Silicon in health:
A beneficial element in reducing the body burden of aluminium

PhD

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Abstract

The essentiality of silicon and the toxicity of aluminium within humans are both topics of much debate; the current conception is that silicon is essential in ameliorating aluminium toxicity, however, the evidence for this remains inconclusive.

To be able to elucidate the connection between silicon and aluminium in neurological disorders, it is first important to understand the relationship between the elements in healthy individuals. This research was designed to investigate whether supplementing the diet with a silicic acid-rich mineral water could be a non-invasive means of reducing aluminium body burden in both healthy individuals and those suffering from multiple sclerosis.

Drinking a silicic acid-rich mineral water significantly enhanced the urinary excretion of aluminium, in both healthy individuals and in multiple sclerosis.

Collecting whole daily urinary excretions for healthy individuals indicated that aluminium was concomitantly excreted with silicon; this was most effective when the mineral water was consumed as a bolus, suggesting that the mineral water has greater influence when consumed in large quantities over a short time. In addition, reductions in urinary aluminium were also witnessed over time, supporting the use of silicic acid-rich mineral water in reducing and maintaining aluminium body burden at a lower level.

Supplementing the diet with a silicon-rich mineral water, for a period of 12 weeks, reduced aluminium body burden in individuals with multiple sclerosis; concomitantly, in this short amount of time, disability scoring showed clinically relevant improvements in 2 out of 15 individuals. Longer-term studies, involving larger study populations, are now needed to see if these effects are long lasting; if improvements are seen over time, it could support the link between aluminium and multiple sclerosis.

This research also presents preliminary evidence that sweat may be a more efficient excretory mechanism in lowering the body burden of aluminium in healthy individuals.

In conclusion, the results suggest that including a silicic acid-rich mineral water into the regular diet, without the need of following strict restrictions, could be a prophylactic therapy against aluminium toxicity in healthy individuals, and in addition, could be beneficial as a chelating agent for endogenous aluminium.

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Contents

List of abbreviations

%D	Percentage Difference
AAS	Atomic absorption spectrometry
AD	Alzheimer's disease
ADAS-Cog	Alzheimer's disease assessment Scale - cognitive
Al	Aluminium
AMC	Age-matched control
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
Cre	Creatinine
CS1	First calibration standard
CS2	Second calibration standard
CS3	Third calibration standard
CS4	Fourth calibration standard
Cu	Copper
dF	Degrees of freedom
DNA	Deoxyribonucleic acid
Fe	Iron
GFAAS	Graphite furnace atomic absorption spectrometry
GFR	Glomerular filtration rate
HAS	Hydroxyaluminosilicate
HCl	Hydrochloric acid
HNO3	Nitric Acid
HV	Healthy volunteers
LoD	Limit of detection
LoQ	Limit of quantification
MW	Mineral water
n	Number of scores/population size
NaOH	Sodium hydroxide
PD	Parkinson's disease
RNA	Ribonucleic acid
SD	Standard deviation
Si	Silicon
SOD	Superoxide dismutase
SSB	Sum of squares between groups
SSW	Sum of squares within groups
TH	Thermally heated
TotBev	Total beverages consumed
UPW	Ultra pure water
UV-Vis	Ultraviolet-visible
v/v	Volume per volume
w/v	Weight per volume
$\bar{x}$	Mean

Chapter 1 – Introduction. The quintessential nature of silicon

Silicon, is a chemical element, with the atomic number of 14. In 1808, the name silicium (from Latin: *silex* meaning a hard stone/flint) was first given to the element (Weeks 1968), with the –ium word ending suggesting a metal like form, a name which is preserved in several languages. The English name, as we know it today was suggested in 1817 to conform to the properties of the physically similar elements, boron and carbon (Weeks 1932). Silicon was first characterised and prepared in its purity in 1823 and is a solid crystalline structure at room temperature with a boiling point of 3,265°C. Like water, its density is greater in the liquid state and expands when it freezes (Hull 1999). Silicon is described to be a metalloid, displaying both metal and non-metal properties. In its crystalline form, it is grey in colour and has a metallic lustre. The outer obital, like carbon, has 4 valence electrons. The 1s, 2s, 2p and 3s subshells are filled completely, there are 2 electrons located in the 3p subshell out of a possible 6. Silicon is a semiconductor with a negative temperature coefficient of resistance, this is due to the increase in the number of free charge carriers with increase temperatures (Hull 1999).

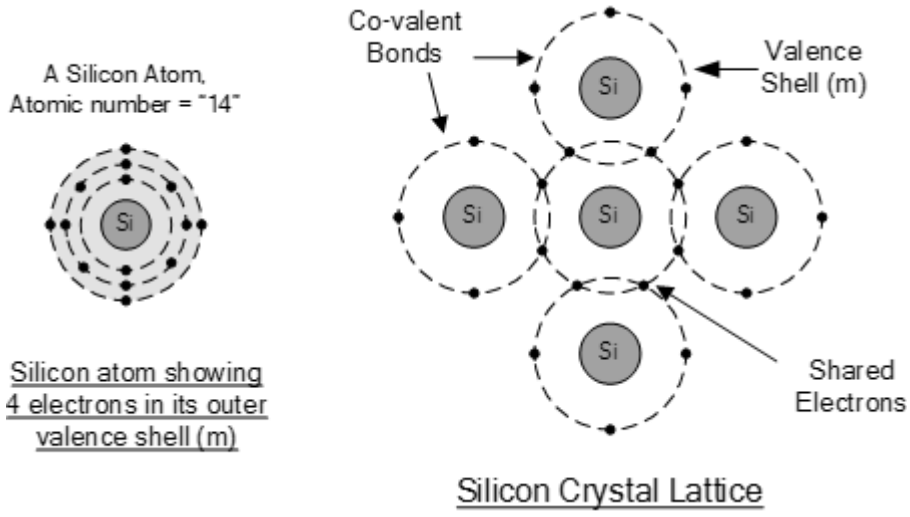

Figure 1.1 Structural image of silicon (Hong, Xiao 1993)

Silicon is the second most abundant in the Earth's crust, but is rarely found in its elemental form due to its high affinity for oxygen, forming rocks and minerals; such as, quartz, aluminosilicates, sedimentary and igneous rocks which are not readily broken down unless exposed to substantial weathering. Chemical and biological weathering of these rocks and minerals, through algae, plants and lichens for example, release the element. This causes the bioavailability of silicon in the natural environment to increase (Jugdaohsingh 2007). The dissolution of silica from these minerals in water results in the formation of soluble silica species below 2mM (Jugdaohsingh 2007), such as orthsilicic acid, $SiOH_4$ (see figure 1.2), this 'free' form of silicon is now in a form that is biologically available and can be actively taken up by plants and animals (Jugdaohsingh 2007).

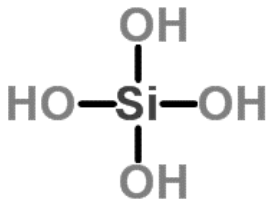

Fig 1.2: Structural view of orthosilicic acid

Si is considered to be a beneficial element in biology (Neilsen 1984). Within the natural world it is known that there are various sea sponges and microorganisms such as diatoms which form skeletal structures composed of silica. Silica is also deposited in many plant tissues, thought to contribute to structural support in the vascular system (Pilon-Smits *et al* 2009). Silicified trichomes of *Cannabis sativa*, horsetails and many grass species are also seen (Cutter 1978).

1.1 Si essentiality in higher animals

There has been much debate over the role of Si in higher animals. Epstein (1999) stated that 'an element is defined as quasi-essential if it is ubiquitous in plants, and if a deficiency of it can be severe enough to result in demonstrable adverse effects or abnormalities in respect to growth, development, reproduction, or viability'. Research has shown that a Si deficient diet can result in poor growth, Carlisle found that by feeding chicks an identical diet accompanied by a Si supplement showed fifty percent higher growth and normal development.

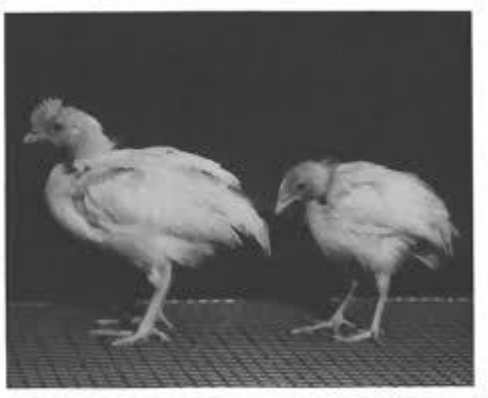

Fig 1.1.1 Carlisle's Chicks: The chick on the right had been given a normal diet, whereas the chick on the left had been supplemented with Si (Carlisle 1972)

Not only was there a clear size difference between the two chicks, see Figure 1.1.1, but the general health of the non-supplemented chick was poorer, with muddyish yellow subcutaneous skin as opposed to the healthy pinkish white of the supplemented chick (Carlisle 1972). Carlisle then began to investigate this further by looking at the effects of silicon supplemented diet on bone formation, looking in particular at the skull and bone (Carlisle 1980). The beaks and legs of the non-supplemented

chicks were thinner, paler, and more easily fractured than those of the supplemented chicks, see Figure 1.1.2.

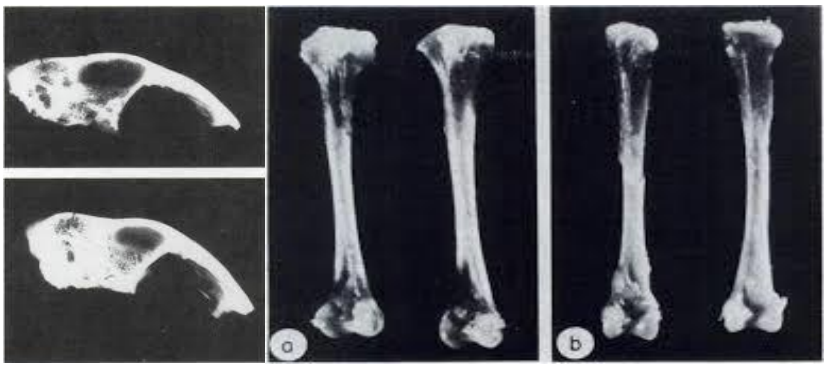

Figure 1.1.2: The top left skull and femur bones B are those of a chick consuming a normal diet, whilst the bottom left skull and femur bones A are taken from a Si supplemented chick of the same age. (Carlisle 1980)

Silicon is regarded as a beneficial element for the human body, and has the primary function of developing and maintaining connective tissue and the structural system. There are a number of studies focusing on the effect of increased silicon on the human body. The effect on bone and cartilage has been explored in humans as early as 1979, when the activity of a soluble Si drink was studied against the trabecular bone volume (TBV) in men. The study noted a significant increase in TBV compared to the controls (Schiano *et al* 1979). Silicon has been described to be 'essential' for the strength and integrity of the tunica intima (Schwartz 1977), the inner layer of an artery, observing that there is an inverse relationship between the concentration of silicic acid in drinking water and the prevalence of cardiovascular disease in Finland.

The effect of silicon on skin, hair and nails appears regularly in literature, Lassus (1993) performed an open study with an oral Si colloidal solution during a 3 month period, from which he saw statistically significant improvements in turgor and thickness of the skin, hair and nails.

This presents the argument whether silicon is beneficial or essential. Humans are exposed to silicon every day, through pharmaceuticals, cosmetics and dust, but, the major and most important source of exposure for the general population, is the diet.

1.2 Si in the diet

The vast majority of silicon we are exposed to comes from our diets. High levels of silicon are found in foods derived from plants, such as; grains, barley and rice, which is to no surprise when the abundance of silicon in the Earth's crust is considered. Silicon in the bioavailable form of orthosilicic acid is at a high exposure in drinking water and beer.

In the western world, an average daily intake of silicon is approximated at 20-50mg/day; (Bowen & Peggs, 1984; Pennington, 1991; Anasuya *et al.* 1996; Uthus & Seaborn, 1996; Van Dyck *et al.* 1999a; Robberecht *et al.* 2009) including around 60% of this value coming from cereals and grains, and 20% from water (Pennington, 1991; Uthus & Seaborn, 1996; Jugdaohsingh *et al.* 2002; Robberecht *et al.* 2009). Due to differences in diet between the sexes, intake is generally lower in women than in men and has been suggested to decrease with age (Pennington, 1991; Jugdaohsingh *et al.* 2002; Bisse *et al.* 2005). As foods have varying levels of silicon, it is clear to see how the different diets around the world affect dietary silicon intake. *Table 1.2.1* is taken from a study (Jugdaohsingh *et al* 2002) showing the top 10 food sources contributing to total silicon intake within the population.

Table 1.2.1 Major (top 10) food sources that contribute to total silicon intake in the population (Jugdaohsingh 2002)

Ranking	Men		Women	
	Food source	Contribution	Food source	Contribution
		%		%
Framingham Offspring cohort[2]				
1	Beer	17.6 ± 23.7	Bananas	10.5 ± 10.1
2	Bananas	9.1 ±10.2	String beans	4.6 ± 4.5
3	White bread	4.6 ± 6.0	White bread	4.6 ± 6.2
4	Cold cereal	4.5 ± 6.3	Cold cereal	4.4 ± 5.8
5	Coffee	3.5 ± 3.7	Dark bread	3.7 ± 5.0
6	Beans and lentils	3.3 ± 4.0	Beans and lentils	3.7 ± 4.6
7	Pizza	3.2 ± 3.5	Coffee	3.5 ± 3.9
8	Dark bread	3.1 ± 4.7	Muffins and bagels	3.5 ± 4.1
9	String beans	3.0 ± 3.1	Beer	3.3 ± 10.0
10	Muffins and bagels	2.7 ± 3.5	Cooked oatmeal	3.3 ± 6.6
Original Framingham cohort[3]				
1	Bananas	13.4 ± 13.1	Bananas	13.9 ± 12.2
2	Beer	10.6 ± 19.6	String beans	5.4 ± 4.9
3	White bread	5.4 ± 7.3	Cooked oatmeal	5.3 ± 9.2
4	Cold cereal	5.0 ± 5.9	Cold cereal	5.3 ± 6.5
5	Cooked oatmeal	4.3 ± 7.5	Dark bread	5.3 ± 6.7
6	String beans	4.1 ± 4.4	White bread	4.7 ± 6.8
7	Beans and lentils	3.6 ± 4.4	Potatoes	3.8 ± 3.3
8	Potatoes	3.2 ± 2.9	Beans and lentils	3.0 ± 4.1
9	Dark bread	3.2 ± 4.4	Muffins and bagels	2.3 ± 3.5
10	Muffins and bagels	2.5 ± 4.5	Coffee	2.2 ± 3.0

[2]Total percentage contribution of the foods listed: 54.6% and 45.1% for men and women, respectively.

[3]Total percentage contribution of the foods listed: 55.3% and 51.2% for men and women, respectively

High fibre diets are linked to being more silicon rich, therefore due to their traditional plant based diet, eastern communities are shown to have higher silicon intake (Chen *et al*. 1994; Anasuya *et al*. 1996). The concentration of silicic acid in drinking water and beer falls below the 2mM saturation, therefore containing a form of Si that is highly bioavailable and absorbable (Sripanyakorn *et al.* 2004). The geographic location at which the water is sourced has a large effect on the silicic acid concentration of the water, with water sourced from more volcanic environments containing the greatest concentrations. Silicon in hard waters is also noted to be at a higher concentration than that of soft waters (Sripanyakorn *et al.* 2004).

As previously discussed, the amount of Si leached from rocks and minerals is variable due to the weathering taking place. *Table 1.2.2* shows a variety of different tap and mineral waters, as well as their source and corresponding silicon contents to represent the differences found in water.

Table 1.2.2: The silicon content of mineral waters and tap waters from different sources.

Author	Country	Source	Silicon content
Dobie, 1982 cited by Birchall, 1991	U.K.	Tap water (n = 23)	7 – 256 µmol L-1
Giammarioli *et al*. 2005	Italy	Mineral waters (n = 207)	77% <10 mg L-1 >25 mg L-1 sourced from volcanic areas
Gillette-Guyonnet *et al*. 2005	France	Tap water (n = 7) Mineral water (n = 8)	4 – 11.2 mg L-1 8.6 – 36.4 mg L-1
Powell *et al*. 2005	U.K.	Tap water (n = 4) Mineral water (n = 3)	0.25 ± 0.11 mg 100g-1 0.54 ± 0.49 mg 100g-1
Robberecht *et al*. 2009	Belgium	Mineral water (n = 24)	0.76 – 19.13 mg kg-

1.3 Absorption and excretion of silicon

As the essentiality of silicon in humans is yet to be confirmed, what can be looked at is the manner in which silicon is absorbed in the body. Studies have shown that the concentration of silicon excreted in the urine is directly proportional to that consumed in a controlled diet, with a very small amount being lost in the faeces (Reffitt 1999). Despite this, it must be understood that the degree of gastrointestinal absorption is relatable to the polymerisation involved (Yokoi & Enomoto, 1979; Reffitt *et al.* 1999; Jugdaohsingh *et al.* 2000; Sripanyakorn *et* al. 2009). For instance, smaller molecules of silica or orthosilicic acid are readily absorbed by the gastrointestinal tract, whereas the larger are broken down using hydrolysis into soluble monomeric repeating units, which can be readily absorbed. From this, it is understood that the absorption of silicon from plant material is low, whereas of silicon in drinking water and beer, in the form of $SiOH_4$, shows high bioavailability and is therefore the most

efficient form of silicon for absorption through the gastrointestinal wall (Bellia *et al.* 1994a). This also provides evidence that the movement of silicic acid follows the water pool.

As silicon is more soluble in near neutral environments than acidic, the majority of the absorption occurs via the intestinal wall, rather than the gastric one, allowing more time for higher silicon complexes to be broken down.

The main excretory organ for silicon is the kidney (Dobbie & Smith, 1982a; Reffitt *et al*. 1999; van Dyck *et al.* 1999b; Sripanyakorn *et al.* 2004). Once absorbed, silicon is then filterable by the kidney. It is worth noting however that there is a very small occurrence of renal reabsorption within the nephron. There is currently no known mechanism behind silicon reabsorption in the kidney (Adler & Berlyne, 1986), however, a significant amount of silicon is seen to be retained in the kidneys of rats after intravenous silicon injections (Mehard & Volcani, 1975; Berlyne *et al.* 1986b; Adler *et al*. 1986). Studies have seen an increase in the blood plasma concentrations of silicon in uremic patients, once again highlighting that the kidney is the main excretory point for silicon.

The excretion value for the renal clearance of Si is reported to be 28.9±13.3 mg L^{-1} (Dhaese *et al.* 1995), which is a value that relates well to the expected dietary intake lying at 20-50mg d^{-1}. This high excretory value further indicates that silicic acid is readily absorbed across the intestinal wall and then swiftly excreted in the urine (Bellia *et al.* 1994b; Popplewell *et al*. 1998), furthermore, peaks in the silicon values were recorded in the urine as little as 4 hours after ingestion (Reffitt *et al.* 1999). Renal clearance is estimated to be between 80 – 100 mL Si min^{-1} (Adler & Berlyne, 1986; Reffitt *et al.* 1999) with a half-life of 2.7 hours (Popplewell *et al.* 1998) further highlighting that urinary excretion is a preferred route for the removal of Si from the body (Reffitt *et al.* 1999; Sripanyakorn *et al.* 2004).

There is a lot of evidence suggesting that the gastrointestinal absorption of silicon is directly proportional to urinary excretion of the element. This absorption is facilitated by aquaporins, protein

channels in the gastrointestinal wall that aid the transfer of water and smaller ions through to the bloodstream. There has been increased attention on this element and its pathway through the body, through strong associations with the beneficial elements, copper and zinc (Henk-Maarten 2007). Silicon is also related to an increase in calcium absorption, again, suggesting a positive effect on bone health (Jugdaohsingh 2007).

1.4 Mechanisms of silicon essentiality

In spite of the fact that silicon is widely regarded as an essential element in lower organisms and is reported to be beneficial to mammals, the biochemistry to explain its requirement in biota remains undefined. Silicic acid is not bound to proteins and exhibits no activity towards organic species at physiological pH (D'Haese *et al*. 1995). Indeed, there is no evidence to support the presence of organic structures of Si-C or Si-O-C bonds in bio-systems and no stable binding of silicic acid in biological molecules has been witnessed (Birchall, 1990).

The only known biological mechanism of silicon is in the interaction with aluminium which is basic at physiological pH (Birchall et al. 1996). It has been proposed that the bioinorganic interaction between silicon and aluminium is the reason behind silicon's essentiality (Exley, 1998).

1.5 Toxicity of aluminium

Aluminium (Al) is a trivalent cation found in its ionic form in most kinds of animal and plant tissues and in natural waters everywhere (Jiang *et* al 2008). Despite being the third most abundant element in the Earth's crust, and being located right next to silicon in the periodic table, aluminium is not tolerated by living organisms. In fact, reports documenting aluminium toxicity in plants, fish and higher

animals have been accumulating. Cases on aluminium toxicity in mammals have been well reported, and many conditions are now associated with the accumulation of this element, specifically neurological conditions. The severity of these conditions seems linked with the route, the quantity introduced, absorption rates, the tissue distribution and the excretion rate (Riihimaki *et al*. 2008; Lemire *et al*. 2009; Hernandez *et al.* 2008).

In contrast to Si, no biological requirements have been attributed to aluminium, more importantly, aluminium is not tolerated by living organisms. Exploration of aluminium toxicity is vast in plants, fish and higher animals. Accounts of aluminium toxicity in mammals and humans include osteomalacia, anemia (Kaiser *et al.* 1984) and a myriad of neurological disorders (discussed later in this section).

1.6 Exposure to aluminium

Aluminium has become particularly isolated from biological systems and generally requires another element to 'deliver' it into the human body, therefore increasing its bioavailability.

Throughout the history, humankind have lived through various 'metal ages' including the Iron Age and the Bronze Age. The world has now entered the 'Aluminium Age', and aluminium is accumulating in the natural world due to recent anthropogenic factors; increased use in industry and deposition of aluminium in soils. This is resulting in elevated aluminium throughout the biosphere (Kawahara *et al*. 2007; Gomez *et al.* 2008; Exley, 2009).

There are a variety of routes from which aluminium can end up entering the body. Dermally it can be absorbed through using anti-persperants (Flarend *et al*. 2001), where aluminium chloride is a

significant component. It can be delivered intravenously, through vaccines containing aluminium adjuvants (Keith et al. 2002) and inhaled from dust in an occupational environment (Rifat *et al*. 1990; McLachlan *et al.* 1995). Oral introduction is also a significant contribution to the overall burden of aluminium, foods such as spinach, potatoes and tea are high in Al (Pennington & Jones, 1989), while the worst culprits are chemicals involved in food additives, water purifiers and medicinal products. (Harris et al. 1996; World Health Organization, 1998).

1.7 Aluminium in the body

Once aluminium has found its way into the body, where does it go?

Aluminium presents low solubility in neutral solutions. This hydrolytic chemistry has meant that this omnipresent element exists in a form of low biological availability and has therefore remained mostly sequestered from biological systems (Exley, 2009). However, recent anthropogenic dynamics, including acid deposition of soils and increased use in industry are increasing the availability of aluminium within the biosphere (Kawahara *et al*. 2007; Gomez *et al.* 2008; Exley, 2009) and resulting in the initiation of aluminium accumulation within living organisms.

Fortunately, only around 0.1% of total aluminium ingested is absorbed through the gastrointestinal tract (Day et al. 1991), of which the majority is excreted rapidly through the kidneys. This fact may signify that aluminium has little benefit to human health. The aluminium that does remain in the body, which is around 5% of that absorbed into the bloodstream, slowly accumulates in various tissues (Priest et al. 1997). Over time, this could lead to morbidity and mortality due to various mechanisms, which is an important implication to consider when studying human disease (Denton et al. 1984; Alfrey et al. 1986; Wilhelm et al. 1990; Day et al. 1991; Yokel, 2006; Verstraeten et al. 2008).

Aluminium is rarely in its free state, however, it can be seen forming biological complexes in the bloodstream, of which the most common reaction is that with the iron transporter ferrin, accounting for around 90% of all aluminium interaction in the blood (Harris & Sheldon, 1990; Wrobel et al. 1995; Milacic et al. 2009). The movement of aluminium to binding sites in the tissue is facilitated by these weak interactions (Priest, 2004).

On average, a healthy individual would contain a total measured body burden of around 60mg aluminium within their bones and tissues (Yokel, 2013). Although research states that aluminium can be found in all tissues in the body, it is very unevenly distributed, showing the greatest accumulation in bone tissue (Ganrot, 1986; Zafar *et al.* 1997). Other favourable spots for aluminium deposition are the skin, kidneys, liver, heart and brain (Alfrey, 1980; Exley, 2001; Xu et al. 1990; Hellstrom *et al*. 2008; Gonzales et al. 2009). On a cellular level, Al accumulation is mostly associated with lysosome, chromatin and cell nucleus (Galle, 1987; Lukiw *et al*. 1992; Karlik *et al*. 1980).

Elimination rate of aluminium is dependents of the location of aluminium in the tissues, as aluminium can move throughout cellular compartments (Exley et al. 1996). When analysing the accumulation of aluminium in bone, it is possible to determine a half-life for aluminium in the body of around 50 years (Yokel, 2013). This 'pool' of aluminium allows to increase accumulation under prolonged exposure (Krewski *et al*. 2007).

Many studies have set about to investigate the mechanism surrounding aluminium's movement across the blood brain barrier. Aluminium is associated with many neurological conditions and can be viewed in the brain tissue histochemically. Modified haemotoxylin or lumigallion are the gold standard for aluminium staining in tissues. A common condition associated with increased brain aluminium pathology is Alzheimers disease, which is usually characterised by the presence of senile plaques, composed of Beta-Amyloid (Jones *et al* 2011). This study highlighted the relationship between aluminium and AB-42. Staining methods were combined to show a clear association between amyloid

protein, stained with Highman's congo red and thioflavin t, and aluminium, stained with modified haemotoxylin, see Figure 1.7.1.

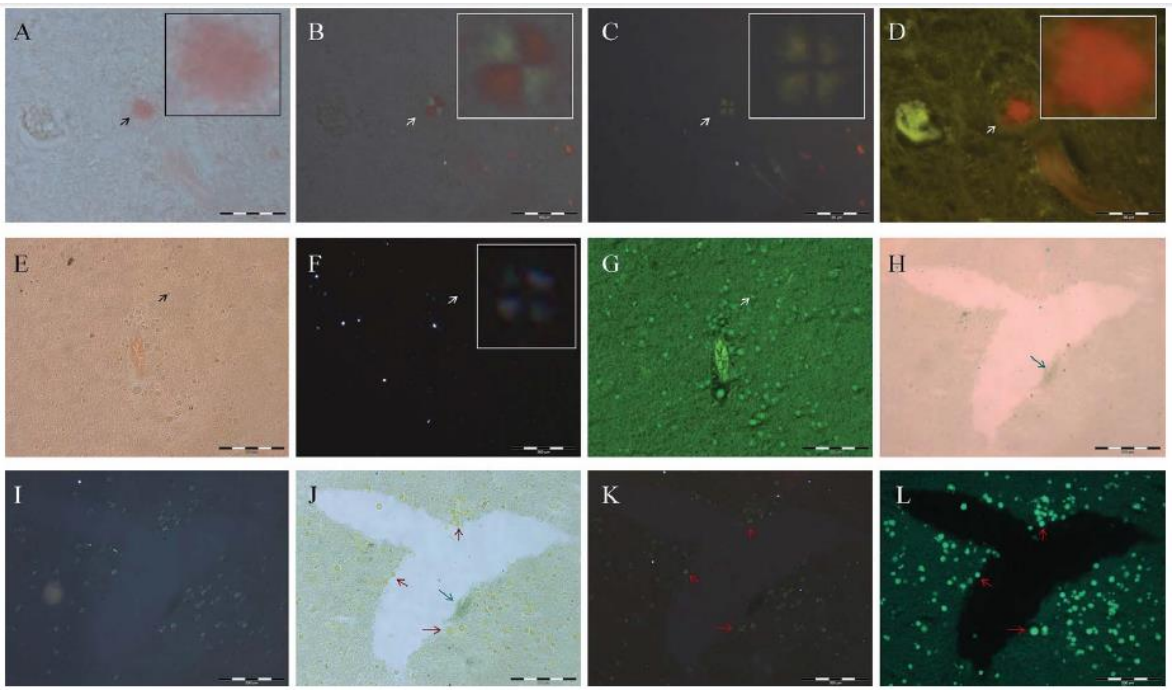

Figure 1.7.1: Brain tissue of Alzheimer's disease patients stained for aluminium and amyloid using modified haemotoxyln, congo red and thioflavin T. Tissue section (20 "m thickness) from occipital lobe (A–D) stained with modified haematoxylin and Highman's Congo red and showing (arrow and insert) a spherulite (ca 20 "m diameter under, A) optical light; B) partially polarized light; C) crossed polarizers; D) fluorescence. Scale bar in A–D is 100 "m. Tissue section (20 "m thickness) from frontal lobe (E–G) stained with modified haematoxylin and thioflavin T and showing (arrows and inserts) numerous spherulites (ca. 5–25 "m diameter) under, E) optical light; F) crossed polarizers; G) fluorescence. Scale bar inE–G is 200 "m. Tissue section (20 "m thickness) from frontal lobe (H–L) stained with Perl's stain for Fe(III) and showing, H) under optical light no obvious spherulites but some 'green' stainingfor Fe(III) (arrow); I) numerous spherulites in the same section under crossed polarizers. Counter staining of the same section with thioflavin T shows numerous spherulites appearing, J) yellowunder optical light; K) as 'green' Maltese crosses under polarized light; L) fluorescent green under fluorescent light. Scale bar in H–I is 200 "m. (Jones et al. 2011)

The mechanism behind the uptake of aluminium into the brain is much more efficient than its release, this indicates that the amount of aluminium residing in our brain tissues increases with age (Exley and House 2011).

1.8 Sources of Al exposure

In this 'Aluminium Age' there are myriad sources responsible for this level of Al exposure. Al can be absorbed dermally through use of antiperspirants (Flarend *et al.* 2001), injected as vaccine adjuvants (Keith *et al*. 2002) or inhaled as dust in an occupational hazard risk (Rifat *et al.* 1990; McLachlan *et al.* 1995).

As explored during this thesis, oral introduction is also a significant source of Al exposure, one that with efforts could be minimised. Plant based foods, such as spinach, potatoes and teas are naturally abundant in Al (Pennington & Jones, 1989); in addition the application of Al in medicines, food additives and as a flocculent in water treatment further increases this oral exposure (Harris *et al.* 1996; World Health Organization, 1998). Typical diets in the western world contain about 20mg d^{-1} (Pennington & Jones, 1989); although Al intake has been noted at up to 5g d^{-1} through the ingestion of antacids (World Health Organization, 1998).

The presence of Al in tap water is relatively small in comparison with the amount contained within the food we eat (Priest *et al.* 1998); a mere 3-8 % of dietary Al comes from municipal water; the guideline for this is given by WHO (1998) and should not exceed 0.2mg L^{-1}. However, it has been proposed that Al may be more bioavailable in water compared to food (Flaten, 2001).

The bioavailability of Al is highly affected by other dietary mechanisms; Al absorption is increased by fatty acids (Aspenstrom-Fagerlund *et al*. 2009) and affiliated with the ingestion of organic acids found in fruits and juices, particularly citrate (Slanina *et al.* 1986; Weberg & Berstad, 1986; Molitoris *et al.* 1989; Walker *et al.* 1990; Day *et al.* 1991; Domingo *et al.* 1991; Taylor *et al*. 1992a; 1998). In direct contrast, silicic acid can reduce the bioavailability of aluminium (discussed later in this section), even in the presence of citrate (Birchall *et al*. 1989; Birchall & Chappell, 1989; Edwardson *et al.* 1993; Birchall, 1995).

1.9 Al distribution around the human body

Despite a number of studies highlighting the danger of large concentrations of Al on our diets, only a fraction (0.1%) of Al ingested by healthy individuals is actually absorbed through the gastrointestinal tract (Day *et al*. 1991) and the bulk of this is readily excreted through the kidneys (Ganrot, 1986; Exley *et al.* 1996; Popplewell *et al*. 1998; Powell *et al*. 1999b). This adds to the hypothesis that Al is of little significance to human health. However, about 5% of this absorbed amount of bioavailable Al is retained in the body (Priest *et al.* 1997), and over time, this accumulates in various tissues; potentially resulting in morbidity and mortality through a variety of mechanisms. This is an important implication for copious human diseases. (Denton *et al*. 1984; Alfrey *et al*. 1986; Wilhelm et al. 1990; Day *et al*. 1991; Yokel, 2006; Verstraeten *et al*. 2008).

Once in the blood, Al can complex with biological molecules (Harris, 1992; Priest, 2004), of which approximately 90% involves the iron transporter transferrin (Harris & Sheldon, 1990; Wrobel *et al*. 1995; Milacic *et al*. 2009). These complexes are bound weakly and therefore the transport of Al to binding sites in our tissues is readily facilitated (Priest, 2004).

Al has been confirmed to accumulate in all tissues in mammals (Gonzales *et al*. 2009); however, the distribution of Al between tissue group is variable, with the highest levels seen in bone (Ganrot, 1986; Zafar *et al*. 1997). Other primary locations of notable Al deposition include the skin, kidneys, liver, heart and brain (Alfrey, 1980; Exley, 2001; Xu *et al*. 1990; Hellstrom *et al*. 2008; Gonzales *et al*. 2009). Al distributions also shows varied distribution within cell organelles, accumulating predominantly in the lysosome (Galle, 1987), cell nucleus (Lukiw *et al*. 1992) and chromatin (Karlik *et al*. 1980).

Total body aluminium has been shown to transfer between different systemic compartments (Exley *et al*. 1996). This is highly dependent on the elimination rate of the tissue (Yokel & McNamara, 1989). The terminal half-life of Al in humans is estimated to be up to 50 years, a value calculated from Al in bone (Yokel, 2013); in addition, this notable high deposition of aluminium within the bone may allow it to serve as a reservoir for prolonged exposure (Krewski *et al*. 2007).

As associations between Al and neurological disease continue, many studies have highlighted that Al may increase the permeability of the blood brain barrier. Uptake of aluminium into the brain is at least an order of magnitude more efficient than its release, which has a half-life of 7 years (Yokel *et al*. 2006). In conclusion, aluminium within the brain increases with age; a review by Exley & House (2011) concluded that 'normal' human brains contain between 0.1 – 4.5 μg g^{-1} dry weight, with typically <2 μg g^{-1} dry weight being associated with non-demented elderly.

Gray matter appears to be the primary site for aluminium deposition in the brain (McDermott *et al*. 1978), a region with a high density of transferrin receptors, such as the cerebral cortex and hippocampus (Edwardson *et al.* 1992). Specific compartments for brain aluminium are identified in *Table 1.9.1*.

Table 1.9.1: Major sinks/sources of aluminium within the brain (reviewed in Exley & House 2011).

Sink/Source for Al	Including
Blood-brain barrier	Endothelia, choroid epithelia, cerebrospinal fluid, pericytes, basal laminas.
Brain interstitial fluid	Proteins (transferrin, albumin), neurotransmitters (glutamate, gamma amino butyric acid, acetylcholine, dopamine), nucleotides (ATP, ADP, AMP), amino acids (aspartate, serine,
Neurons	
Non-neuronal cells	Astrocytes, oligodendrocytes, microglia, mononuclear migratory cells.
Pathological features	Senile plaques, neurofibrillary tangels, Lewy bodies, lipofuscin.

Despite the limited knowledge of the aluminium distribution throughout the tissues, it is known through histochemistry and quantitative analysis that it is present and has a strong association with neurodegenerative disease, specifically; Alzheimer's disease, Parkinsons disease and multiple sclerosis.

1.10 Role of metals: essential or neurotoxic?

A number of metals are involved in the normal functioning and maintenance of the central nervous system. To mention a small number of examples, iron is critically required in the synthesis of neurotransmitters and plays a leading role in oxygen transport and electron transfer (Takeda, 2004); copper is a vital precursor in the electron transport chain involved in adenosine triphosphate synthesis, and finally, copper and zinc biochemistry is connected to messaging events within the synaptic cleft (Duce & Bush, 2010) and are components of superoxide dismutase (SOD).

However, a level of neurotoxicity can result from a deviation in the natural balance of these essential metals (Olanow & Arendash, 1994; Bush, 2000; Sayre *et al.* 2005; Proudfoot, 2009). To measure

toxicity, the underlying mechanisms are examined. These mechanisms of toxicity include alterations in cell membrane permeability, changes in nucleic acid function, and inhibition of protein synthesis resulting in variable enzyme activity.

In multiple sclerosis, the levels of urinary aluminium excretion have been noted as similar to those seen in aluminium intoxication suggested that aluminium may be a hitherto unrecognized environmental factor associated with the aetiology of MS (Exley *et al* 2006).The cellular accumulation of lead and mercury has been associated with the development of autoantibodies against neuronal cytoskeletal proteins, neurofilaments, and myelin basic protein in humans and animals. Overexposure to lead and mercury ions is known to be neurotoxic, particularly to motor neurons. Low-to-moderate levels of lead exposure can cause functional alterations in T-lymphocytes and macrophages that lead to increased hypersensitivity and can alter cytokine production, which increases risk of inflammation-associated tissue damage (Napier *et al.* 2016).

The relationship between these essential trace metals and neurological disease is thought to be directly linked to a disruption in homeostatic mechanisms, leading to significant changes in their distribution and regulation (Duce & Bush, 2010). Evidence advocates that aluminium toxicity promotes the disruption of essential metal homeostasis (Exley, 2001; Kawahara *et al.* 2007; Wu *et al*. 2012).

This has led to the idea that there is a connection between these metals and that their involvement in neurological disease may actually be a result of aluminium toxicity.

1.11 Potential mechanisms of aluminium toxicity

Chemical properties, including charge and size, of aluminium are similar to many elements that the human body sees as essential, this enables it to act as a competitive inhibitor with other metals for target sites on specific enzymes, receptors and signalling molecules, resulting in a manipulation in activity (Exley, 2009).

Magnesium and calcium play a leading role in neuronal reactions, being involved in neurotransmitter release and neuronal growth. Aluminium has been observed to replace magnesium at phosphate at binding sites on cell membranes, ATP, and DNA (Exley, 1992; Harris *et al.* 1996; Kawahara & Kato-Negishi, 2011), and even demonstrated to replace calcium ions within bone, which results in an interference between calcium-based signaling events. This can trigger apoptosis within the tissue (Platt, 1994; Exley, 2001; Kawahara *et al*. 2007; Nday *et al.* 2010).

Biological processes dependent on iron are considered to be a main target of aluminium toxicity. By disturbing iron homeostasis, the presence of aluminium subsequently leads to the production of reactive oxygen species (ROS) (Bondy & Kirstein, 1996; Exley, 2004; Wu *et al.* 2012), contributing to any toxicological effects. An increase in oxidative events have been noticed with interactions between aluminium and copper (Bondy *et al.* 1998; Becaria *et al.* 2003).

In addition to this exploration about aluminium's ability to affect homeostasis of essential metals in the body, other mechanisms for aluminium induced neurotoxicity have been proposed, mainly focussing on multiple sclerosis.

The most commonly discussed suggestions include:

a) Aluminium enhanced oxidative stress caused by lipid peroxidation of nervous tissue phospholipids, which affects the integrity of cell membranes (Sarin *et al.* 1997; Nayak & Chatterjee, 1999; Drago *et al.* 2008; Dua *et al.* 2010).
b) Aluminium promotes neuro-inflammatory events (Platt *et al.* 2001: see also Bondy, 2010).

As it is believed that the occurrence of MS is liked to both genomic and environmental factors, the idea that Al may interact directly with the genomic structure (Nayak & Chatterjee, 1999) further supports this theory. Al is attracted to negative binding sites on nucleic acids (Lukiw et al. 1998). This could have detrimental effects on neuronal activity and could be linked to the several genetic mutations identified in multiple sclerosis. A possible mutation found, in a gene called NR1H3, is a missense mutation that causes loss of function of its gene product, LXRA protein. LXRA controls transcriptional regulation of genes involved in lipid homeostasis, inflammation, and innate immunity. Mice with this gene knocked out are known to have neurological problems, including a decrease in myelin production (Wand *et al*, 2016). A detailed review on the potential mechanisms of Al toxicity can be found in Tomljenovic (2011).

By considering of the variety of biological molecules able to bind to Al and the evidence that Al is able to displace essential metals from their binding sites, the idea can be consulted that Al toxicity could potentially affect any metabolic pathway (Exley, 2009) and may arise from a number of different interactions with the nervous system.

1.12 Evidence for a role of aluminium in neurotoxicity

The first report connecting aluminium and neurotoxicity in animals was reported by Dollken in 1897. However, the first evidential report confirming the connection was provided by Klatzo *et al.* (1965), who noted that rabbits injected with aluminium containing salts over time, formed neurofibrilary tangles in the brain.

Evidence of aluminium neurotoxicity in humans was not presented until almost a century after this first connection (possibly because aluminium wasn't as widely available during this time) when neurotoxicity was observed in individuals receiving dialysis treatment (Alfrey *et al*. 1976), confirming

the primary route of aluminium excretion is the kidney. Individuals with renal insufficiency are unable to effectively excrete aluminium (Flaten *et al*. 1996); dialysis encephalopathy occurred from notably high levels of aluminium in dialysis solutions (Wills & Savory, 1983); stemming from this, brain aluminium was revealed to be 10-fold in dialysis patients (Alfrey *et al.* 1976) compared to normal ranges < 2 μg g^{-1} (Andrasi *et al*. 2005). By treating the water used for dialysis, aluminium levels were decreased and aluminium related conditions were concededly reduced in these uremic individuals (Arenas *et al*. 2008).

Since these investigations, aluminium toxicity has been linked to numerous neurological disorders, including amyotrophic lateral sclerosis, multiple sclerosis, Alzheimer's disease and Parkinson's disease (Harris *et al.* 1996; Jeffery *et al.* 1996; van Landeghem *et al.* 1997; Altmann *et al*. 1999; Van der Voet *et al.* 1999; Jaffe, 2005; Peto, 2010; Bondy, 2010). However, the elements involvement in these conditions remains controversial as the specific role has not yet been concluded, despite increasing research being performed in this area (a full review of this is shown by Kawahara, 2005).

It is thought that a common occupational exposure to aluminium is in dust and is suggested that this may contribute to cognitive impairment (Longstreth *et al*. 1985; Sjogren *et al*. 1990; 1996; White *et al*. 1992; Meyer-Baron *et al.* 2007) and Alzheimer's disease (Kobayashi *et al*. 1987). As a lower urinary aluminium concentration may suggest a higher body burden, studies have related a urinary aluminium concentration below 135μg/L to a reduction in cognitive performance (Meyer-Baron *et al*. 2007). In the mining industry, workers given McIntyre powder, an aluminium rich powder, as a prophylactic treatment against pneumoconiosis, saw a connotation between the on-set of cognitive impairment with the length of their treatment (Rifat *et al.* 1990). However, to

refer back to the controversy surrounding this link, some authors state that there is little or no evidence to relate neurotoxicity to the occupational exposure of aluminium (Graves *et al*. 1998).

The Camelford incident, in July 1988, twenty tonnes of aluminium sulphate was discharged by the South West Water Authority into the drinking water supplied to a large region of North Cornwall, raising the concentration to over three thousand times the admissible amount. Up to 20 000 people were exposed to concentrations of aluminium which were 500–3000 times the acceptable limit under European Union legislation (0.200 mg/l). When the first neuropathological examination of a person who was exposed and died of an unspecified neurological condition was carried out (Exley & Esiri, 2006), a rare form of sporadic early-onset β-amyloid angiopathy in cerebral cortical and leptomeningeal vessels, and in leptomeningeal vessels over the cerebellum was identified. In addition, high concentrations of aluminium were found coincident with the severely affected regions of the cortex. Since then, there have been reports from individuals affected by this event who have developed long-term cerebral impairment (Altmann *et al*. 1999) and severe cerebral congophilic angiopathy (Exley & Esiri, 2006).

A wealth of research has explored the use of aluminium adjuvants in vaccines. Adjuvants are included in vaccines as immunopotentiators (i.e. in order to stimulate the body's immune response to the small amount of coadministered antigen).

