

Non-invasive therapy to reduce the body burden of aluminium in Alzheimer's disease

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Abstract. There are unexplained links between human exposure to aluminium and the incidence, progression and aetiology of Alzheimer's disease. The null hypothesis which underlies any link is that there would be no Alzheimer's disease in the effective absence of a body burden of aluminium. To test this the latter would have to be reduced to and retained at a level that was commensurate with an Alzheimer's disease-free population. In the absence of recent human interference in the biogeochemical cycle of aluminium the reaction of silicic acid with aluminium has acted as a geochemical control of the biological availability of aluminium. This same mechanism might now be applied to both the removal of aluminium from the body and the reduced entry of aluminium into the body while ensuring that essential metals, such as iron, are unaffected. Based upon the premise that urinary aluminium is the best non-invasive estimate of body burden of aluminium patients with Alzheimer's disease were asked to drink 1.5 L of a silicic acid-rich mineral water each day for five days and, by comparison of their urinary excretion of aluminium pre-and post this simple procedure, the influence upon their body burden of aluminium was determined. Drinking the mineral water increased significantly ($P < 0.001$) their urinary excretion of silicic acid (34.3 ± 15.2 to $55.7 \pm 14.2 \mu\text{mol}/\text{mmol}$ creatinine) and concomitantly reduced significantly ($P = 0.037$) their urinary excretion of aluminium (86.0 ± 24.3 to $62.2 \pm 23.2 \text{ nmol}/\text{mmol}$ creatinine). The latter was achieved without any significant ($P > 0.05$) influence upon the urinary excretion of iron (20.7 ± 9.5 to $21.7 \pm 13.8 \text{ nmol}/\text{mmol}$ creatinine). The reduction in urinary aluminium supported the future longer-term use of silicic acid as non-invasive therapy for reducing the body burden of aluminium in Alzheimer's disease.

Keywords: Alzheimer's disease, aluminium, silicic acid, iron, urinary excretion, mineral water, disease therapy

1. Introduction

Although informed opinion has concluded that the role of aluminium (Al) in the aetiology of Alzheimer's disease (AD) remains an open question its solution is, it would seem, accessible neither through animal models nor epidemiology [8]. An alternative approach would be to reduce human exposure to Al and to relate such to relevant indices such as incidence and progression of AD. It might be simpler to purge the body of its burden

of Al and, thereafter, retain it at a lowest possible level. We are aware of only one study in which the objective was to lower the body burden of Al in individuals with AD. This involved the intramuscular injection of desferrioxamine (DFO), a trivalent metal chelator, in individuals with probable AD over 24 months and resulted in significant reductions in the decline of their daily living skills [5]. The authors stated that sustained treatment with low doses of DFO resulted in three-fold increases in urinary Al excretion and slowing of the clinical progression of AD. They concluded that other safe and effective aluminium chelators which could be taken orally should be developed. Over fifteen years

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later we are aware of neither the existence nor application in humans of any such chelators.

Several years prior to the DFO chelation study we showed that silicon protected against the acute toxicity of Al in fish in acid waters and we speculated that such protection might also be afforded to humans [3]. Research during the past decade or so has shown that silicon, as its biologically available form silicic acid ($\text{Si}(\text{OH})_4$), both reduces the gastrointestinal absorption of dietary Al [6] and facilitates the urinary excretion of systemic Al [15]. In addition, silicic acid in potable waters has been linked to reduced incidence of AD [12, 19].

Silicic acid has no known bioorganic chemistry and only limited bioinorganic chemistry [7]. Its interaction with Al to form hydroxyaluminosilicates (HAS) is its only reported inorganic chemistry under physiological conditions [10]. It is possible that this chemistry might explain the unique influence of silicic acid on the absorption and urinary excretion of Al and that it might be used as the basis for a completely non-invasive method to reduce the body burden of Al in AD [2]. To test this possibility we sought and found a readily available source of silicic acid which would not require approval for human consumption. Individuals diagnosed with AD were asked to drink a well-known brand of a silicic acid-rich mineral water and their urinary excretion of Al was monitored. The latter, either in combination with an Al chelator or not, is the most accurate, non-invasive method of estimating the body burden of Al [2, 9,18].

