	0	41.4 (37.1)	40.2 (11.1)	24.7 (13.5)	13.3 (3.6)	19
		1	72.1 (27.2)	88.8 (41.1)	15.2 (7.0)	11.7 (5.4)	↓
		12	54.9 (38.4)	119.4 (16.6)	22.4 (7.1)	17.9 (12.2)	18

'handled' silicon in the diet differently to non-AD controls. The range of urinary Si levels were significantly higher than in our previous silicon-rich mineral water study [15] and this probably reflected the higher content of silicon in the Spritzer mineral water used herein. Coincident with an approximate doubling in the urinary excretion of Si were statistically significant increases in the urinary excretion of Al for individuals with AD and those without AD. The latter excreted significantly more Al in Wk 1 than individuals with AD. Since there were no differences between these groups in their excretion of Si, the lower excretion of Al in the AD group might be indicative of lower accessibility of systemic Al in AD, reducing its rate of excretion via the kidney. Again there were also gender-related effects with females in the non-AD group excreting more Al than females with AD and also more than males with or without a diagnosis of AD. There were no differences between Wk 0 and Wk 1 in the excretion of Fe and Cu in individuals with or without a diagnosis of AD. Drinking a silicon-rich mineral water was effective in increasing the urinary excretion of Si and Al in individuals with and without a diagnosis of AD and importantly this was achieved without influence upon the urinary excretion of the essential metals Fe and Cu.

Urinary Si excretion remained high throughout Wks 2–12 for both non-AD and AD groups and this demonstrated an exceptional level of compliance within the study. If individuals had reduced their intake of Spritzer mineral water, this would have resulted in concomitant falls in urinary silicon excretion. While there were no statistically significant differences in urinary Si excretion between the non-AD and AD groups, the former excreted significantly more Al during week 12 and this difference was due to a statistically significant fall in urinary Al excretion between Wk 12 (median 64.8 nmol/mmol Crt) and Wk1 (median 97.5 nmol/mmol Crt) for the AD group (Wilcoxon signed-rank; $p < 0.001$). There were no statistically significant differences in the urinary excretion of either Fe or Cu between the non-AD and the AD group in Wk 12 or between Wk 12 and Wk1 and so the reduction in the urinary excretion of Al was specific to this element and to the AD group. Regular imbibition of silicon-rich mineral waters have been shown to increase the urinary excretion of Al over the short term and thereafter to slowly reduce Al excretion [16], perhaps reflecting a silicon-rich mineral water-induced reduction in the overall body burden of Al with time [11].

Herein it was demonstrated unequivocally that regular drinking of a silicon-rich mineral water increased the urinary excretion of Si and Al without concomitant effects on Fe and Cu. These effects were observed for individuals with AD and for a similar group of individuals without AD. For the former, there was evidence that longer term drinking of silicon-rich mineral

water reduced the body burden of Al. As a secondary outcome in this study, we used ADAS-Cog to measure cognitive function in both groups before (Wk 0) and after 12 weeks of drinking a silicon-rich mineral water (Wk 12). While no individual within the non-AD group presented with an ADAS-Cog score $\geq$10, if an MCRC of $\geq$3 is applied [19] then of the 14 control subjects 3 showed a deterioration (i.e., an increase in ADAS-Cog score of $\geq$3) in cognitive function, 2 subjects showed an improvement, and 9 subjects showed no change in their cognitive function over the 12 weeks of mineral water therapy. Within the 15 members of the group diagnosed with AD, cognitive function deteriorated in 7 subjects, improved in 3 subjects, and was unchanged in 5 subjects. In 2 of the 3 subjects in which there was an improvement in cognitive function, the ADAS-Cog scores were reduced by a remarkable 8 and 9 units. While less than half of the subjects with AD showed a clinically-relevant decline in their cognitive function during the 13 weeks of the study it is, of course, impossible with such a limited data set to know whether this represents a positive result for the silicon-rich mineral water therapy. However, the group of individuals diagnosed with AD showed a statistically significant reduction in their body burden of Al between Wks 1 and 12 and, concomitantly, evidence that for 8 out of 15 individuals their cognitive function was either unchanged or improved during this same period. Longer term studies are now required to show that any reductions in the body burden of Al can be further improved and sustained and that any cognitive benefits are similarly long-lived.

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