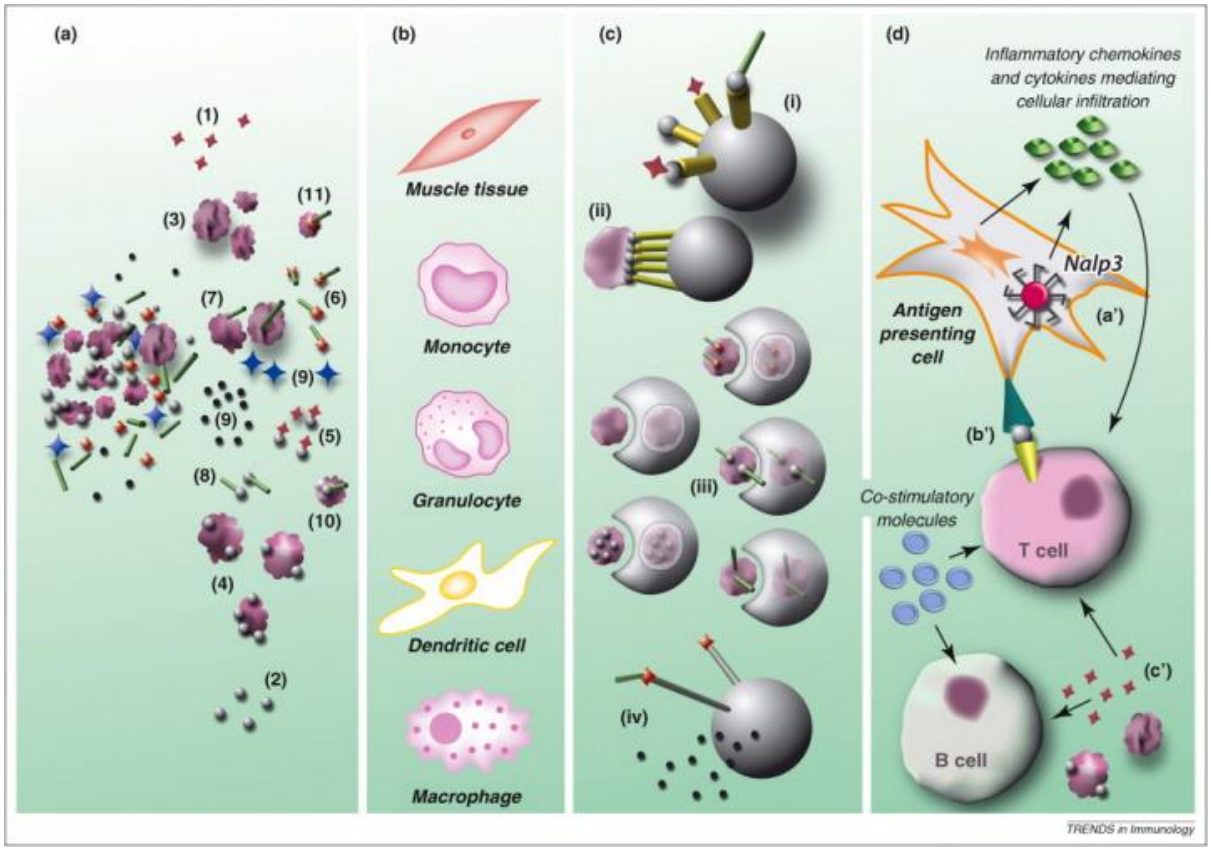

Figure 1.12.1, taken from Exley, Siesjö & Eriksson (2010). The aluminium adjuvant armoury and innate and adaptive immunity. (a) Dilution of the vaccine preparation into the muscle interstitial fluid (MIF) results in an array of potential agonists of the immune cascade, including: (1) $Al^{3+}_{(aq)}$; (2) free antigen (AG); (3) particulate adjuvant (ADJ); (4) ADJ with associated AG; (5) AG-Al complex; (6) MIF ligand-Al complex; (7) ADJ with associated MIF ligand; (8) MIF ligand-AG complex; (9) particulate iron (as contaminant of adjuvant) either free or with adsorbed Al/AG and resultant reactive oxygen species (ROS); (10) ADJ with associated MIF ligand-AG complex; (11) ADJ with associated MIF ligand-Al complex. MIF ligands might include biomolecules such as; ATP, albumin, transferrin, citrate, fibrinogen. (b) The array of agonists act upon a number of cell types including, the resident muscle tissue (potentially causing necrotic and/or apoptotic cell death) and infiltrating innate cells such as, monocytes (potential for Al_{ADJ}-induced differentiation to dendritic cells), granulocytes (potential for Al_{ADJ}-induced eosinophilia acting directly on B cells), macrophages (are known to persist for long periods close to the injection site and may be characterised by inclusions of Al_{ADJ}) and dendritic cells (DC). The latter may be the major antigen presenting cell (APC). (c) There are myriad possible modes of interaction between agonists and innate cells including; (i) toll-like receptor (TLR) binding of AG[2], AG-Al complex[5], MIF ligand-AG complex[8], $Al^{3+}_{(aq)}$[1]; (ii) multiple TLR binding of AG-ADJ[4]; (iii) phagocytosis of ADJ[3], AG-ADJ[4], MIF ligand-ADJ[7], MIF ligand-Al complex-ADJ[11], MIF ligand-AG complex-ADJ[10]; (iv) direct[1] or indirect[6] binding of $Al^{3+}_{(aq)}$ by membrane receptors and extracellular (lipid membrane)

or intracellular (nucleus) activity of ROS[9]. (d) APCs activate adaptive immunity through; (a) Nalp3 inflammasome dependent or independent release of chemokines and cytokines (green saucers) including IL-1β and IL-18; (b) AG presentation by MHC to T cell receptor combined with co-stimulatory molecules; (c) direct action of ADJ and/or $Al^{3+}_{(aq)}$ on B/T cells. The superscripts refer to the numbers in parentheses in the figure.

Neurotoxity has been associated with this use and allied with chronic cognitive dysfunction in macrophagic myofascitis (Couette *et al*. 2009). In one study it was documented that Gulf war veterans who were offered aluminium hydroxide injections as protective measure against anthrax, saw an increase in occurance of motor neuron disease (Shaw & Petrik, 2009). Individual case reports and supporting animal studies in rats have highlighted associations between the use of oral Al-containing antacids during pregnancy and abnormal fetal neurologic development (Gilbert-Barness *et al.* 1998; Shuchang *et al.* 2008).

1.13 The monster inside me: Multiple Sclerosis

Multiple sclerosis (MS) is a potentially debilitating disease of the brain and spinal cord (central nervous system) affecting 2.5 million people worldwide (Fox 2014). MS is an acquired, inflammatory, demyelinating, neurodegenerative disease of the central nervous system (CNS) (Fulgenzi *et* al 2011). MS is referred to as an auto-immune disease. The protective fatty myelin sheath that surrounds the

nerve cells, to increase efficiency of a nerve impulse, is attacked by local immune cells, because it is mistaken for a foreign body, much like an immune reaction (Mcfarlin 1982), see figure 1.13.1. This damage disrupts the conductivity of the nerve cell, delaying and distorting messages, or simply stopping message transmission altogether (Perry 2014). The disease tends to progress in adults at young age (between 20 and 40 years), variably progressing in severity of symptoms for the entirety of life, for which there is no cure (Reiber et al 2009; Ibrahim and Gold 2005). The neurological impairments (attacks) that characterise multiple sclerosis, can be followed by asymptomatic periods, e.g., the pathology can present a relapsing-remitting course. Progression of the disease is generally accompanied by presence in the central nervous system of focal inflammatory lesions which are visualised using magnetic resonance imaging (MRI) (Fulgenzi *et* al 2011). Although the mechanisms involved in the pathogenesis of multiple sclerosis an autoimmune reaction against a myelin-related antigen have been identified, the aetiology of the disease is yet unknown (Exley *et al* 2009). A consensus leads to the opinion that the disorder is the result of a relationship between environmental factors and susceptibility genes. A number of studies propose the association of neurodegenerative diseases with toxic metal (especially aluminium) exposition and accumulation in the CNS (Kumar and Gill 2009; Zahran et al. 2009; Taber and Hurley 2008).

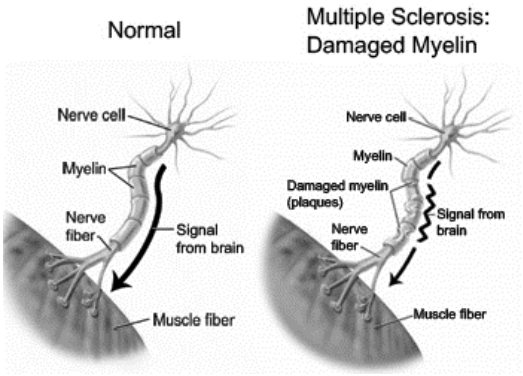

Figure 1.13.1 A normal nerve cell compared to a typical damaged nerve cell of an MS patient (RelayHealth 2009)

1.14 The role of aluminium in MS

In MS, the immune system attacks the protective myelin sheath that coats the nerve fibres. The damaged myelin forms scar tissue (sclerosis), giving the disease its name. The damage to myelin sheath results in nerve impulses traveling to and from the brain and spinal cord becoming distorted or interrupted, producing a wide variety of symptoms (Goldenberg, 2012). The aetiology and pathogenesis of MS has not yet been established although at autopsy, multiple, discrete pink or gray areas that have a hard, rubbery texture are identified within the white matter. The lesions are composed of areas of myelin and oligodendrocyte loss along with infiltrates of inflammatory cells, including lymphocytes and macrophages. The relative preservation of axons and neurons within these lesions helps to differentiate MS from other destructive pathological processes that are accompanied by focal inflammation (Goldenberg, 2012).

More than 30% of MS patients have moderate-to-severe spasticity, mostly in the legs. Initial clinical findings in MS patients are often sensory disturbances, the most common of which are paresthesias (numbness and tingling), dysesthesias (burning and “pins and needles”), diplopia, ataxia, vertigo, and bladder (urinary sphincter) disturbances. A common manifestation of MS is unilateral numbness affecting one leg that spreads to involve the other leg and rises to the pelvis, abdomen, or thorax (Goldenberg, 2012). Sensory disturbances usually resolve but sometimes evolve into chronic neuropathic pain. Another common presenting sign of MS is optic neuritis, highlighted by complete or partial loss of vision. Bladder dysfunction occurs in more than 90% of MS patients and results in weekly or more frequent episodes of incontinence in one-third of patients. At least 30% of patients experience constipation. Fatigue occurs in 90% of patients and is the most common work-related disability associated with MS (Goldenberg, 2012). Sexual problems are often experienced as well.

Both mortality and morbidity data confirm that multiple sclerosis is a geographically-related disease, and thus MS can be thought of as an acquired exogenous illness (Kurtzke, 1977). MS occurrence is high in America, medium in Europe, and low in Asia. Processed foods which are high in aluminium are common in the diets in individuals residing in the western world, and make up a lower proportion of a traditional Asian diet (Pingali, 2007) In addition, an Asian diet is traditionally high in silicon rich foods (Jugdaohsingh, 2002), including rice, further highlighting the importance of the relationship between silicon and aluminium and how there may be a direct link between the prevalence of MS and body bioburden of aluminium.

Several risk factors, including age, genetic disposition and environmental agents, have been connected with MS. Aluminium is a commonly discussed factor and has been associated with a multitude of neurophysiological processes responsible for the characteristic degeneration associated with MS; however, its actual role remains controversial (Exley, 2006). Dietary intake of aluminium from food and chemicals added may also be a noteworthy risk of MS (Rogers & Simon, 1999). Tea, a significant part of the British diet, is estimated to contribute up to 50% of daily intake due to the high levels of aluminium it contains (Yokel, 2013). When greater than four cups of tea were consumed per day, aluminium in the urine was elevated, although not significantly (Forster *et al.* 1995). As aluminium is bound to organic complexes within tea, this may lead to it being poorly absorbed (French *et al.* 1989; Flaten, 2002).

The level of aluminium found in the urine of MS patients is similar to that of aluminium intoxication, ranging from 0.22-0.66 µM (Exley *et al* 2006). There are no previous data on aluminium in MS, however a large incidence of MS was reported in macrophagic myofascitis (Authier *et al* 2001), an

inflammatory myopathy, linked to intramuscular injections of aluminium adjuvant containing vaccines (Gherardi *et al* 2001). Animal studies have shown that the favoured target of aluminium neurotoxicity was via accumulation in the myelin (Verstraeten *et al* 1998; Verstraeten *et al* 1997). Several reports exploring aluminium intoxication demonstrate a significant thinning of the myelin sheath, in both the spinal cord and hippocampus (Golub *et al* 1999; Deloncle *et al* 2001; Miu *et al* 2004). Aluminium has also been seen to increase kinase activity, responsible for the phosphorolation of myelin basic protein and decrease the activity of the myelin specific enzyme, 2'3'-cyclic nucleotide phosphohydrolase (Arroyo-Serralta *et al* 2005; Sarin *et al* 1997). This research indicates clear precedents that in animal research, if not yet in humans, the role of aluminium in MS is important. Those persons with a higher body burden of aluminium may accumulate the metal in oligodendrocytes and myelin, where it can disrupt the chemical pathways and facilitate iron-mediated oxidative damage (Exley 2004). The levels of urinary aluminium that are seen in relapse remissive MS may represent an association with myelin and demyelination, the breakdown of myelin may release aluminium that has accumulated in the protein, this may even mirror with the reactions involved with a relapse (Whitaker *et al* 2001). These neurotoxic results are mirrored in Alzheimer's disease (Bartzokis 2004).

1.15 Treating Al toxicity

Evidence is compiling against aluminium and suggesting that it is a contributory factor in neurological disease. If this indeed is the case, if aluminium is a casual or at least contributory factor, it could be theorised that if the aluminium body burden was reduced and maintained at a lower level, the occurrence of the disease would decrease (Crapper McLachlan et al. 1991; Exley et al. 2006). We can also assume that a lowering of aluminium levels in the body would relieve symptoms of a disease in those previously diagnosed.

If aluminium is contributing so prolifically to neurodegeneration, it is crucial to educate the general population to understand the risks and symptoms surrounding the toxicity of the element. This may result in the population actively avoiding aluminium rich products (anti-persperant) and foodstuffs (tea). Accompanying this with current metal chelation techniques will produce a double whammy effect on aluminium levels in the body, as research has demonstrated chelation therapy as having positive effects on aluminium removal from body stores ((Nakamura *et al*. 2000).

Desferrioxamine (DFO), a common chelating agent typically used as a treatment for ion toxicity, has also shown effectiveness when removing aluminium from the bone and brain (Nakamura *et al*. 2000; Yokel *et al*. 2001). DFO is linked to an increase in the urinary excretion of aluminium as well as reducing the rate of decline in Alzheimer's disease patients suffering with a lowered performance of daily skills (Crapper McLachlan *et al.* 1991). Despite these positive findings, treatments involving the use of DFO is limited due to noticed side effects (Cronin & Henrich, 2006). Despite this, recent studies have suggested that a lower dose may still be effective, while lowering the occurrence of nasty side effects (Kan *et al*. 2010). There are other compounds which are seen to act as aluminium chelators, for example, ascorbate and feralex-G are commonly used in research (Kruck *et al.* 2004). Research into the synthesis of new ligands for aluminium is also increasing (Santos, 2008; Crisponi et al. 2012).

In recent research, silicon has been deduced as an active antagonist towards aluminium. By supplementing the diet with silicon, as bioavailable silicic acid, a reduction in the bioburden of aluminium has been demonstrated, suggesting removal from tissue compartments such as the brain and surrounding nervous tissue (Exley 2009).

1.16 Silicon and its possible protection against aluminium toxicity.

There is a wealth of evidence supporting the effect of silicon on aluminium accumulation in the body. It is also thought that silicon may have an influence on the oral absorption of aluminium (Belle's *et* al 1998).

Once aluminium has found its way into the bloodstream, it is seen to co-localise with silicon, especially in aluminium rich regions such as the plaques characterising Alzheimer's disease (Candy *et al.* 1986). This suggests that perhaps like aluminium, silicon, in its biological form of silicic acid, can cross the blood brain barrier (Yokel 2006). It may in fact be added that silicon in the brain could possess neuroprotective properties, being able to quench aluminium from the brain, hereby relieving the symptoms of AD and other neurodegenerative conditions.

When introducing a silicon rich dietary source to rats, aluminium levels were significantly reduced in all tissues compared to that of the control group. Furthermore, urinary excretion levels of Al were also substantially lower (Belles *et al* 1998). This focuses primarily on the potential role of silicon in preventing oral aluminium absorption and retention in mammals. The results of a number of studies suggest that dietary silicon supplementation could be of therapeutic value for preventing chronic aluminium accumulation in the nervous system, and hence, be a potential therapy for neurodegenerative disease, such as MS (Domongo *et al* 2011).

When processing all of the positive associations of silicon in drinking water, it can be suggested that the further addition of silicon to the diet in this manner, through silicon-rich mineral waters, can provide a non-invasive means to reduce aluminium body burden. The aluminium and silicon research group at Keele University (Exley *et al* 2006) was the leading pioneer in testing this suggestion. The initial study, investigating a potential non-invasive solution of reducing aluminium body burden in

Alzheimer's disease patients, involved the consumption of up to 1L of silicon rich mineral water daily. An association between aluminium and MS has been established, so suggesting an increased silicon uptake, through consuming mineral water naturally high in silicon, is a viable method to reduce the aluminium bioburden in MS patients. The current results corroborate that silicon effectively prevents gastrointestinal aluminium absorption, which may be of concern in protecting against the neurotoxic effects of aluminium.

1.17 Mechanisms behind the chelating effect of silicon.

It was first proposed by Birchall *et al* (1989) that silicon could offer an ameliorating effect. When looking at salmon fry in acidic water, it was shown that acute toxicity of aluminium was eliminated in the presence of silicic acid. This mechanism was believed to involve hydroxyaluminosilicates (HAS) (Birchall *et al*. 1989; Birchall, 1990) and two discrete forms of HAS, called HAS_A and HAS_B. The structures of HAS_A and HAS_B have been determined are shown in Figure 1.17.1. It was theorised that similar mechanisms would apply to humans (Birchall *et al* 1989) and that interactions with human renal tubing may affect aluminium elimination by reducing reabsorption (Birchall, 1992).

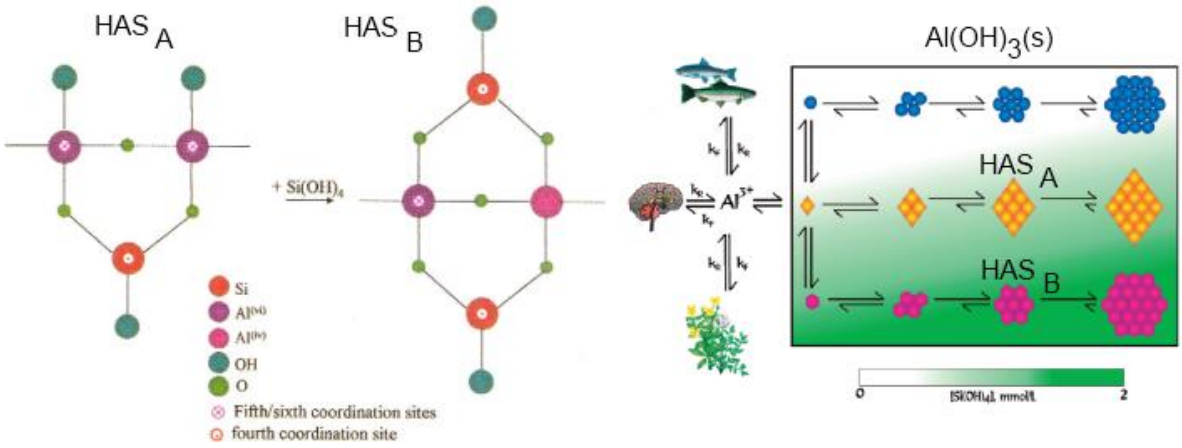

Figure 1.17.1 Hydroxyaluminosilicate structures (left); biological availability and solid phase, HAS_A and HAS_B self-aggregation (right) (Birchall et al 1989)

Investigating these two subspecies of HAS molecules, HAS_A and HAS_B may shine further light onto the protective mechanism of silicon against aluminium (Doucet *et al* 2003). Fig 1.17.2, a schematic showing pH-dependent formation and stability of Al(OH)3(s), HASA and HASB with particular reference to their abilities to carry charge, their pH of minimum solubility and their aggregation towards filterable sizes. (Exley 2012).

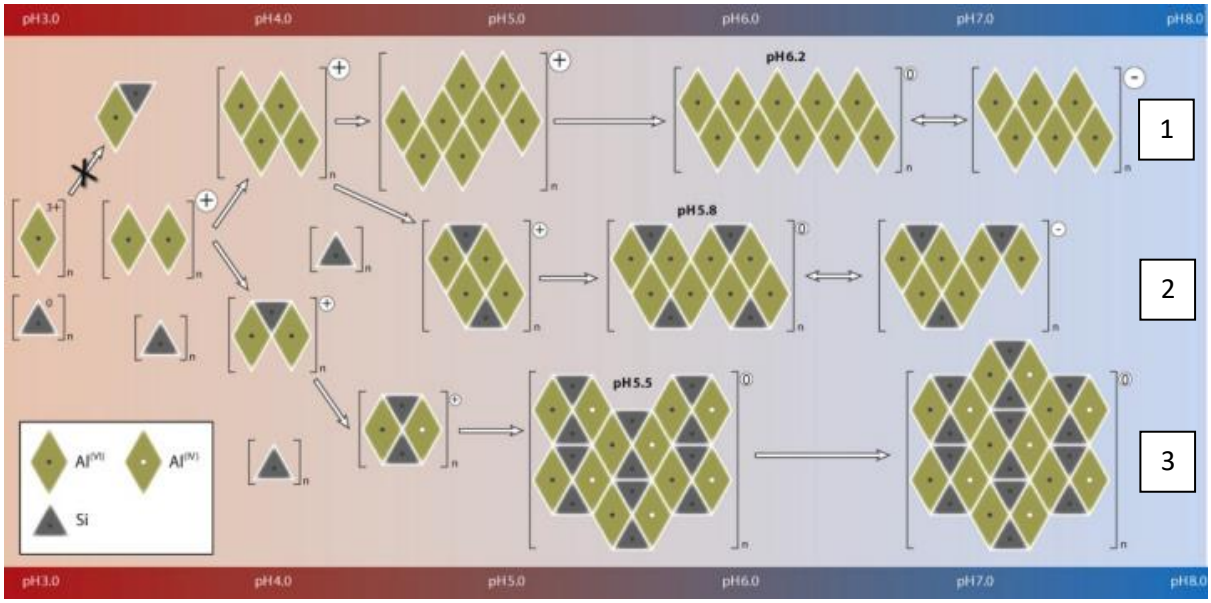

Figure 1.17.2: Schematic showing pH-dependent formation and stability of 1 - Al(OH)3(s), 2 - HAS_A and 3 - HAS_B with particular reference to their abilities to carry charge, their pH of minimum solubility and their aggregation towards filterable sizes. From Exley (2012).

Although it is believed that silicic acid is involved in the homeostasis of aluminium by reducing gastrointestinal absorption and facilitating the renal excretion of aluminium though the inhibition of reabsorption of systemic aluminium (King *et al* 1997), even in the presence of citrate, a natural aluminium binding agent (Birchall and Chappell 1989). Despite these positive findings, some studies show that there is no evidence that the ingestion of silicic acid promotes aluminium excretion (Reffitt *et al* 1999), and the presence of HAS in the body has yet to be proven (Exley, 1998). What is known is that the administration of silicic acid causes a peak in aluminium excretion, followed by a steady

reduction, indicating that aluminium body burden is being reduced (Birchall *et al* 1989; Birchall, 1990; Bellia *et al*. 1996). Work stemming from this, carried out by Birchall and Exley (1992,1993) demonstrated that silicic acid inhibits the growth and nucleation of aluminium hydroxide at physiological levels when the pH is above 4.5, through the formation of filterable molecules of HAS. These studies suggest a minimum silicon concentration of 0.1 mmol L^{-1} is required to deliver this effect (Birchall 1992).

Desouky and colleagues (2003) attempted to clarify the mechanisms behind the chelating effect of silicon on aluminium and how uptake of aluminium is in turn prevented in the water pathway, hereby reducing aluminium in the tissues. These studies involved the pond snail (*Lymnaea stagnalis*) and signified that instead of orthosilicic acid preventing the accumulation of aluminium within the tissues, the protective effect is in fact due to the formation of HAS, either in the water column or in the digestive tract. This idea correlated with notions theorised by Birchall and his colleages (1989).

The Exley group reviewed the role of HAS(s) in controlling the biological availability of aluminium and considered the protective effects of HAS(s), which is known to extend to humans and human physiology, but is very poorly understood.

What did Louis Pasteur have in mind when he said “Effects of silicic acid are destined to play a great and major role in therapy” (Pasteur 1878).

Whatever it was that heightened Pasteur’s interest in the mid-nineteenth regarding the therapeutic properties of $Si(OH)_4$ has remained largely unexplained, although the therapeutic potential of $Si(OH)_4$ still holds significant interest (Martin 2007).

With increasing attention and focus on health, it is a hot topic to explore any method that causes significant health improvements, for such little invasiveness and cost. The fascination with $Si(OH)_4$ and health possibly originates from the reputed health benefits of bathing in spa waters rich in $Si(OH)_4$

(Domino and Gomez, 2011). Nutritional essentiality of silicon has been demonstrated in laboratory animals, using experiments which have given scientific support to theories of silicon essentiality in humans. Another study demonstrated that a higher level of urinary and faecal silicon was present in mice exposed to aluminium, while being administered silicic acid. These excretion levels were higher in those mice dosed with aluminium, suggesting that silicon and aluminium interact and combine to form a species, not taken up by the digestive tract (González-Muñoz *et al*. 2008a). Although, low blood serum correlated with high urinary silicon levels divulge that silicon-aluminium interactions limit reabsorption in the kidney (Bellia *et al*. 1996).

The case for the importance of silicon is strengthened further by evidence that proves that living things grow better and healthier when they are grown in silicon rich environments (Epstein, 1994; Sripanyakorn *et al* 2009). Silicon is recognised as an essential trace element (Carlisle 1982).

1.18 Concluding remarks

The therapeutic mechanism of silicon when coupled with the toxicity of aluminium remains controversial, despite numerous studies supporting these claims. There is a wealth of evidence exploring the protective effects of silicon on the accumulation of aluminium. If biological systems have in fact evolved a protective mechanism, through interactions between silicon and aluminium, heightened also due to the increase in biospheric levels of aluminium (Birchall, 1990), it is understood that sufficient silicon (>0.1 mmol L^{-1} Birchall, 1992) is required to prevent the adverse effect correlated with aluminium intoxication. .

Therefore, silicic acid-rich mineral water may be applied as a prophylactic means of diminishing the accumulation of aluminium within the body, providing an inexpensive and non-invasive method of reducing the risk of aluminium toxicity.

Chapter 2 - Materials and methods

Each study that included sweat and urinary measurements of aluminium and silicon were performed using TH GFAAS. Creatinine concentrations of urine were determined by UV-Vis spectroscopy. Further details of the study protocols are discussed in their relevant chapters.

2.1.0 Background

Techniques involving analysis at the atomic and molecular level are common when determining accurate concentrations of elements.

Atomic techniques involve flame spectroscopy, inductively coupled plasma – optical emission spectroscopy (ICP-OES) and graphite furnace atomic absorption spectroscopy (GFAAS). Molecular techniques involve UV-visible spectroscopy (UV-Vis), infrared (IR) and nuclear resonance spectroscopy (NMR).

This study involves the use of TH GFAAS to determine the concentration of aluminium and silicon in samples of human urine and sweat, these were complemented by creatinine concentrations determined by colourimetric analysis involving UV-Vis.

2.1.1 Spectroscopy

Spectroscopy is a branch of science concerned with the investigation and measurement of the interaction of electromagnetic radiation within matter. Electromagnetic radiation has wave and particle properties. This includes wavelength, frequency and amplitude, while light is composed of photons which possess certain characteristic energies.

There is a relationship between these quantities and it can be represented by the following equation (Eq 2.1.1):

$$E = h\nu = hc/\lambda \quad Eq\ 2.1.1$$

In this equation, E denotes energy (J), c represents the celerity of light (2.99792×10^{8} m s^{-1}) h is the Planck's constant (6.62608×10^{-34} J s), ν and λ denote frequency (s^{-1}) and wavelength (m) respectively.

The electromagnetic spectrum (*Fig. 2.1.1)* is composed of a wide array of wavelengths, ranging from gamma rays (<5nm) to radio waves (>300mm). In this study, only a small part of this spectrum is incorporated: UV-Vis spectroscopy is limited to the range of 190nm-750nm whilst atomic absorption spectroscopy occupies a range of 180nm-900nm. These techniques cover the range of the ultraviolet, visible and near infrared portions of the spectrum.

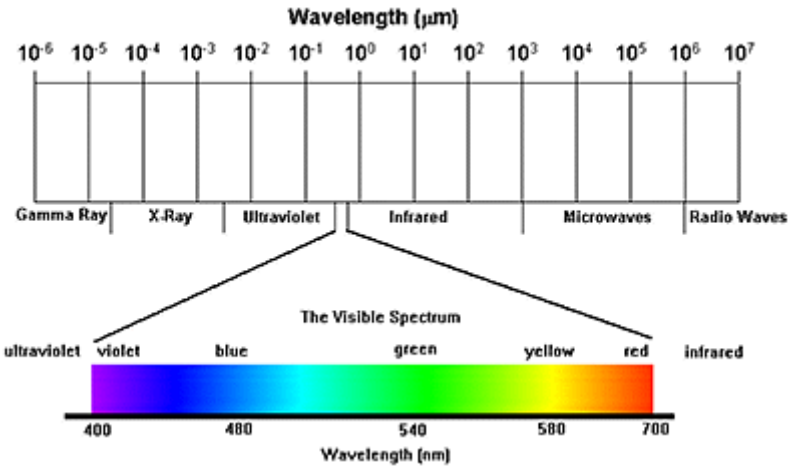

Fig. 2.1.1 The wavelengths of the electromagnetic spectrum (NASA-imagine the universe, 2013).

When light passes through a sample, the energy from the photons promotes a transition within the atoms in the sample. This transition results in the transfer of electrons in the molecule, atom or ion to be relocated to a different energy state.

When an electron is promoted to a higher energy state from a lower energy state, this is known as absorption of energy. When an electron drops from a higher energy state to a lower energy state, this is known as emission of energy.

The Bohr frequency condition shows that the energy absorbed or emitted is equal to that of the energy difference between these levels and as each atom is unique, these values are characteristic to the atom involved and so the amount of radiation needed for these transitions is also characteristic of the element (*Fig 2.1.2)*.

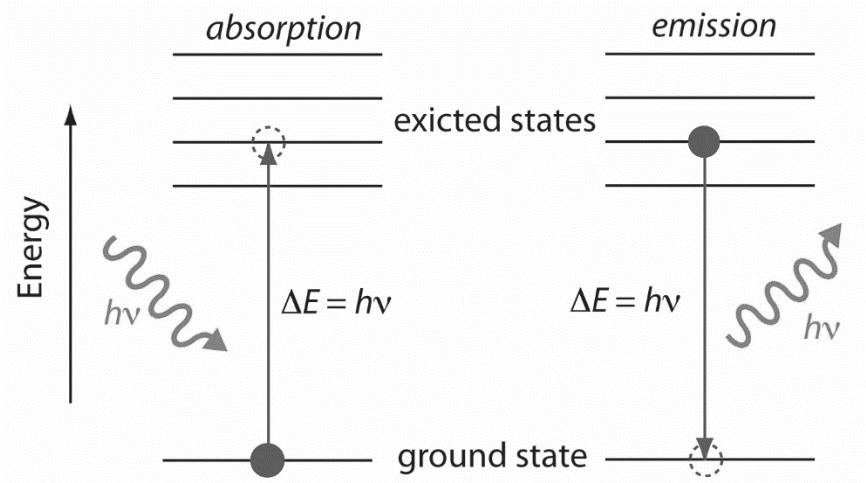

Fig 2.1.2 Transition from a ground state (GS) to an excited state (ES) results in absorption, transitions from an excited state to the ground state results in emission. Energy absorbed or emitted is equal to the energy difference (ΔE) between energy levels.

The two main methods used in this thesis involve types of absorption spectroscopy, therefore promoting electrons from a ground state to an excited state.

These techniques involve a monochromatic light source of intensity (I_o) passing through a cell of analyte (gaseous for GFAAS and liquid for UV-Vis). The analyte in the cell absorbs a quantity of this light which is directly proportional to the concentration of the analyte. The transmitting light (I) is turned into an electrical signal at the detector which is processed to indicate the amount of analyte present in the cell (*Fig 2.1.3).*

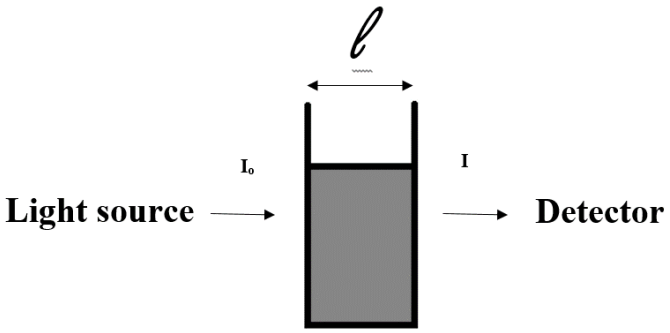

Fig 2.1.3 Schematic diagram of the atomic absorption of analyte in a cell. I_0 is the incident intensity, I is the transmitted intensity and ℓ is the path length.

Transmittance, τ (no units), is the fraction of light that passes through the cell of analyte and is the ratio of intensity transmitted, I, and incident intensity, I_0 (Eq2.1.2).

$$\tau = I/I_0 \qquad \textit{(Eq 2.1.2)}$$

If a high concentration of analyte is present, very little light will be able to pass through the cell, this will result in a transmittance value close to zero; when no analyte is present in the cell, all of the light will pass through and a transmittance value of one occurs. Percentage transmittance can also be used to determine the amount of analyte in a cell.

As there is no linear relationship between transmittance and concentration, quantitative measurements are presented in absorbance, A (no units). Absorbance can be related to transmittance using the following equation (Eq 2.1.3).

$$A = -\log \tau = -\log I/I_0 = \log I_0/I \qquad (\textit{Eq 2.1.3})$$

As concentration of the analyte increases, absorption value increases in consequence. There is a quantitative relationship here between absorbance and concentration and is governed by the Beer Lambert law.

$$A = \varepsilon C\ell \quad (Eq\ 2.1.4)$$

Where ε is the molar absorptivity coefficient ($m^2\ mol^{-1}$); ℓ is the path length of the cell (m) and C is the concentration of the analyte (mol m^{-3}). In AAS, sensitivity is increased by using long illuminated beam paths for the atom cells. This is due to the direct proportionality between path length and absorbance.

As previously discussed, the Beer Lambert law states that there is a linear relationship between absorbance and concentration. However, when investigating larger concentrations, interactions between neighbouring atoms can cause non-linearity by affecting the absorbing ability at the given wavelength. As no light source is truly monochromatic, there are limits to the Beer Lambert law although these can be improved using background correction methods.

2.1.1.1 Atomic absorption spectrometry (AAS)

The principle of AAS is that atoms of different elements absorb characteristic wavelengths of light. To analyse a sample to determine whether it contains a particular element means using light from that element. For example with aluminium, a lamp containing aluminium will emit light from excited aluminium ions, producing the right wavelengths to be absorbed by aluminium ions in the sample. As this light is absorbed, electrons are promoted to an excited state. During the process of AAS, the sample is initially atomised then a beam of electromagnetic radiation which is emitted from the

excited aluminium ions is passed through the vaporised sample. The greater the number of aluminium atoms in the sample, the more radiation is absorbed; therefore, the amount of light absorbed is proportional to the number of aluminium atoms. A unique spectrum is achieved for each element due to a unique set of energy levels.

Kirchhoft's experiment can be used to demonstrate that gases absorb the same radiation they emit, which is a good illustration of atomic absorption. When white light projected through a split is dispersed by a prism, its constituent linear spectrum is observed. If this radiation source were to be replaced by a Bunsen burner, sprinkled with sodium chloride (NaCl) crystals, an emission of sodium is obtained presenting a yellow glow at 589nm and producing faded zones in its emission spectrum. This is the result of a high concentration of Na atoms in the flame which absorb these characteristic frequencies. This phenomenon is a manifestation of atomic absorption.

An AAS requires three components; a light source, a cell compartment to produce gaseous atoms and a method of analysing the specific wavelengths absorbed. The most common light source is a hollow cathode lamp containing a tungsten anode and cylindrical hollow cathode, composed of the element to be quantified. These are sealed in a glass tube, containing an inert gas such as argon.

During normal conditions, atoms are in their ground states (M) and upon light energy exposure are promoted to their excited state (M^*). (*Eq 2.1.1.1*)

$$M_{(g)} + h\nu \rightarrow M^*_{(g)} \quad (Eq2.1.1.1)$$

The ratio between these ground state (N_o) and excited state (N_1) is given by the Boltzmann distribution equation *(Eq2.1.1.2).*

$$\frac{N_1}{N_0} = \frac{g_1}{g_0}\left(e^{\left(-\frac{\Delta E}{kT}\right)} \right) \qquad (Eq\ 2.1.1.2)$$

In this equation, *k* represents the Boltzmann constant (1.381×10^{-23} J K^{-1}). Temperature is noted as T (K) and g_1/g_0 represent the statistical weight of these states, showing each number of degenerative states contributing to the make-up of that level.

Skoog *et al* (1998) shows that the energy difference between the ground (3s) state and the excited (3p) state for sodium is 3.37×10^{-19} J. Within these, there are 2 quantum levels in the 3s state and 6 quantum levels contained by this 3p state.

$$\frac{g_1}{g_0} = \frac{6}{2} = 3$$

When values are submitted into the Boltzmann constant equation, it is shown that at 2500K more than 99.98% of sodium atoms exist in their ground state

$$\text{With} \quad \frac{N_1}{N_0} = 3\exp\left(-\frac{3.37x10^{-19}J}{(1.38x10^{-23}JK^{-1})(2500K)}\right) = 1.72x10^{-4}$$

$$\text{And} \quad \%N_0 = 100 - \left(\frac{N_1}{N_0}x100\right) = 99.98\%$$

When the effect of temperature is examined, even with an increase of 10K, a value of $N_1/N_0 = 1.79 \times 10^{-4}$ is given. This temperature change hardly affects the ground state population and therefore has very little effect on atomic absorption. The temperatures produced in a graphite furnace atomiser coupled to an AAS, ensure maximum sensitivity.

2.1.1.2 Graphite furnace atomic absorption spectrometry (GFAAS)

Using a graphite atomiser is particularly useful when dealing with low analyte concentrations (ppb and ng/mL).

A few millilitres of sample is injected into a graphite tube through a dosing hole (~1-3mm). The tube is heated electronically in several temperature ramping cycles, the highest being up to 3000°C, this is the atomisation step and is required to convert the sample into gaseous atoms.

The entirety of this atomisation protocol consists of four stages: drying, pyrolysis, atomising and cleaning (*Fig 2.1.1.2.1*).The drying stage and pyrolysis stage involve the evaporation of solvent and removal of volatile matrix respectively. During atomisation, an atomic vapour of the analyte is formed during the decomposition of the prepared sample. The final stage concludes a cleaning process between each sample.

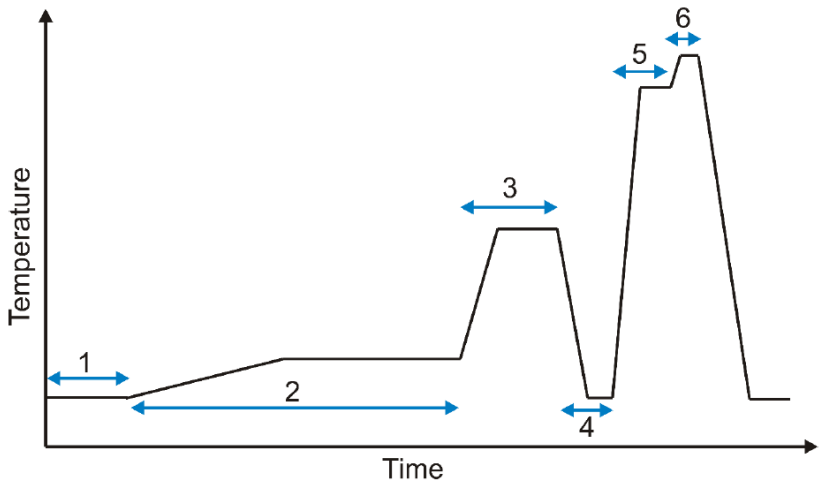

Fig 2.1.1.2.1: Schematic of the temperature cycle employed in GFAAS. 1 = sample introduction, 2 = drying, 3 = pyrolysis, 4 = cooling, 5 = atomisation and 6 = cleansing. Adapted from Butcher and Sneddon (1998).

Throughout the entirety of this step, argon gas is passed through each side of the tube to channel out vaporised sample through the injection site. The gas flow is only ceased for the atomisation step, purely to ensure maximum sample residence time and maintain a higher temperature which coupled, will provide thorough sensitivity.

The specific light energy emitted from the hollow cathode lamp is split and directed along two different paths; one beam is channelled throughout the atomised gaseous analyte, promoting the valence electrons to the higher energy level, while the other beam acts as a reference. These beams then pass through a monochromator which separate the multiple wavelengths receives from those from the analytical line. The detector then coverts the photons of light received at this wavelength into an electrical signal. The ratio of signal detected from the incident beam and transmitted beam respectively is logged and from these values, the absorbance, is recorded over the total analysis time. Following this step, the atoms are removed from the graphite tube. The absorbance value will increase in consequence to more atoms being formed. The concentration can then be accurately determined by using a range of calibration standards of known analyte concentration and compared using fit analysis.

GFAAS offers many advantages over other instruments used for elemental analysis. Very low sample volumes can be used offering a high level of sensitivity therefore lower limits of detection. Despite this, analysis using GFAAS is very slow compared to other conventional methods of elemental detection; one replicate can take up to three minutes for a complete step cycle. The technique may also give a poor quality analysis result on a sample with many interfering matrices. More accurate absorption values may be obtained through a specific temperature design ramping protocol, removing this interfering matrix prior to the atomisation step. This measurement quality may also be improved

if the vaporisation of the analyte is prevented until the wall of the tube reaches a constant temperature. A L'vov platform may improve the efficiency of this step (*Fig 2.1.1.2.2*).

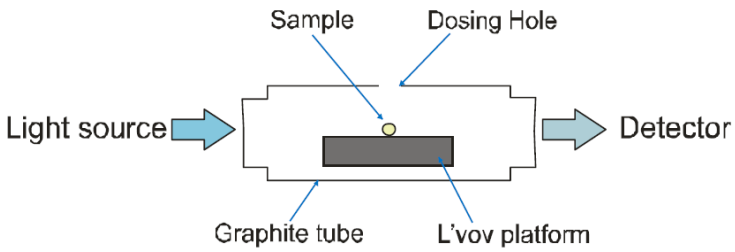

Fig 2.1.1.2.2: L'ov platform inside a graphite furnace

The L'vov platform has minimal connections to the wall of the graphite tube which means that it heats at a steadier rate allowing a more constant temperature to be accomplished prior to the sample becoming atomised, giving a more accurate recording of absorbance. The furnace reaching this uniform temperature reduces any memory effects between readings.

Analyte measurements may also be improved through the incorporation of matrix modifiers. A matrix modifier will increase the volatility of the matrix, thermally stabilising it, therefore reducing any chemical interferences. This is particularly useful when measuring elemental concentration in biological samples, for example silicon in urine; nickel chloride was used as a matrix modifier for the determination of silicon in urine, enhancing sensitivity and reducing background absorption (Kobayashi *et al.* 1997a; Matsusaki *et al.* 1996).

As previously discussed, the Zeeman background correction is used for those spectral interferences. This involved the addition of a 0.8T magnetic field. Measurements are made with and without a magnetic field; upon the addition of a magnetic field, only background readings are measured, whilst when the magnetic field is turned off, background and sample measurements are taken into account. The difference between these two readings equate to the measurement of the sample. The problem here is the interference of the magnetic field, the Zeeman background correction fails to take this into account.

The carbon of the graphite tube can cause refractory carbides to form coupled with a loss of silicon dioxide from the atomisation process, causing a loss of sensitivity and reproducibility. These limitations can be overcome by coating the graphite tube with titanium carbide and as previously discussed, incorporating a matrix modifier into the atomisation step. Matsusaki *et al*. (1996) carried out experiments involving a 0.04 mol L^{-1} cobalt nitrate and 0.01 mol L^{-1} boric acid mixture. The detection limit here was noted to be 0.0006mg L^{-1}, providing high analytical sensitivity.

The graphite tube is pyrolytically coated to minimise analyte diffusion into the material, reduce memory effect and reduce production of carbides (reducing chemical reactivity). This tube is enclosed by a blanket of argon to prevent combustion at the elevated temperatures witness in the graphite tube. The temperatures in this analysis chamber can be controlled up to 2700^{o}C, with ramped heating up to 1500^{o}C/s.

This technique is not suitable for multi element simultaneous sample analysis due to the need for separate cathode lamp and furnace parameters per element. High dilution factors are required for the high sensitivity and limited calibration range of AAS.

2.1.1.3 UV-Vis Spectroscopy

UV-Vis spectroscopy also involves the investigation of the absorption of photons as a principle. All compounds of different colours will absorb different wavelengths of radiation. UV-Vis makes use of this fact and can detect light absorption in the range of 190-900nm, covering ultraviolet, visible and near infra-red portions of the electromagnetic spectrum. The technique can be routinely used to quantify organic compounds by measuring this degree of absorption and the wavelengths at which this absorption occurs. The readings of absorption typically seen using UV-Vis measure between 0 (no absorption) and 2 (99% absorption).

Energy is required to promote an electron to its excited state. When light passes through a 'coloured' complex, this energy corresponds to that particular wavelength and is sufficient to promote or excite a molecular electron to a higher energy orbital. As a rule, energetically favoured electron promotion will be from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). The resulting species referred to as existing in its excited state.

The light transmitted adopts the complementary colour to that of the absorbed wavelength. In the case of the creatinine complex, an absorbance of 500-520nm is observed.

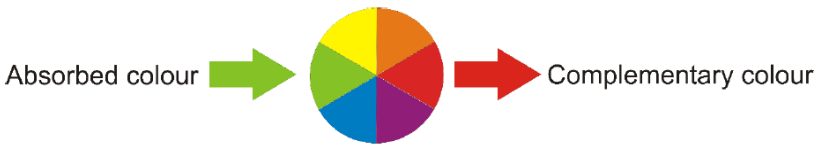

Fig 2.1.1.3.1 Colour wheel representing absorbed colour and their complementary colours

The absorbance value witness corresponds directly to the number of absorbing molecules in the sample. These absorbing molecules are known as chromophores. Chromophore are molecular

identities able to absorb light in the visible region of the spectrum and display colour. Chromophores possess a conjugated system of *pi* electrons or a lone pair of non-bonding valence electrons.

Conjugation is an important concept when analysing chromophores. Conjugation can cause an absorption maximum at a longer wavelength, known as a bathochromic shift phenomenon. During this study, picrate was added to creatinine to form a larger conjugated compound, shifting the absorption to a longer wavelength, inducing a colour change of yellow to orange/red. This phenomenon was also noticed when quantifying silicic acid in water samples, when a colour change was observed from yellow to blue.

2.1.2 Quantification of compounds using UV-VIS Spectroscopy

UV-VIS spectroscopy was used to quantify amounts of creatinine in urine using the Jaffe reaction, and silicic acid concentration in water samples using the molybdenum blue reaction, details of which are provided in the following sections.

2.1.2.1 Creatinine

Creatinine (structure shown in Fig 2.1.2.1.1), is the major breakdown product of creatine. Creatine is a component of muscle of which its main role is to facilitate recycling of adenosine triphosphate (ATP), the energy currency of the cell.

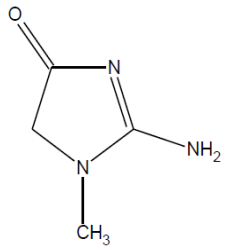

Fig 2.1.2.1.1 Molecular structure of creatinine

Creatinine however, has no known function and is readily excreted from the body in urine at a reasonably consistent rate (Rodriguez *et al*. 2004). Detecting levels of creatinine is an important diagnostic medical tool to monitor glomerular filtration rate (GFR), which has clinical importance in measuring kidney function as well as muscular function (Costa *et al.* 2007; Spierto *et al.* 1997). In this study however, creatinine will be measured to account for dilution factors in the sampling protocol.