The results have confirmed that regular drinking of silicic acid-rich potable waters may be an effective mechanism whereby Al might be purged from the body and the body burden of Al retained at a lowest possible level.

2. Methods

2.1. Patients and protocol

After obtaining ethical permission for the study from the local research ethics committee (North Staffordshire LREC) ten individuals diagnosed as having AD according to DSM-IV-TR criteria [1] and their carer were recruited via the Memory Clinic in the Department of Geriatric Medicine, University Hospital of North Staffordshire. All participants provided written informed consent and agreed to comply with the study requirements including the completion of report cards.

Study exclusion criteria included; patients taking any form of Al-based medication including antacids; patients in whom co-morbidity would render the consumption of large volumes of fluid potentially dangerous, e.g. heart failure; patients with significant urinary incontinence.

The study protocol was divided between two consecutive weeks. The primary objective in Week 1 was to obtain an estimate of the patient's normal urinary Al excretion. Patients were provided with pristine airtight sample bottles and asked to collect their first urine of each of five consecutive days. It was neither practical nor ethically acceptable to ask patients to collect 24 hour urine samples. However, the use of 'spot' samples to estimate urinary Al excretion was validated as explained in Section 2.2 below. The urine samples were stored in appropriate biohazard bags in the patient's refrigerator before being collected, transported to the laboratory and frozen at -20°C . In Week 2 patients were asked to drink up to 1.5 L of silicic acid-rich mineral water (hereafter referred to by its brand name 'Volvic') per day for each of five consecutive days. Once again, beginning with the first day which followed drinking the first 1.5 L of Volvic, the patients were asked to collect their first urine sample of the day and to repeat this for five consecutive days. Storage, collection, transport and treatment of urine samples were as per Week 1. In this way we collected five pre-Volvic (Week 1) and five post-Volvic (Week 2) urine samples for each of the ten patients. Patients completed record cards on which they indicated how much Volvic they drank on each day and whether or not they drank it neat or used it to make other beverages. Patients were not asked to drink the 1.5 L of Volvic as an additional constituent of their daily diets but to include it as part of their diets for that week. Apart from drinking Volvic in Week 2 the patients were encouraged to carry out their normal daily routines and to adhere to their usual diets.

2.2. Measurement of Al, Si, Fe and creatinine in urine

Apart from urinary Al, we also measured Si, iron (Fe) and creatinine. The latter measurements were used to correct for differences in glomerular filtration rates and allowed for accurate normalisation of urine concentrations of Al, Fe and Si [11]. The measurement of urinary Si indicated whether or not drinking Volvic resulted in increased excretion of Si whereas measurement of Fe indicated whether drinking Volvic influenced the excretion of a metal with no known chemistry with silicic acid.

Thawed urine samples were thoroughly mixed and acidified to 17% *v/v* with 14 M HNO₃. Total Al and total Fe were determined by graphite furnace atomic absorption spectrometry (GFAAS) using programmes developed in our laboratory [20]. The levels of quantitation achieved were 0.04 and 0.03 $\mu\text{mol/L}$ for Al and Fe respectively. Total Si was determined by inductively coupled plasma emission spectroscopy (ICP-OES) using a modification of a published programme [21]. 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 were always within 5%. Creatinine in thawed samples of urine was determined by RP-HPLC using a modification of a published method [13].

2.3. Statistical analyses

Data were normalised by log transformation and analysed by repeated measures one-way analysis of variance to obtain significance for the urinary excretion of Si, Al and Fe between Week 1 and Week 2. In addition data were normalised by log transformation and analysed by two-way analysis of variance using weekly means for each patient with Week 1, Week 2 and gender as the main factors and age and duration of disease as co-variables (MINITAB[®] Statistical Software Version 13). Regression analyses were carried out on all data and all combinations of variables to determine the significance of Pearson's product-moment correlation coefficients [23].

3. Results

Patient compliance with the study protocol was extremely good. There were no spoiled data. Eight out of ten patients drank more than 90% of the 1.5 L of Volvic supplied each day whereas the remaining two, 8 and 9, drank at least 50% of this volume on each of the five days. The 'silica' content of Volvic is given on commercial bottles as 31.7 mg/L which equates to ca 530 μM Si(OH)₄. We found the [Si(OH)₄] of Volvic to be $580 \pm 21 \mu\text{M}$ ($n = 5$). [Al] and [Fe] in Volvic were 0.14 ± 0.01 and $0.03 \pm 0.01 \mu\text{M}$ respectively. Other main inorganic constituents of Volvic are given by the manufacturers as; Ca 11.5 mg/L; Mg 8.0 mg/L; Na 11.6 mg/L; K 6.2 mg/L; chlorides 13.5 mg/L; nitrates 6.3 mg/L; sulphates 8.1 mg/L; bicarbonates 71.0 mg/L.

Urinary concentrations of Si and creatinine which related to Week 1, Week 2 or the combined data were significantly ($P < 0.001$) positively correlated for each of these combinations whereas there were no significant correlations between creatinine and either Al or Fe for the same combinations. The highly significant positive correlations between Si and creatinine were used to confirm 'normal' glomerular filtration in the kidney in the patients and to validate normalisation of urine concentrations of Si, Fe and Al by expressing them per mmol of excreted creatinine. Differences in urinary concentrations of Si, Al and Fe between Week 1 and Week 2 were compared statistically using both repeated measures analysis and two way analysis of variance using weekly means for each patient. We obtained the same statistical significance (expressed as P values) using either approach and for convenience we have only quoted those gained using two-way analysis of variance.

Urinary excretion of Si increased for each patient in Week 2 (Table 1; Fig. 1). Applying data for all ten patients the mean excretion of Si increased significantly ($P < 0.001$) from $34.3 \pm 15.2 \mu\text{mol/mmol}$ creatinine in Week 1 to $55.7 \pm 14.2 \mu\text{mol/mmol}$ creatinine in Week 2. Combining data for Week 1 and Week 2 showed that females ($51.5 \pm 18.4 \mu\text{mol/mmol}$ creatinine) excreted significantly ($P = 0.008$) more Si than males ($35.2 \pm 13.1 \mu\text{mol/mmol}$ creatinine).

Urinary excretion of Al decreased for eight out of ten patients in Week 2 (Table 1; Fig. 2). Two male patients, 3 and 6, were 'super-excreters' of Al in both Week 1 and Week 2 and heavily biased any statistical analyses. When these patients were excluded the data for the remaining eight showed that the mean excretion of Al decreased significantly ($P = 0.037$) from $86.0 \pm 24.3 \text{ nmol/mmol}$ creatinine in Week 1 to $62.2 \pm 23.2 \text{ nmol/mmol}$ creatinine in Week 2. Combining data for these eight individuals for Week 1 and Week 2 showed that females ($82.3 \pm 21.1 \text{ nmol/mmol}$ creatinine) excreted significantly ($P = 0.007$) more Al than males ($49.6 \pm 26.3 \text{ nmol/mmol}$ creatinine).

Urinary excretion of Fe showed no clear trend between Week 1 and Week 2 (Table 1; Fig. 3). Applying data for all ten patients mean excretion in Week 1 ($20.7 \pm 9.5 \text{ nmol/mmol}$ creatinine) was not significantly ($P > 0.05$) different to Week 2 ($21.7 \pm 13.8 \text{ nmol/mmol}$ creatinine). However, combining data for Week 1 and Week 2 it was found that females ($25.6 \pm 12.8 \text{ nmol/mmol}$ creatinine) excreted significantly ($P = 0.010$) more Fe than males ($14.6 \pm 4.6 \text{ nmol/mmol}$ creatinine).