The concentration of creatinine observed is sensitive to age and lean body mass, it can naturally range between 500-2000mg/day (Dugdale, 2009). Individuals that regularly carry out a lot of sport will naturally have a higher proportion of lean body mass, therefore excreting a larger amount of creatinine per day. Vegetarians however, will on average excrete less creatinine per day as they will consume less creatinine in their diets (Smith-Palmer, 2002).

Creatinine excretion remains quite linear for each individual, ranging only slightly from any variations in diet and exercise. Creatinine values can fluctuate throughout the day, corresponding to dietary intake, work load and volumes of liquids consumed (Clarke, 1961).

2.1.2.2 Jaffe reaction for the quantification of creatinine

The Jaffe reaction was used for the colourmetric measurement of creatinine in urine. In 1886, Max Jaffe (1841–1911) wrote about the basic principles of the paper 'Über den Niederschlag, welchen Pikrinsäure in normalem Harn erzeugt und über eine neue Reaction des Kreatinins' (1886) in which he described the properties of creatinine and picric acid in an alkaline solution. The colour change that is observed is directly proportional to the concentration of creatinine

The method using in the investigations presented in this thesis was adapted from Gentaur (2004) and Accura Diagnostics Inc. (2009) although other current methods can include; capillary electrophoresis (CE), biosensors and HPLC.

In this Jaffe reaction, an alkaline picric reagent is added to a prepared sample, producing a red coloured complex when combined with structures of creatinine. The intensity of this red colouring formed in the reaction mixture directly correlates with the concentration of creatinine and can be accurately quantified using UV-Vis spectroscopy, measured at 520nm (Clarke, 1961; Cambridge Biomedical Research Group, 2009).

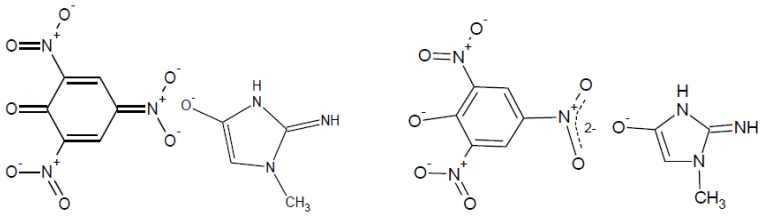

Fig 2.1.2.2.1 Possible interactions of the creatinine – picrate complex (Vasiliades, 1976)

The Jaffe reaction is a reproducible and reliable method of quantifying creatinine in urine samples, as interferences from picrate interactions with glucose and proteins are not as high in urine as they are in serum.

2.1.2.1.1 Stability of creatinine

During the urine collection protocol, patients were asked to keep urine samples in a cool place, such as a fridge. Once the samples had been collected and transported to the University they were stored in the appropriately labelled laboratory refrigerator until removed for analysis. It was imperative to investigate the stability of creatinine in urine and consider how long the sample could be kept for and what conditions must be maintained. Readings of creatinine may be lower in older samples, if creatinine were to be unstable.

A study looking into the decomposition of creatinine in sheep urine by Van Niekerk et al. (1963), showed a rapid decay when urine is stored above room temperature, however, when he stored his samples below 4°C there was no notable change in the concentration of creatinine over a 5 month period.

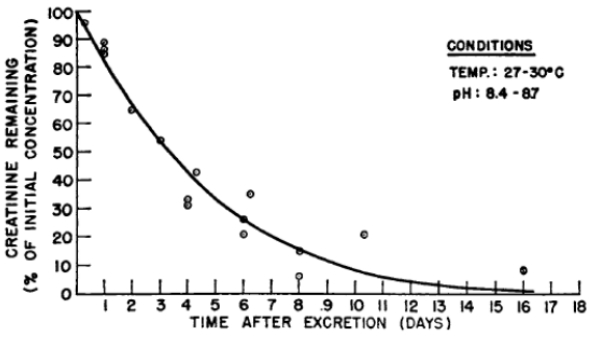

Fig. 1 The rate of loss of creatinine from sheep urine stored at 27 to 30° C at its normal pH (8.4 to 8.7).

Fig 2.1.2.1.1 The percentage reduction in creatinine concentration over time in sheep urine stored between 27-30ºC (Van Niekerk et al. 1963).

It is worth noting that the pH of sheep urine is higher than that of humans, ranging from 8.4-8.7, therefore being slightly alkaline (Van Niekerk *et al,* 1963). Human urine has a larger range, due to a more varied diet, of 4.6-8, although is typically around 6.5 (Smith-Palmer 2002). More reliable methods of analysis can be obtained without treating the urine with acid, therefore creatinine concentration is acquired prior to acid digestion.

As with sheep's urine, when human urine is maintained at a higher temperature, the creatinine value depletes at a faster rate. When human urine is kept at <4°C, it is much more stable over that same period of time (*Fig 2.1.2.1.2).* In fact, at room temperature, a creatinine reduction of 0.5% was witness compared with a 3% reduction at 55°C (Spierto *et al*. 1997).

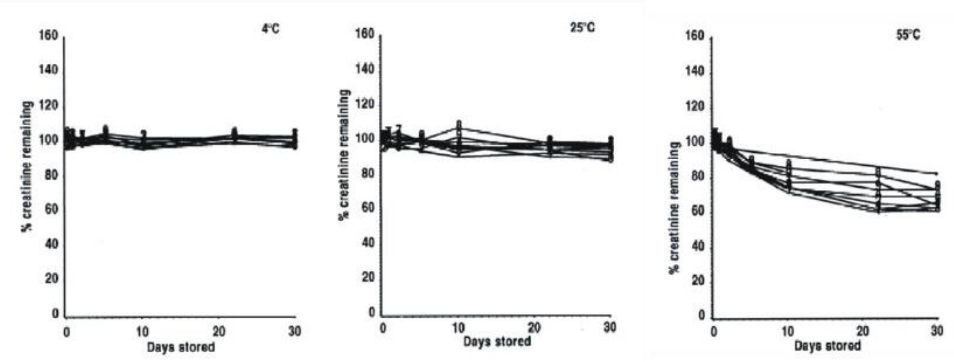

Fig 2.1.2.1.2 Creatinine levels (% of initial values) in human urine stored at 4, 25 and 55˚C for 1hour to 30 days (Spierto et al. 1997)

In summary, literature indicates that the manner in which urine is stored leaves creatinine concentration virtually affected. The urine should also be left untreated prior to analysis ((Miki & Sudo 1998; Ng *et al*. 1984). The National Committee for Clinical Laboratory Standards (Lockitch *et al.* 1997)

advise that sample analysis should take place no more than 2 hours after sample collection, and if this cannot be accomplished, the sample must be kept refrigerated.

2.1.2.3 Quantification of silicic acid by molybdenum blue

The molybdenum blue method is generally used to determine the concentration of soluble silica in water samples due to its high degree of sensitivity, allowing for the determination of trace concentrations of silicon (*Karge Weitkamp, 2002*). The determination of silicon is an example of the use of heteropoly-molybdenum blue in analytical chemistry. Silicic acid ($SiOH_4$) combined with an acidic solution of Mo^{VI}, in this case ammonium molybdate, produces the yellow complex $SiMo_{12}O_{40}^{3-}$, which has an α-Keggin structure (Eq 2.1.2.3.1).

$$7Si(OH)_4 + 12H_6Mo_7O_{24}\cdot 4H_2O + 17H_2O \Leftrightarrow 7H_4SiMo_{12}O_{40}\cdot 29H_2O \quad (Eq\ 2.1.2.3.1)$$

The reaction could be stopped here and transferred to a UV-Vis cell for analysis, with readings taken at 568nm but a further step and colour change is often used to observe increased sensitivity and accuracy. The $SiMo_{12}O_{40}^{3-}$ anion can be then reduced by ascorbic acid to form the blue coloured β-keggin ion, $SiMo_{12}O_{40}^{7-}$, a silicomolybdic acid cluster (Fig 2.1.2.3.1). The amount of the blue coloured ion produced is proportional to the amount of $Si(OH)_4$ present. The blue analyte can then be measured using UV-VIS Spectroscopy, set at 700nm, to determine the concentration of $Si(OH)_4$.

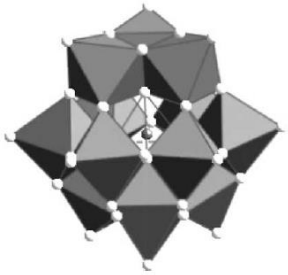

Fig.2.1.2.3.2 Structure of the silicomolybdic acid cluster. The silicon atom (in grey) is caged by twelve MoO_6 octahedra (oxygen atoms in white). (Coradin et al. 2004)

2.1.3 TH GFAAS analysis

2.1.3.1 Reagents

Laboratory reagent grade HCl (12.4M, Fisher Scientific, UK) was used in solutions for rinsing and storing of polyethylene equipment and storage containers. An analytical grade HNO_3 (15.9M, Fisher Scientific, UK) was used for all acid digests and acidification of samples. Ultrapure water from and Elga option R 7/15 (conductivity ≤ 6.8×10^{-6} S m^{-1}) was used throughout.

2.1.3.2 Treatment of microwave vessels

In an effort to limit contamination, microwave vessels (PFA Teflon©) were stored in 5% HCl and rinsed 3 times with UPW then allowed to dry before use. In the case of urinary analysis, 5mL of urine samples (20% v/v HNO_3) and 1.25mL sweat samples (20% v/v HNO_3 / 30% w/v H_2O_2) were added to the

microwave vessels which were weighed before and after the digest to check for any loss of sample. One method blank acidified to 20% v/v HNO_3 was also added for approximately every 10 samples for quality assurance. Used vessels were cleaned well with detergent (Decon 90) and rinsed 3 times with UPW. Each vessel was then put through the microwave digest programme with 1mL of HNO_3, rinsing a final 3 times with UPW before being transferred back into the 5% HCl acid bath.

2.1.3.6 Microwave digest programme

The above described digestions of sweat and urine samples were carried out using the Microwave Accelerated Reaction System, Model MARSXpress (CEM Mircowave Technology Ltd, UK). Prepared samples were heated from room temperature to 180ºC over a 10minute period; they were then held at 180ºC for 15minutes (1600W) before a final cooling period over 30minutes (0W). Vessels were then removed from the system and left on the work top to cool to room temperature.

2.1.3.7 Instrumentation

Concentrations of total Si and Al were measured using an AAnalylist 600 atomic absorption spectrometer with a Transversely Heated Graphite Atomiser (THGA). This was equipped with a longitudinal Zeeman effect background corrector and an AS-800 autosampler (Perkin-Elmer UK). Standard THGA pyrolytic-coated graphite tubes with an integrated L'vov platform were used (Perkin Elmer UK).

2.0mL acrylic TH GFAAS sample cups (Gradco International Ltd) were used and were rinsed with the sample before use. Each measurement was performed using lumina hollow cathode lamps and the software employed by the system was Winlab 32 (Perkin Elmer, UK). The TH GFAAS instrument parameters are shown in Table 2.1.3.7.1 and the temperature programmes are shown in Table 2.1.3.7.2The Zeeman background corrected peak area of the atomic absorption signal was used for the determination of each experimental reading was the mean of three injections with relative standard deviation <10% accepted for each sample.

Table 2.1.3.7.1 TH GFAAS instrument parameters for the measurement of aluminium and silicon in human urine and sweat samples.

Instrument Parameters	Aluminium	Silicon
Lamp current (mA)	25	30
Wavelength (nm)	309.3	251.6
Bandwidth (nm)	0.7	0.2
Injection Volume (µL)	30	30
Injection Temperature (°C)	20	20
Pipette speed (%)	100	100
Read Time (s)	2	3
Delay Time (s)	0	0
BOC Time (s)	0	0

Table 2.1.3.7.2: TH GFAAS atomisation programme for aluminium and silicon.

Stage	Temperature (^oC)	Ramp Time ($^oC/s^{-1}$)	Hold Time (s)	Internal Flow ($Lmin^{-1}$)
Aluminium				
Drying	110	10	40	250
Drying	130	15	40	250
Pyrolysis	500	10	10	250
Pyrolysis	1200	10	15	250
Atomisation	2300	0	5	0
Cleansing	2300	1	5	250

Stage	Temperature (^oC)	Ramp Time ($^oC/s^{-1}$)	Hold Time (s)	Internal Flow (Lmin-1)
Silicon				
Drying	110	10	40	250
Drying	130	15	40	250
Pyrolysis	1200	10	20	250
Atomisation	2350	0	5	0
Cleansing	2450	1	5	250

2.1.3.8 TH GFAAS calibration standards

TH GFAAS was calibrated by automated serial dilution from 300, 60, 100, 40 ppb (*w/v*) standard solutions of Si, Al, Fe and Cu respectively, with 1% HNO_3 to give the standards outlined in Table 2.1.3.8. Solutions were prepared by the dilution of 10 ppm (*w/v*) stock solutions with 1% HNO_3 in 10 mL borosilicate volumetric flasks. The 10 ppm (*w/v*) stock solutions were prepared by dilution of 1000 ppm (*w/v*) (2% HNO_3) pure atomic spectrometry certified standards (Perkin Elmer, UK) with 1% HNO_3 in 100mL polyethylene volumetric flasks.

Table 2.1.3.8: TH GFAAS calibration standard concentrations

Analyte	Concentration of calibration standards (ppb w/v)
Silicon	50, 100, 200, 300
Aluminium	10, 20, 40, 60

Blank correction was performed using 1% HNO_3. Non-Linear through zero WinLab 32 generated calibration fits were applied (Perkin Elmer, UK).

2.1.3.9 Dilutions of urine samples

Urine samples were diluted to achieve concentrations within the calibration range. Analysis for Al, Fe and Cu were made using a 1:1 dilution (digested sample: 1% HNO_3); silicon required higher dilutions of: 1:50, 1:100 and 1:200. Therefore, dilution effects were investigated for silicon.

2.1.3.9 Dilution effects on silicon determination

24-hour treatment samples from three participants were used to investigate the effects of dilution on the Si readings. These samples were chosen because the lowest dilution (1:50) was within the calibration range. The results of these measurements are shown in Table 2.1.3.9.1.1.

Table 2.1.3.9.1.1: The effect of dilution on the measured mean (n = 3) silicon concentrations

Sample ID	Measured Means (ppb w/v) for dilutions of			Percentage Difference from the 1:50 Dilution		
	1:50	1:100	1:200	1:50	1:100	1:200
24F8W1-3	299.72	153.04	71.15	0.00	-2.10	5.18
24F1W12-1	301.52	152.71	76.27	0.00	-1.29	-1.17
24M4W1-4	272.5	134.49	65.36	0.00	1.30	4.14

As the Si samples are highly diluted in order to obtain an absorbance reading in the middle of the calibration range, it was not considered necessary to use matrix modifiers as high dilutions will reduce matrix effects.

2.1.3.10 Quality control for TH GFAAS

As some error is inherent to all analytical techniques, it is important to quantify this error and ensure that it does not exceed a tolerable level.

2.1.3.10.1 Limit of detection (LoD)

The LoD is the smallest quantity of analyte which is significantly different from the blank. TH GFAAS LoD for Si and Al were determined from three times the standard deviation (*n* = 3) of the absorbance of the 1% HNO_3 calibration blank divided by the slope of the calibration fit. Means and SD of the LoD for each element are provided in *Table 2.1.3.10.1.1.*

Table 2.1.3.10.1.1: Mean (SD) limit of detection of silicon and aluminium

TH GFAAS Limit of Detection µg L-1		
Element	**Silicon**	**Aluminium**
Mean	4.53	0.43
SD	3.5	0.71
n	77	84

2.1.3.10.2 Limit of quantification (LoQ)

LoQ is the amount of analyte which carries a reasonable degree of statistical certainty. TH GFAAS LoQ for Si and Al were determined from ten times the standard deviation (n = 3) of the absorbance of the 1% HNO3 calibration blank divided by the slope of the calibration curve. Means and SD of the LoQ for each element are provided in *Table 2.1.3.10.2.1*

Table 2.1.3.10.2.1: Mean (SD) limit of quantification of silicon and aluminium

TH GFAAS Limit of Quantification µg L-1		
Element	**Silicon**	**Aluminium**
Mean	15.1	1.42
SD	11.66	2.35
n	77	84

2.1.3.11 Method blanks

Method blanks were used to determine the level of contamination which occurs from the procedure; the blanks were treated in exactly the same way as the samples, i.e. acidified to 20% *v/v* HNO_3 and digested, they were then diluted with 1% HNO_3 before analyses in relation to the element being determined. Frequency distributions are given for the method blanks after dilution factors were taken into account (*Figure 2.1.3.11.1*). The mean was taken from measured values as a source of contamination.

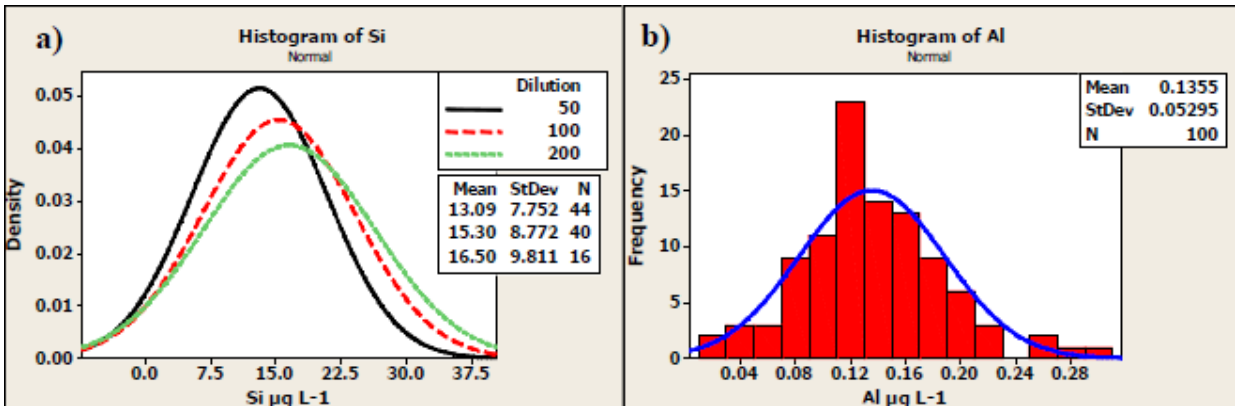

Figure 2.1.3.11.1: Frequency distributions of method blanks for ***a)*** *Si at dilutions 50, 100 and 200,* ***b)*** *Al.*

2.1.3.12 Routine calibration standards

Throughout analysis, standards of known concentrations are analysed to determine any changes in signal. A reading is considered adequate when it is within 10% of the known concentration value and subsequent measurements fall within 15% of the initial reading. Frequency distributions have been expressed for the initial calibration standards (CS1) and the continuing calibration standards (CS2, CS3 and CS4) (Fig 2.1.3.12.1). Percentage differences from these initial calibration values are given in *Table 2.1.3.12.1*.

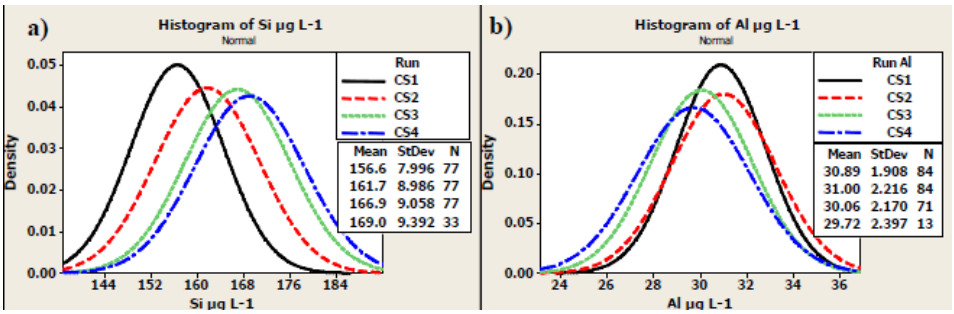

*Figure 2.1.3.12.1: Frequency distribution of calibration standards for **a)** Si and **b)** Al. This figure represents CS1 as the initial calibration standard and CS2, CS3 and CS4 are the continuing calibration standards.*

Table 2.1.3.12.1: Mean concentrations (µg L^{-1}) of calibration standards (M) with standard deviations (SD) and percentage difference (%D) from initial concentration standard (CS1).

	Silicon			Aluminium		
	M	SD	%D	M	SD	%D
CS1	157	8	N/A	30.9	1.91	N/A
CS2	162	8.89	3.23	31	2.22	0.29
CS3	167	9.06	6.41	30.1	2.17	2.84
CS4	169	9.39	5.42	29.7	2.4	3.27

2.1.3.13 Replicate samples

Replicates of the same sample were also measured to evaluate the precision of the equipment. The precision of the instrument was accepted if the replicate samples were within a percentage difference of 5. Examples are given in Appendix 1.

2.1.3.14 Duplicate samples

Duplicates of samples were independently analysed to determine the precision of the preparation methods. The precision of the preparation methods was accepted if the duplicate samples were within a percentage difference of 5. Examples are given in Appendix 2.

2.1.3.15 Spike recovery

A known concentration of analyte was added to a sample as a spike, the recovery of which allowed determination of the amount of analyte lost and may suggest the occurrence of any interferences. Spikes were added to selected urine samples and method blanks (reagent spikes). The quantity of spike added were selected to gain a concentration at the mid-point of the calibration curves at 100% recovery (*Table 2.1.3.15.1*).

Table 2.1.3.15.1: Spike recovery from reagents and urine samples

	Reagent Spike		Urine Sample Spike	
Element	**Mean recovery % (n = 10)**	**SD**	**Mean recovery % (n = 10)**	**SD**
Silicon	99.73	3.42	103.00	2.39
Aluminium	98.23	2.68	100.52	2.29

On summary of this data, a high reliability of the analytical methods is shown through approximately 100 % of the reagent and sample spikes being recovered; in this instance the need to account for interferences is not required.

2.1.3.16 Contamination control

Treatment of samples was performed in the clean room. Blank samples (UPW) were stored in plastic vessels and analysed in order to test for contamination. All plastic ware was washed and stored in acid bins to reduce adsorption of metals.

2.2.0 Materials and methods for the determination of creatinine by UV-Vis spectroscopy

2.2.1. Reagents

Reagent grade sodium hydroxide (2M, Fisher-Scientific, UK) and reagent grade picric acid (1%, Sigma-Aldrich, UK) were used in the determination of creatinine concentration in urine. Anhydrous creatinine (Sigma-Aldrich, UK) was used in the calibration. UPW was used throughout.

2.2.2 Treatment of samples

Urine samples were diluted by 10 fold with UPW and mixed (1:10) with a freshly prepared reactant comprising of equal amounts of 1% picric acid and 0.75M NaOH; these were then agitated and left to react for 20 minutes before analysis by UV-Vis spectroscopy. Each sample was analysed three times and an average was taken.

2.2.3 Instrumentation

Creatinine concentration was determined using a Lambda 14 UV-Vis spectrometer (Perkin Elmer, UK), set to read wavelengths between 400–600 nm; maximum absorbance was at 520 nm.

2.2.4 Determination of creatinine concentration

An 8 mM stock solution of creatinine was prepared by addition of anhydrous creatinine to UPW. This stock solution was serially diluted to give calibration standards containing 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 8 mM creatinine in UPW. The absorption of each calibration standard at 520 nm (25°C, cuvette path length 1 cm) was recorded. An example of a typical Beer Lambert calibration curve is shown in Figure 2.2.4.1.

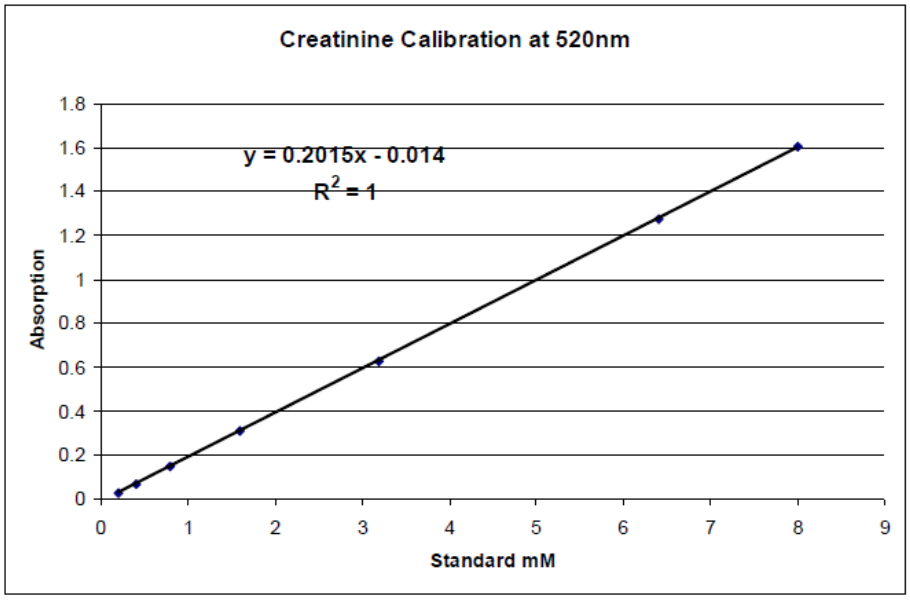

Figure 2.2.4.1: A standard Beer Lambert calibration curve for the creatinine-picrate complex: the absorbance of aqueous solutions containing creatinine concentrations between 0.2 and 8 mM measured using UV-Vis at 520nm.

The Beer-Lambert calibration curve (*Fig. 2.2.1.3.1)* was linear (R^2=1) up to and including the highest concentration (8mM) indicating that its application is suitable for the determination of creatinine

concentration. However, the standards 6.4 and 8 mM gave absorbance readings > 1.0 and so 3.2 mM was chosen as the highest calibration standard during testing.

2.2.5 Quality control for the determination of creatinine

2.2.5.1The limit of detection

The LoD was determined to be 0.00324 ± 0.00168 A.

2.2.1.5.2 The limit of quantification

The LoQ was determined to be 0.0108 ± 0.00559 A.

2.2.1.5.3 Precision

Precision was determined by sampling five replicates of 5 separate samples (Table 2.2.1.5.3.1). Each sample was diluted by 1:10, removing matrix interference and ensuring that they were within this measured detectable range of calibration. The reproducibility of each sample, highlighted by the low standard deviations, indicates that the precision of the technique is acceptable.

Table 2.2.1.5.3.1: sampling of five replicates of 5 separate samples to determine precision in the quantification of creatinine using the Jaffe reaction and UV-Vis Spectroscopy.

Sample	Measured Creatinine Concentration mM					Mean	SD
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5		
F001-24-1	2.25	2.27	2.32	2.34	2.29	2.3	0.004
M004-24-3	6.24	6.19	6.27	6.25	6.19	6.2	0.036
F009-24-3	4.32	4.35	4.31	4.36	4.28	4.3	0.032
F013-24-4	4.22	4.26	4.17	4.25	4.19	4.2	0.038
M007-24-1	3.98	3.92	3.95	3.97	4.05	4	0.048

2.2.1.5.4 Spike recovery

A known concentration of creatinine was added to selected urine samples as a spike and the recovery determined (*Table 2.2.1.5.4.1).*

Table 2.2.1.5.4.1 Spike recovery in creatinine analysis.

Sample	Spike Concentration (mM)	Total Creatinine Concentration (mM)		Recovery (%)
		Expected	Measured	
F015-24-1	4.00	10.1	9.92	92.77
	8.00	14.1	14.44	102.64
	16.00	22.1	22.48	101.85

Recovery was within the 10% acceptable limit, indicating the reliability of the analytical methods without the need to account for interferences.

2.3.0 Detection of elements within mineral waters

The brand of mineral water used within the healthy volunteer study and MS study was provided by the Malaysian company Spritzer. This mineral water, Spritzer, is commercially available within Malaysia, but was not available within the U.K during the time of the study. It is now commercially available under the name ACILIS.

The amount of Si and Al within a variety of mineral waters was determined by TH GFAAS (Table 2.3.1).

Table 2.3.1: Mean and SD of the amount of Si and Al detected in the mineral waters (n = 10).

	Amount in 1L of the water	
Brand of Water	**Silicon (µmol)**	**Aluminium (nmol)**
Spritzer	1053 ± 16	84 ± 6
Volvic	576 ± 17	124 ± 6
Evian	24 ± 7	81 ± 4
Buxton	31 ± 4	69 ± 5
Fiji	1879 ± 32	127 ± 6
Island Chill	768 ± 18	131 ± 8

2.4.0 Study Protocols

These studies aimed to investigate if silicic acid-rich mineral waters could be used as a completely non-invasive tool of 'everyday' diets to lower the body burden of aluminium. For this reason all volunteers, for each procedure, were asked to continue as normal with their daily routine and were not requested to avoid any products other than mineral waters not provided for the study. Specific aims for each study are provided at the beginning of the relevant results section.

The technique of measuring the urinary excretion of Al has been used as a non-invasive indicator of the body burden of Al (Bellia *et al.* 1996; Exley *et al.* 1996; Roberts *et al.* 1998; Exley *et al*., 2006).

Control measurements were always used to obtain estimates of volunteer's 'normal' urinary excretion of Al. Comparisons of the urinary excretion of aluminium, before and after consumption of Si-rich mineral water, enabled accurate determination of the influence of the mineral water on the body burden of Al (see for example, Exley *et al*. 2009). Witnessing an initial increase in aluminium excretion would propose the removal of systemic aluminium from body stores. A reduction in aluminium excretion over time would suggest that aluminium body burden has been reduced (see Exley, 2012). Details of methods used here are provided in the relevant chapters.

2.4.1 Silicic acid-rich mineral water as a non-invasive method of reducing the aluminium body burden in healthy individuals

Nineteen healthy volunteers, comprising of 9 males and 10 females, with a mean age of 24 years (range 18-34 years), were recruited through Keele University. This was attained by personal invitations to volunteer via email, and through oral communication. All volunteers provided written consent prior to participation. Ethical approval for the study was obtained from Keele University Research Ethics Committee (favourable outcome is included in the appendix).

The inclusion criteria for participation consisted of individuals between the ages of 18-35 years, who were considered to be in good health, could carry out a mild exercise regime and would not suffer any practical difficulties from the ingestion of up to 1.5L mineral water over a short time period.

The exclusion criteria removed any individuals with any medical conditions or reoccurring ailments (e.g. bladder infections) which may lead to discomfort and/or influence results of the study. Those taking any medications containing the examined elements in the study, such as antacids, were excluded from the study as this may affect the results.

The project protocol was divided into two objectives (detailed below); each volunteer was requested to participate in both parts of the study. Therefore the same group is used for both of the following procedures.

2.4.1.1 Record Sheets

It was a crucial aspect of the study to ensure that participants did not change their diets or lifestyles in any way for the study. They were asked to complete a Life Style Questionnaire which was devised to give an approximate value of their daily exposures to Al. Dietary record sheets were also completed for the duration of each 24 hour urine collection procedure to observe the amount and type of beverages consumed within these periods, this information is provided for each volunteer in the appendix of this thesis. It was decided too much to ask for a full dietary analysis (as silicic acid will be released from food during digestion); the most bioavailable form of silicon is in the form of silicic acid found in the water. Therefore a general diet analysis and beverage record sheets were decided to give ample information.

Volunteers were asked to consume the full amount of mineral water suggested, 1.5L per protocol. Although if this wasn't possible, they were asked to record the volume that they could comfortably manage to consume within the given time period.

2.4.1.2 Objectives

Each of these protocols involved a time period in which the participant consumed the silicon rich mineral water and provided the urine sample for analysis.

Sweat samples were collected at the time of the study.

a) 24 hour trial (Chapter 3)

Investigation of total urinary excretion of Si and Al in 24h samples.

Nineteen participants, 9 male and 10 female (age range 18–34) were recruited from the student population at Keele University and ethical approval was obtained from the University's Life Sciences Ethical Review Body (Details of which are provided in the appendix). All participants were self-assessed as healthy and agreed to partake in a mild exercise program as part of the procedure for collecting the urine samples

Participants provided a 24h urine sample before and following the consumption of up to 1.5L of mineral water, these before and after periods are hereafter referred to as control and treatment periods.

The treatment period involved two different aspects, a loading and dosing mechanism. Following the first urinary excretion after waking, the volunteers were requested to collect all subsequent urinary excretions up to and including the first sample the following morning. This is a standard procedure for the collection of a 24-hour urine sample (CLS, 2007). For the loading treatment period, participants were asked to consume as much of the 1.5L of mineral water as they could manage between their first and second urinary excretion of the day. For the second part, referred to as the dosing protocol, the participants were instructed to consume approximately 150mL of the water each hour for 10 hours of the day. This way, the most effective measure of silicon delivery could be established.

Differences in the urinary excretions of Si and Al between control and treatment periods were determined using the paired *t*-test, in addition comparisons between males and females were performed using the 2-sample *t*-test.

Results for the 24 hour studies are displayed as being corrected for total amount excreted in the 24 hours. These results display the total excretion of the elements in the urine sample. These results

provide the most effective information on the urinary excretions of the elements and are regularly used in similar studies (CLS 2007).

Both creatinine-corrected data and 24 hour corrected data were collected for each individual.

b) 30 – minute exercise trial (Chapter 4)

Investigation of sweat excretion of Si and Al collected after 30 minutes of mild exercise

Nineteen participants, 9 male and 10 female (age range 18–34) were recruited from the student population at Keele University and ethical approval was obtained from the University's Life Sciences Ethical Review Body (Details of which are provided in the appendix). All participants were self-assessed as healthy and agreed to partake in a mild exercise program as part of the procedure for collecting the sweat samples.

An appropriate material was selected for the absorption of sweat, details of this analysis is presented in Chapter 4.3.1. This material, cut into 5 x 5cm sections, were soaked in 5% HCl for 2 hours, UPW for 24 hours, and then placed in a drying oven overnight before use. Prior to the exercise protocol, the upper back was cleaned using a pad immersed in 100% ethanol, then six pieces of this prepared material was transferred to the upper back of each individual, covered with a plastic film and secured with an adhesive medical tape to prevent any allergic reaction to adhesives. The individual was then instructed to complete a total of thirty minutes cycling on an exercise bike, including five minutes initial fast paced cycling, twenty minutes moderate paced cycling and a final five minutes fast paced cycling. Once this program was completed, the sweat soaked material was transferred to 7mL centrifuge tubes and span down at 6000RPM for 6 minutes to extract the volume of sweat collected in the material to the bottom of the tube, this series of steps is shown in Figure 2.4.1.2.1.

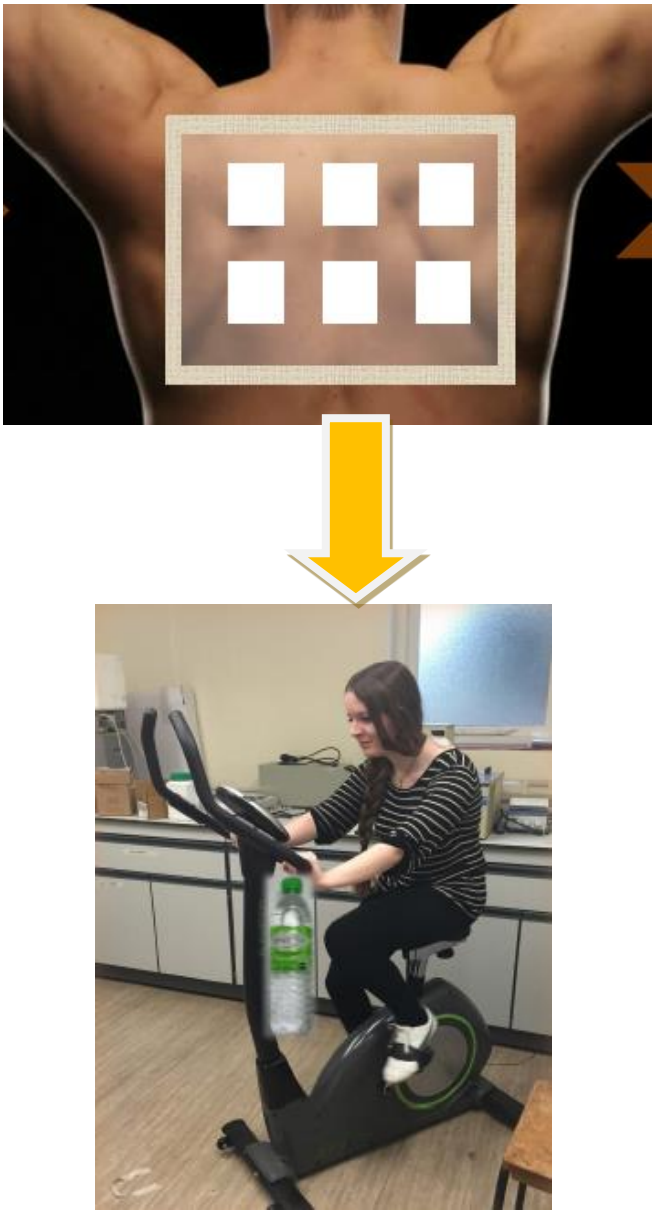

Figure 2.4.1.2.1: Sweat collection apparatus and exercise protocol

Differences in the excretions of Si and Al in sweat between control and treatment periods were determined using the paired *t*-test, in addition comparisons between males and females were performed using the 2-sample *t*-test.

2.5.0 Silicic acid-rich mineral water as a non-invasive method of reducing the aluminium body burden in multiple sclerosis participants

Fifteen multiple sclerosis patients were recruited through the University Hospital of North Staffordshire (UHNS, now Royal Stoke) through the MS department by a qualified clinician following the inclusion and exclusion criteria. Ethical approval was attained by myself, from the national research ethics committee (North Staffordshire NREC). All volunteers provided written consent prior to participation, which was obtained by a qualified clinician.

All SPMS patients attending the Neurology clinics were screened for possible inclusion in the study. Suitable and interested patients will be posted a participant information pack, including the PIS and coversheet, explaining the outline of the study. This was followed up via telephone calls/postal letters by a study clinical to confirm their interest and participation. Those who were interested were asked to be come back to a research clinic where a screening assessment was carried out by a study clinician and eligible patients were asked to sign the consent form. A standard time period of 24 hours was given between the receipt of the study information sheet and taking informed consent.

The inclusion criteria included: All patients must have a confirmed diagnosis, by a study clinician, of secondary progressive multiple sclerosis (SPMS) according to the McDonald inclusion criteria. Patients must be willing and able to give informed consent in line with the Mental Capacity Act. Patients had a carer to help ensure that the protocol was followed accordingly. Carer was willing and able to give informed consent. Patient would not suffer any ill effects or practical difficulties on the consumption of up to 1L of mineral water and had no restriction on fluid ingestion. As well as any patients not satisfying the above inclusion criteria, a potential study participant would be excluded; those patients on disease modification treatment, patients with current urinary infections and patients with a history of impaired renal function. The patient could be initiated onto the study once an infection free urine

sample had been provided. During recruitment, a Kurtjze EDSS score was given to each patient. This was followed up at the end of the study to look for improvements in mobility, as well as a questionnaire querying general wellness.

One patient (008) had to be withdrawn from the study due to a series of urine infections. The mean age of the remaining 15 MS patients who completed the study, was 72 (range 56 - 81) and 8 were female.

2.5.1 Protocol

The project protocol was divided into two objectives; the primary objective was to measure the urinary excretions of Si and Al over a 24 week period during which patients consumed up to 1.5L of silicon-rich mineral water each day. The first 12 weeks of the study, referred to as the control, required each participant to provide urine samples following their normal diets, thus acting as their own control to determine individual changes. The secondary objective was to observe changes in mobility using EDSS scores at the beginning and end of the study.

2.5.2 Urinary excretions

This study examined the effects of long-term consumption of mineral water on the urinary excretion of Si and Al. The study duration was split up into two parts, the first 12 weeks (W1-W12), known as the control period and the second 12 weeks (W13-W24) referred to as the treatment period.

Participants collected all of their urine (24 hour samples) for the 5 days in W1, W12, W13 and W24 into 3L sterile containers. During the rest of the study weeks, the first morning sample of the day on the same day of each week was collected into sterile 50mL containers. These collections were made for the baseline (W1 to W12 - no mineral water consumption) and treatment periods (W13 to W24 - including consumption of up to 1.5L of mineral water each day).

Differences in the urinary excretions of Si and Al between control and treatment weeks 1, 2, 13 and 24 for each individual were determined using one-way ANOVA and Tukey's paired comparison tests. Differences for control and treatment data were measured using repeated measures ANOVA and Wilcoxon signed-rank tests.

Results were displayed as creatinine-corrected data in order to account for the deviations in urine volumes of spot samples. Volunteers were requested to consume as much as the 1.5L of the provided mineral water as they could comfortably manage. This volume is the same as the healthy individual urine study because the consumption of 1.5L every day for a period of 12 weeks seemed acceptable to the patients, although, unlike the loading treatment in the healthy volunteer study, patients in this study were able to make up their normal drinks with the water, essentially replacing other water consumed in their daily diets with the Spritzer.

It was thought to be inconvenient for the patients to provide record sheets as in the health volunteer study. Instead, the patients were provided with a tick sheet to recognise their water consumption and sample collection.

As in the healthy volunteer study, results are recorded both before and following correction for creatinine concentrations, and 24 hour samples were expressed in both formats.

The requirements for this study were maintained at a minimum so not to make participation too complexed for individuals suffering from MS. Therefore, dietary record sheets, as collected in the healthy individual, study were not required during this study. The length of the control period satisfied this as it meant that a thorough indication of normal Si and Al excretion could be deduced.

Differences in the urinary excretions between the control and treatment periods were determined using the paired t-test, and comparisons between males and females were performed using the 2-sample t-test. An aged matched healthy control group was not sought after for this study, but instead data available from other studies was used (Minshull *et al* 2014).

2.5.3 EDSS Score

The Kurtzke Expanded Disability Status Scale (EDSS) is a method of quantifying disability in multiple sclerosis. The scale has been developed by John F. Kurtzke (1983).

Each individual completed the EDSS test prior to and following the treatment of the mineral water, this was performed by the qualified clinician. Comparisons between the initial and final score were used to determine whether the consumption of the mineral water had any influence on their mobility. It is accepted that an EDSS score reduced by 0.5 or more points (Kurtjze 1983) indicates an improvement in mobility performance.

Overall scores of the EDSS tests were provided by the clinical nurse on the completion of the urinary analysis and are noted in the appendix of this thesis, referenced by patient ID. Detailed reports of the performance of the tests (i.e. scores of each section) were not made available for this thesis due to patient confidentiality. Patient questionnaires were collected post study to determine any improvements in health, wellbeing and general feedback.

2.6.0 Biostatistical analysis

As discussed within the study protocols, the purpose of these analyses were to assess the use of silicic acid-rich mineral waters as a potential non-invasive therapy to reduce aluminium body burden. The primary null hypothesis was that the consumption of silicic acid-rich mineral waters would have no effect on the urinary excretion of aluminium, thereby, would have no effect on aluminium body burden.

Analysis of the biological data has been statistically examined using the software Minitab ® 15. This section includes a description of the general statistical tests that were conducted to identify changes in urinary excretions of Si and Al. Details on the statistical tests used within this thesis can be found in (Zar, 1996).

The results are ordered by study (HV U, HV S and MS) and within each study are ordered by element (Si andAl). Comparisons are made between control and treatment periods, male and female populations using the appropriate tests.

2.6.1 Power analysis

Power analysis with values of 80% and 90% were calculated using Minitab's Power and Sample Size calculation for a single sample. This was in order to provide an indication of the sample size needed for each study to conduct a statistically reliable experiment (*Table 2.6.1.1*). Expected differences (SD) were derived from Exley *et al*. (2006) and used to calculate the effect size (Difference/SD).

Table 2.6.1.1: Sample size needed for each study.

Expected difference	SD	Effect size	Sample size	
			Power 80%	Power 90%
24	24	1	10	13

2.6.1 P-value

The P-value was accepted as statistically significant when it was below P = 0.05. All confidence intervals were set to 95%. P-values were noted to 3 decimal places and a P-value less than 0.001 was reported as < 0.001.

2.6.2 Normality and transformations

Parametric tests assume that the data has a normal distribution; these were always attempted before the non-parametric tests. They are more powerful and offer increased accuracy in determining statistical significance. However, if the data wasn't to follow a normal distribution, these parametric tests may not provide reliable results.

Anderson-Darling normality checks were performed on the residual data for each data set; $P > 0.05$ directed that the data followed a Gaussian distribution and could therefore be considered as being normally distributed. Data sets which did not follow a normal distribution were rendered normal using logarithmic transformations.

If the transformation of the data didn't normalise the distribution, non-parametric tests were used as these are distribution free, reducing the influence that outlying data points have on the overall data set.

Minitab highlighted outliers in the data but these points were only removed from the data set if there was reason to assume that the results were unreliable (i.e. contamination); it's possible to suggest that some outliers represented natural biological variability. Where a direct comparison was made between data sets and at least one set was not normally distributed the selection of non-parametric tests were used.

2.6.3 Study variables

Independent variable = urinary/sweat excretion under control conditions (i.e. before consumption of the mineral water).

Dependent variable = urinary/sweat excretion under treatment conditions (i.e. after consumption of the mineral water).

Confounding variables = differences in dietary intake, including the amount of mineral water the individual managed to consume.

2.6.4 Statistical tests used

The different parametric tests used within this study are highlighted in Table 2.6.10.1. Details of the test and their non-parametric equivalents are described below.

2.6.5 Pearson's correlation coefficient and linear regression

Purpose - Test for relationships between two variables

Rational - Simple linear regression and correlations performed to determine a relationship between variables and to describe this particular witnessed relationship. The best fit was determined by Minitab using the available method of least squares.

The Pearson's correlation coefficient (r) measures the strength of linear relationships. Values are between -1, which indicates a perfect negative relationship and 1, which indicates a perfect positive relationship. The non-parametric analogue used was the Spearman's rank correlation.

2.6.6. Paired t-test

Purpose - Compare the means of two measurements within a single group.

Rational - The paired t-test calculates the difference between two measurements for each individual member of a population and determines whether the mean difference is different from zero. This test was used to compare urinary excretions before and after the treatment of mineral water. Wilcoxon signed rank is the non-parametric equivalent to the paired t-test. This test compares the medians of the two groups.

2.6.7 One-way ANOVA

Purpose – Compare the variance in the mean for more than two measurements between groups.

Rational - One-way ANOVA uses the F-distribution to compare the variance between the means of three or more measurements and to determine whether or not there is a difference. The F-distribution is related to the F-crit value to determine significance of the data. This test was used to determine whether an individual showed a difference in their urinary excretions between the two control and treatment weeks.

The F-distribution is calculated by dividing the sum of squares between each group (SSB) by the sum of squares within each group (SSW).

$$F = \frac{\text{Variance due to difference between means}}{\text{Variance due to difference within means}}$$

Tukey's paired comparison was used in conjunction with ANOVA to distinguish where the differences lie between specific pairs. Kruskal-Wallis test is the non-parametric equivalent to one-way ANOVA.