Table 1

Information on each of the ten AD patients including urinary excretion of Al, Si and Fe for Week 1 (Pre-Volvic) and Week 2 (Post-Volvic). Mean and SD are given, $n = 5$

Patient	Sex M/F	Age	Disease duration, months	[Al]	[Si]	[Fe]	[Al]	[Si]	[Fe]
				nmol/mmol Creatinine Week 1	$\mu\text{mol/mmol}$ Creatinine Week 1	nmol/mmol Creatinine Week 1	nmol/mmol Creatinine Week 2	$\mu\text{mol/mmol}$ Creatinine Week 2	nmol/mmol Creatinine Week 2
1	F	78	42	88.0 (37.3)	30.9 (8.0)	21.4 (13.8)	43.0 (10.9)	66.7 (14.7)	12.2 (3.3)
2	F	84	18	73.8 (17.5)	37.6 (9.1)	23.3 (7.1)	52.5 (18.6)	61.4 (7.9)	30.5 (9.9)
3	M	76	12	301.1 (237.5)	16.9 (3.2)	11.1 (3.8)	242.3 (171.5)	39.7 (12.3)	14.5 (0.7)
4	M	70	<12	36.9 (20.3)	35.7 (10.8)	7.4 (2.3)	26.8 (23.7)	56.4 (13.6)	9.9 (3.8)
5	M	68	<12	86.9 (39.6)	29.8 (14.6)	17.4 (8.5)	47.7 (16.6)	48.0 (8.1)	17.0 (8.1)
6	M	79	30	268.1 (33.5)	20.8 (2.2)	18.5 (14.1)	417.6 (147.3)	34.5 (3.8)	20.6 (7.5)
7	F	70	24	102.0 (24.2)	21.5 (3.7)	16.1 (3.2)	87.9 (28.8)	62.1 (15.4)	18.4 (6.6)
8	F	76	12	78.4 (45.4)	44.7 (8.8)	39.6 (15.2)	90.5 (34.4)	60.8 (16.8)	57.4 (34.5)
9	F	53	18	111.9 (57.6)	34.8 (14.1)	32.8 (13.8)	80.0 (36.3)	44.7 (20.2)	15.8 (5.4)
10	F	65	18	110.4 (57.8)	70.1 (38.0)	19.1 (10.6)	69.3 (16.2)	82.2 (17.9)	20.7 (17.3)

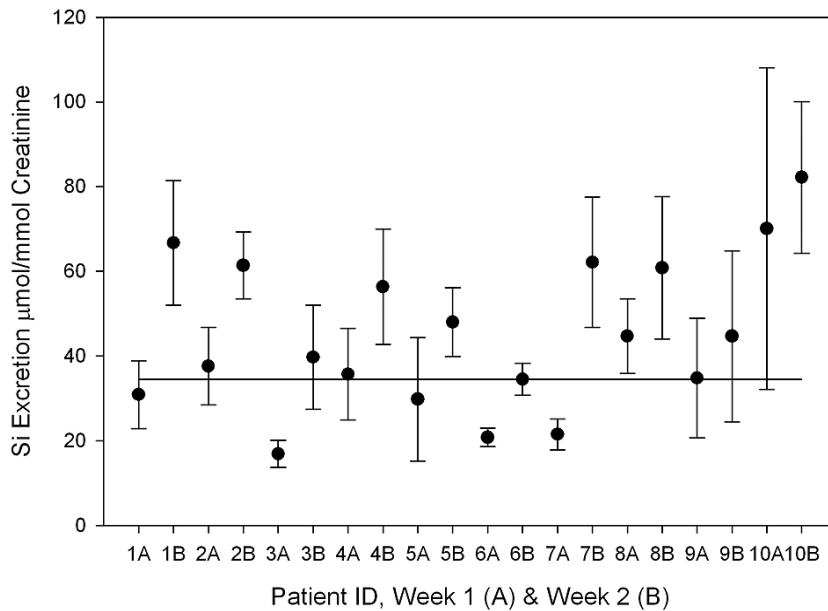


Fig. 1. Urinary Si excretion for each patient pre- (Week 1) and post- (Week 2) Volvic therapy. Mean and SD are plotted, $n = 5$. Solid line shows mean urinary Si excretion for a control population (see text).

4. Discussion

Regular drinking of a mineral water, Volvic, which contained a high concentration of silicic acid, significantly increased urinary excretion of Si. In eight out of ten patients higher excretion of Si was coincident with lower excretion of Al. In two out of ten patients, 6 and 8, increased excretion of Si resulted in increased excretion of Al. We have used 'spot' samples of urine taken at the same time (first urine sample of the morning) on each of five consecutive days to estimate that drinking Volvic reduced the body burden of Al in eight patients and increased it in two. We have not used these

data to estimate the daily excretion of Al. For the latter the 'spot' samples would have to be representative of Al excretion throughout the day. We actually know very little about the dynamics of urinary Al excretion whether following the ingestion of silicic acid-rich mineral water or not. We have shown that Al was labilised from body stores and excreted as a 'bolus' shortly after a fasting healthy male volunteer drank 1.5 L of Volvic (Fig. 4). However, for the present study we do not know when or how often in the day patients drank the 1.5 L of Volvic. The healthy single volunteer study would suggest that a bolus of Al might be excreted following each drink and that the size of this bolus could

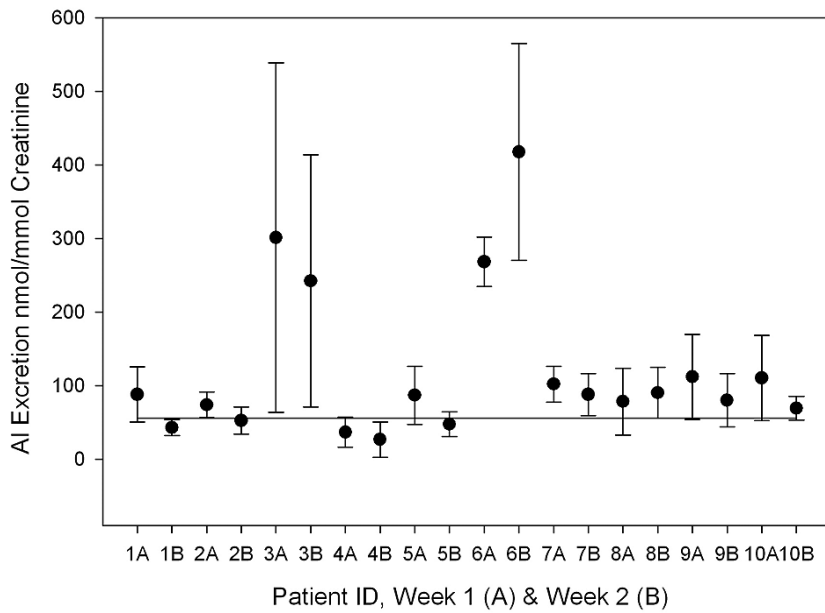


Fig. 2. Urinary Al excretion for each patient pre- (Week 1) and post- (Week 2) Volvic therapy. Mean and SD are plotted, $n = 5$. Solid line shows mean urinary Al excretion for a control population (see text).