2.6.8 Repeated measures ANOVA

Purpose – Compare variance in the mean for more than two measurements for a single group.

Rational – Repeated measures ANOVA uses the F-distribution (as described above) to compare the variance between the means of three or more measurements and to determine whether or not there

is a significant difference. This test was performed in Excel, and was used to determine whether the group showed a difference in their urinary excretions between the control weeks and treatment weeks.

2.6.9 2-sample t-test

Purpose - Compare the means of a single measurement between two different populations.

Rational - The 2-sample *t*-test calculates the difference between the means of two populations which are independent of each other. The difference in means is compared with an approximation of the standard error calculated between the two populations. This test was used to compare the urinary excretion of the elements between different groups, i.e. between males and females, BMI data and relationships between age. Mann-Whitney U test is the non-parametric equivalent to the 2-sample *t*-test. This compares the medians of the two groups.

Table 2.6.10.1: Summary of the different parametric tests used within the statistical analyses

Statistical test	Study		
	HV		MS
	Urine	Sweat	
Pearson's Correlation Coefficient	✓	✓	✓
paired *t*- test	✓	✓	✓
2 sample *t*- test	✓	✓	✓
One way ANOVA			✓
Repeated measures ANOVA			✓

Chapter 3. - Silicic acid mineral water as a non-invasive method of reducing aluminium body bioburden in healthy individuals.

3.1.0 Aims

The aim of this chapter is to investigate if regular drinking of a silicon-rich mineral water can facilitate the removal of Al from the body of healthy individuals.

3.2.0 Introduction

Previous studies have demonstrated that silicic acid reduces the gastrointestinal absorption of aluminium (Edwardson *et al.* 1993), facilitating the removal of systemic aluminium within the body (King *et al*. 1997; Exley *et al*. 2006). Certain commercial mineral waters, such as Volvic, provide a good source of silicic acid and could potentially be utilized as a preventative measure against the accumulation of aluminium within the body. This could be exploited in young, healthy individuals, which could in turn reduce the rate and slow the progression of aluminium-related disorders. However, the role of silicon in drinking water and how humans handle this silicon has not been widely studied, particularly with respect to healthy individuals.

The purpose of this work was to elucidate the effect of drinking silicic acid-rich mineral waters on the urinary excretion of silicon in healthy individuals. The main aim of this research was to investigate the influence of a silicic acid-rich mineral water, as a non-invasive therapy, on the urinary excretion of aluminium. This was investigated following the objectives detailed in Chapter 2. Data for each individual are provided in Appendix 3.

3.3.0 Methodology

Nineteen healthy volunteers, comprising of 9 males and 10 females, with a mean age of 24 years (range 18-34 years), were recruited through Keele University. This was attained by personal invitations to volunteer via email, and through oral communication. All volunteers provided written consent prior to participation. Ethical approval for the study was obtained from Keele University Research Ethics Committee (See Appendix for favourable outcome letter).

The inclusion criteria for participation consisted of individuals between the ages of 18-35 years, who were considered to be in good health, could carry out a mild exercise regime and would not suffer any practical difficulties from the ingestion of up to 1.5L mineral water over a short time period.

The exclusion criteria removed any individuals with any medical conditions or reoccurring ailments (e.g. bladder infections) which may lead to discomfort and/or influence results of the study. Those taking any medications containing the examined elements in the study, such as antacids, were excluded from the study as this may affect the results.

The project protocol was divided into two objectives (detailed below); each volunteer was requested to participate in both parts of the study. Therefore the same group is used for both of the following procedures.

3.3.1 Treatment of urine samples

Urine samples were collected and stored directly into pre-acid washed 3L polypropylene containers for each twenty four hour trial. The twenty four hour sample volumes were measured, vortex to ensure uniformity, and a portion transferred to a washed 50mL Falcon™ tube for storage in a clearly marked refrigerator. The remaining sample was disposed of down the toilet and the jug thoroughly cleaned with decon. An aliquot of un-acidified urine sample was taken and diluted to 10% with UPW for creatinine (Crt) analysis using the Jaffe method (Toora, 2002). The remaining urine was acidified using 15.8M HNO_3 to 20% *v/v* and subjected to microwave digestion to prepare samples for measurement of total silicon and aluminium by THGFAAS.

3.3.1 Record Sheets

It was a crucial aspect of the study to ensure that participants did not change their diets or lifestyles in any way for the study. They were asked to complete a Life Style Questionnaire which was devised to give an approximate value of their daily exposures to Al (see Appendix).

Dietary record sheets were also completed which indicated the amount and type of foods consumed by each participant in their average daily diet. It was decided that it was too much to ask for a full dietary analysis (as silicic acid will be released from food during digestion); the most bioavailable form of silicon is in the form of silicic acid found in the water. Full beverage record sheets were decided on to give the most appropriate information.

Volunteers were asked to consume the full amount of mineral water suggested during the loading protocol, 1.5L in 1 hour. Although if this wasn't possible, they were asked to record the volume that they could comfortably manage to consume within the given time period.

3.3.2 Investigation of total urinary excretion of Si and Al in 24h samples.

Participants provided a 24h urine sample before and following the consumption of up to 1.5L of mineral water, these before and after periods are hereafter referred to as control and treatment periods.

The treatment period involved two different aspects, a loading and dosing mechanism. Following the first urinary excretion after waking, the volunteers were requested to collect all subsequent urinary excretions up to and including the first sample the following morning. This is a standard procedure for the collection of a 24-hour urine sample (CLS, 2007). For the loading treatment period, participants were asked to consume as much of the 1.5L of mineral water as they could manage between their first and second urinary excretion of the day. For the second part, referred to as the dosing protocol, the participants were instructed to consume approximately 150mL of the water each hour for 10 hours of the day. This way, the most effective measure of silicon delivery could be established.

Differences in the urinary excretions of Si and Al between control and treatment periods were determined using the paired *t*-test, in addition comparisons between males and females were performed using the 2-sample t-test.

Results for the 24 hour studies are displayed as being corrected for total amount excreted in the 24 hours. These results display the total excretion of the elements in the urine sample. These results

provide the most effective information on the urinary excretions of the elements and are regularly used in similar studies.

Methods of analysis are detailed in Chapter 2.

3.4.0 Results

This chapter includes all the statistical comparisons between the control and treatment data for the 24-hour trial. Comparisons were made for the overall group and for males and females separately; however, male and female data are only presented within the main text when showing a noteworthy difference from the overall group.

Results are displayed as total amounts excreted and expressed as 24-hour data.

The group size (n) is equal to 19, 9 male and 10 female.

3.4.1 Urinary creatinine and sample volume

Creatinine (Crt) concentrations were measured for each sample in order to correct for differences in renal functioning between individuals and to take any dilution effects of the urine into consideration.

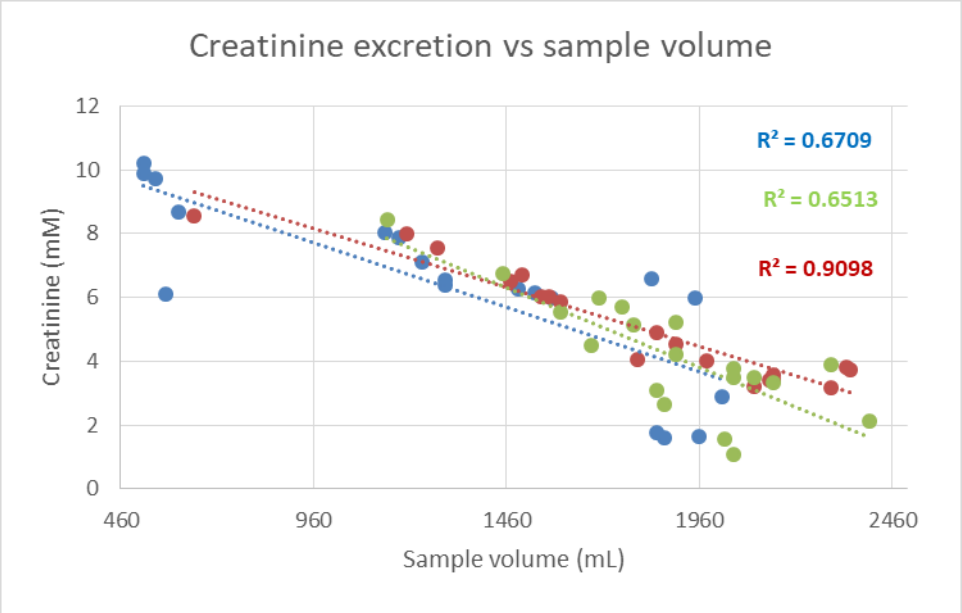

Figure 3.4.1.1: Crt concentration (mM) against sample volume (mL) for control (●) and treatment samples, loading (●) and dosing (●) in a 24-hour urine sample.

There was no significant difference between males and females in mean Crt concentrations within all protocols; the control protocol gave an average Crt measurement of 4.7mM for males and 7.7mM for females (t = 2.87: P = 0.012), the loading treatment gave an average Crt measurement of 5.3 mM for males and 4.9mM for females (t = 0.82: P = 0.424) and dosing treatment periods gave an average Crt measurement of 4.0 mM for males and 4.4mM for females (t = 1.09: P = 0.293). There was also no significant difference between males and females in the mean volumes collected within both treatment protocols; the loading protocol gave an average volume of 1866mL for males and 1708mL for females (t = -1.1: P = 0.29), and the dosing treatment gave an average volume of 1963mL for males and 1811mL for females (t = -2.38: P = 0.031) periods (*Table 3.4.1.1).*

Table 3.4.1.1 Mean (SD) Crt concentration (mM) and sample volume (mL) provided in a 24-hour urine sample for the control and loading/dosing treatment periods for males (n = 9) and females (n = 10).

	Creatinine Concentration (mM/24h)		Sample Volume (mL/24h)	
	Male	Female	Male	Female
Control	4.7 (2.7)	7.7 (1.7)	1705 (336.8)	968 (446.5)
Loading Treatment	4.9 (1.5)	5.3 (2.0)	1866.7 (385)	1709 (509.5)
Dosing Treatment	4 (1.9)	4.4 (1.9)	1963.3 (357.9)	1810.5 (219.4)

Females revealed a higher Crt in their urine, specifically for the control period, equating to an average of 7.7mM (1.7) compared with an average male Crt concentration of 4.7mM (2.7).

In comparison, urinary volume collected for the 24 hour period was, on average, larger in males than females. This difference was significant during the baseline collection period (t = -3.97: P = 0.001), when an average urine collection was measured at 1705mL in males and 968mL in females. During the treatment protocol, the amount of urine collected during the 24 hours was comparable, 1867mL (385) collected for males and 1709mL (SD=510) collected for females.

3.4.2 Comparison between the urinary excretion of silicon with the volume of silicon rich mineral water consumed

The mean amount of all beverages imbibed was significantly increased for the loading treatment period compared to the control period for the overall group, increasing from 1527mL (±501mL) to 2579mL (±139mL), (t = -5.84, P < 0.001). When considered separately (*Table 3.4.2.1*), this increase was less significant for males (t = -3.61, P = 0.006), presenting an increase of beverages consumed from 1918mL (±245) than for females (t = -4.47, P = 0.001), who in the control consumed 1175mL (±398mL) and in the loading treatment consumed 2592mL (±154mL). Consumption of the mineral water provided approximately half of the total volume of beverages consumed in the loading treatment period for the group, total beverages equalled 2579mL (±139mL) and each individual consumed on average 1357mL of the provided mineral water during this protocol. When completing the mineral water dosing protocol, the mineral water provided approximately three quarters of the total volume of beverages as total consumed equalled 2112mL (±177mL) and each individual consumed all 1500mL of mineral water during this protocol.

3.4.2.1 Total mineral water excreted vs amount of urinary volume and silicon excreted

Males consumed significantly more than females for both the control (t = 2.53, P = 0.020), and both loading and dosing treatment (t = 2.05, P = 0.060) periods, males consumed 1918mL (±245mL), 2564mL (±128mL) and 2142mL (±229mL) respectively compared to females who consumed 1175mL

(±398mL), 2592mL (±154mL) and 2086mL (±119mL) respectively. In addition, males consumed a greater volume on average of the mineral water than females (t = 4.31, P = 0.001), 1447mL (±81mL) compared to 1437mL (±137mL).

There was a significant relationship (Figure 3.4.2.1.1) between the urinary excretion of Si with the total urine collected for the loading treatment period (r = 0.48, P = 0.022) and dosing treatment period (r = 0.44, 0.036), suggesting that a higher urinary volume produces a larger Si excretion. The relationship was not significant for the control (r = 0.28, P = 0.200) where no additional Si was being added to the diet.

When looking into the difference in Si excretion between the control and combined treatment periods (i.e. excretion of Si in the treatment group minus excretion of Si in the control group), this overall relationship was considered as insignificant (r = 0.26, P = 0.226) when considering the total amount of beverages consumed over the 24-hour periods (i.e. total volume of beverages consumed in the treatment group minus total volume of beverages consumed in the control portion of the study).

The amount of Si measured in the provided mineral water was 865±17µmol L^{-1}. There was no correlation (r = 0.35, *P* = 0.099) between the difference in Si excretion (i.e. Si excreted in either treatment period minus Si excreted in control period) and the amount of Si present in the imbibed mineral water.

Table 3.4.2.1.1: Total volume (mL) of all beverages consumed over the 24-hour period

Volume of beverages consumed (mL)					
	Control	Loading treatment		Dosing treatment	
	Total	MW	Total	MW	Total
Group	**1526** (501)	**1442** (111)	**2579** (139)	**1500**	**2112** (177)
Males	**1917** (245)	**1447** (81)	**2564** (128)	**1500**	**2142** (229)
Females	**1175** (398)	**1437** (137)	**2592** (155)	**1500**	**2086** (119)

Total refers to the sum of all beverages consumed over the 24-hour period. MW is the volume of mineral water consumed; this counts towards the total in the treatment period.

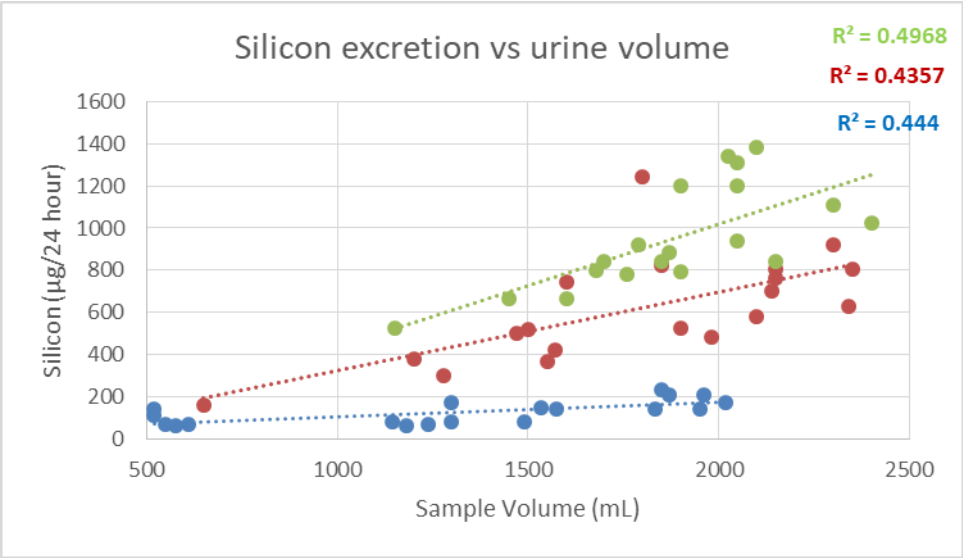

Figure 3.4.2.1.1: Amount of Si excreted (µg/24h) against total urine excreted during the 24 hours for the control (●) and loading treatment (●) and dose treatment (●) samples.

Table 3.4.2.1.2: Percentage of Si consumed as mineral water which equates to the difference in Si excretion between the control and treatment period.

Ref	Amount of silicon consumed as mineral water (µmol)	Difference in silicon excretion (Loading treatment – Control, µmol)	Difference in silicon excretion (Dose treatment – Control, µmol)	Percentage of mineral water which equates to the difference in silicon - Loading	Percentage of mineral water which equates to the difference in silicon - Dose
M1	764	215	372	28	43
M2	865	511	1154	59	133
M3	865	634	939	73	109
M4	865	621	882	72	102
M5	865	665	800	77	93
M6	865	612	993	71	115
M7	865	711	993	82	115
M8	793	223	803	28	93
M9	761	363	782	48	90
F1	865	533	751	62	87
F2	865	91	597	10	69
F3	865	447	720	52	83
F4	865	322	607	37	70
F5	643	499	761	78	88
F6	724	1160	702	160	81
F7	865	432	771	50	89
F8	865	372	1197	43	138
F9	865	382	1200	44	139
F10	865	488	657	56	76
ẍ	831 (64)	488 (232)	825 (215)	59 (31)	95 (25)

ẍ represents the mean values. Pink represents females and blue represents males.

The concentration of Si in the urine collected from the dosing treatment protocol was larger than that collected from the loading dosing protocol, equating to 951.1µmol/24hr (±245.8 µmol/24hr) compared to 614.2 µmol/24hr (253.0 µmol/24hr). This equated to a difference in Si

excretion of between 59 ± 31% for the loading treatment and 95± 25% for the dosing period of the amount of Si consumed from the mineral water. An elevated Si excretion in the dosing protocol was seen for both male and female participants.

Patient ID F6 was an anomaly to this trend who excreted a much larger amount of Si during the loading protocol compared to the dosing protocol, measuring 1160 µmol/24hr and 702 µmol/24hr respectively, this individual was notably one of the two female patients who didn't consume the suggested volume of mineral water. All remaining participants were shown to excrete more Si during the dosing protocol than the loading treatment protocol (*Table 3.4.2.1.2)*.

3.4.3 Urinary excretion of silicon and aluminium in control and treatment periods

24-hour collections provide the most reliable information on urinary excretions and were collected for 2 consecutive days. One week was allowed between protocols. Data from each individual is considered below and presented in concentrations of nmol/24 hr for Al excretion and µmol/24 hr for Si excretion. When examined as a group (patient F6 omitted), Al excretion correlates positively with Si excretion ($r = 0.76$) during the loading protocol. This relationship indicates statistical significance for the healthy treatment protocol for long term Si consumption as a method of removing bioavailable Al ($P < 0.001$).

Silicon

There is a weak positive correlation between the urinary excretion of Si in the control and loading treatment data ($r = 0.20$, $P = 0.015$). When an individual presents a small urinary Si concentration during their baseline, a comparatively small concentration is noticed during the addition of a high dose of Si to the diet (*Figure 3.4.3.1)*. This is true for all of the data except for one patient, ID F007 presenting the largest concentration of Si in their loading treatment compared to a comparatively small Si concentration in the baseline quantification, equating to 1160µmol and 724 µmol respectively. Removing this outlier produced a stronger correlation between this data set ($r = 0.76$, $P = 0.012$). When considering the whole group data, correlation was stronger between the control and dosing treatment ($r = 0.40$, $P = 0.011$).

The mean excretion of Si during both treatment protocols is significantly higher ($t = -5.13$, $P < 0.001$) than the control period (Table 3.4.3.1); no individuals excreted more Si prior to drinking the mineral water.

Males excreted significantly more Si than females for the treatment period ($t = 2.95$, $P = 0.008$), but not for the control ($t = 1.30$, $P = 0.208$).

Table 3.4.3.1: ***Mean*** *(SD) urinary excretion of Si (µmol/24hr) for the healthy volunteer study population for both loading and dosing treatment protocols (n=7)*

Healthy Volunteer Urine Study Mean (SD) Si concentration (n=5)			
ID	**Si Concentration - Control (µmol/24 Hr)**	**Si Concentration - Loading (µmol/24 Hr)**	**Si Concentration - Dose (µmol/24 Hr)**
M1	150.7	365.2	523.2
M2	230.7	741.4	1384.4
M3	170.2	804.4	1109.2
M4	140.5	761.0	1022.6
M5	140.9	806.1	941.1
M6	210.2	822.6	1202.8
M7	210.7	921.4	1203.4
M8	80.3	302.8	883.3
M9	61.0	424.0	842.8
F1	170.3	703.5	921.6
F2	70.0	160.7	667.1
F3	70.3	517.2	789.9
F4	60.4	382.0	667.6
F5	80.9	580.1	841.7
F6	80.0	1240.3	781.9
F7	70.1	502.0	841.4
F8	111.0	483.2	1307.7
F9	140.5	522.6	1340.7
F10	140.7	628.4	798.0
Mean (SD)	**125.8 (55.3)**	**614.2 (253.0)**	**951.1 (245.8)**

Data presented for the whole study population in *figure 3.4.3.2* is a visual representation of the difference in Si excretion between the baseline and the loading protocol. This figure shows a similarity in this for fifteen out of the nineteen participants. Participant F7 presents a clear peak in this data, excreting more Si than other participants. Individuals F2, M2 and M8 excreted a smaller difference in Si between the control and treatment periods.

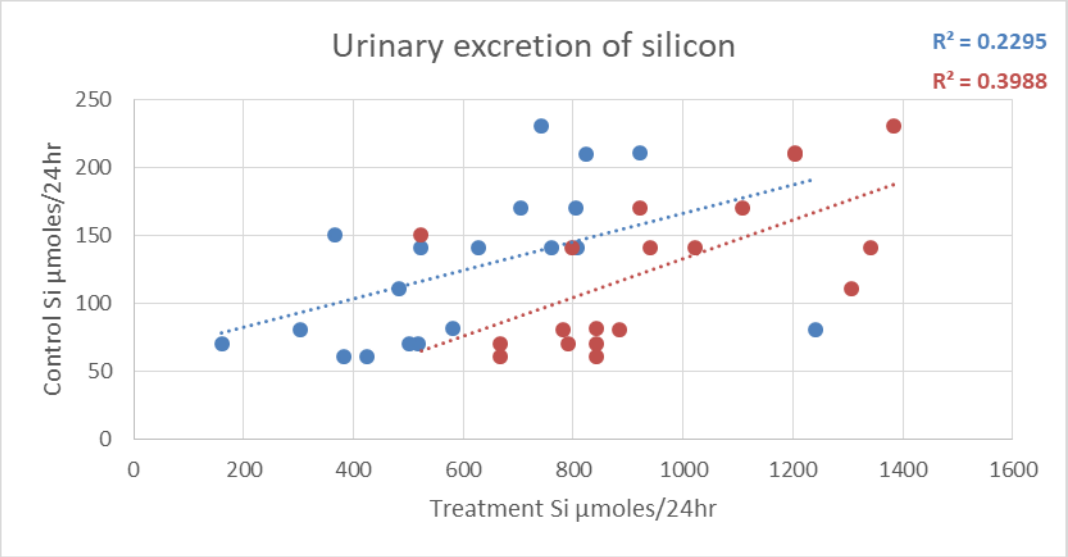

Figure 3.4.3.1: Urinary excretion comparison of Si (µmol/24h) during control and treatment periods. Control vs loading (●) and control vs dosing treatment (●)

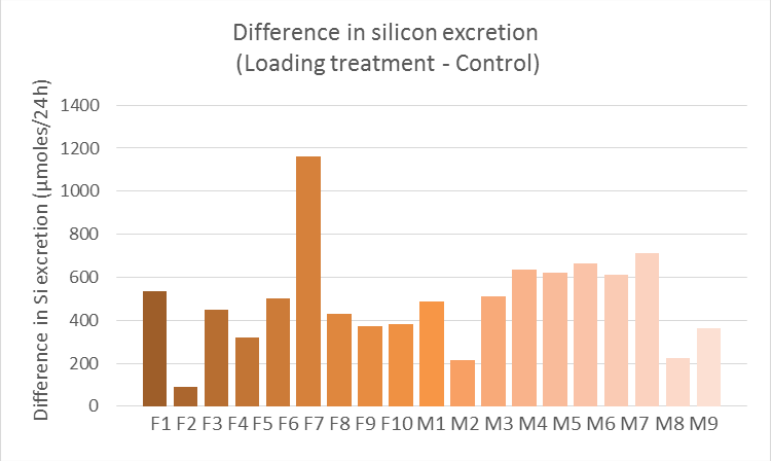

*Fig 3.4.2.2 **Difference** in Si excretion (µmol/24h) between control and treatment data for each individual (loading treatment – control) (n=5).*

Values collected for the difference in Si excretion between the dosing protocol and the baseline are consistently higher than the loading protocol in *Figure 3.4.3.2*. When visualising this data, it represents a similar pattern in that fifteen out of the nineteen participants present a similar difference in Si excretion. Patients F9, F10 and M13 excrete a notably higher amount of Si during this protocol, while patient M2 presents a much lower excretory value of Si, see *Figure 3.4.3.3.*

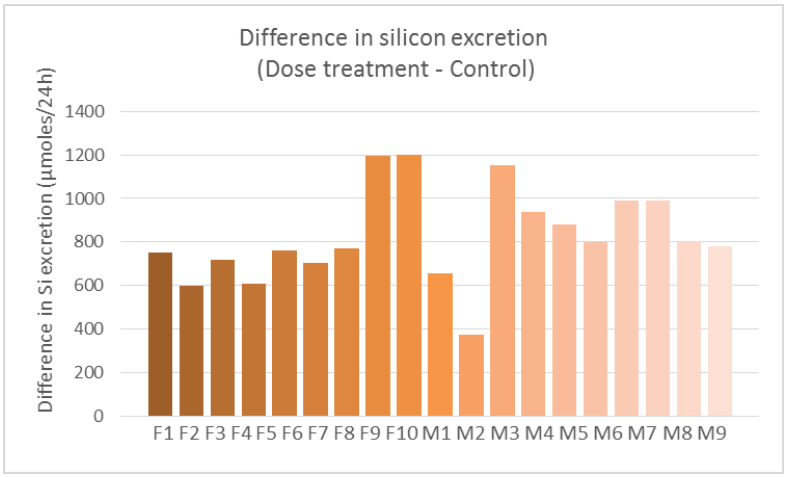

*Fig 3.4.3.3: **Difference** in Si excretion (µmoles/24h) between control and treatment data for each individual (dosing treatment – control) (n=5)*

Aluminium

The mean excretion of Al during both treatment protocols is significantly higher ($t = -4.87$, $P < 0.001$) than the control period (*Table 3.4.3.2*); no individual excreted more Al prior to drinking the mineral water. Males excreted significantly more Al than females for the treatment period ($t = 3.16$, $P = 0.006$), and the control ($t = 3.84$, $P = 0.011$).

Al excretion was highest during the loading protocol. Considering the whole study population, Al excretion rose from 934.4nmol/24 hr to 2889.1nmol/24 hr and 2049.6nmol/24 hr during the loading treatment and dosing treatment respectively.

Patient M1 and F6 saw a large increase in Al excretion during the loading protocol, although presented a urinary Al excretion similar to that of the baseline amount during the dosing protocol. Patient M1 revealed a mean baseline Al urinary concentration of 645.3±34.1nmol/24hr, giving rise to a urinary Al concentration of 1602.6±143.7nmol/24hr during Si loading. This value falls back to 741.6±28.5nmol/24hr during the dosing protocol.

*Table 3.4.3.2: **Mean** (SD) urinary excretion of Al (nmol/24hr) for the healthy volunteer study population for both loading and dosing treatment protocols (n=7)*

Healthy Volunteer Urine Study Mean (SD) Al concentration (n=5)			
ID	**Al Concentration - Control (nmol/24 Hr)**	**Al Concentration - Loading (nmol/24 Hr)**	**Al Concentration - Dose (nmol/24 Hr)**
M1	645.3	1602.6	741.6
M2	2044.4	3147.4	3042.5
M3	2021.6	4551.6	3726.4
M4	1949.1	4527.0	4216.6
M5	1367.6	4652.7	3144.6
M6	1842.0	3702.5	2961.0
M7	1944.8	5016.9	2643.5
M8	300.0	960.4	1004.8
M9	392.9	1723.6	1208.6
F1	1047.3	1531.0	1144.4
F2	661.5	1605.1	1271.5
F3	343.0	1647.6	1406.5
F4	622.5	2762.2	2408.2
F5	360.5	2003.1	1102.4
F6	287.5	1684.2	551.5
F7	567.1	3111.4	2762.4
F8	467.1	2781.7	2198.5
F9	546.9	3641.3	2000.1
F10	342.8	4240.6	1407.6
Mean (SD)	**934.4 (682.8)**	**2889.1 (1297.1)**	**2049.6 (1065.6)**

There was a significant positive correlation between the urinary excretion of Al in the control and loading treatment ($r = 0.5$, $P < 0.001$) as well as control and dosing treatment ($r = 0.6$, $P < 0.001$), as shown in *Figure 3.4.3.4*. When an individual presents a small urinary Al concentration during their baseline, a comparatively small concentration is noticed during the addition of a high dose of Si to the diet.

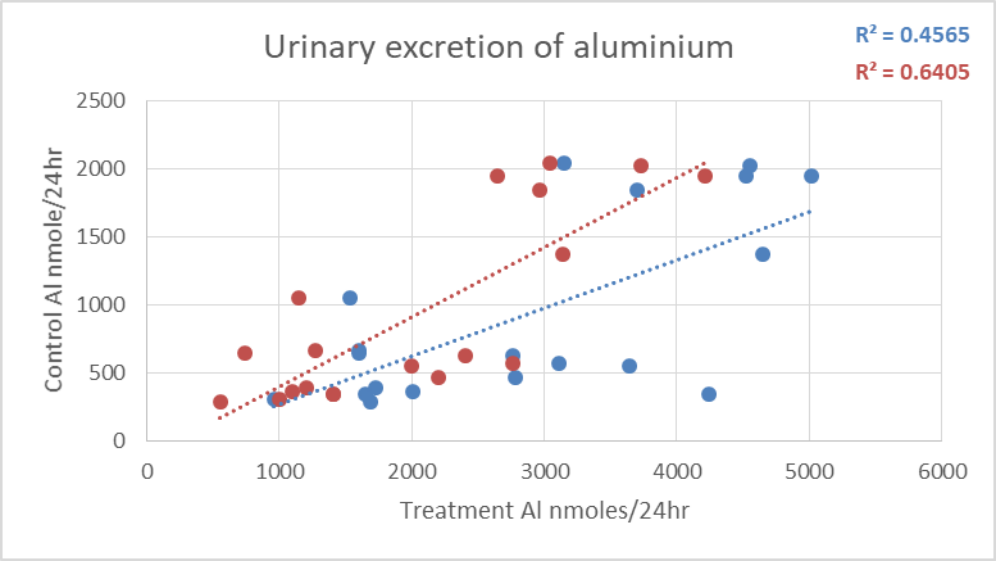

Figure 3.4.3.4: Urinary excretion comparison of Al (nmol/24hr) between the control and treatment periods. Control vs loading (●) and control vs dosing treatment (●)

Urinary excretion of silicon and aluminium in control and treatment periods

Urinary excretion of Al and Si differed greatly when a Si rich mineral water was added to the diet of nineteen healthy volunteers. The excretion of Si was higher during the dosing protocol, whereas the

concentration of Al in urine was higher during the loading protocol. These differences were more predominant in males than females.

*Table 3.4.3.3: **Mean** (SD) urinary excretion of Si (µmol/24hr) and Al (nmol/24hr) for the healthy volunteer study population for both loading and dosing treatment protocols*

Healthy Volunteer Urine Study Mean (SD) Si (µmol/24hr) and Al (nmol/24hr) concentration (n=5)						
ID	Control		Loading Treatment		Dose Treatment	
	Si	Al	Si	Al	Si	Al
M1	150.7	645.3	365.2	1602.6	523.2	741.6
M2	230.7	2044.4	741.4	3147.4	1384.4	3042.5
M3	170.2	2021.6	804.4	4551.6	1109.2	3726.4
M4	140.5	1949.1	761.0	4527.0	1022.6	4216.6
M5	140.9	1367.6	806.1	4652.7	941.1	3144.6
M6	210.2	1842.0	822.6	3702.5	1202.8	2961.0
M7	210.7	1944.8	921.4	5016.9	1203.4	2643.5
M8	80.3	300.0	302.8	960.4	883.3	1004.8
M9	61.0	392.9	424.0	1723.6	842.8	1208.6
F1	170.3	1047.3	703.5	1531.0	921.6	1144.4
F2	70.0	661.5	160.7	1605.1	667.1	1271.5
F3	70.3	343.0	517.2	1647.6	789.9	1406.5
F4	60.4	622.5	382.0	2762.2	667.6	2408.2
F5	80.9	360.5	580.1	2003.1	841.7	1102.4
F6	80.0	287.5	1240.3	1684.2	781.9	551.5
F7	70.1	567.1	502.0	3111.4	841.4	2762.4
F8	111.0	467.1	483.2	2781.7	1307.7	2198.5
F9	140.5	546.9	522.6	3641.3	1340.7	2000.1
F10	140.7	342.8	628.4	4240.6	798.0	1407.6
Mean (SD)	**125.8 (55.3)**	**934.4 (682.8)**	**614.2 (253.0)**	**2889.1 (1297.1)**	**951.1 (245.8)**	**2049.6 (1065.6)**

Data for the three protocols are shown in *Table 3.4.3.3* with means and deviations for the data population shown at the bottom of the table. Comparisons between male and female individual data are made later on in chapter 3.

The variance between the data points is smaller for Si than Al. In the control period, standard deviation in Si concentration is 55.3, whereas the deviation between the same data points is 682.8

for Al. The differences between the standard deviation values for both treatment protocols remains similar for Si, at 253.0µmol/24hr and 245.8 µmol/24hr respectively, this variance represents the difference in Si handling between individuals. Despite a significantly larger excretion in Al during the loading protocol, variability between the data for both loading and dosing protocol does not vary largely, equating to 1297.1 nmol/24hr and 1065.5 nmol/24hr respectively. Baseline excretion of Si ranged from 60.4-230.7µmol/24hr, this range increases to 365.2-1240.3µmol/24hr during the loading protocol and 523.2-1384.4µmol/24hr. This range further highlights the difference in Si handling between individuals. This difference may promote a similar affect in Al excretion, which is seen in the range of data points seen in urinary Al concentration throughout the three protocols. Baseline Al excretion was large and ranged from 287.5-2044.4nmol/24hr, this range increased to 960.4-5016.9nmol/24hr and 551.5-4216.6nmol/24hr for the loading and dosing treatment protocols respectively.

The small variance between Si excretion data points and large deviation between Al excretion data points is shown in *Figure 3.4.3.5.*

*Table 3.4.3.4: **Mean** (SD) excretion of Si (µmol/24h) and Al (nmol/24h) in control and treatment urine samples for the healthy volunteer group (n = 19).*

Urinary excretions in a 24-hour urine sample						
	Silicon (µmol/24h)			Aluminium (nmol/24h)		
	Mean	SD	Range	Mean	SD	Range
Control	126	55	60-231	967	687	288-2044
Loading treatment	614	253	161-1240	2814	1292	960-5017
Dosing treatment	951	246	583-1384	2085	1085	552-4217

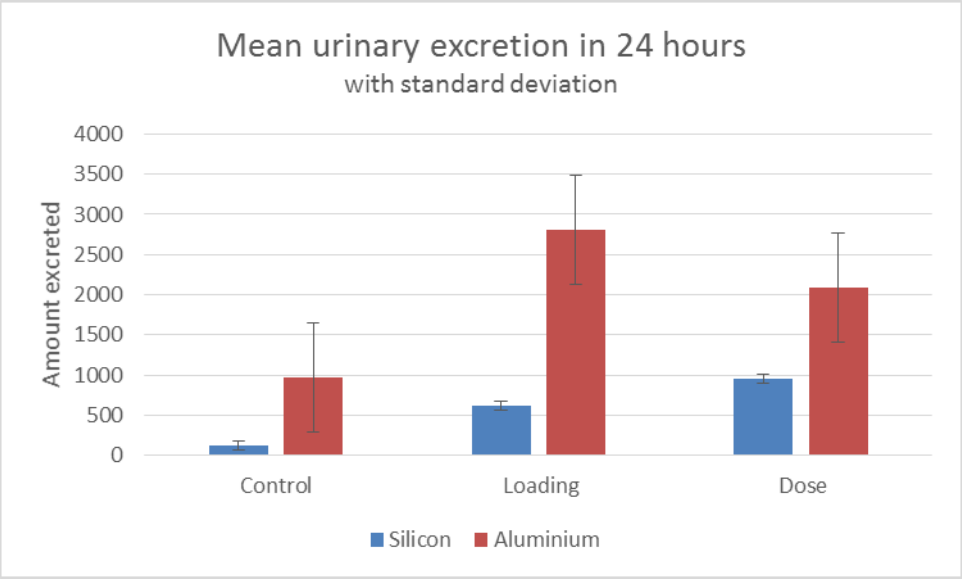

*Figure 3.4.3.5 **Mean** and standard deviation bars of excreted Si (µmol/24h) and Al (nmol/24h) in the control and treatment samples (n=19)*

3.4.4 Gender comparisons

When comparing male and female data, increases in Si and Al were significant for both treatment protocols. When comparing the amount of Si consumed, the p-value between genders for these weeks are below 0.04 (P = 0.038, Pearson) which is consistent with the idea that all patients are consuming the same amount of silicon during this time. The excretion of Al is more sporadic and p-value between each treatment protocol shows a clear difference and variation between the means for loading and dosing (P = 0.56 and P = 0.52, Wilcoxon signed ranked), showing a large variation between genders during this treatment period (*Table 3.4.4.2).*

T*able 3.4.4.1:* ***Mean*** *(SD) excretion of Si (µmol/24h) and Al (nmol/24h) in control and treatment urine samples for males (n=9) and females (n=10) in the healthy volunteer group.*

Urinary excretions in a 24-hour urine sample				
	Silicon (µmol/24h)		**Aluminium (nmol/24h)**	
	Male	**Female**	**Male**	**Female**
Control	155 (58)	99 (39)	1390 (741)	525 (225)
Loading treatment	661 (230)	572 (277)	3321 (1536)	1501 (956)
Dosing treatment	1013 (253)	896 (239)	2521 (1244)	1625 (687)

Differences between gender elemental excretions are smaller for Si than Al. Although males consistently excrete more Si and Al in each of the protocols, this difference is more notable for Al excretion. During the control protocol, Si excretion was noted to average at 155µmol/24hr while

this value for females is 99µmol/24hr, this difference is comparably larger for Al excretion during the same time period, varying from 1390nmol/24hr for males and 525nmol/24hr for females.

During the loading protocol, Males excrete more than double the amount of Al than females, 3321nmol/24 hr compared with 1501nmol/24 hr, despite only showing a slight positive increase in Si excretion, 661µmol/24hr compared with 572 µmol/24hr for females.

These relationships were confirmed with Wilcoxon signed-rank statistical testing. Differences between the means were smaller for Si than Al and data was considered statistically similar (P = 0.08) between males and females for baseline Si excretion, indicating that there is no notable difference in the metabolism of Si between genders in a normal population. However, the large difference between the means between genders considering Al excretion suggests that there may be a difference in Al body burden in this pool of individuals.

*Table 3.4.4.2: Summary of **Wilcoxon signed-rank** - differences between pairs of means of excretion for male (n = 9) and female groups (n = 10).*

Male v Female Healthy Volunteers		
	Silicon	Aluminium
	P - Value	P - Value
Control	0.08	0.37
Loading	0.22	0.56
Dosing	0.27	0.52

3.4.5 Urine volume

Urinary excretion of Si demonstrated a significant relationship, using Pearson correlation analysis, with sample volume for both the control (r = 0.56, *P* = 0.005), and treatment periods (r = 0.45, *P* = 0.030); however, this correlation was not seen in the treatment period when males were examined separately (r = 0.04, *P* = 0.901).

A significant relationship was highlighted between urinary excretion of Al and sample volume for both the control (r = 0.45, *P* = 0.030), and treatment periods (r = 0.46, *P* = 0.029); however, no correlation was seen when males (Control, r = 0.29, *P* = 0.414; Treatment, r = 0.43, *P* = 0.214) and females (Control, r = 0.48, *P* = 0.099; Treatment, r = 0.42, *P* = 0.156) were considered separately.

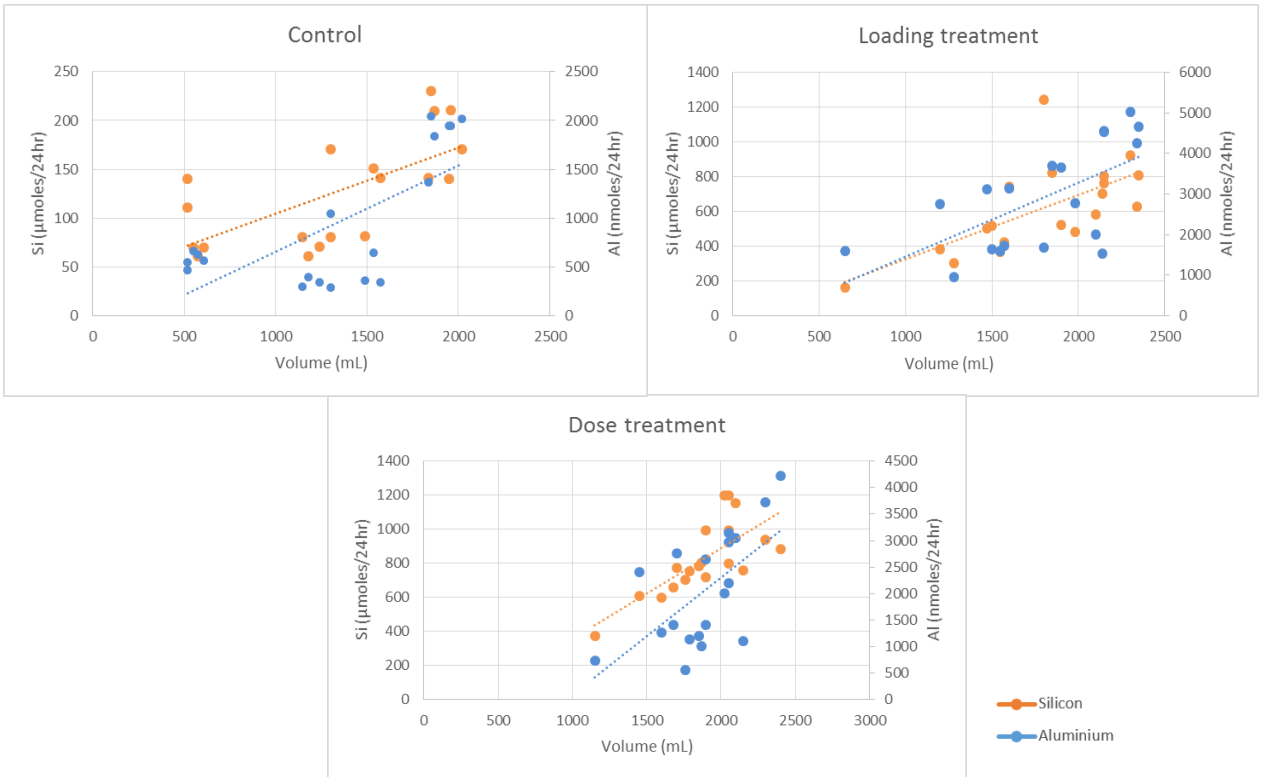

Figure 3.4.5.1: Excretion of Si (µmol/24h) and Al (nmol/24h) in the control and the treatment periods, loading and dosing, against sample volume (mL).

3.4.5.1 Silicon

Urine volume increased as Si excretion increased within the female population, however, three out of the nine males saw a decrease in urine volume correlated with an increase in Si during one of the treatment protocols. Patient ID M1 gave a baseline 24 hour urine sample of 1535mL which contained 150.7µmol Si, while during the dosing treatment protocol the same patient passed 1150mL urine during this 24 hour period despite excreting 523.2µmol Si (see *Table 3.4.5.1)*.

*Table 3.4.5.1: **Mean** (SD) urinary excretion of Si (µmol/24hr) total urine volume (mL) for the healthy volunteer study population for both loading and dosing treatment protocols*

Healthy Volunteer Urine Study Mean (SD) Si (µmol/24hr) and Volume (mL) (n=5)						
ID	**Control**		**Loading Treatment**		**Dose Treatment**	
	Si	**Volume**	**Si**	**Volume**	**Si**	**Volume**
M1	150.7	1535	365.2	1550	523.2	1150
M2	230.7	1850	741.4	1600	1384.4	2100
M3	170.2	2020	804.4	2150	1109.2	2300
M4	140.5	1950	761.0	2150	1022.6	2400
M5	140.9	1835	806.1	2350	941.1	2050
M6	210.2	1870	822.6	1850	1202.8	2050
M7	210.7	1960	921.4	2300	1203.4	1900
M8	80.3	1145	302.8	1280	883.3	1870
M9	61.0	1180	424.0	1570	842.8	1850
F1	170.3	1300	703.5	2140	921.6	1790
F2	70.0	550	160.7	650	667.1	1600
F3	70.3	1240	517.2	1500	789.9	1900
F4	60.4	575	382.0	1200	667.6	1450
F5	80.9	1490	580.1	2100	841.7	2150
F6	80.0	1300	1240.3	1800	781.9	1760
F7	70.1	610	502.0	1470	841.4	1700
F8	111.0	520	483.2	1980	1307.7	2050
F9	140.5	520	522.6	1900	1340.7	2025
F10	140.7	1575	628.4	2340	798.0	1680
Mean (SD)	**125.8 (55.3)**	**1317 (541)**	**614.2 (253.0)**	**1783 (450)**	**951.1 (245.8)**	**1883 (295)**

Overall, an increase in urine volume was coupled with an increase in the excretion of Si. A mean increase in urine volume from 1317mL in the control period to 1783mL in the loading treatment period was twinned with an increase of silicon from 125.8µmol to 614.2 µmol. A larger increase still was seen during the dosing protocol, showing an increase in urine volume from 1317mL to 1883mL correlating with an increase in Si from 125.8µmol to 951.1µmol. This increase was consistent between males and females.

Aluminium

Urine volume increased as Al excretion increased within the female population, however, three out of the nine males saw a decrease in urine volume correlated with an increase in Al in their urine during at least one of the treatment protocols. Patient ID M1 gave a baseline 24 hour urine sample of 1535mL which contained 645.3nmol Al, while during the dosing treatment protocol the same patient passed 1150mL urine during this 24 hour period despite excreting 1602.6nmol Al (see *Table 3.4.5.2)*.