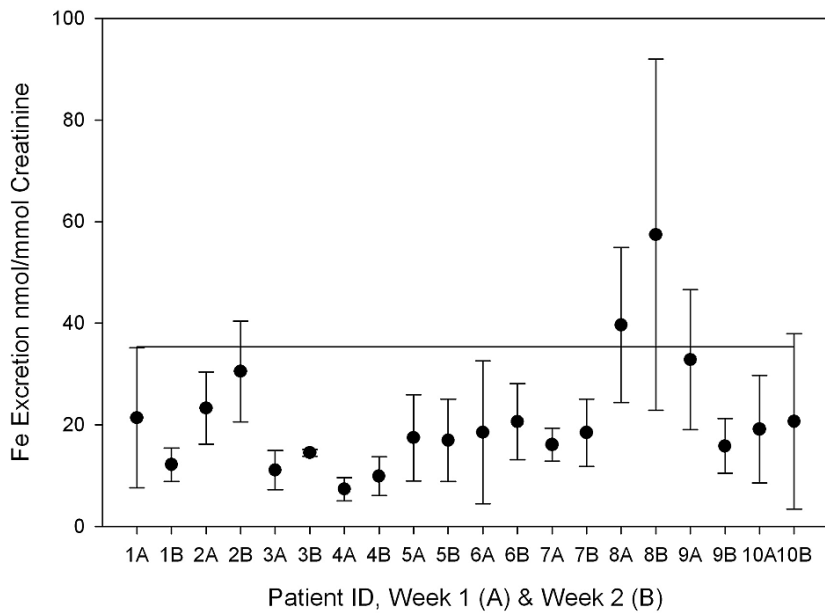


Fig. 3. Urinary Fe excretion for each patient pre- (Week 1) and post- (Week 2) Volvic therapy. Mean and SD are plotted, $n = 5$. Solid line shows mean urinary Fe excretion for a control population (see text).

depend upon the volume of Volvic ingested each time. This is supported by data which showed that ingested silicic acid was absorbed and excreted rapidly [17] and that identified the coincidence of the renal clearance of Al and Si [2,15]. We have no data concerning possible correspondence between when Volvic was drunk

and collection of the first urine sample of the day. As such the latter could be a measure of Al excretion at any point in an ongoing cycle of Volvic-facilitated Al excretion. In addition, contradictory excretion data in Week 2 for patients 3 and 6 (Table 1; Fig. 2), both of whom showed very high levels of Al excretion in Week

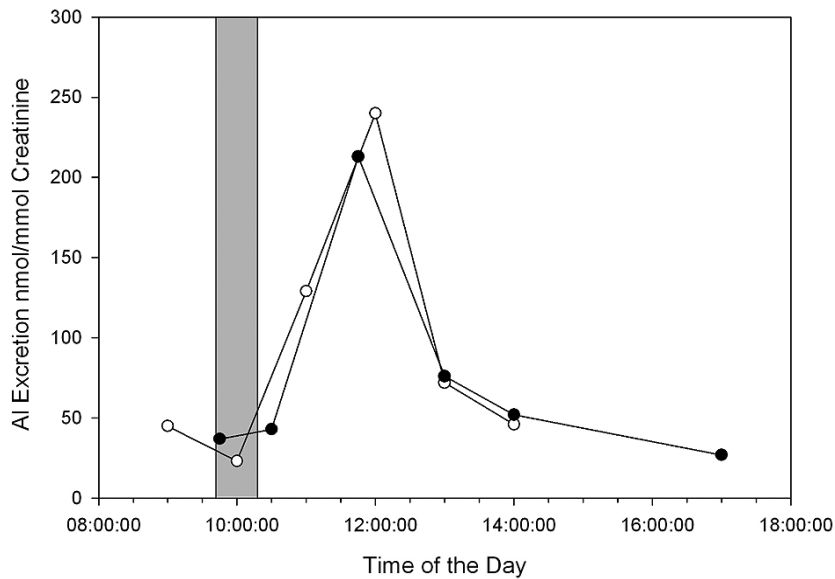


Fig. 4. Time-dependent urinary excretion of Al in a healthy male volunteer following drinking of 1.5 L of Volvic. The shaded area indicates the approximate time over which the Volvic was drunk whereas the solid and open circles represent replicate experiments.