*Table 3.4.5.1: **Mean** (SD) urinary excretion of Al (nmol/24hr) total urine volume (mL) for the healthy volunteer study population for both loading and dosing treatment protocols*

Healthy Volunteer Urine Study Mean (SD) Al (nmol/24hr) and Volume (mL) (n=5)						
ID	Control		Loading Treatment		Dose Treatment	
	Al	Volume	Al	Volume	Al	Volume
M1	645.3	1535	1602.6	1550	741.6	1150
M2	2044.4	1850	3147.4	1600	3042.5	2100
M3	2021.6	2020	4551.6	2150	3726.4	2300
M4	1949.1	1950	4527.0	2150	4216.6	2400
M5	1367.6	1835	4652.7	2350	3144.6	2050
M6	1842.0	1870	3702.5	1850	2961.0	2050
M7	1944.8	1960	5016.9	2300	2643.5	1900
M8	300.0	1145	960.4	1280	1004.8	1870
M9	392.9	1180	1723.6	1570	1208.6	1850
F1	1047.3	1300	1531.0	2140	1144.4	1790
F2	661.5	550	1605.1	650	1271.5	1600
F3	343.0	1240	1647.6	1500	1406.5	1900
F4	622.5	575	2762.2	1200	2408.2	1450
F5	360.5	1490	2003.1	2100	1102.4	2150
F6	287.5	1300	1684.2	1800	551.5	1760
F7	567.1	610	3111.4	1470	2762.4	1700
F8	467.1	520	2781.7	1980	2198.5	2050
F9	546.9	520	3641.3	1900	2000.1	2025
F10	342.8	1575	4240.6	2340	1407.6	1680
Mean (SD)	**934.4 (682.8)**	**1317 (541)**	**2889.1 (1297.1)**	**1783 (450)**	**2049.6 (1065.6)**	**1883 (295)**

Overall, an increase in urine volume was coupled with an increase in the excretion of Al. A mean increase in urine volume from 1317mL in the control period to 1883mL in the dosing treatment correlating with an increase in Al from 934.4nmol to 2049.6nmol. A larger increase still was seen during the loading protocol, showing an increase in urine volume from 1317mL to 1783mL,

twinned with an increase of silicon from 934.4nmol to 2889.1 nmol. This increase was consistent between males and females.

3.4.6 Comparisons between the urinary excretions of silicon and aluminium

Relationships between the excretions of Si with the metal Al was explored to determine variability in handling between individuals. Correlations between the compounds were also considered. The data showed that there was no significant correlation between Al excretion (*Fig 3.4.6.1*) with Si excretion for the control and both treatment periods. The strongest correlation was seen in the control period (r = 0.68), but as amounts of both elements increased, the strength of the relationship between them decreased. These differences highlight the variance in individual biochemistry.

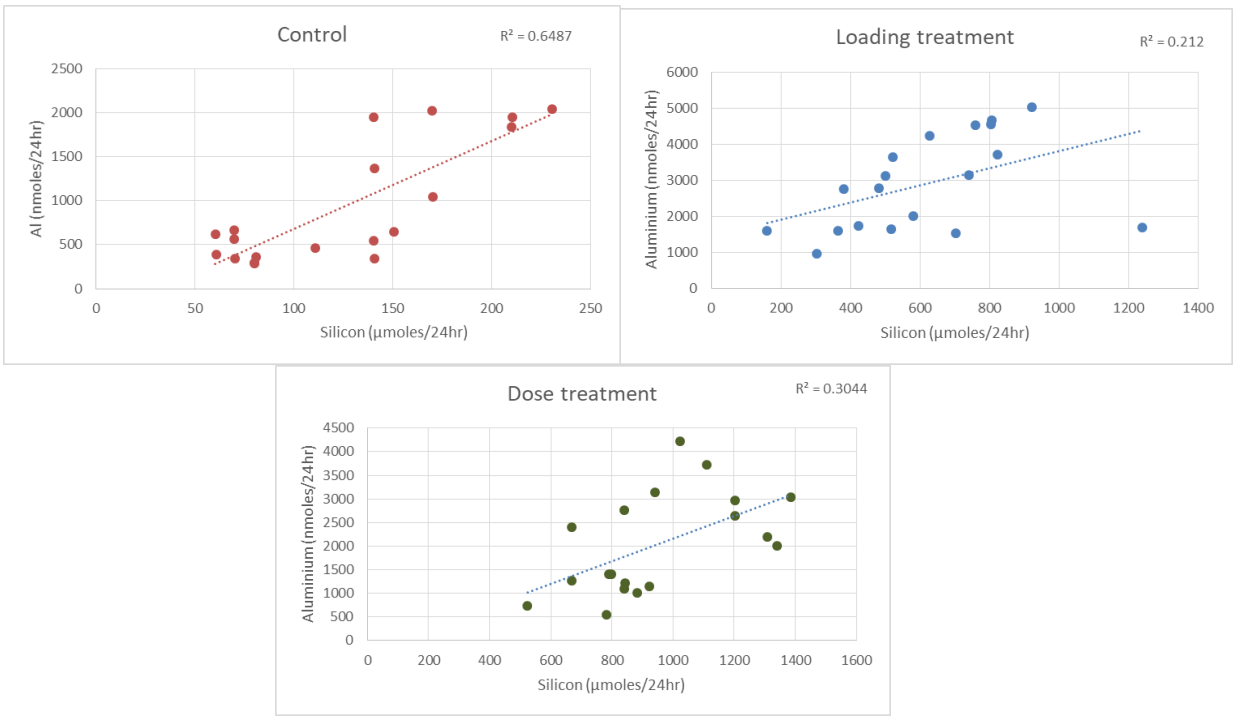

Figure 3.4.6.1: Comparative correlation of the excretion of Al (nmol/24h) against excretion of Si (µmol/24h) during the three study protocols

*Table 3.4.6.1: **Pearson** correlation analysis between the urinary excretions of Al (nmol/24h) with the excretion of Si (µmol/24h).*

Relationship with silicon		
	Aluminium	
	r Value	p Value
Control	0.65	0.64
Loading treatment	0.21	0.69
Dose treatment	0.4	0.64

3.4.7 Baseline aluminium excretion compared to metadata.

The average consumption of beverages during the control period are presented in *Figure 3.4.7.1* Bottled water (neat or with cordial) and coffee make up the majority of beverages consumed (18% and 18% respectively). Thirteen out of nineteen individuals consumed tea during the control period. There was a very weak positive relationship (r = 0.02; P = 0.77) between the amount of tea consumed with Al excretion (Figure 3.4.7.2). Nine out of nineteen individuals consumed bottled water during the control period. Four out of nine males consumed beer during the control period, as discussed in chapter 1, beer and some bottled waters of which are potentially rich sources of Si.

There was no significant relationship (r = 0.21; P = 0.605) between body mass index (BMI) with Al excretion (Figure 3.4.7.2).

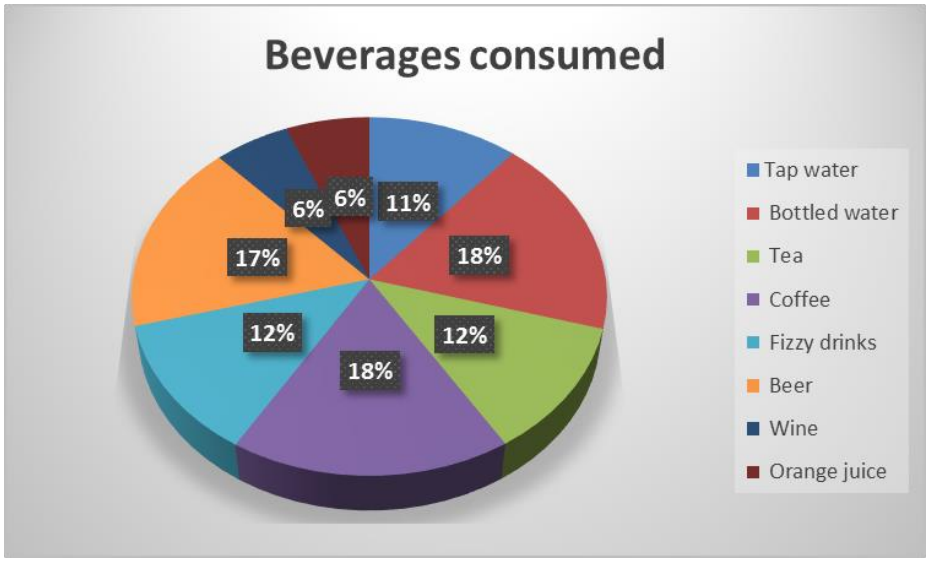

Figure 3.4.7.1: Average consumption of beverages consumed during the control 24 hour period (n = 19)

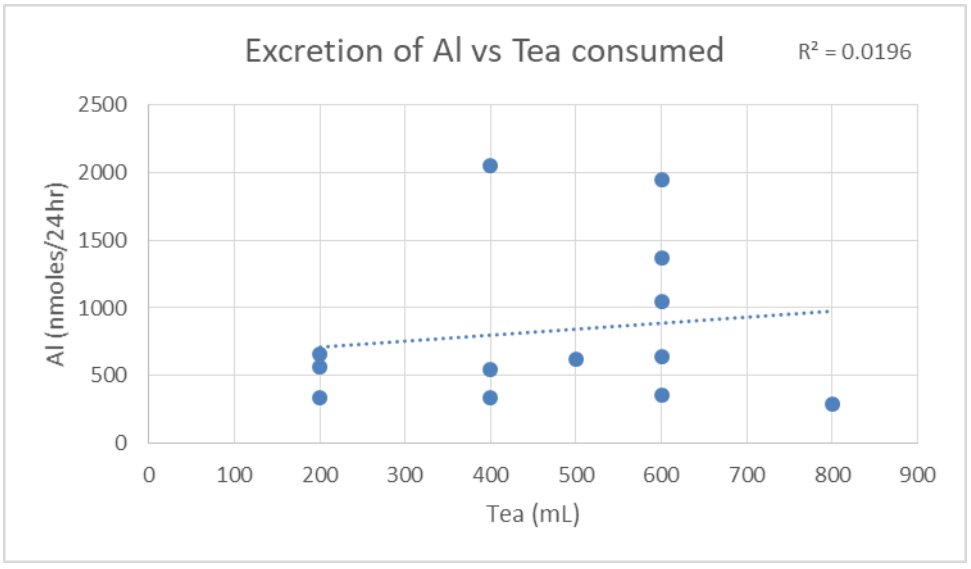

Figure 3.4.7.2 Excretion of Al at baseline against volume of tea consumed by each healthy volunteer (n=19)

Most of the healthy volunteers were of a normal BMI, ranging from 20-25. Figure 3.4.7.3 shows how the individuals with a larger BMI differ from the trend and excrete an Al concentration either side of the general trend. The majority of individuals with a normal BMI excrete a lower concentration of Al.

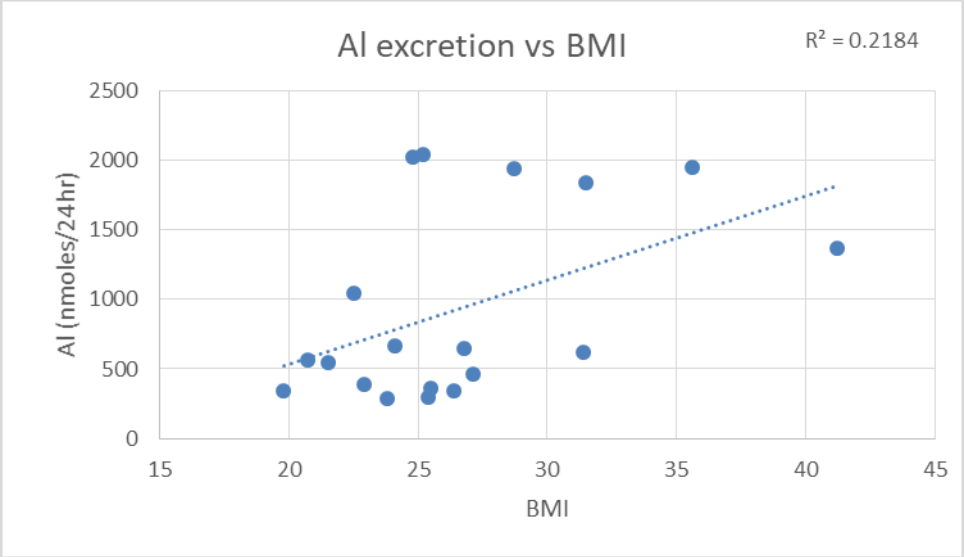

Figure 3.4.7.3 Excretion of Al baseline compared with the body mass index score calculated for each participant.

3.5 Summary of results for healthy individuals

• Creatinine concentration was negatively correlated with sample volume (Section 3.4.2.).

• The difference in Si excretion between the loading treatment and control periods corresponded to 59± 31% of the amount of Si consumed as the mineral water. (Section 3.4.2).

•The difference in Si excretion between the loading treatment and control periods corresponded to 95± 25% of the amount of Si consumed as the mineral water. (Section 3.4.2).

• Urinary excretions of Si and Al are significantly increased following the consumption of the mineral water (Section 3.4.3).

• Si and Al showed weak positive correlation with sample volume (Section 3.4.5).

•Weak positive correlation was demonstrated between Si and Al excretion (Section 3.4.6)

• No correlation was noted between BMI and urinary excretion of Al and Si during the control protocol (Section 3.4.7)

3.6 Discussion of results

This research was undertaken with the aim of elucidating the utilisation of a commercial silicic acid-rich mineral water as a non-invasive method of reducing Al body burden, when used as part of an individual's regular diet. The primary objectives were to investigate Si handling and the consequent effects on urinary Al excretion.

Despite extensive evidence suggesting a beneficial role of Si in biota, there is very little reliable information on Si handling in humans, particularly regarding healthy individuals. In order to determine whether there is any change in Si handling in disease it is first important to elucidate Si handling in 'normal' individuals. Thorough investigations concerning healthy individuals were therefore performed, using three different protocols. As Si is known to be rapidly absorbed and excreted (Popplewell *et al*. 1998), it was considered that a period of one week between each protocol would be sufficient to minimize any influence on Si excretion between each study. However, it is likely that Al body burden would have been influenced, if not lowered, after each protocol.

Al intake in the diet is approximately 20 mg day-1 (Pennington & Jones, 1989); only 0.1% of this is absorbed (Day et al. 1991), meaning that around 140 µg of Al could be added to Al body burden from

dietary exposure each week. With this in mind, it is unlikely that the time periods between each different protocol were sufficient to replenish the Al body burden removed from the preceding Si dose.

However, as each protocol consisted of a control and treatment period in which the urinary excretions were compared, the overall effect on Al excretion would not have been negated by the preceding protocols.

Comparing urinary volume and creatinine concentration

24-hour collections provide the most reliable information on urinary excretions; however, creatinine corrected measurements were also documented. The negative correlation between creatinine concentration and sample volume (*Figure 3.4.1.1)* verifies that creatinine concentration provides an acceptable indication of dilution effects. The mean creatinine concentration at baseline (5.2 ± 2.5 mM) and volume of urine produced (1687 ± 542 mL) correlates well with the literature values (*Table 7.1*), confirming that the healthy participants had normal kidney function. A high level may mean that the kidneys are not working as they should. The amount of creatinine in the blood depends partly on the amount of muscle tissue present in the body. Men generally have higher creatinine levels than women. Although, in this study, Crt values were higher throughout the study for females than males (Table 3.4.1.1).

Silicon handling was variable between individuals

Urinary excretion of Si was variable between individuals, for example, the range of urinary Si at baseline was 60.4-230.7µmol/24hr (Table 3.4.5.1). As urinary Si is a good marker of absorbed Si, thus dietary differences between individuals would have contributed to these variations (Jugdaohsingh et al. 2002; Sripanyakorn et al. 2009).

Overall, an increase in urine volume was coupled with an increase in the excretion of Si. A mean increase in urine volume in the control period to the loading treatment period was twinned with an increase of Si in the urine. This relationship was further strengthened during the dosing protocol, showing an even greater increase in urine volume correlating with an increase in Si excretion. An increase that was consistent throughout the study between males and females.

It must be noted that overall fluid consumption increased for each volunteer between the control and either treatment protocol. To improve the outcome of this investigation, the amount of fluids consumed each day must be kept constant to minimise variability.

The effect of the mineral water on urinary excretions

Consumption of the mineral water significantly increased the amount of Si excreted in the urine ($P < 0.001$, Section 3.4.3), this was coincident with increased Al excretion ($P < 0.001$). This signifies that the consumption of silicic acid-rich mineral water successfully enhanced the urinary excretion of Al in healthy individuals.

The mean amount of Al excreted increased for all healthy volunteers after the addition of the Si rich mineral water. Furthermore, Al excretion was highest during the loading protocol than the dosing protocol, while Si excretion was consistently larger during the dosing protocol. Considering the

whole study population, Al excretion was larger in males, however females saw a notable increase in each treatment period (Table 3.4.3.3). Co-excretion of Si and Al was apparent in this data; the relationship between Si and Al along with the rapid excretion of Si demonstrates that Al is excreted as a bolus following the consumption of the mineral water.

Conclusions

To summarise the healthy individual study; consumption of the mineral water significantly increased the urinary excretions of Si and Al. Si and Al were rapidly excreted as a bolus and treatment over a long term period may result in reduced Al body burden.

Baseline 24h urinary excretions of Si and Al were compared with literature values (Table 7.2). With the exceptions of the studies by Roberts et al. (1998) and Kazi et al. (2008), who used large study populations, the group sizes used in the other studies were considerably lower. These differences would have contributed to the high variations seen in urinary excretions between the literature sources. For instance, the urinary excretion of Al appears to be higher compared to other studies using a similar age range (Morie et al. 1996; Reffitt et al. 1999), however, these studies had population sizes of 6 and 5 respectively. In addition, the low excretion of Al in the study by Reffitt et al. (1999) could be attributed to participant fasting.

The higher excretion of Al seen within the present study could also be a reflection of a university student's diet, typically high in processed foods and alcohol (Devine *et al*. 2006), these food are generally high in Al (Pennington & Jones, 1989) and alcohol is noted to increase gut permeability (Barchfeld & Deamer, 1988), possibly enhancing Al uptake. Other dietary factors, such as citrate

(Taylor *et al.* 1998) also effects the absorption of Al, therefore, an individual who regularly consumes fruit juice may have an increased absorption of Al.

The majority of individuals in the present study were also regular tea drinkers 13/19. Tea is a diuretic which contains high levels of Al (Forster *et al*. 1995). Even though the bioavailability of Al in tea is considered to be low (Powell *et al*. 1993; Gardner & Gunn, 1995), regular consumption could be linked to an increased absorption and to the high amount of Al present (Nieboer *et al*. 1995).

In the study by Reffitt *et al.* (1999), the participants fasted in order to investigate whether silicic acid promotes the urinary excretion of endogenous Al. Their results demonstrated no significant change in Al excretion; however, considering the young age of the group (and the small population size) it is unlikely that Al accumulation was particularly high and so any effects on removing Al from body stores would have been minimal.

Chapter 4 - Silicic acid rich mineral water accompanied by exercise to reduce body burden of aluminium via sweat excretion in healthy volunteers.

This chapter includes all the statistical comparisons between the control and treatment data for the 30 minute exercise trial. Comparisons were made for the overall group and for males and females separately.

4.1.0 Aims

The aim of this chapter is to investigate if regular drinking of a silicon-rich mineral water can facilitate the removal of Al from the body of healthy individuals in sweat.

4.2.0 Introduction

There are few data to describe human excretion of systemic aluminium and almost no reliable data which relate to aluminium in sweat, although recent data has suggested that perspiration might be a significant route of excretion of systemic aluminium (Exley, 2014).

Certain commercial mineral waters, provide a good source of silicic acid and could potentially be utilised as a preventative measure against the accumulation of aluminium within the body. This could be exploited in young, healthy individuals, which could in turn reduce the rate and slow the progression of aluminium related disorders, including multiple sclerosis, Alzheimer's disease and Parkinson's disease. However, the role of silicon in drinking water and how humans handle this silicon has not been widely studied, particularly with respect to healthy individuals.

The purpose of this work was to elucidate the effect of drinking silicic acid-rich mineral waters on the excretion of silicon in the sweat of healthy individuals. The main aim of this research was to investigate the influence of a silicic acid-rich mineral water, as a non-invasive therapy, on the excretion of aluminium in sweat. Data for each individual are provided in Appendix 3.

4.3.0 Methodology

Details are provided for the specific methods involved during the collection of sweat during a thirty minute exercise protocol. Further details regarding sample analysis of Al and Si are presented in Chapter 2.

4.3.1 Method blank material analysis

It was paramount to design a sweat collection apparatus that would be effective in absorbing as much sweat as possible during a 30 minute duration of exercise, would be comfortable for the healthy volunteers and that wouldn't produce any contamination to the final eluate.

In previous methods, sweat was collected using measured squares of Whatman's 541 hardened ashless filter paper. When testing materials for their suitability to this study, it was seen that the filter paper resulted in a significant contamination of Si and therefore wasn't suitable for this investigation, of which the accurate measurement of silicon was paramount. Other materials were tested with solutions of known Al and Si concentrations, to determine contamination level and leachability of these elements into the centrifuged eluate. A summary of these results are provided in *Table 4.3.1.1.* Images of the materials tested in addition to the filter paper are taken from Lennard Jones Laboratories at Keele University and shown in Figure 4.3.1.1.

Figure 4.3.1.1 Materials analysed for Al and Si contamination for use during the sweat collection program.1) Cotton wool, 2) Dressing 3) Foam armpit pad

A 5mM solution of citric acid was used due to its known ability to bind Al and so potential to leach Al from the material being tested. Table 4.3.1.1 shows how larger amounts of Al were drawn out of the materials when immersed with citric acid; an example being filter paper where 6.2µg/L was quantified in the citric acid eluate compared with 1.2 µg/L being quantified in the eluate of the UPW test. This is important when considering the absorption of sweat, and how sweat may leach Al from the material. Al 'hiding' in the material may be drawn out with the eluate, giving a falsely strong signal. 1mL of each solution was added to the materials prior to centrifuging. The solutions drawn out of the material were diluted 1:1, although dilution corrections were made for this analysis.

Table 4.3.1.1 Comparative summary of materials tested for elemental contamination (n=18)

ID	Neat contamination µg/L (SD)							
	UPW		5mM Citric Acid		100µg/L Al		100µg/L Si	
	Al	Si	Al	Si	Al %	Si	Al	Si %
Solutions Alone	0.0034	0.012	0.022	0.011	99.82	0.065	0.017	99.981
Filterpaper	1.321	4.533	6.2	4.889	104.53	4.341	1.209	103.618
Cotton wool	0.809	1.244	4.218	3.042	103.56	1.206	0.754	102.312
Foam	0.012	0.026	0.508	0.278	100.52	0.038	0.018	100.192
Dressing	0.112	0.098	0.708	0.498	101.27	0.174	0.154	101.78

The foam material, cut from an armpit sweat collection pad, provided the smallest contamination values, see *Table 4.3.1.1*, and this result was comparable to the control metadata (P = 0.011, R=0.96, Pearson) so was therefore selected for the investigation. Method blanks, using this foam material, were immersed in 1mL UPW alongside each experiment and treated in the same manner to the samples. This control was treated in the same manner as the samples and subjected to the microwave digestion program (details of this are given in Chapter 2) to establish the final level of contamination that should be considered when processing the experimental data. This value was calculated to be 57ng/digest (n=50) for aluminium and 92ng/digest for silicon (n=50). These values were subtracted from each sweat sample before the aluminium and content of the sweat samples were analysed.

3.4.2 Protocol

Nineteen participants, 9 male and 10 female (age range 19–34) were recruited from the student population at Keele University and ethical approval was obtained from the University's Life Sciences Ethical Review Body (details of which are provided in the appendix). All participants were self-assessed as healthy and agreed to partake in a mild exercise program as part of the procedure for collecting the sweat samples.

Foam pads, taken from an armpit sweat collection apparatus were selected as they delivered a good absorbency and minimal aluminium and silicon contamination. Pads were soaked in 5% HCl for 2 hours, ultra-pure water for 24 hours, then placed in a drying oven overnight before use. Prior to the exercise protocol, the upper back was cleaned using a pad immersed in 100% ethanol, then six pads were transferred to the upper back of each individual, covered with a plastic film and secured with an adhesive medical tape to prevent any allergic reaction to adhesives. The

individual was then instructed to complete a total of thirty minutes cycling on an exercise bike, including five minutes initial fast pace cycling, twenty minutes moderate paced cycling and a final five minutes fast paced cycling. Once this program was completed, the pads were transferred to 7mL centrifuge tubes and span down at 6000RPM for 6 minutes to extract the volume of sweat collected in the material to the bottom of the tube, this series of steps is shown in Figure 4.3.2.2.

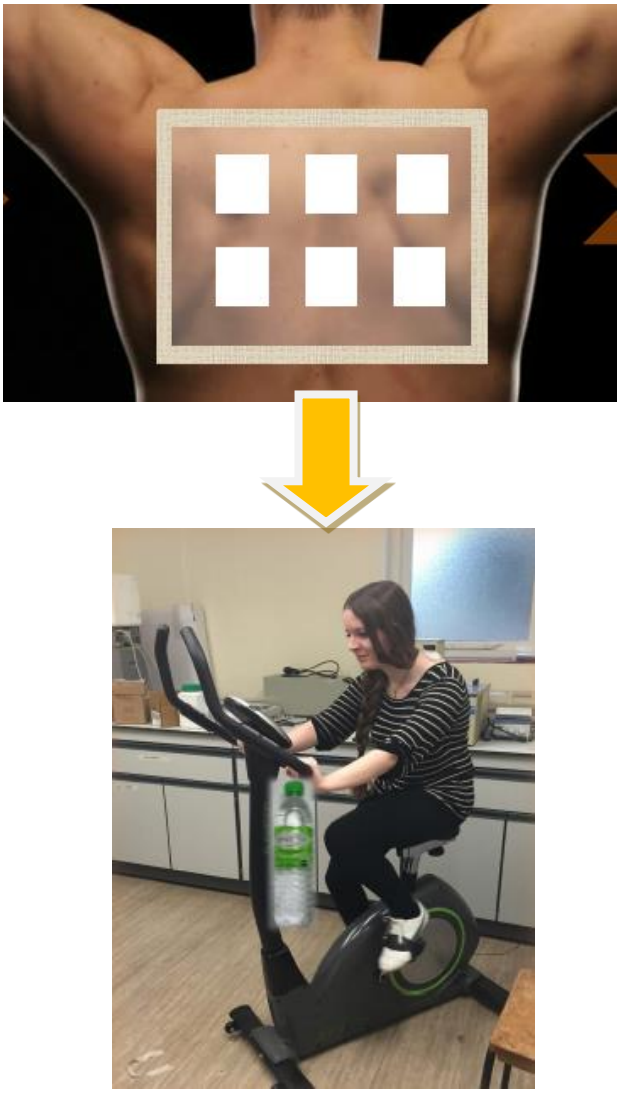

Figure 4.3.2.1 Sweat collection apparatus and exercise protocol

Figure 4.3.2.2: Removing sweat from the collection pad

The only difference between the control and treatment protocols was the addition of the Si rich mineral water one hour prior to the exercise period. Each protocol was repeated three times for each healthy volunteer.

Concentrations of aluminium and silicon in collected sweat were adjusted to take account of the volume of sweat produced by men (1342 mL/24 h) and women (712 mL/24 h) in this age range (Manz *et al,* 2012).

The aluminium content of sweat was measured in 19 healthy volunteers (9 male and 10 female) following 30 minutes of mild exercise, with and without the addition of theSi rich mineral water, hereafter referred to as the control and treatment period. Research has strongly suggested that perspiration is the major route of excretion of systemic aluminium in humans (Exley *et al* 2013).

The amount of Si in the Spritzer mineral water provided was determined to be 26.1mg/L

The mean aluminium content of method blanks equated to a contamination level of 57 ng/digest (mean±1.654 SD) and this value was subtracted from each sweat sample before the aluminium content of sweat samples were calculated.

The volumes of sweat collected using this method ranged from 0.3 to 1.4 mL. Method blanks which consisted of the same squares of prepared foam material to which 1 mL of ultrapure water had been added were centrifuged and the solution extracted was collected in acid-washed centrifuge tubes and frozen prior to analysis. These method blanks were estimates of contamination by extraneous aluminium of the procedure used for collecting and processing the sweat.

4.4.0 Results

This chapter includes all the statistical comparisons between the control and treatment data for the controlled exercise experiments. Comparisons were made for the overall group and for males and females separately; however, male and female data are only presented within the main text when showing a noteworthy difference from the overall group.

Results are displayed as amounts excreted per L^{-1} and extrapolated for literature values of sweat production for males and females per 24 hours (Manz *et al,* 2012).

The group size (n) is equal to 19, 9 male and 10 female.

4.4.1 Sweat Volume

The amount of sweat collected by the foam pad material is representative of the amount of sweat produced during the 30 minute exercise period. In general, Males excreted more sweat than females during this time. When comparing the data as a whole, the total sweat volume from men was around double that of females, for the control period gaining total volumes (n=6) of 8.82mL (±1.91mL) and 3.96mL (±1.42mL) for the control and treatment period respectively.

Sweat production was slightly higher in data collected post Si rich mineral water consumption than in the control. In the control period, females excreted an average total volume of 3.96mL, compared to a total volume of 4.5mL (±1.77mL) in the treatment period. This relationship was seen for male participants, during the control, a total volume of 8.82mL was collected, while in the treatment period, this value increased to 9.29mL (±1.81).

Table 4.4.1.1: Collection volume average (men and women) including individual sweat collection pad total as well as combining all six sweat collection pad volumes (n=3)

Volume of sweat collected mL (SD)				
	Control		Treatment	
ID	Mean total collected	Mean Individual Volume	Mean total collected	Mean Individual Volume
F1	5.62	0.94 (0.07)	5.87	0.98 (0.11)
F2	2.63	0.44 (0.04)	3.02	0.5 (0.04)
F3	3.76	0.63 (0.03)	4.29	0.72 (0.06)
F4	2.66	0.44 (0.04)	3.40	0.57 (0.03)
F5	5.15	0.86 (0.05)	5.82	0.97 (0.06)
F6	3.31	0.55 (0.03)	3.32	0.5 (0.03)
F7	6.24	1.04 (0.04)	8.44	1.41 (0.04)
F8	1.99	0.33 (0.04)	2.64	0.44 (0.03)
F9	3.34	0.56 (0.03)	4.12	0.69 (0.05)
F10	4.85	0.81 (0.02)	5.42	0.9 (0.02)
Mean	**3.96 (1.42)**	**0.66 (0.24)**	**4.5 (1.77)**	**0.77 (0.3)**
M1	7.94	1.32 (0.09)	8.96	1.49 (0.06)
M2	11.01	1.84 (0.06)	11.35	1.89 (0.05)
M3	7.5	1.25 (0.06)	8.23	1.37 (0.03)
M4	6.04	1.01 (0.08)	6.42	1.07 (0.03)
M5	6.69	1.12 (0.04)	7.31	1.22 (0.03)
M6	11.36	1.89 (0.07)	11.59	1.93 (0.04)
M7	9.79	1.63 (0.04)	10.23	1.71 (0.05)
M8	8.69	1.45 (0.04)	8.77	1.46 (0.04)
M9	10.33	1.72 (0.06)	10.71	1.79 (0.04)
Mean	**8.82 (1.91)**	**1.47 (0.32)**	**9.29 (1.81)**	**1.55 (0.3)**

Males had a higher variance in their data for sweat volume; in the control period, total volumes were 6.04-11.36mL, resulting in a difference of 5.32mL, while for females, sweat volumes during the control period were measured of 1.99-6.24mL, a range of 4.25mL. Although, females had a greater variance in their sweat volume during the treatment protocol; measured at 2.64-8.44mL, a difference of 5.8mL, while for males, sweat volumes during the treatment period were measured of 6.42-11.59mL, a difference of 5.17mL.

4.4.2 The effect of a Si rich mineral water on Si excretion in sweat

During the baseline, Si excretion equated to a mean of 748 µg/L (±140 µg/L) when considering the whole group, while during the treatment protocol, this mean increased to 1660 µg/L (±303 µg/L). In the control period, Si concentration in sweat varied from 576-1050µg/L between the study population, this value increased to 1110-2327µg/L during the treatment protocol. The smallest increase between control and treatment period was seen in individual M3, where the sweat Si content increased from 874 µg/L to 1281µg/L after the consumption of the mineral water (Table 4.4.2.1).

Table 4.4.2.1 Mean (SD) content of Si (µg/L) in sweat collected and measured after 30 minutes of mild exercise for both treatment and control patient data (n=6)

Excretion of Si in sweat		
ID	**[Si] Sweat control (µg/L)**	**[Si] Sweat treatment (µg/L)**
F1	812 (170)	1466 (172)
F2	647 (8)	1609 (23)
F3	601 (9)	1332 (72)
F4	626 (27)	1683 (113)
F5	576 (5)	1476 (22)
F6	668 (37)	1612 (56)
F7	616 (12)	1110 (17)
F8	732 (87)	1634 (323)
F9	787 (57)	1778 (18)
F10	691 (9)	1938 (17)
M1	944 (31)	1661 (18)
M2	1050 (10)	2167 (28)
M3	874 (22)	1281 (35)
M4	743 (34)	1870 (80)
M5	810 (25)	1348 (52)
M6	783 (20)	2327 (73)
M7	994 (15)	1941 (5)
M8	578 (31)	1588 (28)
M9	674 (17)	1726 (41)
Mean (SD)	**748 (140)**	**1660 (303)**

The mean amount of Si excreted in sweat is higher for all study participants during the treatment protocol than the baseline. Males excreted statistically similar amounts of Si to females during the control period ($r = 0.81$, $P = 0.009$ Pearson Correlation analysis used for comparing means), presenting mean sweat Si concentrations of 828 µg/L (±153 µg/L) and 676µg/L (±80 µg/L) respectively.

Concentrations of Si in sweat during the treatment protocol indicated little variance between males and females (r = 0.69, P = 0.013 Pearson Correlation analysis) equating to 1768 µg/L and 1564 µg/L respectively.

Table 4.4.2.2: Mean (SD) content of Si(µg/L) in sweat collected and measured after 30 minutes of mild exercise for males in both treatment and control patient data (n=6)

Excretion of Si in sweat in males		
ID	**[Si] Sweat control (µg/L)**	**[Si] Sweat treatment (µg/L)**
M1	944 (31)	1661 (18)
M2	1050 (10)	2167 (28)
M3	874 (22)	1281 (35)
M4	743 (34)	1870 (80)
M5	810 (25)	1348 (52)
M6	783 (20)	2327 (73)
M7	994 (15)	1941 (5)
M8	578 (31)	1588 (28)
M9	674 (17)	1726 (41)
Mean (SD)	**828 (153)**	**1768 (333)**

The variance seen between male and females in regard to Si excretion in sweat was relatively small. Although, this variance was larger during the treatment period than the control period, for males, the range in concentrations was 1281 µg/L to 2327 µg/L, and for females, this range was noted at 1110 µg/L to 1938 µg/L. The SD value increased for each gender during the treatment people, suggesting higher variability within the data, increasing from 153 to 333 in males (Table 4.4.2.2), and 80 to 232 in females (Table 4.4.2.3). This larger variation in treatment protocol Si excretion is consistent throughout the healthy volunteer studies and can be contributed to individual biochemistry.

Table 4.4.2.3: Mean (SD) content of Si (µg/L) in sweat collected and measured after 30 minutes of mild exercise for males in both treatment and control patient data (n=6)

Excretion of Si in sweat in females		
ID	**[Si] Sweat control (µg/L)**	**[Si] Sweat treatment (µg/L)**
F1	812 (170)	1466 (172)
F2	647 (8)	1609 (23)
F3	601 (9)	1332 (72)
F4	626 (27)	1683 (113)
F5	576 (5)	1476 (22)
F6	668 (37)	1612 (56)
F7	616 (12)	1110 (17)
F8	732 (87)	1634 (323)
F9	787 (57)	1778 (18)
F10	691 (9)	1938 (17)
Mean (SD)	**676 (80)**	**1564 (232)**

4.4.3 The effect of a Si rich mineral water on Al excretion in sweat

The mean amount of Al excreted in sweat is higher for all study participants during the treatment protocol than the baseline. Males excreted significantly more Al than females for the treatment period ($t = 3.26$, $P = 0.007$), presenting mean sweat Al concentrations of 1208 µg/L (±433 µg/L) and 834 µg/L (±334 µg/L) respectively. Contrary to this, baseline sweat concentrations did not show large variance between males and female ($t = 1.07$, $P = 0.46$), equating to 341 µg/L and 353 µg/L respectively.

An increase in sweat Al concentration was seen for all study participants during the treatment protocol however, this increase was more predominant in some members of the study population. The sweat of individual M2 contained 72 µg/L, the lowest concentration of Al of all study participants, although,

presented the largest increase in sweat Al content after consumption of a Si rich mineral water, increasing to 1030 µg/L.

In the control period, Al concentration in sweat varied from 71-574 µg/L between the study population, this value increased to 381-1677 µg/L during the treatment protocol. The smallest increase between control and treatment period was seen in individual F2, where the seat Al content increased from 217 µg/L to 381µg/L after the consumption of the mineral water.

Table 4.4.3.1 Mean (SD) content of Al (µg/L) in sweat collected and measured after 30 minutes of mild exercise for both treatment and control patient data (n=6)

Excretion of Al in sweat		
ID	**[Al] Sweat control (µg/L)**	**[Al] Sweat treatment (µg/L)**
F1	467 (96)	1184 (169)
F2	217 (11)	381 (33)
F3	270 (10)	1081 (55)
F4	255 (17)	986 (20)
F5	187 (9)	619 (13)
F6	300 (16)	424 (42)
F7	266 (57	427 (29)
F8	520 (120)	1035 (131)
F9	585 (8)	994 (51)
F10	461 (9)	1215 (85)
M1	444 (88)	772 (15)
M2	71 (4)	1030 (42)
M3	526 (6)	1589 (113)
M4	574 (26)	1677 (28)
M5	464 (12)	1666 (33)
M6	183 (36)	1276 (87)
M7	152 (57)	1536 (88)
M8	400 (31)	690 (18)
M9	255 (34)	639 (25)
Mean (SD)	**347 (156)**	**1012 (419)**

As discussed earlier in this section, the excretion of Al in sweat is significantly higher in males than females throughout the treatment protocol.

Table 4.4.3.2: Mean (SD) content of Al (µg/L) in sweat collected and measured after 30 minutes of mild exercise for males in both treatment and control patient data (n=6)

Excretion of Al in sweat in males		
ID	**[Al] Sweat control (µg/L)**	**[Al] Sweat treatment (µg/L)**
M1	444 (88)	772 (15)
M2	71 (4)	1030 (42)
M3	526 (6)	1589 (113)
M4	574 (26)	1677 (28)
M5	464 (12)	1666 (33)
M6	183 (36)	1276 (87)
M7	152 (57)	1536 (88)
M8	400 (31)	690 (18)
M9	255 (34)	639 (25)
Mean (SD)	**341 (245)**	**1208 (433)**

Table 4.4.3.3: Mean (SD) content of Al (µg/L) in sweat collected and measured after 30 minutes of mild exercise for females in both treatment and control patient data (n=6)

Excretion of Al in sweat in females		
ID	**[Al] Sweat control (µg/L)**	**[Al] Sweat treatment (µg/L)**
F1	467 (96)	1184 (169)
F2	217 (11)	381 (33)
F3	270 (10)	1081 (55)
F4	255 (17)	986 (20)
F5	187 (9)	619 (13)
F6	300 (16)	424 (42)
F7	266 (57	427 (29)
F8	520 (120)	1035 (131)
F9	585 (8)	994 (51)
F10	461 (9)	1215 (85)
Mean (SD)	**353 (141)**	**835 (334)**

The variance in data is smaller for females than males, in both the control period at 141 µg/L and 245 µg/L respectively, and the treatment period, at 334 µg/L and 433 µg/L . Al excretion doubled in participant F8, increasing from 520 µg/L to 1035 µg/L, while Al excretion quadrupled in participant F3, who had a baseline Al sweat excretion of 270 µg/L which rose to 1081 µg/L post Si rich mineral water consumption.

Table 4.4.3.4 Mean (SD) content of Al (µg/L) and (µg/L) Si in sweat collected and measured after 30 minutes of mild exercise for both treatment and control patient data (n=6)

Excretion of Al and Si in sweat					
ID	Age	[Al] Sweat control (µg/L)	[Si] Sweat control (µg/L)	[Al] Sweat treatment (µg/L)	[Si] Sweat treatment (µg/L)
F1	24	467 (96)	812 (170)	1184 (169)	1466 (172)
F2	25	217 (11)	647 (8)	381 (33)	1609 (23)
F3	19	270 (10)	601 (9)	1081 (55)	1332 (72)
F4	20	255 (17)	626 (27)	986 (20)	1683 (113)
F5	24	187 (9)	576 (5)	619 (13)	1476 (22)
F6	23	300 (16)	668 (37)	424 (42)	1612 (56)
F7	18	266 (57	616 (12)	427 (29)	1110 (17)
F8	25	520 (120)	732 (87)	1035 (131)	1634 (323)
F9	20	585 (8)	787 (57)	994 (51)	1778 (18)
F10	22	461 (9)	691 (9)	1215 (85)	1938 (17)
M1	27	444 (88)	944 (31)	772 (15)	1661 (18)
M2	34	71 (4)	1050 (10)	1030 (42)	2167 (28)
M3	26	526 (6)	874 (22)	1589 (113)	1281 (35)
M4	24	574 (26)	743 (34)	1677 (28)	1870 (80)
M5	21	464 (12)	810 (25)	1666 (33)	1348 (52)
M6	28	183 (36)	783 (20)	1276 (87)	2327 (73)
M7	21	152 (57)	994 (15)	1536 (88)	1941 (5)
M8	29	400 (31)	578 (31)	690 (18)	1588 (28)
M9	25	255 (34)	674 (17)	639 (25)	1726 (41)

4.4.4 Comparing the study population

Elemental sweat excretions were comparable within the group, with low variance between the collated data. Excretions of Si and Al during the control period were 748 µg/L (±140 µg/L) and 347

µg/L (±156 µg/L) respectively, while during the treatment period, these values were shown to increase to 1660 µg/L (±103) and 1012 µg/L (±419) respectively (*Table 4.4.4.1).*

*Table 4.4.4.1: **Mean** (SD) and **median** sweat concentrations of Si (µg/L) and Al (µg/L) for patients (n = 19)*

Mean (SD) elemental sweat concentration				
	Silicon (µg/L)		Aluminium (µg/L)	
	Control	Treatment	Control	Treatment
Group	747.6 (140.4)	1660.3 (303.2)	347.2 (156.0)	1011.5 (419.4)

When comparing the relevance of these means within the study population, a t-test was performed to establish the distribution of this data. This test revealed that the means between the control were statistically different for Al ($t = 1.52$, $p = 0.08$) and Si ($t = 1.22$, $p = 0.01$), this difference was even more notable during the treatment period for Al ($t = 2.45$, $p < 0.001$) and Si ($t = 3.75$, $p < 0.001$) are statistically different, highlighting that Si and Al biochemistry varies significantly between individuals.

*Table 4.4.4.2: Summary of **t-Test for paired means** - differences in the urinary excretion of Al and Si in control and treatment protocols in the healthy volunteer population (n=19)*

	t-Test: Paired Two Sample for Means Aluminium (n=19)	
Function	**Control**	**Treatment**
t Stat	1.52	2.45
P one-tail	0.08	3.65E-05
t Crit one-tail	1.71	1.91
P two-tail	0.15	7.29E-05
t Crit two-tail	2.17	2.23
Pearson Correlation	0.68	0.93

	t-Test: Paired Two Sample for Means Silicon (n=19)	
Function	**Control**	**Treatment**
t Stat	1.22	3.79
P one-tail	0.0100	0.00
t Crit one-tail	0.97	2.14
P two-tail	0.0200	0.00
t Crit two-tail	1.07	3.45
Pearson Correlation	0.78	0.96

The One-way ANOVA statistical testing (*Table 4.4.4.3*) revealed that data was significant ($P < 0.001$) for all nineteen healthy volunteers for Al and Si between control exercise period and treatment exercise period for the study group.

*Table 4.4.4.3 Summary of **One-way ANOVA** - differences in the urinary excretion of Si (µg/L) and Al (ug/L) between the control and treatment period of the volunteer group.*

Patient	Difference in sweat elemental excretion between control and treatment (dF=6)							
	Aluminium				Silicon			
REF	F Crit/F	Significant	P	Tukeys	F Crit/F	Significant	P	Tukeys
F1	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
F2	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
F3	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
F4	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
F5	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
F6	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
F7	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
F8	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
F9	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
F10	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
M1	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
M2	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
M3	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
M4	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
M5	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
M6	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
M7	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
M8	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control
M9	F Crit < F	✓	P<0.001	Treatment > Control	F Crit < F	✓	P<0.001	Treatment > Control

Means within the data displayed significance when comparing the F crit and F values. F – crit was smaller than F for all study participants for Al and Si and when applying Tukey testing, the treatment period contained higher elemental excretion of Si and Al in sweat for all individuals.

4.4.5 Comparing gender within the study population

Wilcoxon signed-rank was used to determine where any differences within the data lie. The differences between the mean sweat excretion of Al and Si for the study group are shown in *Table 4.4.5.1*. Correlation between the treatment data was stronger than the control, presenting r-values of 0.96 and 0.51 respectively, highlighting that these two independent variables were selected from populations with the same distribution.

*Table 4.4.5.1: Summary of **Wilcoxon signed-rank** - differences between pairs of means of excretion for male (n = 9) and female groups (n = 10).*

Relationship with Silicon		
	Aluminium	
	r-Value	**p-Value**
Control	0.51	<0.05
Treatment	0.96	<0.05

Males generally excreted more Si and Al, which was statistically significant during the entire study duration (P = 0.001). An example of a treatment study replicate showing a mean excretory Al value of 619µg/L was seen for female F5, compared to 1276 µg/L for male M5. A further example

for a mean excretory Si value of a treatment study replicate of 1476µg/L was seen for female F5, compared to 2327µg/L for male M5.

*Table 4.4.5.2 Summary of **Wilcoxon signed-rank** - differences between pairs of means of excretion for male (n = 9) and female groups (n = 10).*

Male v Female Patients		
	Silicon	Aluminium
	P-Value	P-Value
Control	0.018	0.962
Treatment	0.268	0.039

The Mann-Whitney U test confirms a statistical significance (W=0, P < 0.05) for the study population when comparing amount of Al and Si sweat. *Table 4.4.5.3* shows this statistical relationship and the difference in medians between the control and treatment period.

*Table 4.4.5.3: Significance of the differences in sweat excretions of Si (µg/L) and Al (µg/L) between control and treatment (**Mann Whitney U test**).*

Silicon (ug/L)						
Difference	**Male**			**Female**		
	W-Value	**P-Value**	**Median**	**W-Value**	**P-Value**	**Median**
Control-Treatment	0	0.014	810-1726	0	0.009	657-1611

Aluminium (ug/L)						
Difference	**Male**			**Female**		
	W-Value	**P-Value**	**Median**	**W-Value**	**P-Value**	**Median**
Control-Treatment	0	0.014	400-1276	0	0.009	285-990

Data (*Table 4.4.5.4)* showing the excretion of Si in sweat during the control phase was statistically similar for males and females (P=0.018) although data held less statistical relevance between the same pool of individuals for the treatment period (P = 0.268). In addition to this, the data showed similarities between males and females during the treatment period for Al, indicating that people show a similar relationship in Al excretion (P = 0.039), however, during the control, this relationship was statistically dissimilar different between males and females (P=0.962).

Table 4.4.5.4 Differences in the median excretions of Si (µg/L) and Al (µg/L) between males and females for the healthy volunteer group.(Mann-Whitney U test).

Silicon (µg/L)			
	Patient		
	Male (n=9)	Female (n=10)	P-value
Control	826	676	0.018
Treatment	1767	1564	0.268
ΣW	1298	1120	0.143
Aluminium (µg/L)			
	Patient		
	Male (n=9)	Female (n=10)	P-value
Control	341	353	0.962
Treatment	1208	835	0.039
ΣW	775	594	0.5

When looking at the difference between amounts of Si and Al excreted in the sweat of the healthy volunteers (*Table 4.4.5.5)*, seventeen out of the nineteen individuals excreted 50% more Si in their sweat after consuming the Si rich mineral water. Seventeen out of nineteen participants excreted 50% more Al in their sweat after the consumption of the mineral water, the two individuals (M3 and M5) presenting less than 50% were different for the lower percentage difference in Si excretion (F6 and F7). It is interesting to note that for Al these individuals were both male, while for Si these individuals were both female.

*Table 4.4.5.5: **Mean** creatinine corrected urinary excretions of Si (µg/L) and Al (µg/L) for the control and treatment groups and the **percentage difference** between the groups.*

	Silicon			Aluminium		
	Control	Treatment	% Difference	Control	Treatment	% Difference
F1	812	1466	57.4	467	1184	86.9
F2	647	1609	85.3	217	381	54.8
F3	601	1332	75.6	270	1081	120.1
F4	626	1683	91.6	255	986	117.8
F5	576	1476	87.7	187	619	107.2
F6	668	1612	82.8	300	424	34.3
F7	616	1110	57.2	266	427	46.5
F8	732	1634	76.2	520	1035	66.2
F9	787	1778	77.3	585	994	51.8
F10	691	1938	94.9	461	1215	90
M1	944	1661	55	444	772	53.9
M2	1050	2167	69.4	71	1030	174.2
M3	874	1281	37.8	526	1589	100.5
M4	743	1870	86.3	574	1677	98
M5	810	1348	49.9	464	1666	112.9
M6	783	2327	99.3	183	1276	149.8
M7	994	1941	64.5	152	1536	164
M8	578	1588	93.3	400	690	53.2
M9	674	1726	87.7	255	639	85.9

The correlation between aluminium and silicon excretion in sweat during the baseline and treatment period is shown in figure 4.4.5.1.

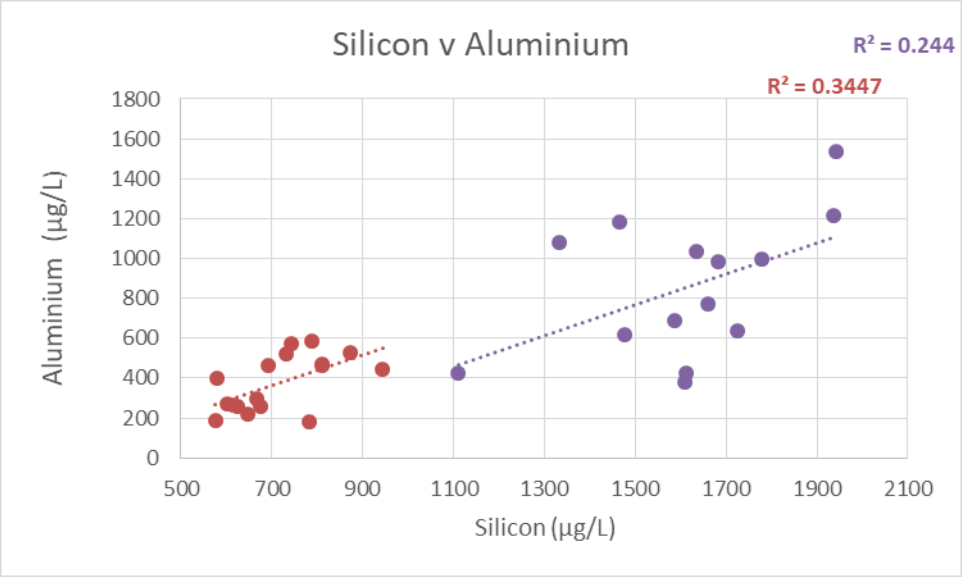

Figure 4.4.5.1 Sweat excretion of Al (µg/L) for the control (●) and treatment (●) periods against Si excretion (µg/L).

4.4.6 Comparison between the excretion of silicon in sweat with the volume of silicon rich mineral water consumed

All healthy volunteers were instructed to consume as much of the 1500mL bottle of Spritzer mineral water as possible 1 hour before the exercise programme was initiated. The final volumes consumed were noted for each control and treatment experimental replicate (n=6) and the amount of Si taken on board prior to the exercise was calculated (*Table 4.4.2.1*). This value was then compared to the difference between control and treatment Si excretion values so any influence of the water on Si excretion was established.

Despite the loading of the mineral water 1 hour before exercise, the difference in Si values represents a small proportion of the Si consumed in the water, with low variability between both male and female participants, 2.4 and 2.1% respectively.

The concentration of silicon in sweat increased significantly during the treatment period, a trend seen for all volunteers ($P < 0.005$, ANOVA). Further to sweat volume being larger for male participants, on average, silicon excretion was larger in males than females, despite more males not consuming the entirety of the Spritzer water provided to them. Five out of the ten females and six out of the nine males failed to consume the suggested volume of 1500mL in 1 hour.

The maximum amount of Si in 1500mL of the mineral water was calculated to be 44.26mg and eight out of the nineteen volunteers consumed this full amount.

When the study population was considered as a group, sweat Si concentration increased after the consumption of the mineral water an hour before exercise. During the control, Si excretion was calculated at 748µg/L (±140 µg/L) and during the treatment protocol, this value increased to 1660 µg/L (±303 µg/L). This increase was seen for both males and females (*Table 4.4.6.1)*, although, the difference was more significant for the male subjects, when comparing the two means ($P < 0.001$ and $P = 0.002$ respectively, Pearson correlation analysis). Males excreted 828 µg/L (±153 µg/L) Si in their sweat as a baseline and 1767 µg/L (348 µg/L) during the treatment protocol, while females produced 676 µg/L (±79 µg/L) of Si in the baseline period and 1564 µg/L (±244 µg/L).

Table 4.4.6.1: Comparison between the excretion of Si in sweat with the volume of silicon rich mineral water consumed (mL) and percentage of Si consumed as mineral water.

ID	Spritzer consumed (mL)	Si consumed (µg)	Difference in silicon excretion (Treatment – Control, µg/L)	Percentage of mineral water which equates to the difference in Si
F1	1258	37091	654	1.8
F2	1460	43047	962	2.2
F3	1329	39184	731	1.9
F4	1500	44226	1057	2.4
F5	1376	40570	900	2.2
F6	1500	44226	944	2.1
F7	1247	36767	494	1.3
F8	1500	44226	902	2.0
F9	1500	44226	991	2.2
F10	1500	44226	1247	2.8
Mean	1417	41779	888	2.1
M1	1320	38919	717	1.8
M2	1500	44226	1117	2.5
M3	754	22231	407	1.8
M4	1343	39597	1127	2.8
M5	1022	30133	538	1.8
M6	1500	44226	1544	3.5
M7	1500	44226	947	2.1
M8	1429	42133	1010	2.4
M9	1300	38329	1052	2.7
Mean	1296	38224	940	2.4

During the healthy volunteer study in Chapter 3, a weak positive correlation was witnessed between the amount of Si taken into the body and the amount excreted. This is seen in sweat too. When more Si is consumed, more Si is excreted (R=0.39). Eight out of the nineteen volunteers consumed all 1500mL silicon rich mineral water, but those that didn't enabled us to see this relationship in *Figure 4.4.6.1*.

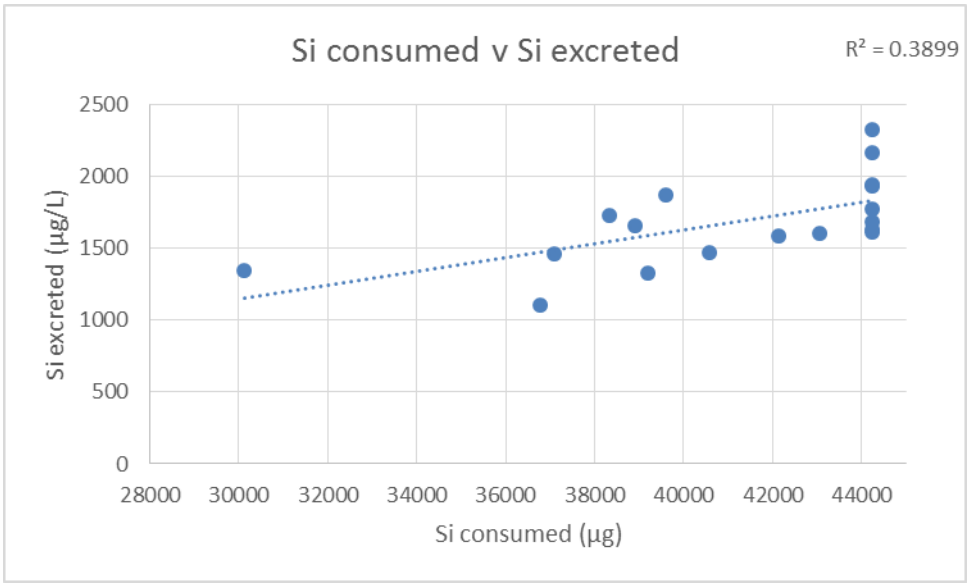

Figure 4.4.6.1 Comparing the amount of Si taken on by each participant in the form of Si rich mineral water in relation to the amount excreted by each study participant.

4.4.7 Twenty-four hour data correction

The data was corrected for 24 hour sweat excretion using literature values. Concentrations of aluminium and silicon in collected sweat were adjusted to take account of the volume of sweat produced by men (1342 mL/24 h) and women (712 mL/24 h), values established by Manz *et al* (2012).

4.4.7.1 Silicon

When corrected for 24 hour perspiration, Si excretion increased for males and reduced for females over this time (*Table 4.4.7.1.1)*, making these differences more prominent than measurements

calculated per L^{-1}. The amount of Si excreted in the sweat of males increased from 1110.9 µg (±205.8 µg) during the control 24 hour period to 2372.1 µg (±467.7 µg). The variance in this male data was from 905 µg to 1409 µg during the control 24 hour period, and 1719.1 µg to 3123.2 µg during the 24 hours for the treatment protocol.

Table 4.4.7.1.1: 24-hour mean (SD) excretion of Si in the perspiration of males within the study population (n=9)

[Si] in 24 hr sweat excretion in males		
ID	**[Si] Sweat control (µg/24hr)**	**[Si] Sweat treatment (µg/24hr)**
M1	1267.3	2228.5
M2	1409.0	2907.7
M3	1172.9	1719.1
M4	997.1	2509.7
M5	1086.3	1808.7
M6	1050.1	3123.1
M7	1334.5	2605.0
M8	776.2	2131.0
M9	905.0	2316.6
Mean (SD)	1110.9 (205.8)	2372.1 (467.7)

The amount of Si excreted in the sweat of females increased from 486.3 µg (±57.2 µg) during the control 24 hour period to 1125.9 µg (±167.3 µg). The variance in this female data was from 414.9 µg to 584.6 µg during the control 24 hour period, and 799.2 µg to 1395.2 µg during the 24 hours during the treatment protocol (*Table 4.4.7.1.2)*.

Table 4.4.7.1.2: 24-hour mean (SD) excretion of Si in the perspiration of females within the study population (n=9)

[Si] in 24 hr sweat excretion in females		
ID	**[Si] Sweat control (µg/24hr)**	**[Si] Sweat treatment (µg/24hr)**
F1	584.6	1055.4
F2	465.5	1158.6
F3	432.6	959.1
F4	450.4	1211.6
F5	414.9	1062.9
F6	480.7	1160.6
F7	443.4	799.2
F8	527.1	1176.5
F9	566.6	1279.8
F10	497.5	1395.2
Mean (SD)	486.3 (57.2)	1125.9 (167.3)

After 24-hour correction, a larger difference can be seen between elemental excretion in sweat between males and females within the healthy volunteer study (*Table 4.4.7.1.3)*. When considering Si excretion, the baseline Si concentration in sweat for males equated to 1110.0µg/L, while for females, less than half of this amount was recorded at 486.3µg/L. This ratio was consistent to the treatment protocol, as for males, an average sweat Si content was 2372.1 µg/L, while for females, this value equated to 1125.9 µg/L.

Table 4.4.7.1.3: 24-hour mean (SD) excretion of Si in the perspiration of healthy volunteers within the study population (n=19)

[Si] in 24 hr sweat excretion in healthy volunteers		
Gender	**[Si] Sweat control (µg/24hr)**	**[Si] Sweat treatment (µg/24hr)**
Male	1110.9 (205.8)	2372.1 (467.7)
Female	486.3 (57.2)	1125.9 (167.3)

4.4.7.2 Aluminium

When corrected for 24 hour perspiration, Si excretion increased for males and reduced for females over this time, making these differences more prominent than measurements calculated per L^{-1}. Data in Table 4.4.7.2.1 shows how the amount of Al excreted in the sweat of males increased from 457.6 µg (±241.1 µg) during the control 24 hour period to 1621.5 µg (±581.4 µg). The variance in this male data was from 95.8 µg to 769.8 µg during the control 24 hour period, and 857.1 µg to 2250.0 µg during the 24 hours for the treatment protocol.

Table 4.4.7.2.1: 24-hour mean (SD) excretion of Al in the perspiration of males within the study population (n=9)

[Al] in 24 hr sweat excretion in males		
ID	**[Al] Sweat control (µg/24hr)**	**[Al] Sweat treatment (µg/24hr)**
M1	595.8	1036.0
M2	95.8	1382.5
M3	705.2	2132.3
M4	769.8	2250.0
M5	623.0	2236.0
M6	245.9	1712.0
M7	203.7	2061.8
M8	536.5	925.7
M9	342.5	857.1
Mean (SD)	457.6 (241.1)	1621.5 (581.4)

The amount of Al excreted in the sweat of females increased from 254 µg (±101.6 µg) during the control 24 hour period to 600.8 µg (±240.4 µg). Table 4.4.7.2.2 shows the variance in this female data, ranging from 191.2 µg to 421.1 µg during the control 24 hour period, and 305.1 µg to 874.7 µg during the 24 hours during the treatment protocol.

Table 4.4.7.2.2: 24-hour mean (SD) excretion of Al in the perspiration of females within the study population (n=9)

[Al] in 24 hr sweat excretion in females		
ID	**[Al] Sweat control (µg/24hr)**	**[Al] Sweat treatment (µg/24hr)**
F1	336.5	852.3
F2	156.4	274.1
F3	194.3	778.5
F4	183.6	709.6
F5	134.9	445.8
F6	215.8	305.1
F7	191.2	307.4
F8	374.5	745.1
F9	421.1	715.9
F10	331.6	874.7
Mean (SD)	254.0 (101.6)	600.8 (240.4)

After 24-hour correction, a larger difference can be seen between elemental excretion in sweat between males and females within the healthy volunteer study (*Table 4.4.7.2.3)*. When considering Al excretion, the baseline Al concentration in sweat for males equated to 457.6µg/L, while for females, just more than half of this amount was recorded at 254.0µg/L. This ratio was consistent to the treatment protocol, as for males, an average sweat Al content was 1621.5 µg/L, while for females, this value equated to 600.8 µg/L, less than half of the amount calculated for males.

Table 4.4.7.2.3: 24-hour mean (SD) excretion of Si in the perspiration of healthy volunteers within the study population (n=19)

[Al] in 24 hr sweat excretion in healthy volunteers		
Gender	**[Al] Sweat control (µg/24hr)**	**[Al] Sweat treatment (µg/24hr)**
Male	457.6 (241.1)	1621.5 (581.4)
Female	254.0 (101.6)	600.8 (240.4)

Once corrected, weak positive correlation was noticed between Si and Al excretion in sweat during both control (r = 0.4) and treatment (r = 0.5) protocols (*Figure 4.4.7.2.1).*

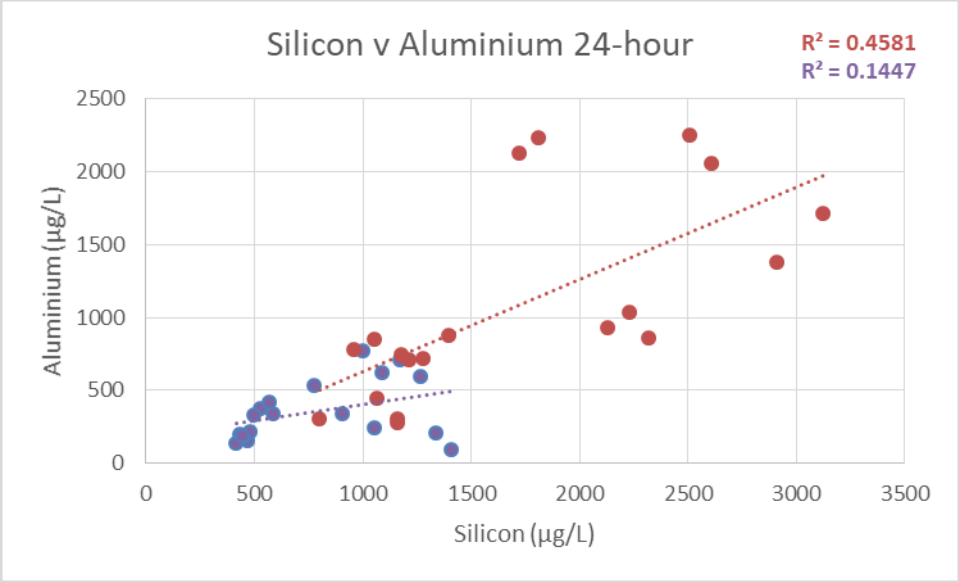

Figure 4.4.7.2.1: Corrected 24 hour sweat excretion concentrations of Al (µg/L) for the control (●) and treatment (●) periods against Si excretion (µg/L).

Pearson correlation analysis (*Table 4.4.6.7)* indicated that this weak positive correlation between Si and Al excretion in sweat was significant for both control and treatment protocols ($P < 0.005$).

Table 4.4.6.7: Correlation analysis between the 24hr corrected excretion of Al (µg/24hr) with the excretion of Si (µg/24hr).

Relationship with silicon		
	Aluminium	
	r-Value	p-Value
Control	0.4	<0.05
Treatment	0.5	<0.05

All data analysis highlights that consuming the Si rich mineral water had a positive effect on the excretion of Si and Al in sweat, supporting the observation that levels of Si and Al in sweat approximately doubled after the incorporation of 1500mL of mineral water 1 hour before exercise. This is visually represented in *Figure 4.4.6.2.*

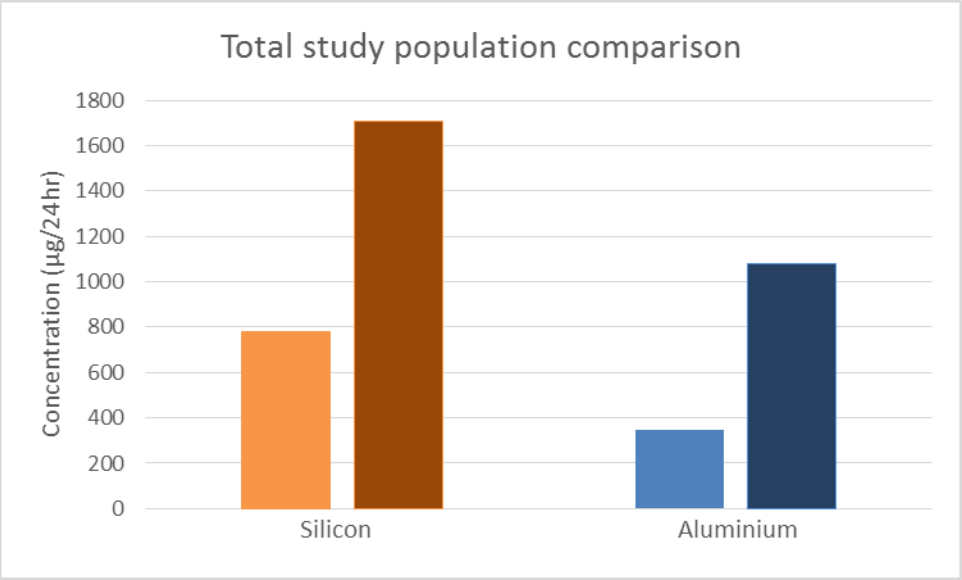

Figure 4.4.6.2: 24 hour corrected excretion of Al (µg/24hr) for the control (Si ● Al ●) and treatment period (Si ● Al ●) compared to Si excretion (µg/24hr) for the study group (n=19)

4.4.7 Age and BMI comparisons within the study group

After 24-hour correction, the elemental sweat excretions were compared with age. Healthy volunteers in this study ranged from 18-34 years and despite low significant correlation between age and Si excretion in sweat (P > 0.3), correlation between Al excretion in sweat and age were more significantly correlated (P <0.05), using Pearson correlation analysis (*Table 4.4.7.1*).

Table 4.4.7.1: Correlation analysis between age and excretion of Si and Al (µg/L) between the control and treatment periods

Age v 24hr corrected sweat excretion		
	Silicon	Aluminium
	p- value	
Control	0.4	0.007
Treatment	0.3	0.004

Excretion of Si and Al were plotted against age in *Figure 4.4.7.1.* The mean age for the study was 24 years and only one individual over 30 years of age took part in the study. Weak positive correlation can be seen for all relationships, although, the relationship between elemental excretion and age is stronger during the control period.

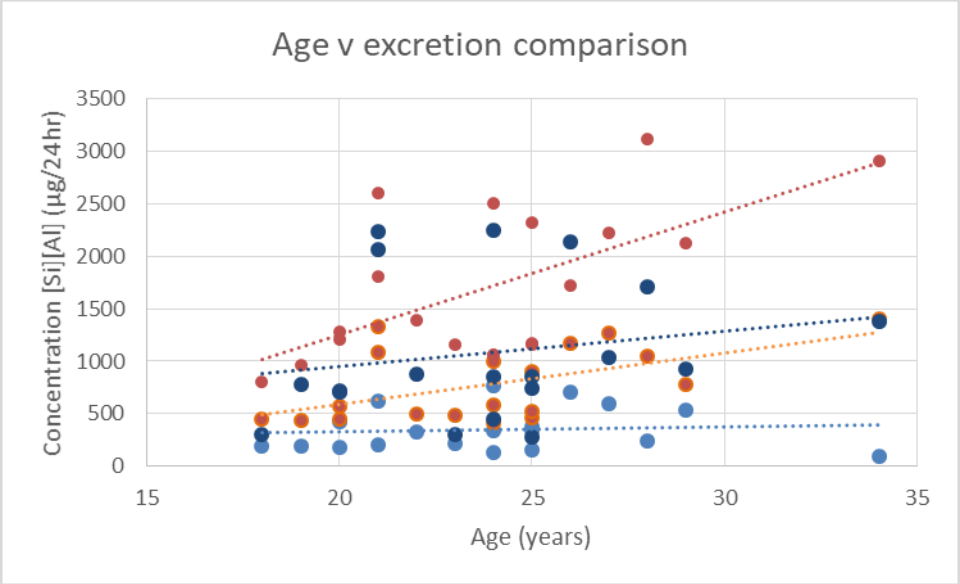

Figure 4.4.7.1: 24 hour corrected excretion of Al (µg/24hr) against age for the control (Si ● Al ●) and treatment period (Si ● Al ●) compared to Si excretion (µg/24hr) against age for the study group (n=19)

Pearson correlation analysis, shown in *Table 4.4.7.2* confirms the relationship between age and elemental excretion in sweat. Stronger correlation and higher r – values were seen during the control protocol for both Si and Al, at 0.59 and 0.79 respectively. This correlation decreases during the treatment protocol, signifying a unique relationship between each individual and their handling of Si at high doses.

Table 4.4.7.2: 24-hour corrected comparison between elemental excretion in sweat and subject age (n=19)

Age v sweat elemental excretion (n=19)		
Study	**Silicon**	**Aluminium**
	r - Value	
Control	0.59	0.79
Treatment	0.44	0.26

After 24-hour correction, the elemental sweat excretions were compared with BMI. Healthy volunteers in this study ranged BMI values from 20-41.5, although most of the healthy volunteers were of a normal BMI, ranging from 20-25. When analysed, the data followed a similar trend to age comparisons. The statistical significance between elemental excretion was larger for the control protocol than the treatment protocol, Pearson correlation analysis revealed a p- value of 0.022 and 0.17 for these populations respectively.

Despite low significant correlation between BMI and Si excretion in sweat ($P > 0.2$), correlation between Al excretion in sweat and BMI were significantly correlated ($P < 0.05$) in the control period, using Pearson correlation analysis (*Table 4.4.7.3)*.

Table 4.4.7.3: Correlation analysis between BMI and excretion of Si and Al (µg/L) between the control and treatment periods

BMI v 24hr corrected sweat excretion		
	Silicon	Aluminium
	p - value	
Control	0.022	0.018
Treatment	0.17	0.13

Excretion of Si and Al were plotted against BMI in *Figure 4.4.7.2.* The mean BMI for the study was 26.4 and only one individual with a BMI over 40 took part in the study. Weak positive correlation can be seen for all relationships, although, the relationship between elemental excretion and BMI is stronger during the control period.

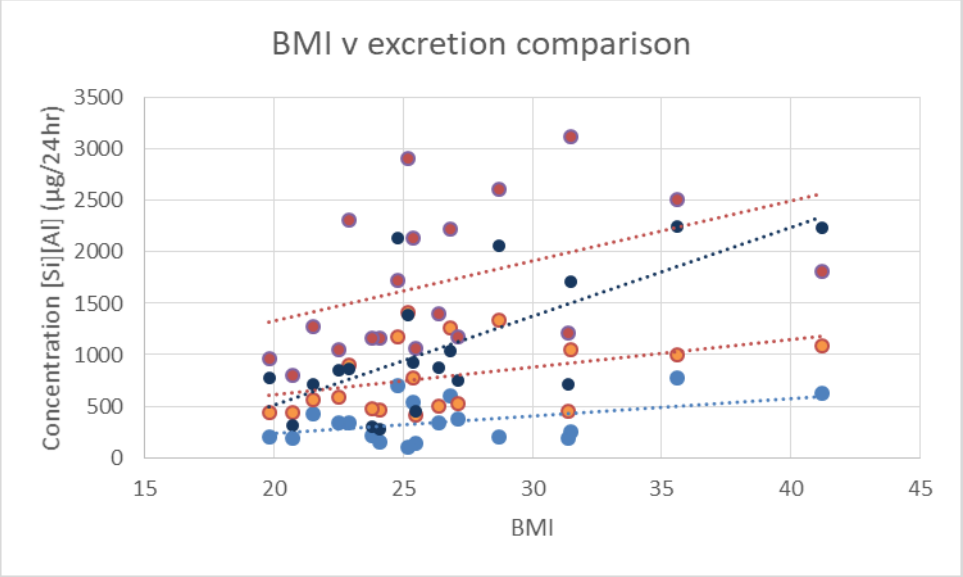

Figure 4.4.7.2: 24 hour corrected excretion of Al (µg/24hr) against BMI for the control (Si ● Al ●) and treatment period (Si ● Al ●) compared to Si excretion (µg/24hr) against age for the study group (n=19)

Pearson correlation analysis, shown in *Table 4.4.7.4,* confirms the relationship between BMI and elemental excretion in sweat. Stronger correlation and higher r – values were seen during the control protocol for both Si and Al, at 0.55 and 0.68 respectively. This correlation decreases during the treatment protocol, signifying a unique relationship between each individual and their handling of Si at high doses.

Table 4.4.7.4: 24-hour corrected comparison between elemental excretion in sweat and subject BMI (n=19)

BMI v sweat elemental excretion (n=19)		
Study	**Silicon**	**Aluminium**
	r - Value	
Control	0.55	0.68
Treatment	0.31	0.18

4.5 Summary of results for healthy individuals

• Sweat volume collected was larger for males than females (Section 4.4.1.).

• The amount of Si from the Si rich mineral water was determined to be only around 2% of that consumed (4.4.2)

• Excretions of Si and Al in sweat are significantly increased following the consumption of the mineral water (Section 4.4.3).

•Weak positive correlation was demonstrated between Si and Al excretion (Section 4.4.5)

• Si and Al showed weak positive correlation with age during the control period (Section 4.4.7).

• Weak positive correlation was noted between BMI and urinary excretion of Al and Si during the control protocol (Section 4.4.7)

4.6 Discussion of results

The mean aluminium content in the control period was higher in females (341 ± 245 µg/L) than males (353 ± 141 µg/L) though the data for each group were not found to be significantly different from one another ($P > 0.05$). Contrary to this the mean aluminium content in the treatment protocol was higher in males (1208 ± 433µg/L) than females (835 ± 334µg/L) and the data for each group were found to be significantly different from one another ($P < 0.05$). The volume of sweat collected ranged from 0.66 to 1.55 mL and corresponded well with literature values for perspiration rates (Chen *et al.* 2012). The method blanks demonstrated a very low level of possible contamination by aluminium and were within the range of a previous study (Exley *et al* 2014).The latter demonstrated a high degree of

confidence in the concentrations of aluminium measured herein which were towards the higher end of previous literature values (House *et al* 2012).

When the concentrations of aluminium in collected sweat were adjusted to take account of the volume of sweat produced by men (1342 mL/24 h) and women (712 mL/24 h) in this age range (Manz *et al* 2012) and undergoing mild exercise the amount of aluminium excreted over 24 h ranged from 135 to 421 μg/24 h for women to 96–770 μg/24 h for men during the control and from 274 to 875 μg/24 h for women to 857–2250 μg/24 h for men (*Table 4.4.7.2.1 and Table 4.4.7.2.2*) with men excreting significantly more aluminium than women ($P < 0.05$). These data are significantly higher than those that describe the daily excretion of aluminium in urine, up to 100 μg/24 h (Exley, 2013) and therefore they heavily implicate sweating as the major route of excretion of systemic aluminium in humans, especially after the consumption of a Si rich mineral water. In doing so it may be that men, through perspiration, excrete aluminium from the body more effectively than women and it can be suggested that regular exercise might be a way to increase the excretion of aluminium from the body. If sweating is the major route for the removal of systemic aluminium from the body then this observation puts into question the practice of disrupting or blocking perspiration using antiperspirants, specifically, aluminium-based antiperspirants.

Chapter 5 - Silicic acid-rich mineral water as a non-invasive method of reducing the aluminium body burden in individuals diagnosed with multiple sclerosis.

5.1.0 Aims

The aim of this chapter is to investigate if regular drinking of a silicon-rich mineral water can help in the removal of Al from the body of individuals with a diagnosis of MS. Secondary aims were to determine if drinking silicic acid-rich mineral water had any effect on mobility index scores and general patient wellbeing.

5.2.0 Introduction

The link between aluminium and multiple sclerosis has been one of tremendous dispute for several decades. Despite a number of studies suggesting a potential involvement, the link still remains controversial (see section 1.1). By highlighting the effect of silicic acid in the removal of aluminium from the human body, unequivocal data can be obtained to support this link (Exley *et al* 2006).

Multiple sclerosis (MS) is a chronic, immune-mediated, demyelinating disease of the central nervous system of as yet unknown aetiology (see section 1.14). A consensus of opinion has suggested that the disorder is the result of an interplay between environmental factors and susceptibility genes. Exley *et al* (2006) used a battery of analytical techniques to determine if the urinary excretion of i) markers of oxidative damage and ii) the environmental toxin aluminium and its antagonist, silicon, were altered in secondary progressive MS (SPMS). Urinary concentrations of aluminium were significantly increased in 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 is lower in MS and significantly so in SPMS ($P<0.05$). Levels of urinary aluminium excretion were similar to those seen

in aluminium intoxication and 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.

In Chapter 3 of this thesis it was demonstrated that for healthy individuals (Chapter 3.0), silicic acid-rich mineral waters could be exploited as a non-invasive means of reducing burden of aluminium and long term treatment by regular consumption could maintain aluminium body burden at a reduced level.

5.3.0 Methodology

Fifteen multiple sclerosis patients were recruited through the University Hospital of North Staffordshire (UHNS, now Royal Stoke) through the MS department by a qualified clinician following the inclusion and exclusion criteria. Ethical approval was attained by myself, from the national research ethics committee (NREC reference 14/YH/1115). All volunteers provided written consent prior to participation, which was obtained by a qualified clinician.

All SPMS patients attending the Neurology clinics were screened for possible inclusion in the study. Suitable and interested patients were posted a participant information pack, including the PIS and coversheet, explaining the outline of the study. This was followed up via telephone calls/postal letters by a study clinician to confirm their interest and participation. Those who were interested were asked to be come back to a research clinic where a screening assessment was

carried out by a study clinician and eligible patients were asked to sign the consent form. A standard time period of 24 hours was given between the receipt of the study information sheet and taking informed consent.

The inclusion criteria included: All patients must have a confirmed diagnosis, by a study clinician, of secondary progressive multiple sclerosis (SPMS) according to the McDonald inclusion criteria. Patients must be willing and able to give informed consent in line with the Mental Capacity Act. Patients had a carer to help ensure that the protocol was followed accordingly. Carer was willing and able to give informed consent. Patient would not suffer any ill effects or practical difficulties on the consumption of up to 1.5L of mineral water and had no restriction on fluid ingestion. As well as any patients not satisfying the above inclusion criteria, a potential study participant would be excluded if those patients were on disease modification treatment, patients with current urinary infections and patients with a history of impaired renal function. The patient could be initiated onto the study once an infection free urine sample had been provided. During recruitment, a Kurtjze EDSS score (Kurtjze 1983) was given to each patient. This was followed up at the end of the study to look for improvements in mobility, as well as a questionnaire querying general wellness.

One female patient (008) had to be withdrawn from the study due to a series of urine infections. The mean age of the remaining 15 MS patients who completed the study, was 72 (range 56 - 81) and 8 were female.

5.3.1 Protocol

The project protocol was divided into two objectives; the primary objective was to measure the urinary excretions of Si and Al over a 24 week period during the second 12 week period of which patients consumed up to 1.5L of silicon-rich mineral water each day. The first 12 weeks of the study, referred to as the control, required each participant to provide urine samples following their normal diets, thus acting as their own control to determine individual changes. The secondary objective was to observe changes in mobility using EDSS scores at the beginning and end of the study.

5.3.2 Urinary excretions

This study examined the effects of long-term consumption of mineral water on the urinary excretion of Si and Al. The study duration was split up into two parts, the first 12 weeks (W1-W12), known as the control period and the second 12 weeks (W13-W24) referred to as the treatment period. Participants collected all of their urine (24 hour samples) for the 5 days in W1, W12, W13 and W24 into 3L sterile containers. During the rest of the study weeks, the first morning sample of the day on the same day of each week was collected into sterile 50mL containers. These collections were made for the baseline (W1 to W12 - no mineral water consumption) and treatment periods (W13 to W24 - including consumption of up to 1.5L of mineral water each day).

Differences in the urinary excretions of Si and Al between control and treatment weeks 1, 2, 13 and 24 for each individual were determined using one-way ANOVA and Tukey's paired

comparison tests. Differences for control and treatment data were measured using repeated measures ANOVA and Wilcoxon signed-rank tests.

Results for the daily collections are presented as total amount excreted per 24 hours (µg/24 hr for Si and ng/24 hr for Al), however, these concentration are also displayed as creatinine-corrected data in order to make comparisons with the spot samples, allowing for any deviations in urine volume. Volunteers were requested to consume as much as the 1.5L of the provided mineral water as they could comfortably manage. This volume is the same as the healthy individual urine study because the consumption of 1.5L every day for a period of 12 weeks seemed acceptable to the patients, although, unlike the loading treatment in the healthy volunteer study, participants in this study were able to make up their normal drinks with the water, essentially replacing other water consumed in their daily diets with the Si rich mineral water.

It was thought to be inconvenient for the patients to provide record sheets as in the healthy volunteer study (see Chapter 3). Instead, the patients were provided with a tick sheet to record their water consumption and sample collection.

As in the healthy volunteer study, results are recorded both before and following correction for creatinine concentrations, and 24 hour samples were expressed in both formats.

The requirements for this study were maintained at a minimum so not to make participation too complexed for individuals suffering from MS. Therefore, dietary record sheets, as collected in the healthy individual, study were not required during this study. The length of the control period

satisfied this as it meant that a thorough indication of normal Si and Al excretion could be deduced.

5.3.2.1 Treatment of urine samples

Samples were collected and stored in pre acid-washed polypropylene containers (5% HCl), rinsed with UPW. Urine samples were collected directly into these pre-washed 3L containers for the 24 hour trial. Pre-washed jugs were provided for the 24 hour-long term trials for the purpose of convenience. The 24 hour sample volumes were measured and a portion transferred to the 50mL container. The remaining sample was disposed of down the toilet and the jug rinsed with Decon. Samples were directly transferred to the trace element lab where there were stored in a clearly labelled refrigerator. Samples were vortexed to ensure uniformity and an aliquot of urine sample was taken and diluted to 10% with UPW for creatinine analysis using the Jaffe method (Toora, 2002).The remaining urine was acidified using 15.8M HNO_3 to 20% *v/v* and subjected to microwave digestion to prepare samples for measurement of total silicon and aluminium by THGFAAS.

5.3.3 Statistics

One-way ANOVA at ά = 0.05 was carried out on each individual data set to compare the means of Wk 1, Wk12, Wk 13 and Wk 24 (5 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, male and female was valid because of equal variance. The non-parametric Mann-Whitney U test was used to compare pairs of medians. Analyses were carried out using Minitab® 15.

Differences in the urinary excretions between the control and treatment periods were determined using the paired t-test, and comparisons between males and females were performed using the 2-sample t-test. An aged matched healthy control group was not sought after for this study, but instead data available from other studies was used.

5.3.4 EDSS Score

The Kurtzke Expanded Disability Status Scale (EDSS) is a method of quantifying disability in multiple sclerosis and monitoring changes in the level of disability over time. It is widely used in clinical trials and in the assessment of people with MS. The scale has been developed by John F. Kurtzke, (1983) (See Appendix 5 for the scale).

Each individual completed the EDSS test prior to and following the treatment of the mineral water, this was performed by the qualified clinician. Comparisons between the initial and final score were used to determine whether the consumption of the mineral water had any influence on their mobility. An increase in EDSS score would indicate that the level of disability is worsening whereas a reduction in score would highlight an improvement in mobility and reduction in disability.

Overall scores of the EDSS tests were provided by the clinical nurse on the completion of the urinary analysis. Detailed reports of the performance of the tests (i.e. scores of each section) were not made

available for this thesis as these reports carry confidential patient information. Patient questionnaires were collected post study to determine any improvements in health, wellbeing and general feedback.

5.4.0 Results

This chapter includes the statistical comparisons to the baseline period (week 1 to week 12) and the study period (week 13 to week 24). Comparisons were made for each participant using one-way ANOVA to echo individual responses to the mineral water. Repeated-measures ANOVA were then performed for each patient to provide a general depiction of the effect of the mineral water on the urinary excretions.

All spot sample results shown in this chapter have been creatinine corrected. Data for the 24 hour collections in Wk 1, 12, 13 and 24 have been creatinine corrected and expressed as total amount excreted in this 24 hour period. Individuals collected one urine sample a week for weeks 2-11, then 14-23.

Fifteen individuals, diagnosed as SPMS patients completed the study and informal indices, such as conversations and completion of questionnaires, suggested that compliance with study protocols was excellent. Data are summarized according to treatment group, gender and analysed below as treatment groups and as individuals.

5.4.1 Creatinine measurements

Creatinine concentrations were measured for each sample in order to correct for differences in renal functioning between individuals and to take any dilution effects of the urine into consideration. There was no significant difference between males and females in mean creatinine concentrations, for both the control (t = 2.87: P = 0.012), and treatment (t = 1.71: P = 0.424) period, 4.44mM and 4.45mM respectively.

Table 5.4.1.1. Mean (SD) urine creatinine concentrations (mM) collected from the whole MS study population for both baseline and treatment periods (n=20)

Mean (SD) urinary creatinine concentration for each MS patient		
ID	**Baseline (mmol)**	**Treatment (mmol)**
F001	2.3 (1.0)	2.6 (1.1)
M002	5.1 (1.8)	4.1 (2.1)
F003	4.5 (2.0)	4.6 (3.4)
M004	6.2 (2.8)	6.2 (3.0)
M005	8.2 (3.8)	4.8 (2.1)
F006	3.4 (0.8)	3.5 (1.4)
M007	4.2 (1.4)	4.1 (2.2)
F009	2.4 (0.5)	2.9 (1.1)
M010	3.8 (1.0)	4.4 (1.6)
M011	5.9 (2.6)	7.4 (3.0)
F012	3.4 (1.7)	2.3 (0.6)
F013	4.0 (1.6)	4.4 (1.6)
M014	6.7 (2.4)	7.5 (2.8)
F015	2.5 (1.6)	3.0 (1.6)
F016	4.0 (1.9)	4.5 (1.9)
ẍ	**4.44**	**4.45**

Mean urinary creatinine concentration remained constant for gender comparisons, although remaining higher for males throughout the studies. Mean baseline creatinine concentration for males and females was 5.7mM and 3.2mM respectively and mean creatinine concentration during the treatment period was noted at 5.5mmol and 3.5mmol, decreasing for males and increasing for females, this however was not a significant change (t = 1.05, p = 0.71).

5.4.2 Volume measurements

Total volume for the 24 hour collections periods were measured to accurately quantify amounts of Si and Al excreted over this time. Total urine volume was larger during the treatment period than the baseline study for 11 out of 15 participants, although these differences were notably small (P = 0.028). Urine volume increased significantly for one patient, ID F015, who saw their urine increase from 1867mL/24 hr to 3033ml/24 hr (Table 5.4.2.1).

Table 5.4.2.1. Mean urine volumes collected from the whole MS study population during the 24 hour studies for both baseline and treatment periods (n=7)

Mean (SD) urinary volume for the baseline and treatment 24 hour collections			
ID	**Baseline volume (mL)**	**Treatment volume (mL)**	**Difference in Volume**
F001	1825 (466)	1931 (494)	106
M002	1473 (331)	1875 (643)	402
F003	1161 (199)	1133 (574)	-28
M004	1398 (267)	1639 (352)	241
M005	1202 (200)	1056 (420)	-146
F006	1313 (256)	1362 (307)	49
M007	2239 (597)	2391 (636)	152
F009	2029 (390)	2017 (321)	-12
M010	2156 (408)	2403 (206)	247
M011	1757 (272)	1944 (423)	187
F012	1598 (636)	2568 (345)	970
F013	2035 (521)	1853 (653)	-182
M014	1090 (212)	1461 (303)	371
F015	1867 (984)	3033 (63)	1166
F016	718 (139)	1207 (821)	489
ẍ	**1591 (392)**	**1858 (199)**	**267**

There was no significant difference between males and females in the mean volumes excreted for the control (t = -3.61: P = 0.001) and treatment (t = -1.44: P = 0.29) period (*Table 5.4.2.2).*

Table 5.4.2.2. Mean urine volumes compared by gender in MS study population during the 24 hour studies for both baseline and treatment periods (n=7)

Gender comparisons between urine volume in 24 hour collection weeks (n=7)				
Gender	**Volume Wk 1** (mL)	**Volume Wk 12** (mL)	**Volume Wk 13** (mL)	**Volume Wk 24** (mL)
Male	1620 (559)	1612 (535)	1857 (679)	1690 (558)
Female	1587 (709)	1550 (620)	1918 (797)	1787 (762)

The amount of urine increased for both genders between baseline Wk 1, giving a mean value for males and females of 1620mL and 1587mL respectively. This value increases in both genders in the initial treatment Wk 13 to 1857mL and 1918mL respectively.

5.4.3 Individual data for urinary Si excretion from week 1 to week 24 in the MS study group

Si excretion in Wk1 ranged from 26-198µmol/mmol Crt with a mean value of 102µmol/mmol Crt. Si excretion in Wk 13 ranged from 118-481 µmol/mmol Crt with a mean of 253 µmol/mmol Crt (Table 5.4.4.1and figure 5.4.4.1) Data for Wk13 showed an increase in Si excretion compared to Wk1 for 15 out of 15 individuals (P <0.001). Mean urinary excretion of Si remained statistically consistent between control Wk 1 to control Wk 12 (P = 0018). Si excretion in Wk 12 ranged from 22-224µmol/mmol Crt with a mean value of 84µmol/mmol Crt, see Table 5.4.4.1.

Mean urinary excretion of Si for the final treatment week, Wk 24, ranged between 92-552 µmol/mmol Crt with a mean value of 225 µmol/mmol Crt. These values remain similar between treatments Wk 13 and Wk 24, although when considered individually, in five out of the fifteen patients, Si excretion reduced significantly (>50%). In a further five of the fifteen patients, the concentration of urinary Si excretion remained statistically consistent ($P < 0.001$)., for example, patient ID M002 ranging from 135 µmol/mmol Crt to 127.7 µmol/mmol Crt between Wk 13 and Wk 24. The excretion of Si continued to increase between treatment Wk 13 and Wk 24 in three of the fifteen patients, to highlight an example, patient ID M011 presented an increase in Si excretion of 328 -552 µmol/mmol Crt between Wk 13 and Wk 24 (Table 5.4.4.1).

5.4.4 Creatinine corrected individual data for urinary Al excretion from week 1 to week 24 in the MS study group

Al excretion in Wk1 ranged from 46-340nmol/mmol Crt with a mean value of 165nmol/mmol Crt. Al excretion in Wk 13 ranged from 42-735 nmol/mmol Crt with a mean of 280 nmol/mmol Crt, see table 5… Data for Wk13 showed an increase in Al excretion compared to Wk1 for 13 out of 15 individuals ($P <0.001$). For a single patient, ID M002, Al excretion between Wk 1 and Wk 13 did not significantly alter ($P = 0.008$).