1, would suggest that the shape (breadth and height) of Volvic-facilitated excretion profiles for Al were dependent upon the amount of Al to be excreted (or the body burden of Al of the patient). The present study was designed with the overall objective of reducing the body burden of Al in individuals with AD. Urinary excretion of Al, with or without concomitant chelation therapy, is considered to be the most representative non-invasive indicator of body burden of Al and as such, drinking Volvic was herein shown to be successful in reducing Al body burden in patients with AD. The observation that drinking Volvic did not influence the urinary excretion of Fe and the knowledge that none of the other constituents of Volvic could have influenced Al excretion via the kidney suggested very strongly that the removal of Al was linked to the increase in excretion of Si in Week 2 and was not simply a generalised increase in urinary excretion of metals. Dietary silicic acid might both reduce the gastrointestinal absorption of dietary Al and facilitate the urinary excretion of systemic Al. The former might be explained by the formation of insoluble hydroxyaluminosilicates in the gut whereas the latter must suggest the repartitioning of body stores of Al to allow their passage from blood to urine via the kidney. While we know from our own fasting healthy volunteer study (Fig. 4) and the literature [2,15] that increased urinary excretion of Al appeared coincident with commensurate increases in urinary excretion of Si we do not as yet have a bioinorganic explanation for this effect as the formation of hydroxyaluminosilicates

(of a size which would not be filtered out by the kidney) has not been demonstrated *in vivo*. We have assumed that both reduced gastrointestinal absorption and increased urinary excretion contributed towards lowering the body burden of Al in AD patients in this study.

We made the consistent observation that urinary excretion of Si, Al and Fe was in each case significantly higher in women than in men. Our data set was too small to determine if the differences were related to drinking Volvic in Week 2. Higher excretion of Al could be interpreted as a higher body burden of this metal. There is a higher incidence of AD in women [22] though whether this might be linked to a higher body burden of Al remains to be determined.

Data on the urinary excretion of Si, Al and Fe are scarce. Mean urinary excretion of Al in five healthy aged individuals was reported as ca 60 nmol/mmol creatinine (represented by a solid line on Fig. 2) compared to ca 200 nmol/mmol creatinine for five individuals with AD [16]. Our data are consistent with these results and may indicate that urinary Al excretion approached 'normal' values in Week 2 for eight out of ten patients. We have not been able to find comparative data for urinary excretion of either Si or Fe in AD. The solid line in Fig. 1 indicates mean urinary Si excretion in a middle-aged control population drawn from the same geographical area as this AD study [11]. If one takes account of the observation that plasma Si is reduced with ageing [4] then urinary Si excretion was normal for seven out of ten patients in Week 1 and elevated in

nine out of ten patients in Week 2. It may be significant that the unusually low values in Week 1, in particular patients 3 and 6, corresponded with high excretion of Al and that this relationship between the urinary excretion of Al and Si was also recently observed in individuals with secondary progressive multiple sclerosis [11]. The urinary excretion of Fe appeared to be very low though we have not been able to compare it with age-matched control data (Fig. 3). It was low compared with a middle-aged control population (solid line in Fig. 3) and compared to urinary iron excretion in another neurodegenerative disease, multiple sclerosis (ca 70–100 nmol/mmol creatinine) [11]. Iron is accumulated in the brain in AD [14] and this may be related to our observation of very low urinary excretion of Fe in AD?

We have carried out the first study to determine if silicic acid in a potable water might be used to reduce the body burden of Al in individuals with AD. While the small number of participants must imply that our results are preliminary the statistical significance achieved for the Volvic-facilitated reduction of the body burden of Al is encouraging and future research will both increase the number of study participants and extend the period of time over which therapy is administered. A final aim is, of course, to determine if lowering the body burden of Al has any influence upon the incidence, progression and aetiology of AD.

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