Two out of fifteen patients revealed a lower Al excretion post Si water consumption. Patient ID F006 indicated a significant ($P = 0.004$) reduction of Al between Wk 1 and Wk 13 of 219 – 72 nmol/mmol Crt. This is also seen in the data collected from patient ID F009 who indicated a drop from 101 – 42 nmol/mmol Crt between control Wk 1 and treatment Wk 13.

Mean urinary excretion of Al remained statistically consistent between control Wk 1 to control Wk 12 (P = 0.029, Pearson Correlation). Al excretion in Wk 12 ranged from 75-555nmol/mmol Crt with a mean value of 134nmol/mmol Crt (*Table 5.4.4.1*).

Mean urinary excretion of Al for the final treatment week, Wk 24, ranged between 254-827 nmol/mmol Crt with a mean value of 429 µmol/mmol Crt. This value shows significant difference between (p = 0.008, ANOVA) treatments Wk 13 and Wk 24. When considered individually, nine out of the fifteen patients showed a significant increase in Al excretion over the treatment weeks and excretion was larger in Wk 24 then Wk 13. An example of this is shown when looking at patient ID M002; this patient displays an increase in urinary Al concentration from 52 to 427 nmol/mmol Crt between treatment Wks 12 and 24.

In the remaining six of the fifteen patients, the concentration of urinary Al reduced significantly (P = 0.012, ANOVA), for example, patient ID M007 ranging from 358 nmol/mmol Crt to 295 nmol/mmol Crt between Wk 13 and Wk 24.

*Table 5.4.4.1: **Mean** (SD) urinary excretion of Si (µmol/mmol Crt) and Al (nmol/mmol Crt) for the 24 hour data collected in Wk 1, Wk 12, Wk 13 and Wk 24.*

Patient Mean (SD) Concentrations excreted (n = 5)								
	Silicon (µmol/mmol creatinine)				Aluminium (nmol/mmol creatinine)			
REF	W1	W12	W13	W24	W1	W12	W13	W24
P1	93.8	223.8	376.5	331.9	167.0	180.9	361.3	376.1
	(9.8)	(25.0)	(77.4)	(23.8)	(60.1)	(85.8)	(158.9)	(112.1)
P2	59.4	70.3	135	127.7	48	91.6	51.9	426.5
	(14.5)	(13.6)	(43.2)	(64.9)	(25.4)	(40.3)	(44.7)	(123.7)
P3	197.8	91.8	326.7	120	105.4	125.6	164.3	354.5
	(22.7)	(20.8)	(104)	(61.9)	(29.7)	(37.2)	(51.2)	(146.6)
P4	87.8	69.8	121.5	194.5	75.3	78.8	108.1	827
	(34.8)	(25.9)	(16.8)	(117.6)	(10.6)	(30.5)	(67.6)	(356.3)
P5	25.7	26.5	283.5	92	46.2	226.2	123.2	347.6
	(2.8)	(17.2)	(90.3)	(18.4)	(23.2)	(254.8)	(54.6)	(112.6)
P6	94.3	107.6	476.1	267	219.3	133.4	71.5	699.2
	(11.8)	(49.5)	(99.3)	(52.7)	(89.0)	(74.6)	(67.2)	(80.9)
P7	43.1	45.5	302.7	166.5	114	124.5	358	295.2
	(20.3)	(8.9)	(83.5)	(31.3)	(25.1)	(49.4)	(115.2)	(88.7)
P8	165.1	111.1	481.1	272	100.9	102.2	42.4	803.1
	(46.9)	(26.4)	(73.2)	(48)	(42.1)	(93.5)	(37.3)	(102.1)
P9	139.3	136.3	273.7	299.9	248.6	127.9	363.6	276.6
	(29.2)	(37.6)	(15.7)	(42.5)	(86.8)	(106.7)	(110.1)	(69.2)
P10	65.1	83.1	128.1	209	125.9	197.9	233.5	256.8
	(17.1)	(32.1)	(29.0)	(81.9)	(42.9)	(85.9)	(77.1)	(114.7)
P11	84.2	77.4	328.3	552.4	283.7	554.8	649.9	510.2
	(29.5)	(35.8)	(103.7)	(168.6)	(75.3)	(76.5)	(156.2)	(403.6)
P12	153.5	21.9	299.2	179.8	334.2	100.8	735.3	404.8
	(141.7)	(4.5)	(69.2)	(79.6)	(86.0)	(33.4)	(309.2)	(110.1)
P13	44.1	15.3	130.3	131.7	195.2	75.1	456.3	253.7
	(12.0)	(5.8)	(36.1)	(31.5)	(24.3)	(20.8)	(143.4)	(134.2)
P14	159.6	88.3	220.1	328.9	339.7	95.9	472.2	297.5
	(22.6)	(22.8)	(25.1)	(85.0)	(53.1)	(31.4)	(42.3)	(75.9)
P15	89.6	94.2	118.4	127.5	166.5	157.7	420.5	468.1
	(28.5)	(23.7)	(7.8)	(35.6)	(52.8)	(45.7)	(212.9)	(317.1)

5.4.5 Group data for urinary Si and Al excretion from week 1 to week 24 in the MS study group

When comparing the group data, excretion of Si is significantly higher in the treatment weeks than the control week (P <0.001, 2-way ANOVA). By collating the data, the mean Si excretion for the control period is calculated at 92.8 µmol/mmol Crt and Si excretion for the treatment weeks is calculated at 238.85 µmol/mmol Crt (*Table 5.4.5.1)*, this value is over two fold more than the control value.

Table 5.4.5.1: Mean (SD) and median urinary concentrations of Si (µmol/mmol Crt) and Al (nmol/mmol creatinine) for patients (n = 15)

Mean (SD) urinary concentrations for W1, W12, W13 and W24								
	Silicon (µmol/mmol Cre)				Aluminium (nmol/mmol Cre)			
	W1	W12	W13	W24	W1	W12	W13	W24
Patient	101.6 (42.8)	84.0 (52.7)	253.2 (126.8)	224.5 (142.8)	165.3 (103.5)	126.3 (134.1)	280.2 (212.3)	429.1 (265.2)

When comparing the group data, excretion of Al is significantly higher in the treatment Wks 13 and 24 than the control Wks 1 and 12 (P <0.001, ANOVA). Considering the data as a whole, the mean Al excretion for the control period is calculated to lie between 165nmol/mmol Crt and 126nmol/mmol Crt. The standard deviation between these values is quite large, further indicating that Al is handled differently between patient. Al excretion under the influence of a high influx of bio-available Si was more than doubled in the first treatment Wk 13 at 280.2nmol/mmol Crt, then

almost quadrupled by the final treatment Wk 24, presenting a mean value of 429nmol/mmol Crt. This data was normally distributed and statistically significant (t = 3.86, P = 0.012, t test).

5.4.6 One way ANOVA analysis for Si and Al excretion between control and treatment weeks

One-way ANOVA (*Table 5.4.6.1)* indicated that the increase in Si excretion between the baseline weeks (Wk 1 and Wk 12) and the initial treatment week (Wk 13) was statistically significant (P < 0.001) for all but one individual from the patient group. The One-way ANOVA statistical testing revealed that data was significant (P < 0.03) for 14 out of 15 patients for Si. One-way ANOVA was manipulated for two data sets to see where the main differences were. The control weeks (Wk 1 and Wk13) were compared with the first treatment week (Wk 13) and the two treatment weeks (Wk 13 and Wk 24) were compared with the last control week.

*Table 5.4.6.1 Summary of **One-way ANOVA** - differences in the urinary excretion of Si (µmol/mmol Crt), and Al (nmol/mmol creatinine) between Wk1, Wk12 and Wk13 for each individual in the **study** group*

Patient

Difference in urinary excretion btween weeks 1, 12 and 13 (dF=14)

	Aluminium					Silicon				
REF	**F Crit/F**	**Significant**	**P**	**Tukeys**		**F Crit/F**	**Significant**	**P**	**Tukeys**	
P1	F Crit < F	✓	P=0.028	W1<W12	W12<W13	F Crit < F	✓	P<0.001	W1<W12	W12<W13
P2	F Crit > F	×	P=0.17	W1<W12	W12>W13	F Crit < F	✓	P=0.002	W1<W12	W12<W13
P3	F Crit > F	×	P=0.104	W1<W12	W12<W13	F Crit < F	✓	P<0.001	W1>W12	W12<W13
P4	F Crit > F	×	P=0.445	W1<W12	W12<W13	F Crit < F	✓	P=0.03	W1>W12	W12<W13
P5	F Crit > F	×	P=0.2	W1<W12	W12>W13	F Crit < F	✓	P<0.001	W1<W12	W12<W24
P6	F Crit < F	✓	P=0.033	W1>W12	W12>W13	F Crit < F	✓	P<0.001	W1<W12	W12<W13
P7	F Crit < F	✓	P<0.001	W1<W12	W12<W13	F Crit < F	✓	P<0.001	W1<W12	W12<W13
P8	F Crit > F	×	P=0.27	W1<W12	W1>W12	F Crit < F	✓	P<0.001	W1>W12	W12<W13
P9	F Crit < F	✓	P=0.011	W1>W12	W12<W13	F Crit < F	✓	P<0.001	W1>W12	W12<W13
P10	F Crit > F	×	P=0.09	W1<W12	W12<W13	F Crit < F	✓	P=0.009	W1<W12	W12<W13
P11	F Crit < F	✓	P=0.005	W1<W12	W12<W13	F Crit < F	✓	P<0.001	W1>W12	W12<W13
P12	F Crit < F	✓	P<0.001	W1>W12	W12<W13	F Crit < F	✓	P<0.001	W1>W12	W12<W13
P13	F Crit < F	✓	P<0.001	W1<W12	W12<W13	F Crit < F	✓	P<0.001	W1>W12	W12<W13
P14	F Crit < F	✓	P<0.001	W1>W12	W12<W13	F Crit < F	✓	P<0.001	W1<W12	W12<W13
P15	F Crit < F	✓	P=0.011	W1>W12	W12<W13	F Crit > F	×	P=0.124	W1<W12	W12<W13

The One-way ANOVA statistical testing revealed that data was significant (P < 0.04) for 13 out of 15 patients for Si (*Table 5.4.6.2)*. Si excretion was greater in W13 than in W24 for 8 of the patients, although in some cases this value simply wasn't considered as a significant difference (P = 0.81), therefore values may remain similar during W13 and W24 and these discrepancies can be related to Si content in the diet.

*Table 5.4.6.2 Summary of **One-way ANOVA** - differences in the urinary excretion of Si (µmol/mmol Crt), and Al (nmol/mmol Crt) between W12, W13 and W24 for each individual in the **study** group*

Patient

Difference in urinary excretion btween weeks 12, 13 and 24 (dF=14)

	Aluminium				Silicon			
REF	**F Crit/F**	**Significant**	**P**	**Tukeys**	**F Crit/F**	**Significant**	**P**	**Tukeys**
P1	F Crit < F	✓	P=0.049	W13>W12 W24>W13	F Crit < F	✓	P=0.001	W13>W12 W24<W13
P2	F Crit < F	✓	P<0.001	W13<W12 W24>W12	F Crit > F	×	P=0.087	W13>W12 W24<W13
P3	F Crit < F	✓	P=0.004	W13>W12 W24>W13	F Crit < F	✓	P<0.001	W13>W12 W24<W13
P4	F Crit < F	✓	P<0.001	W13>W12 W24>W13	F Crit < F	✓	P=0.047	W13>W12 W24>W13
P5	F Crit > F	×	P=0.14	W13<W12 W24>W12	F Crit < F	✓	P<0.001	W13>W12 W24<W13
P6	F Crit < F	✓	P=0.002	W13<W12 W24>W13	F Crit < F	✓	P<0.001	W13>W12 W24<W13
P7	F Crit < F	✓	P=0.004	W13>W12 W24<W13	F Crit < F	✓	P<0.001	W13>W12 W24<W13
P8	F Crit < F	✓	P<0.001	W13<W12 W24>W13	F Crit < F	✓	P<0.001	W13>W12 W24<W13
P9	F Crit < F	✓	P=0.007	W13>W12 W24<W13	F Crit < F	✓	P<0.001	W13>W12 W24>W13
P10	F Crit > F	×	P=0.62	W13<W12 W24>W13	F Crit < F	✓	P=0.9	W13>W12 W24>W13
P11	F Crit > F	×	P=0.68	W13<W13 W24<W13	F Crit < F	✓	P<0.001	W13>W12 W24>W13
P12	F Crit < F	✓	P<0.001	W13>W12 W24<W13	F Crit < F	✓	P<0.001	W13>W12 W24<W13
P13	F Crit < F	✓	P<0.001	W13>W12 W24<W13	F Crit < F	✓	P<0.001	W13>W12 W24>W13
P14	F Crit < F	✓	P<0.001	W13>W12 W24>W13	F Crit < F	✓	P<0.001	W13>W12 W24>W13
P15	F Crit > F	×	P=0.098	W13>W12 W24>W13	F Crit > F	×	P=0.14	W13>W12 W24>W13

One-way ANOVA (*Table 5.4.6.1 and Table 5.4.6.2*) indicated that there was a significant difference in Si excretion between control Wks (Wk1 and Wk 12) and treatment Wks (Wk 13 and Wk 24) the patient ($P < 0.001$) group.

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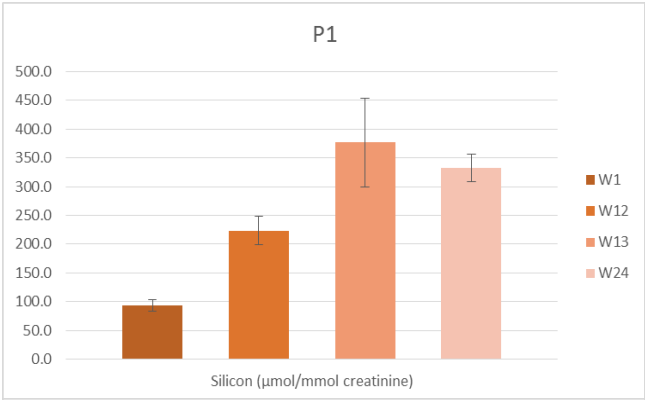
Wilcoxon signed-rank was used to determine where any differences within the data lie. Males generally excreted less silicon, this was statistically significant during the entire study duration (P = 0.001). An example of Wk 12 shows a mean excretory value of 56.19µmol/mmol creatinine in W12 was seen for males compared to 99.46µmol/mmol Crt in W12 for females.

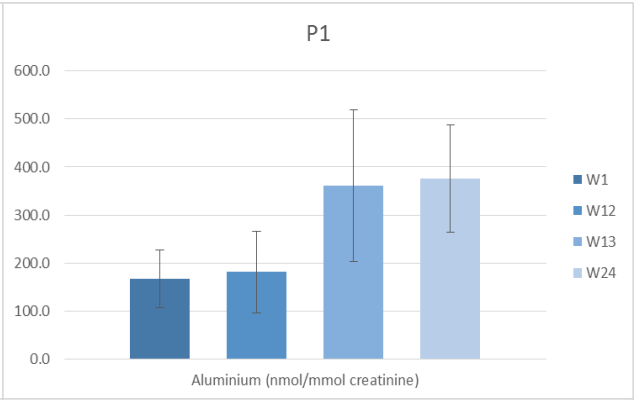
5.4.7 Long term effect of consuming a silicon rich mineral water

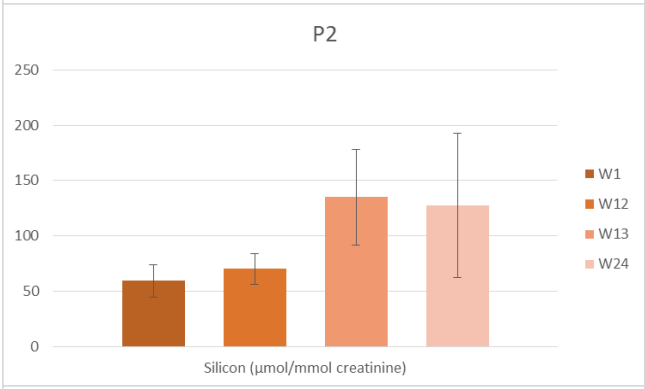
Data from each patient show statistically linear trends in that Si and Al concentration are increased after the addition of a Si rich mineral water to the diet ($P < 0.001$). A visual representation of this relationship is shown in figure 5.4.7.1. Patients 7 and P12 present a trend of Si and Al excretion, implying that Si concentration in the gut may be directly linked to excretion of Al in these individuals, the amount of Si excreted directly correlated with the amount of Al excreted ($P = 0.005$).

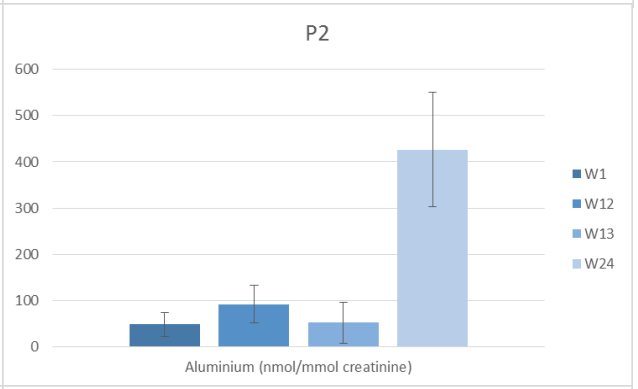
Si data is presented in orange, while Al data is presented in blue and weekly data is presented for each MS study participant.

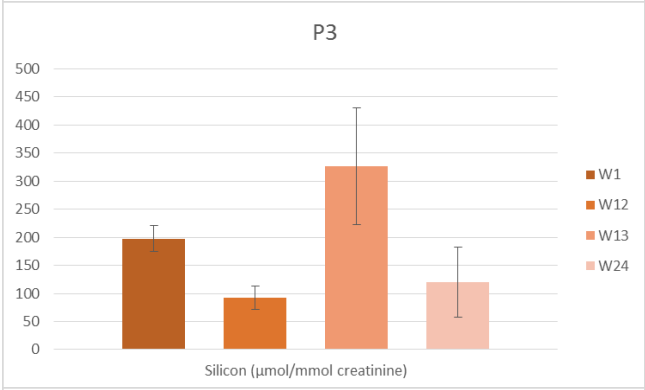
Visual representation of changes in mean urinary excretions

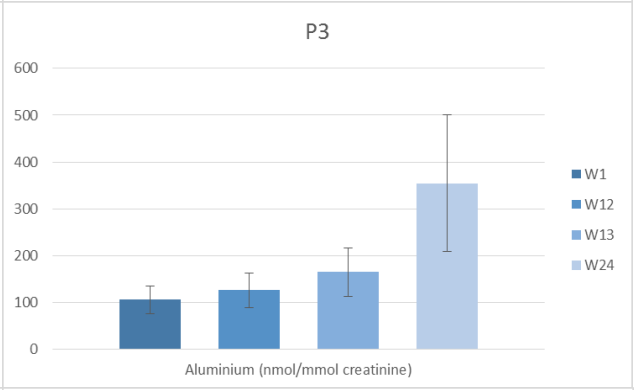


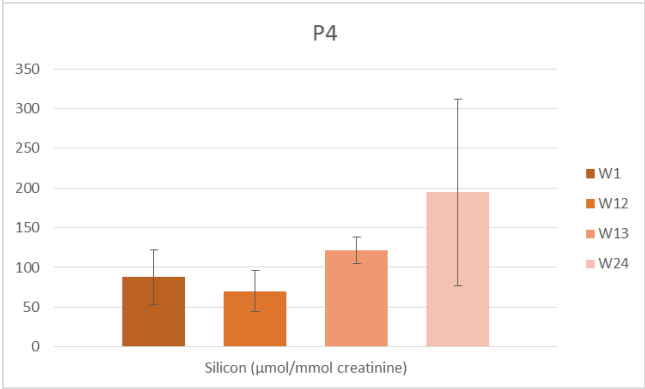


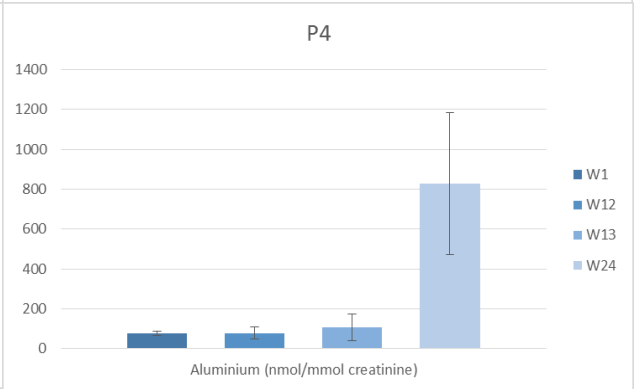


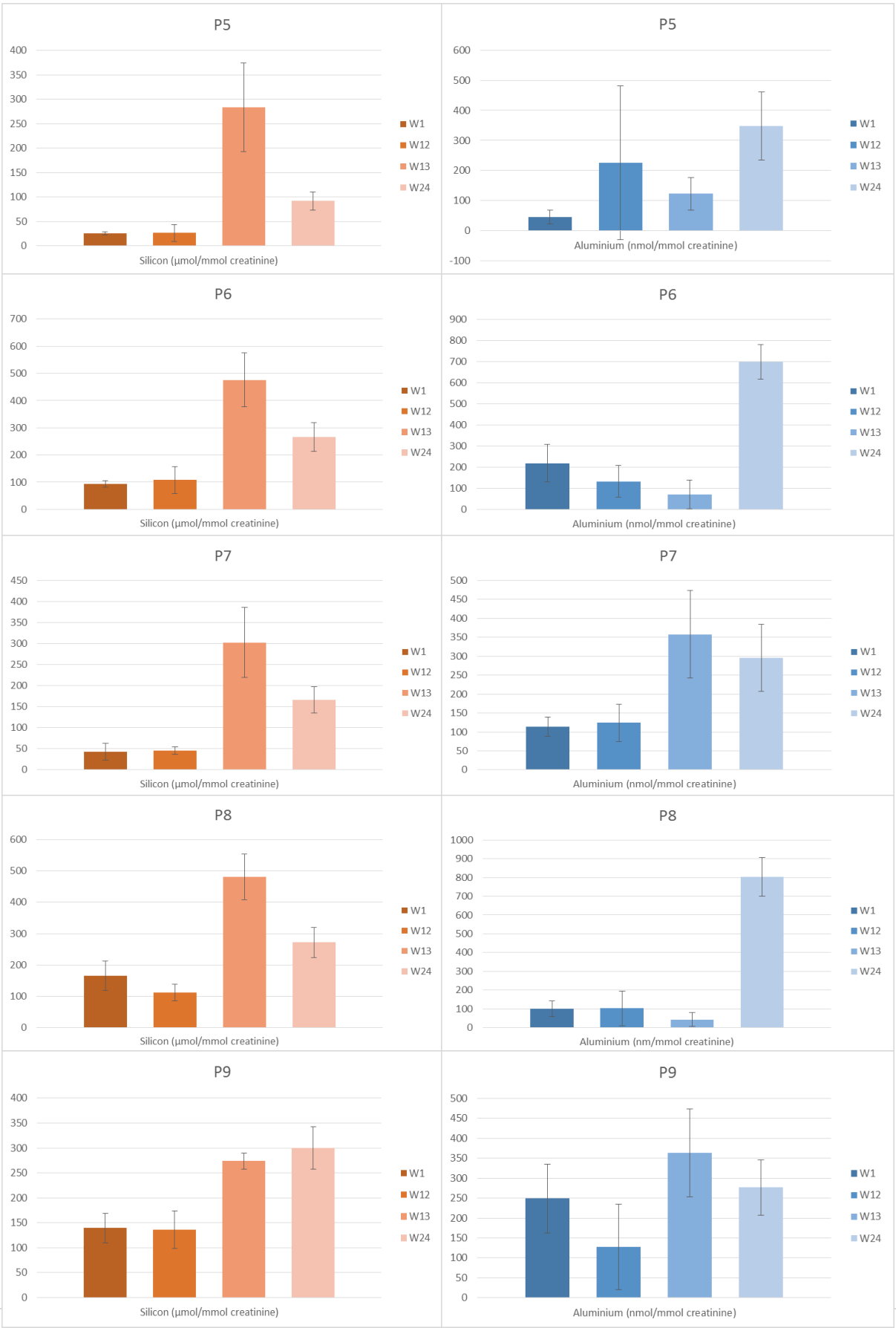


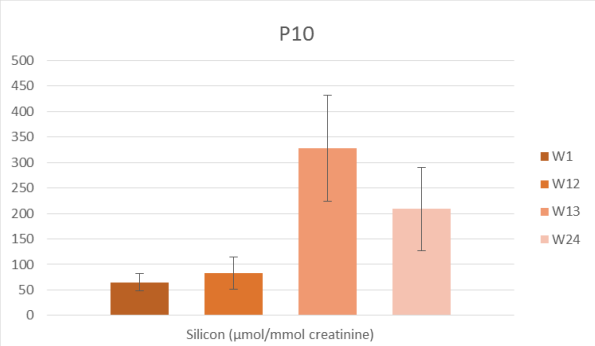


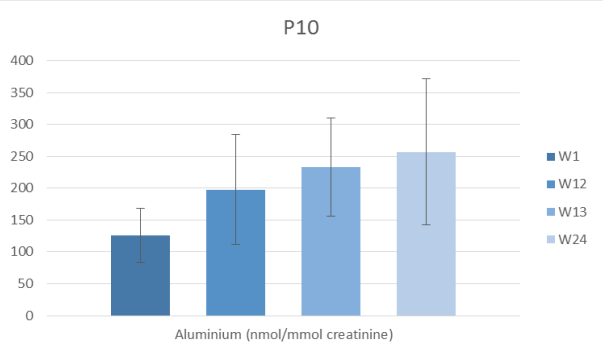


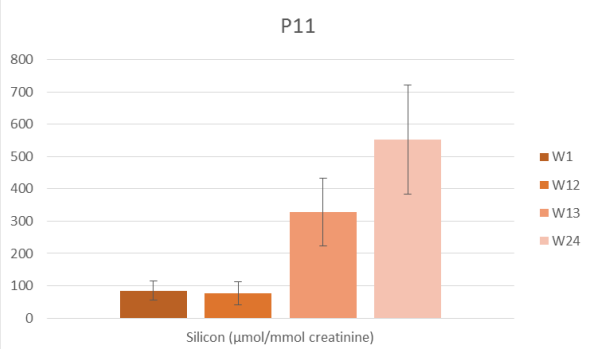


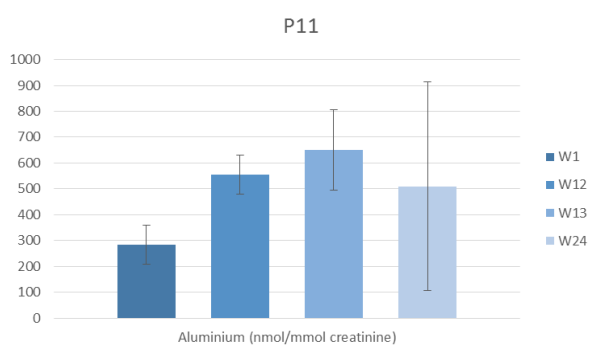


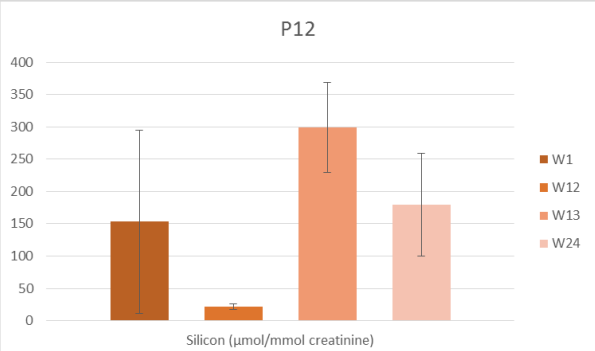

P5
Silicon (µmol/mmol creatinine)
W1
W12
W13
W24
P5
Aluminium (nmol/mmol creatinine)
W1
W12
W13
W24
P6
Silicon (µmol/mmol creatinine)
W1
W12
W13
W24
P6
Aluminium (nmol/mmol creatinine)
W1
W12
W13
W24
P7
Silicon (µmol/mmol creatinine)
W1
W12
W13
W24
P7
Aluminium (nmol/mmol creatinine)
W1
W12
W13
W24
P8
Silicon (µmol/mmol creatinine)
W1
W12
W13
W24
P8
Aluminium (nm/mmol creatinine)
W1
W12
W13
W24
P9
Silicon (µmol/mmol creatinine)
W1
W12
W13
W24
P9
Aluminium (nmol/mmol creatinine)
W1
W12
W13
W24

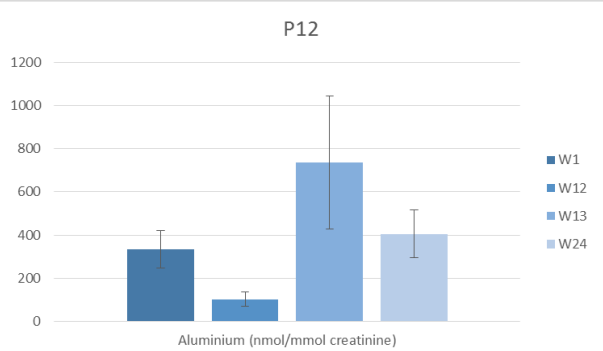

P10
500
450
400
350
300
250
200
150
100
50
0
W1
W12
W13
W24
Silicon (µmol/mmol creatinine)

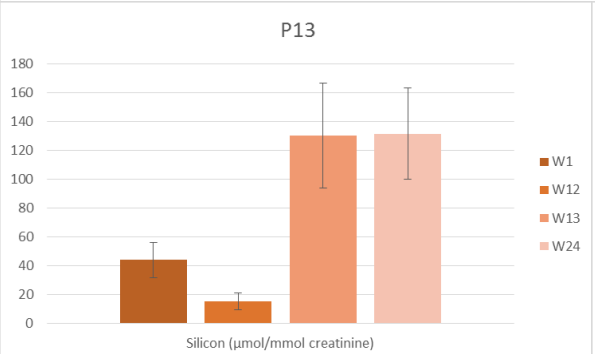

P10
400
350
300
250
200
150
100
50
0
W1
W12
W13
W24
Aluminium (nmol/mmol creatinine)

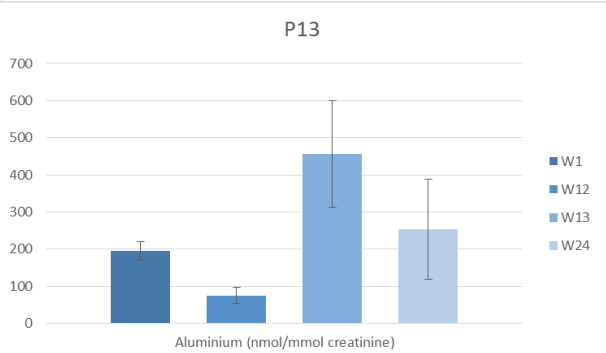

P11
800
700
600
500
400
300
200
100
0
W1
W12
W13
W24
Silicon (µmol/mmol creatinine)

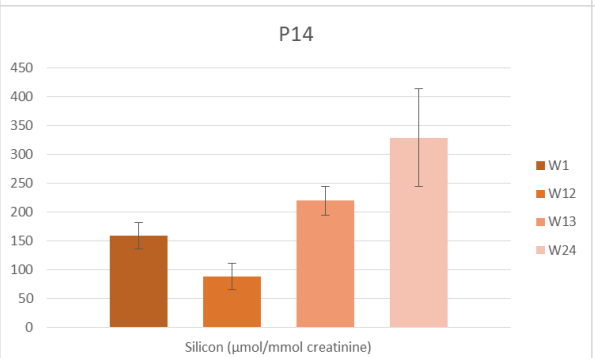

P11
1000
900
800
700
600
500
400
300
200
100
0
W1
W12
W13
W24
Aluminium (nmol/mmol creatinine)

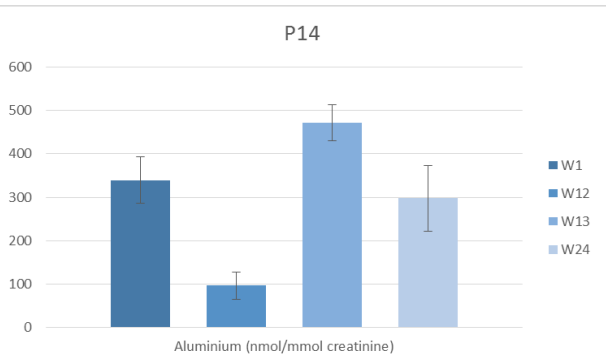

P12
400
350
300
250
200
150
100
50
0
W1
W12
W13
W24
Silicon (µmol/mmol creatinine)


P12
1200
1000
800
600
400
200
0
W1
W12
W13
W24
Aluminium (nmol/mmol creatinine)


P13
180
160
140
120
100
80
60
40
20
0
W1
W12
W13
W24
Silicon (µmol/mmol creatinine)


P13
700
600
500
400
300
200
100
0
W1
W12
W13
W24
Aluminium (nmol/mmol creatinine)


P14
450
400
350
300
250
200
150
100
50
0
W1
W12
W13
W24
Silicon (µmol/mmol creatinine)


P14
600
500
400
300
200
100
0
W1
W12
W13
W24
Aluminium (nmol/mmol creatinine)

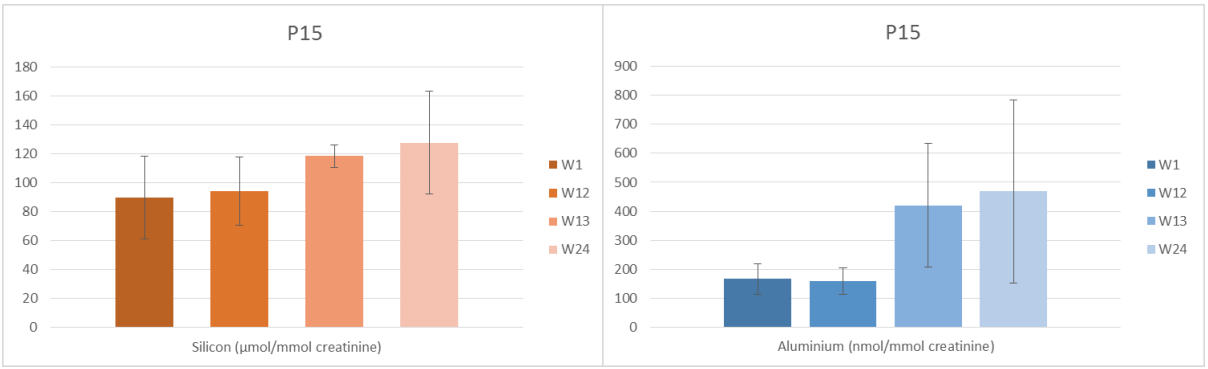


Fig. 5.4.7.1: Mean and SD (n=15) urinary excretion of Si (µmol/mmol Crt) and Al (nmol/mmol Crt) for weeks 1, 12, 13 and 24 for each patient.

When comparing the data as a whole, the means between W1 and W12 remain at a comparable value. Al excretion during W24 is on average much larger (429.1 nmol/mmol creatinine) than that of the initial treatment week, W13 (280.2nmol/mmol creatinine), although as in all cases here, the larger the average, the larger the deviation present between the values.

Si dependent t-stat values between control Wk 1 and Wk 12 were calculated to be 4.14 with a P value < 0.001 and Pearson Correlation of 0.96. Patient t-stat values between treatment Wk 13 and Wk 13 were calculated to be 2.63 with a P – value < 0.02 and Pearson correlation of 0.96 (*Table 5.4.7.1)*. Values for the treatment period are less correlated due to Si handling alternating between individuals.

*Table 5.4.7.1: Summary of **t-Test for paired means** - differences in the urinary excretion of Si between baseline period Wk1 and Wk12 then study period Wk13 and Wk24 for each study participant*

	t-Test: Paired Two Sample for Means Silicon (n=15)	
Function	**Week 1- Week 12**	**Week 13-Week 24**
t Stat	4.14	2.63
P one-tail	0.0005	0.01
t Crit one-tail	1.76	1.76
P two-tail	0.0010	0.02
t Crit two-tail	2.14	2.14
Pearson Correlation	0.96	0.96

Al dependent t-stat values between control Wk 1 and Wk 12 were calculated to be 1.52 with a P value < 0.15 and Pearson Correlation of 0.68. Patient t-stat values between treatment Wk 13 and Wk 24 were calculated to be -5.54 with a P – value < 0.001 and Pearson correlation of 0.93 (*Table 5.4.7.2)*. Values for the control are less correlated due to naturally fluctuating levels of Al in body biochemistry, levels of Al in the treatment period are more statistically linear ($p < 0.001$, $P = 0.93$).

*Table 5.4.7.2: Summary of **t-Test for paired means** - differences in the urinary excretion of Al between baseline period Wk1 and Wk12 then study period Wk13 and Wk24 for each study participant*

	t-Test: Paired Two Sample for Means Aluminium (n=15)	
Function	**Week 1- Week 12**	**Week 13-Week 24**
t Stat	1.52	-5.54
P one-tail	0.08	3.6468E-05
t Crit one-tail	1.76	1.76
P two-tail	0.15	7.29359E-05
t Crit two-tail	2.14	2.14
Pearson Correlation	0.68	0.93

5.4.8 Gender comparisons

When comparing male and female data, the values were significant for weeks W13 and W24 for silicon for both genders. The p-value between genders for these weeks are below 0.04 (0.038) which is consistent with the idea that all patients are consuming the same amount of silicon during this time. The excretion of Al is more sporadic and p-value between these treatment weeks shows insignificance for W13 (P = 0.83), showing a large variation between genders during this treatment period (*Table 5.4.8.1).*

*Table 5.4.8.1: Summary of **Wilcoxon signed-rank** - differences between pairs of means of excretion for male (n = 7) and female groups (n = 8).*

Male v Female Patients		
	Silicon	Aluminium
	P-Value	P-Value
W1	0.175	0.134
W12	0.1	0.175
W13	0.038	0.83
W24	0.038	0.432

When comparing the differences in excretion of Si and Al between genders, females appear to consistently excrete more Si and Al than males in both the control and treatment periods. These values were compared using Mann-Whitney U-test analysis to determine the significance of these differences (*Table 5.4.8.2).* In the first baseline Wk 1, males excreted a mean Si amount of 75.7µmol/mmol CrT while females excreted a mean Si amount of 124.2µmol/mmol CrT. This difference was consistent throughout the study and in Wk 24, the final treatment week, males presented a mean Si excretion of 156.8µmol/mmol CrT while this value was noted at 206.1µmol/mmol Crt for females.

Table 5.4.8.2: Differences in the excretions of Si (µmol/mmol Crt) and Al (nmol/mmol Crt) between males and females for the patient group.

(Mann-Whitney U test).

Silicon (µmol/mmol Crt)			
	Patient		
	Male (n=7)	Female (n=8)	P-value
W1	75.7	124.2	0.175
W12	65.3	103.3	0.1
W13	184.9	313	0.038
W24	156.8	283.7	0.038
ΣW	120.7	206.1	0.09
Aluminium (nmol/mmol Crt)			
	Patient		
	Male (n=7)	Female (n=8)	P-value
W1	118.6	206.2	0.134
W12	80.9	166	0.175
W13	248.6	307.8	0.83
W24	313.6	530.04	0.432
ΣW	190.4	302.5	0.39

The relationship between Al and Si excretion is shown for females (*Figure 5.4.8.1*) and males (*Figure 5.4.8.2*). In five out of the eight female MS patients (F1, F2, F4, F5, F8) and 4 out of the seven male MS patients (M1, M2, M3 and M4), a clear relationship can be seen between Si and Al excretion. The first half of the study, the baseline period, produces lower peaks in the data compared with the second half of the study, the treatment period, when the

incorporation of the Si rich mineral water in the diet commenced. Patient M5 would follow this trend but a sharp Si peak was noticed at 281µmol/mmol Crt during baseline Wk 7.

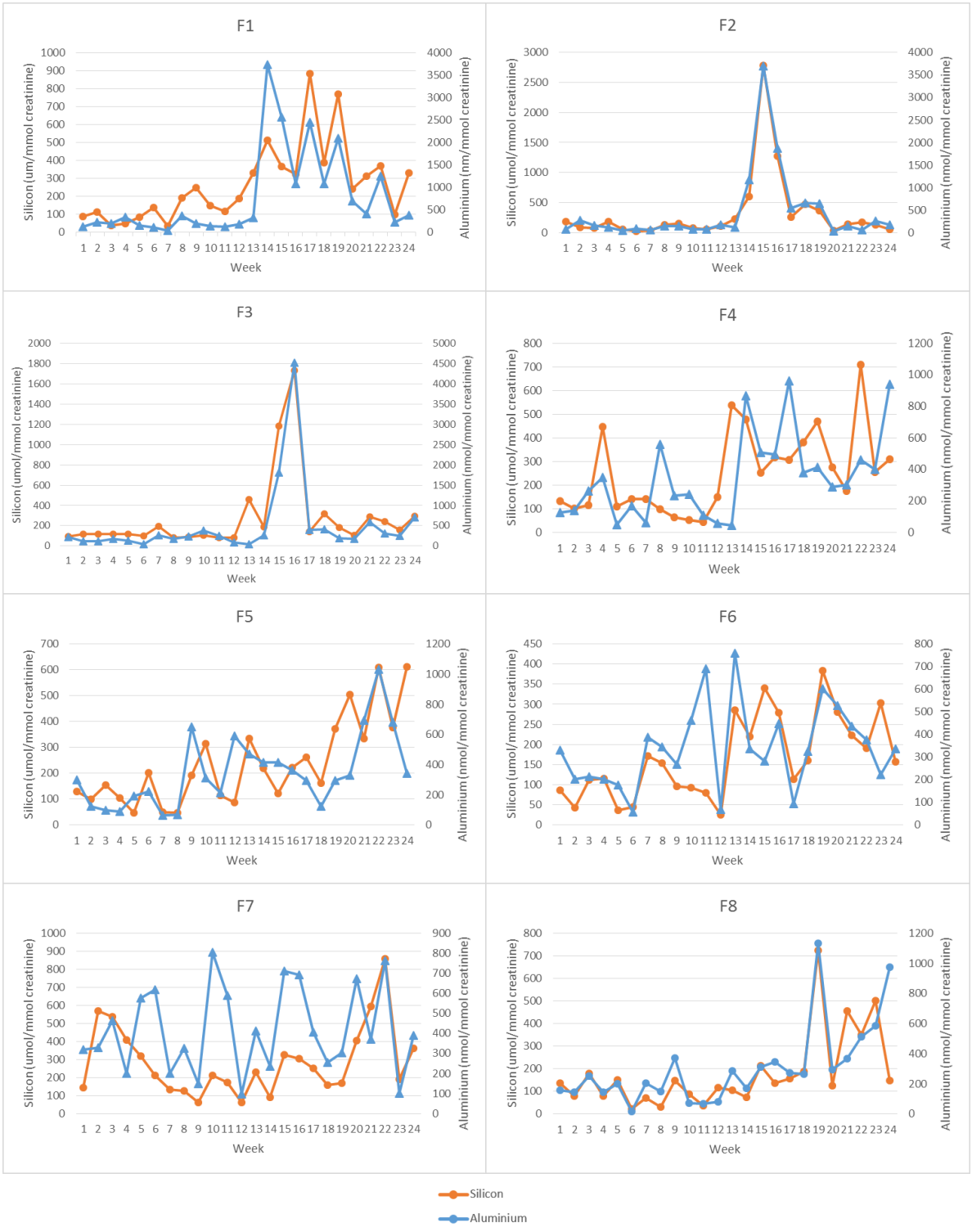


Figure 5.4.8.1: Creatinine corrected urinary excretion of Si (µmol/mmol Crt) and Al (nmol/mmol Crt) over the 24 week collection period – Female

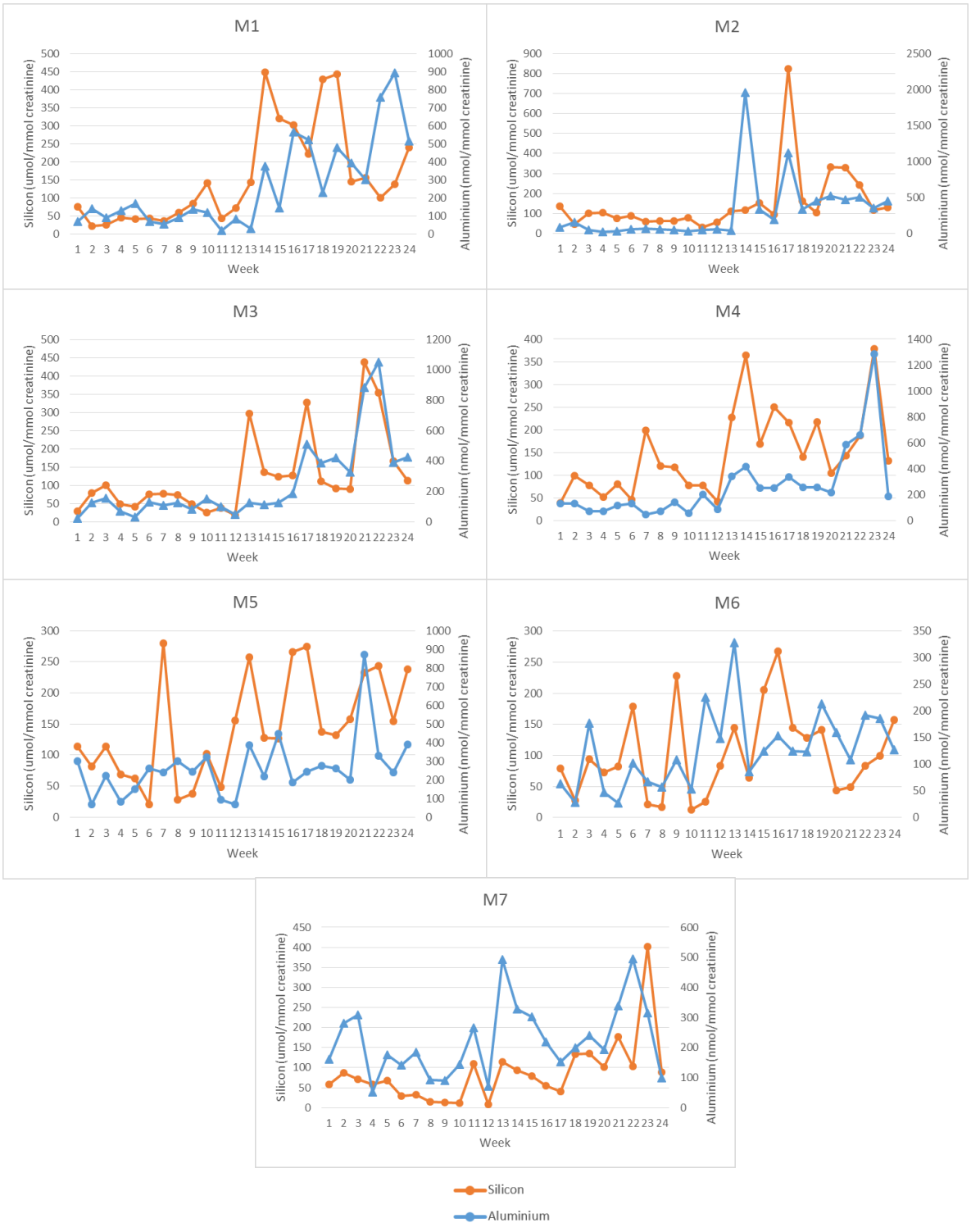
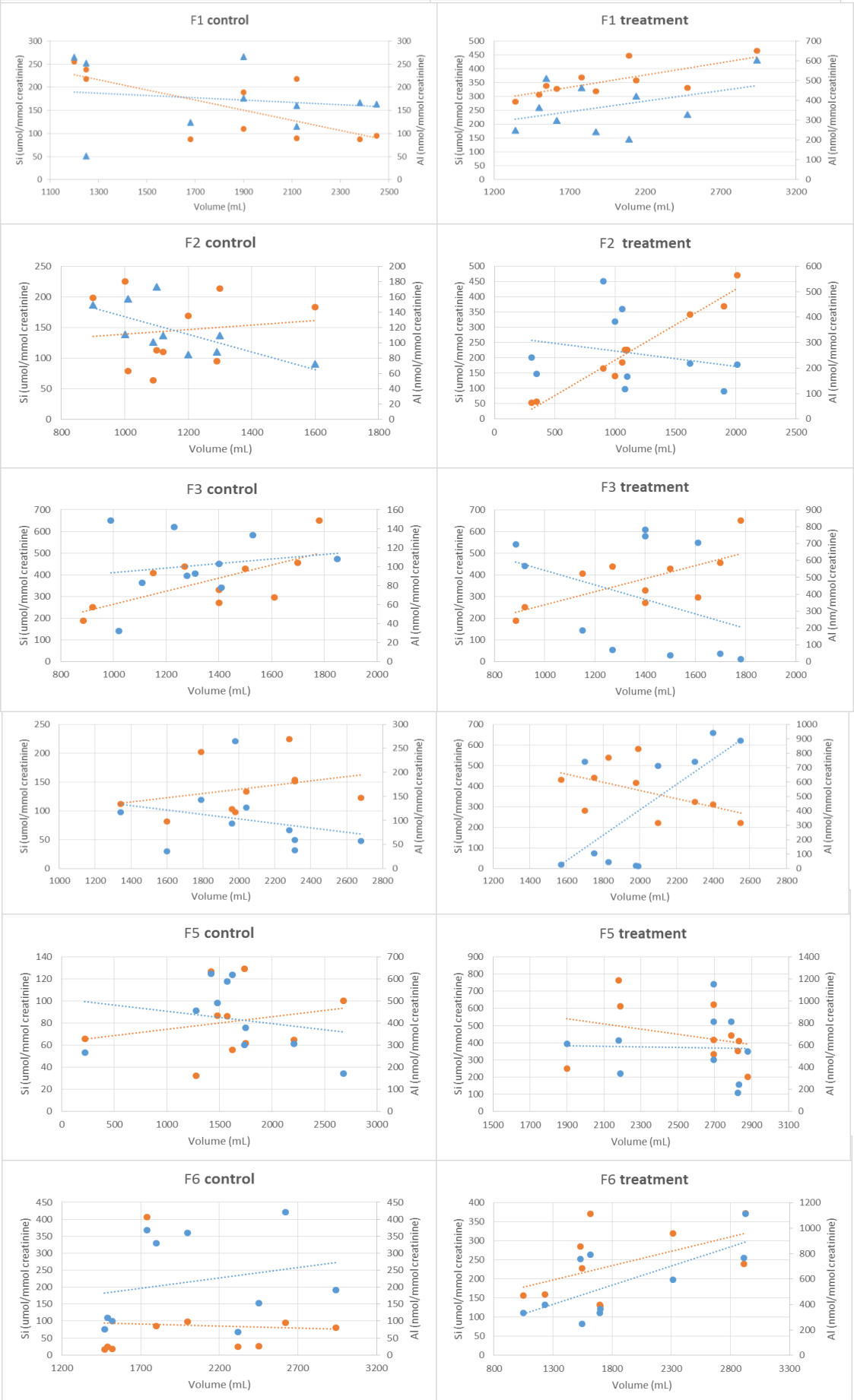


Figure 5.4.8.2 Crt corrected urinary excretion of Si (µmol/mmol Crt) and Al (nmol/mmol Crt) over the 24 week collection period – Male

Excretions of Al and Si were compared with the urine volumes collected throughout the 24 hour baseline and treatment collection periods. Genders were considered individually, although there was no correlation between urine volume and amounts of Si and Al in either male (r = 0.24) (*Figure 5.4.8.4)* or female (r = 0.18) (*Figure 5.4.8.3)* study populations. Patient F4 indicted the strongest positive correlation between urine volume and Si (r = 0.81) and Al (r = 0.75) excretion, although this is seen for the control period only.


F1 control
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
F1 treatment
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
F2 control
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
F2 treatment
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
F3 control
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
F3 treatment
Si (umol/mmol creatinine)
Al (nm/mmol creatinine)
Volume (mL)
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
F5 control
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
F5 treatment
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
F6 control
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
F6 treatment
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)

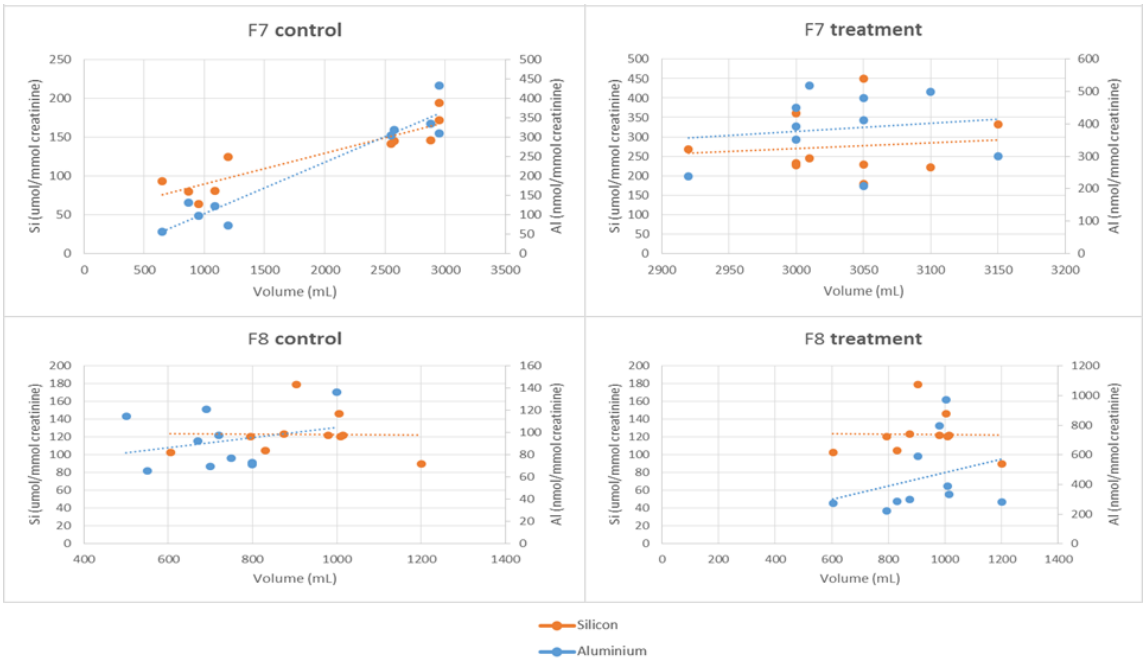
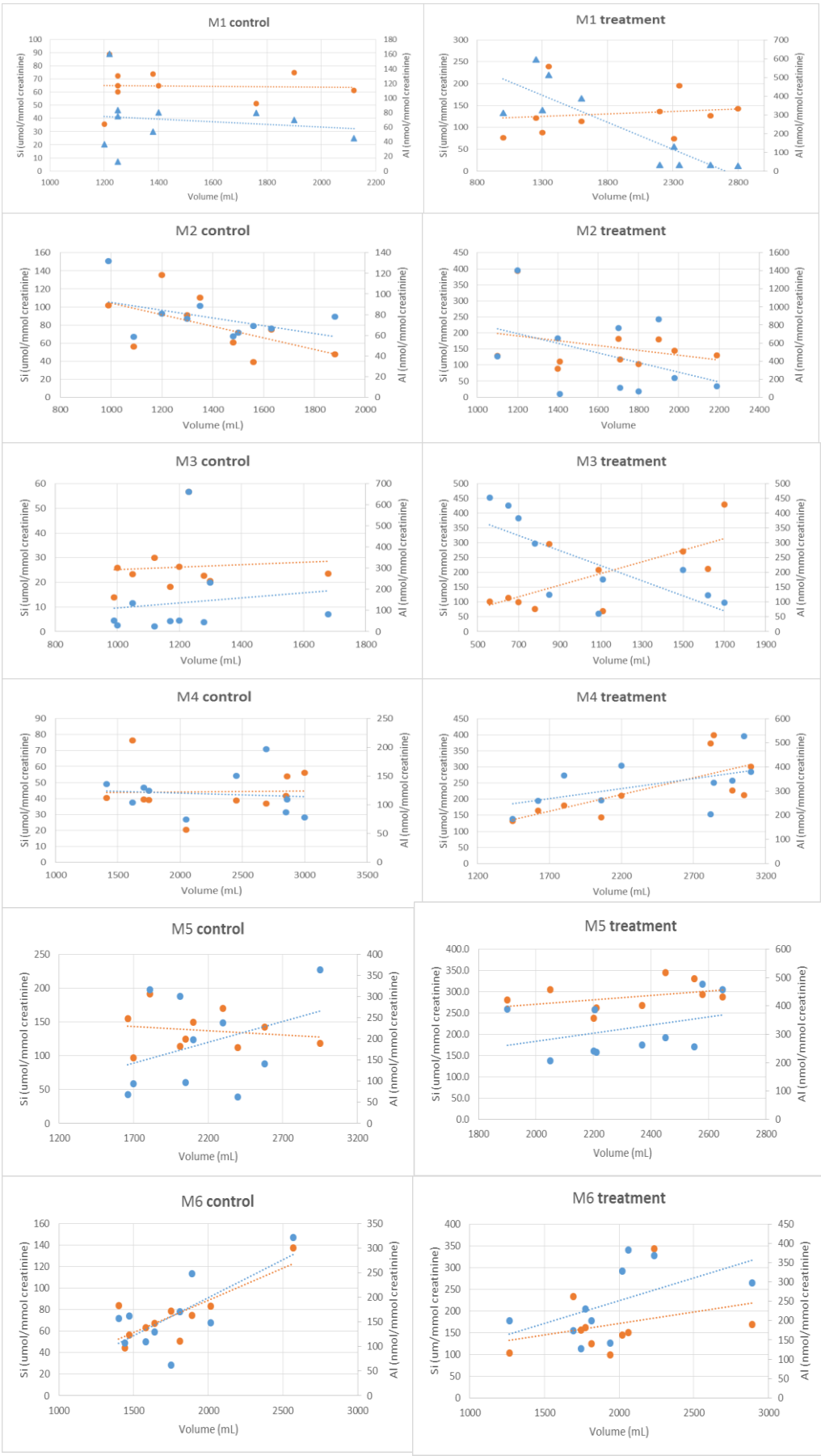


*Figure 5.4.8.3 Creatinine corrected elemental excretion comparisons to volume of urine excreted over the **control** and **treatment** period for each individual **female**. **W1** and **W12** 24 hour collection data points are included for the **control** then **W13** and **W24** 24 hour collection data are presented in the **treatment** study. Primary y-axis = Si (µmol/mmol Crt), secondary y-axis = Al (nmol/mmol Crt).*


M1 control
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
M1 treatment
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
M2 control
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
M2 treatment
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume
M3 control
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
M3 treatment
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
M4 control
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
M4 treatment
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
M5 control
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
M5 treatment
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
M6 control
Si (umol/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)
M6 treatment
Si (um/mmol creatinine)
Al (nmol/mmol creatinine)
Volume (mL)

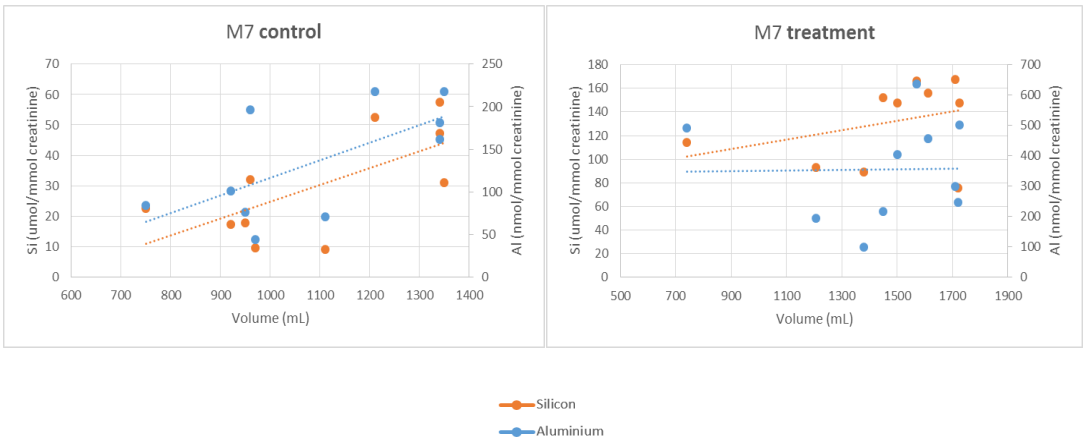


Figure 5.4.8.4 Crt corrected elemental excretion comparisons to volume of urine excreted over the **control** and **treatment** period for each individual male. **W1** and **W12** 24 hour collection data points are included for the **control** then **W13** and **W24** 24 hour collection data are presented in the **treatment** study. Primary y-axis = Si (µmol/mmol Crt), secondary y-axis = Al (nmol/mmol Crt).

Silicon
Aluminium

5.4.9 Comparisons between creatinine corrected urinary excretions in the control and treatment periods – group data

All study participants that excreted a larger concentration of silicon after consuming the Si rich mineral water correlated this with an increase in Al excretion ($P < 0.001$). The percentage difference between the control and treatment period of Si ranged from -0.2% to 158%. This figure is equivalent to the addition having no effect of Si excretion to it encouraging eight fold the excretion of Si compared to the normal expected value (*Table 5.4.9.1)*. A single patient produced a minus percentage difference value of -0.2%. Patient F8 excreted less Si on average in their urine during the treatment period (Wks 13-Wk24) than the control period (Wks 1-Wks12). This value was not significant ($P = 0.78$).

Table 5.4.9.1: ***Mean*** *Crt corrected urinary excretions of Si (µmol/mmol Crt) and Al (nmol/mmol Crt) for the control and treatment groups and the* ***percentage difference*** *between the groups.*

Mean of the creatinine corrected urinary excretions over a 24-hour period						
REF	Silicon (µmol/mmol creatinine)			Aluminium (nmol/mmol creatinine)		
	Control	Treatment	% Difference	Control	Treatment	% Difference
M1	73.7	191	88.6	76.8	271.1	111.7
M2	95.8	120.1	22.5	69.7	245.4	111.5
M3	24.05	204.9	158.0	36.8	276.1	153.0
M4	40.6	180	126.1	108.5	263.8	83.4
M5	149.8	247.8	49.3	184.7	387.7	70.9
M6	81	150.5	60.0	105.5	227.6	73.3
M7	33.3	101.5	101.2	116.1	296.3	87.4
F1	137.95	331.8	82.5	150.4	352.5	80.4
F2	148.1	140.8	5.1	122.75	146.6	17.7
F3	87.8	375.8	124.2	153.9	376.3	83.9
F4	142.2	424	99.5	93	491.4	136.3
F5	107.6	472.8	125.8	444.2	406.4	-8.9
F6	55.3	221.2	120.0	198.5	547.1	93.5
F7	104.7	295.2	95.3	208.1	401.4	63.4
F8	125.5	125.3	-0.2	118	630.2	136.9

All patients, excluding one, excreted more aluminium in the treatment period than the control period (P = 0.004). Patient F5 was the only member of the study to excrete less aluminium as a whole during the treatment period W13 to W24 compared to the control period, Wks 1 and Wk 12, decreasing excretion by -9%. This value was not significant (P = 0.81).

There is strong positive correlation between aluminium and silicon excretion for the treatment period (r = 0.96). Figure 5.4.9.1 highlights the relationship between aluminium and silicon, demonstrating the control period in dark grey (r = 0.51) and the treatment period in pale grey (r = 0.96). Correlation between the excretions of the elements is stronger during the treatment period (P < 0.005), although a higher quantity may encounter a lower error margin, resulting in a more linear relationship. R-values and p- values for these relationships are shown in table 5.4.9.2.

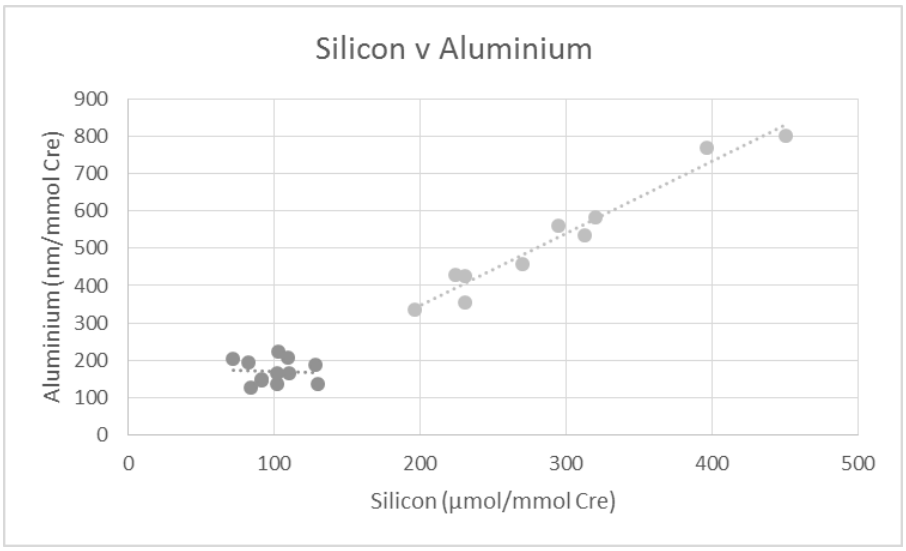


Figure 5.4.9.1: Crt corrected excretion of Al (nmol/mmol Crt) for the control (●) and treatment (●) periods against Si excretion (µmol/mmol Crt).

Table 5.4.9.2: Correlation analysis between the Crt corrected excretion of Al (nmol/mmol Crt) with the excretion of Si (µmol/mmol Crt).

Relationship with Silicon		
	Aluminium	
	r-Value	p-Value
Control	0.51	<0.05
Treatment	0.96	<0.05

5.4.10 Comparisons between total Al and Si excreted during 24 hour urinary collections in the control and treatment periods

24-hour collections provide the most reliable information on urinary excretions and were collected for 5 consecutive days in two of the control weeks and two of the treatment weeks. Data from each individual is considered below and presented in concentrations of ng/24 hr for Al excretion and µg/24 hr for silicon excretion. When examined as a group, Al excretion shows strongly positive correlation with Si excretion ($r = 0.965$). This relationship indicates statistical significance for the MS treatment protocol for long term Si consumption as a method of removing bioavailable Al ($P < 0.001$).

5.4.10.1 Individual data for 24 hour urinary Si excretion from week 1 to week 24 in the MS study group

Si excretion in Wk1 (Baseline) ranged from 84-1153µg/24 hr with a mean value of 536 µg/24 hr (SD=317 µg/24 hr, n=15). Si excretion in Wk 13 (Treatment) ranged from 489-2391 µg/24 hr with a mean of 1710 µg/24 hr (SD=612 µg/24 hr, n=15), (Table 5.4.10.1). Data for Wk13 showed an increase in Si excretion compared to Wk1 for 15 out of 15 individuals (P <0.001).

Mean urinary excretion of Si remained constant between control Wk 1 to control Wk 12, with means of 536 µg/24 hr and 432 µg/24 hr respectively (P = 0014). Si excretion in Wk 12 ranged from 110-882 µg/24 hr with a mean value of 432µg/24 hr (SD=254 µg/24 hr, n=15), see Table 5.4.10.2.1.

Mean urinary excretion of Si for the final treatment week, Wk 24, ranged between 475-2465 µg/24 hr with a mean value of 1482 µg/24 hr (SD=682 µg/24 hr, n=15). This value remains consistent (**Pearson correlation analysis**, P = 0.018) between treatments Wk 13 and Wk 24, with a small distribution in the data (SD = 617 µg/24 hr in Wk13 -619 µg/24 hr in Wk 24) compared with differences in Si between the baseline weeks. Although, when considered individually, In seven out of the fifteen patients, Si excretion increased significantly between treatment Wk 13 and Wk 24, increasing from an average of 1264 µg/24 hr to 1904 µg/24 hr. In a further seven of the fifteen patients, the concentration of urinary Si excretion dropped by a statistically significant amount during the treatment period (P < 0.001), from 1878 µg/24 hr in Wk 13 to 934 µg/24 hr in Wk 24. Patient ID's M002 and M004 gave the greatest reduction of Si excretion during this time. Patient M002 excreted an average of 2391 µg/24 hr Si in Week 13 compared to 831 µg/24 hr in Wk 24, and Patient M004 excreted an average of 2095 µg/24 hr of Si in Wk 13 compared with 972 µg/24 hr in Wk 24.

When considered as a group, the amount of silicon in the urine increased significantly during the entire treatment period (P < 0.001).

5.4.10.2 Individual data for 24 hour urinary Al excretion from week 1 to week 24 in the MS study group

Al excretion in Wk1 ranged from 204-2235 ng/24hr with a mean value of 939 ng/24 hr (SD=564ng/24 hr, n=15). Al excretion in Wk 13 ranged from 275-2458 ng/24 hr with a mean of 2214 ng/24 hr (SD =1184ng/24hr, n=15), see table 5.4.10.2.1 Data for Wk13 showed an increase in Al excretion compared to Wk1 for 13 out of 15 individuals (P <0.001). For those patients of which Al decreased, the values altered from 859 to 275 ng/24 hr and 495 to 199 ng/24 hr between baseline Wk 1 and initial treatment Wk 13. Both of these patients were female and reductions of Al were significant between these weeks (P = 0.008 and P = 0.013 respectively).

This is also seen in the data collected from patient ID F009 who indicated a drop from 101 – 42 ng/24 hr between control Wk 1 and treatment Wk 13. Of the remaining individuals, ten out of the thirteen indicated a strong increase of Al after the addition of the Si rich mineral water, Al excretion more than doubled for these individuals, with an average increase of 898 to 2812 ng/24hr (P < 0.001). The last three participants, still presented a significant increase between this baseline and treatment week, with a mean increase of 1368 to 2203 ng/24 hr (P=0.014).

Mean urinary excretion of Al remained statistically consistent between control Wk 1 to control Wk 12 (P = 0.017). Al excretion in Wk 12 ranged from 278-2300 ng/24 hr with a mean value of 862 ng/24 hr, see table 5.4.10.2.1.

Mean urinary excretion of Al for the final treatment week, Wk 24, ranged between 1670 – 6252 ng/24 hr with a mean value of 2505 nmol/24 hr. This mean value is consistent between treatments Wk 13 and Wk 24 (**Pearson correlation analysis,** P = 0.002). When considered individually, eight out of the fifteen patients showed a significant increase in Al excretion over the treatment weeks and excretion was larger in Wk 24 then Wk 13. Patient ID F009 displays an unusual increase in urinary Al

concentration from 199 ng/24 hr in treatment Wk 13 (compared to 508 ng/24 hr for their baseline average) to 6252 ng/24 hr in treatment Wk 24. Other patients showed a marked reduction in Al excretion over this time (P = 0.27) and patient ID F001 showed a very statistically similar excretion throughout this treatment period (P = 0.004) by excreting on average 2183 ng/24 hr in Wk 13 and 2137 ng/24 hr in Wk 24.

Deviation within this data was larger for the treatment period than the baseline study. Deviation ranged from 564-632 ng/24 hr between Wk 1 and Wk 12, whereas, between treatment Wk 13 and Wk 24, deviation ranged from 1184-1440 ng/24 hr.

Visual representation of this relationship throughout the study is shown in figure 5.4.10.2.1. During the control period, these values remain similar **(Pearson correlation** analysis, P = 0.011), although between treatment periods these values differ (P = 0.28). There is more variability between data collected during the treatment period.

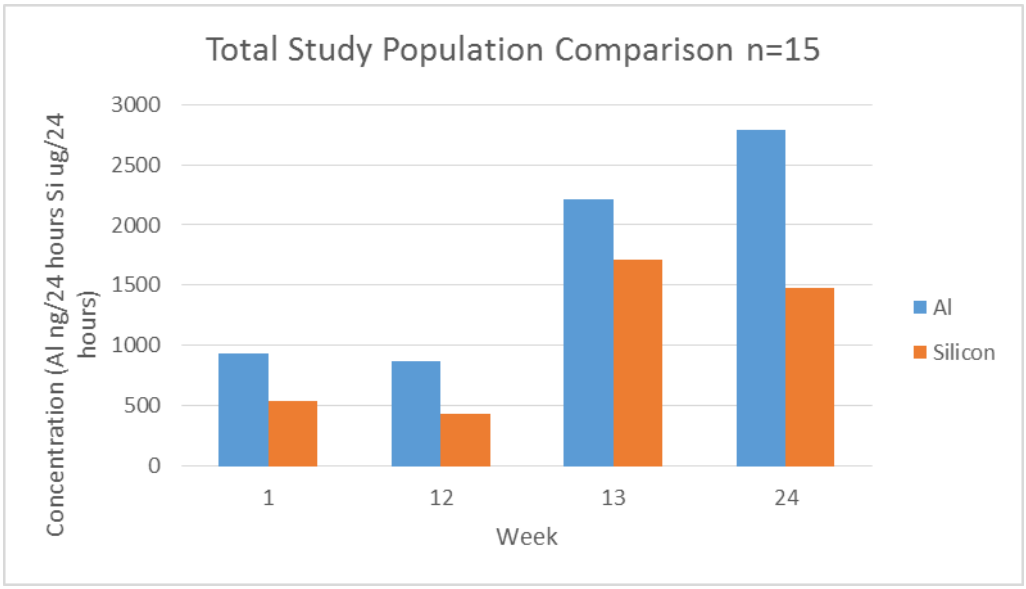


Figure 5.4.10.2.1: Twenty four hour excretion of Al (ng/24hr) for the control W1 and W12 and treatment W13 and W24 compared to Si excretion (µg/24hr) for the study group (n=15)

Table 5.4.10.2.1: ***Mean*** *(SD) urinary excretion of Si (µg/24 hr) and Al (ng/24 hr) for the 24 hour data collected in Wk 1, Wk 12, Wk 13 and Wk 24.*

Patient ID	Mean of the 24 hour urinary excretions over a 24-hour period (n=5)							
	Wk 1		Wk 12		Wk 13		Wk 24	
	Al	Si	Al	Si	Al	Si	Al	Si
F001	204	84	810	880	2183	495	2137	1951
M002	459	562	641	499	820	2391	2790	831
F003	300	573	707	513	918	1844	1674	590
M004	374	379	628	545	1855	2095	4454	972
M005	478	255	1548	224	649	1415	1670	475
F006	859	363	544	419	275	1892	3431	1321
M007	789	294	949	355	2769	2230	2481	1414
F009	495	854	516	561	199	2211	6252	2088
M010	1921	1086	836	882	3017	2263	2088	2289
M011	1247	648	1685	711	3362	1895	2616	2063
F012	1247	410	2300	284	4409	1264	2104	2426
F013	2235	1153	810	176	4458	1810	2828	1219
F014	1225	277	558	110	3654	1087	2639	1437
F015	1704	805	193	172	2944	1354	2199	2465
F016	551	299	278	157	1698	489	2505	696
Mean	**939 (564)**	**536 (317)**	**862 (632)**	**432 (254)**	**2214 (1184)**	**1649 (612)**	**2791 (1440)**	**1482 (689)**

5.4.11 Silicon consumed compared to silicon excreted

The amount of Si excreted can be directly related to that taken on in the form of Si rich mineral water. The amount of Si consumed per day in each study participant can be up to 24.19mg (864µmol), calculated from the average amount of Si in 1L of Spritzer mineral water, the amount being consumed by each patient per day of the treatment period. By calculating the difference in Si excretion between the control and treatment period, the difference in mineral water equating to that difference in Si can

be calculated. This value ranged from 41-205% and there was no significant difference between genders (p = 0.79). Amounts were compared using measurements in µmol. Patient F5 saw the largest difference in Si, between the control and treatment period, Si increased by 1769µmol, allowing for a difference in Si of 204%. Patient M1 saw the smallest increase of 41% by silicon values increasing by 352µmol between control and treatment periods. All patients saw an increase in Si excretion during the treatment period compared to the baseline study (*Table 5.2.11.1)*.

Table 5.2.11.1 Percentage of Si (µmol) consumed as mineral water which equates to the difference in Si excretion between the control and treatment period (n=15)

REF	Amount of silicon consumed as mineral water (µmol)	Difference in silicon excretion (Treatment – Control, µmol)	Percentage of mineral water which equates to the difference in silicon
M1	864	352.3	40.8
M2	864	592.9	68.6
M3	864	721.4	83.5
M4	864	858.5	99.4
M5	864	1176.7	136.2
M6	864	1183.8	137.0
M7	864	809.4	93.7
F1	864	741.05	85.8
F2	864	703.6	81.4
F3	864	902.4	104.4
F4	864	1233.6	142.8
F5	864	1768.7	204.7
F6	864	657.5	76.1
F7	864	548.8	63.5
F8	864	538.7	62.3

Percentage difference of Si excreted varied between participants, there was very weak positive correlation between Si consumed and Si excreted (r = 0.71), when analysing these differences.

5.4.12 Secondary objective – measurement of disability

A secondary outcome of the study was to measure the Kurtzke expanded disability status score for each participant before and after the study period. The results of this and other comparisons are made in this section.

There is no significant relationship between disability level and excretion of Al and Si, as shown in figure 5.4.12.1 This was explored for both control and treatment periods. The r-value for the relationship between Si excretion and disability score for the control and treatment study was 0.084 and 0.06 respectively. For Al, this relationship was weaker still, the corresponding r-value for the control period and treatment period was 0.04 and 0.001. At this point it was of interest to compare the creatinine levels in the urine to the disability scores.

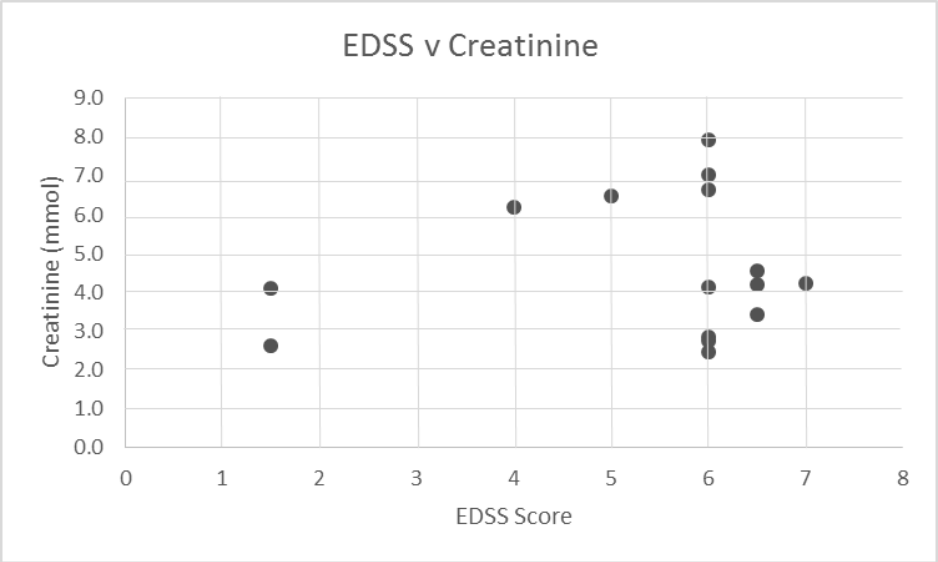


Figure 5.4.12.1 Correlation analysis between elemental excretion (n= 60) and EDSS scores of study participants (n=15)

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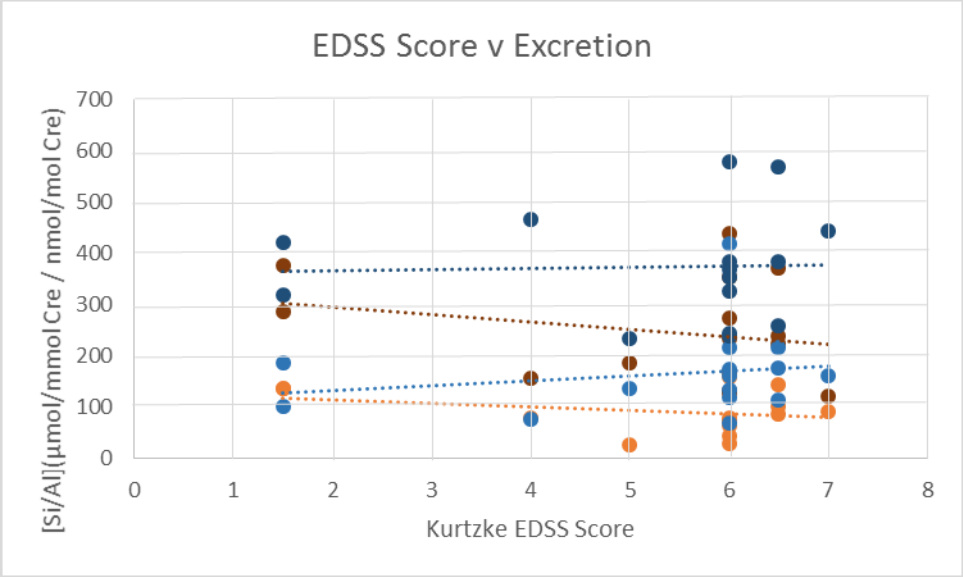


Figure 5.4.12.2 Correlation analysis between average elemental excretion (n= 60) and EDSS scores of study participants (n=15) for control Si ● and Al ● excretion and treatment Si ● and Al ● excretion.

There is no significant relationship between disability level and urinary creatinine concentration (r = 0.012). Thirteen out of the fifteen patients had an EDSS score between 4 and 7. Two of the patients had an EDSS score of 1.5. The lower the score, the less the disability level. Although the CrT values of these patients were amongst the lowest (between 2.5 and 4.5mmol), patients with a more severe disability score of 6 presented with similar levels of CrT excretion in their urine. These values can be seen in table 5.4.12.1.

Table 5.4.12.1 Average creatinine content (n= 60) and EDSS scores of study participants (n=15).

	Correlation analysis between EDSS Score and Creatinine production (n=15)	
ID	**EDSS Score**	**Mean creatinine (mmol)**
P1	6	2.5
P2	6	7.9
P3	6.5	4.6
P4	4	6.2
P5	5	6.5
P6	6.5	3.4
P7	6	4.1
P8	1.5	2.6
P9	1.5	4.1
P10	6	6.6
P11	6	2.9
P12	6.5	4.2
P13	6	7.0
P14	6	2.8
P15	7	4.2

Two out of the fifteen study participants showed marked improvements in their EDSS scores (>0.5 point difference), while thirteen out of the fifteen patients showed no change during these 12 weeks. No patients showed any decline in disability measure.

5.5 Summary of results for MS individuals

• Creatinine concentration was negatively correlated with sample volume (Section 5.4.1.).

• Males produced a larger urine volume during the control period while females produced a larger urine volume during the treatment period (Section 5.4.2).

•Creatinine concentration was larger in males than females (Section 5.4.4)

• Urinary excretions of Si and Al are significantly correlated during the treatment protocol (Section 5.4.9).

• Urinary excretions of Si and Al are significantly increased following the consumption of the mineral water (Section 5.4.10).

• The difference in Si excretion between the treatment and control periods corresponded to 99± 42% of the amount of Si consumed as the mineral water. (Section 5.2.11).

•Two out the fifteen participants indicated an improved EDSS score after the study period (Section 5.2.12)

• No significant relationship was noted between EDSS score and urinary creatinine concentration (Section 5.4.12)

5.6 Discussion

Comparing urinary volume and creatinine concentration

24-hour collections provide the most reliable information on urinary excretions; however, creatinine corrected measurements were also documented. The negative correlation between creatinine concentration and sample volume verifies that creatinine concentration provides an acceptable indication of dilution effects. Creatinine concentration remained constant for males and females throughout the control and treatment protocol, at 4.44mM and 4.45mM respectively. A high level may mean that the kidneys are not working as they should. The amount of creatinine in the blood depends partly on the amount of muscle tissue present in the body. Men generally have higher creatinine levels than women. The measurements of volume and creatinine were comparable to literature values provided in Chapter 7.1, indicating that kidney function was normal for all study participants.

EDSS scores

There was no significant change in EDSS score for either the patient group. However, despite the relatively short treatment period, disability performance was improved (EDSS scores ≥ 0.5 points; Kujzke 1983) for 2 patients (F1 and F6), which highlights the potential use of silicic acid-rich mineral water in relieving the symptoms of multiple sclerosis. Longer term studies on larger populations are now needed to fully elucidate these findings and double blind studies should be used to determine any placebo effects.

The baseline Al excretion was variable between the individuals showing an improvement in EDSS score, there was also no pattern in the excretion of Al between these individuals. Participant F1 showed a gradual decrease in Al over the treatment time after an initial sharp increase while participant F6 demonstrates a sporadic relationship with Al excretion, excreting high levels throughout the control and treatment, which fluctuate weekly. It may be suggested that this individual collected their spot samples at different periods of the day, leading to such fluctuations in elemental urine concentration. Urinary excretion of Al was higher in the treatment period than the baseline for both of these individuals. A linear relationship can be seen when looking at the excretion of Al and Si in participant F1, where a high urinary excretion for Si is noticed, a high urinary excretion is present also.

Not one of the individuals in the study had a relapse during the entire study period, which over a 6 month period is notable as individuals suffering with secondary progressive multiple sclerosis tend to suffer a relapse 1-2 times a year (Scalfari *et al.* 2010).

Silicon handling was variable between individuals

There is minimal data on the excretion of Si in urine, specifically concerning individuals suffering with MS. Urinary excretion of Si was variable between individuals, for example, the range of urinary Si at baseline was 84-1086µg/24hr (Table 5.4.10.2.1). As urinary Si is a good marker of absorbed Si, thus dietary differences between individuals may have contributed to these variations (Jugdaohsingh et al. 2002; Sripanyakorn et al. 2009).

Overall, an increase in urine volume was coupled with an increase in the excretion of Si. A mean increase in urine volume in the control period to the loading treatment period was twinned with an increase of Si in the urine. Si excretion decreased over the treatment period, in the initial treatment week, Si excretion was 1649µg/L (±612 µg/L) while by the final treatment week, Si

excretion had fallen to 1482µg/L (±619 µg/L). This may suggest that participants were not as enthusiastic when it came to drinking the mineral water as they initially were. This trend is not seen when looking at aluminium when considering the group as a whole. The urinary concentration of aluminium increases over the course of the treatment study, from 2214 µg/L (±1184 µg/L) in wk 13 to 2791 µg/L (±1440 µg/L) during Wk 24. There is however, large variation in this data.

When looking at individual data, it is evident that aluminium follows silicon in the body of these individuals. When looking at Figure 5.4.8.1 and Figure 5.4.8.2, concurrent peaks can be seen for both aluminium and silicon throughout the study period. Participant F2 shows a very low plateau of silicon and aluminium urinary concentration up until Wk 12, then a sharp increase in silicon and aluminium excretion during initial treatment Wk 13. Over just a couple of weeks, this value returns to a level similar to the plateau we saw in the baseline period, suggesting that systemic aluminium had been 'flushed out' of stores during the spike.

Consumption of the mineral water significantly increased the amount of Si excreted in the urine ($P < 0.001$, Section 5.4.10), this was coincident with increased Al excretion ($P < 0.001$). This signifies that the consumption of silicic acid-rich mineral water successfully enhanced the urinary excretion of Al in people with multiple sclerosis.

Chapter 6 - Comparisons between the healthy volunteer and multiple sclerosis studies.

This chapter compares the urinary excretions between the different study groups.

The MS study (*Chapter 5*) was compared to the 24-hour protocol of the healthy volunteer study (*Chapter 3*). The Mann Whitney U test was used to determine whether there was any difference in the median urinary excretions between the groups before and following the treatment of the mineral water.

It should be noted, as mentioned in the methods chapter, that the brand of mineral water provided is Spritzer. This brand of water was maintained for all investigations to ensure thorough consistency of data and controls. That the amount of Si taken in remained consistent between all volunteers.

6.1 Creatinine

Creatinine concentration was higher in MS patients ($P = 0.003$) and the younger age group of the healthy individual study ($P = 0.007$). Creatinine measurement wasn't as crucial during the healthy volunteer study due to the fact that only 24 hour samples were collected, whereas spot samples were collected for the long term MS study. Creatinine values were collected for consistency.

6.2 Sample Volume

Total 24-hour urine volume was larger in the MS study population than the healthy volunteers for females, healthy males excreted more urine than males with SPMS. On average, males excreted on average 1702mL/24 hr while females excreted on average 1710mL/24hr. In the healthy subject study, males excreted on average 1845mL/24hr while females excreted 1496ml/24hr. This difference remained consistent for both genders and in both sets of data, female urine volume was consistently larger for both control and treatment periods.

In the healthy volunteer study, urine volume collected over the 24 hours was consistently larger for the dosing treatment than the loading treatment, for both males and female.

6.3 Silicon

Each study indicated an increase in the excretion of Si in the treatment period compared to the control.

Urinary silicon concentration was significantly larger in the MS study population than in the healthy volunteers for the control and treatment periods. The median Si excretion for the MS patient control period was 484µg/24hr compared to 124µg/24hr for the HV group. This was consistent among genders. Men consistently excreted less Si than females ($p = 0.002$). Participants of the HV loading study were less likely to consume all of the total 1.5L volume of spritzer, leading to a lower Si excretion.

The percentage of Si from the mineral water which equated to the difference in the urinary excretion between the control and treatment periods (i.e. treatment minus control) was 99 ± 42%, this was

statistically higher (P = 0.018) compared to the healthy volunteer study, 59 ± 31% for the loading treatment and 95± 25% for the dosing period.

Silicon excretion in the healthy volunteer study was higher during the dosing treatment than the loading treatment (*Table 6.4.1)*.

The mean excretion of Si in the HV treatment period was significantly higher (t = -5.13, P < 0.001) than the control period; no individuals excreted more Si prior to drinking the mineral water. In the HV study, males excreted significantly more Si than females for the treatment period (t = 2.95, P = 0.008), but not for the control (t = 1.30, P = 0.208).

There was a significant difference in the median Si excretion between males and females for the MS patient groups in both the control (t = 3.15, P = 0.008) and treatment (t = 2.98, P = 0.004) section of the investigation. Males generally excreted more silicon, in the control and treatment period.

6.4 Aluminium

Each study indicated an increase in the excretion of Al in the treatment period compared to the control. Excretion of Al in the healthy volunteer group was mostly smaller for the treatment period (P < 0.001) than that of patients in the MS study. MS patient excreted more Al during the treatment period.

There was no significant difference in the median urinary excretion of Al between the MS group and the healthy individual group for the control period; however, healthy volunteers excreted significantly less ($P < 0.001$) Al in the treatment period compared to the MS group.

It is most sensible to relate the dosing HV data to the MS study data, this is because the MS study population were consuming the water throughout the day in a similar manner. Therefore, direct comparisons are made between dosing treatment data for the HV population and the MS treatment period. Loading treatment data is included for consistency.

However, the manner in which these groups handled Al following the treatment of the mineral water were different (*Table 6.4.2)*; the healthy individual group demonstrated a large increase in Al excretion (P = 0.004) during the Si loading treatment, which was coincident with an increase in Si excretion (P = 0.001). This was not the case for the MS study, where a smaller change in Al (P = 0.41) was witnessed at the beginning of the long term treatment study with an alternating increase in Si (P = 0.28). In general, Al excretion was larger for the last treatment week than the initial treatment week (P = 0.009), shown in *Table 6.4.3*, suggesting a long term treatment with Si is more beneficial for lowering body bioburden of Al.

Table 6.4.1: Mean urinary excretions of Si before and after consuming the mineral water in healthy individuals (HV) and individuals with multiple sclerosis (MS)

Urinary excretion of silicon (µg/24hr)					
Group	Therapy	Control	Treatment	Brand of water	Maximum water consumed
HV	Loading	126	614	Spritzer	1.5L
HV	Dose	126	951	Spritzer	1.5L
MS	Dose	484	1596	Spritzer	1.5L

Table 6.4.2: Mean urinary excretions of Al before and after consuming the mineral water in healthy individuals (HV) and individuals with multiple sclerosis (MS)

Urinary excretion of Al (ng/24hr)					
Group	Therapy	Control	Treatment	Brand of water	Maximum water consumed
HV	Loading	967	2814	Spritzer	1.5L
HV	Dose	967	2085	Spritzer	1.5L
MS	Dose	903	2503	Spritzer	1.5L

Table 6.4.3 Differences in the median excretions of Si and Al during the control and treatment weeks between healthy individuals (n = 19) and patients with multiple sclerosis (n = 15) - ***Mann Whitney U test***

Comparison of median excretions						
	Silicon (µg/24hr)			Aluminium (ng/24hr)		
Week	HV	MS	P - value	HV	MS	P - value
Control	84	296	0.002	742	658	0.001
Treatment	804	1224	0.096	1754	2206	0.004

Chapter 7 – Discussion: Integrating silicic acid-rich mineral water into the diet is an effective, non-invasive method of reducing body burden of Al.

This research was undertaken with the aim of illuminating the application of a commercial silicic acid-rich mineral water as a non-invasive method of reducing Al body burden, when used as part of an individual's normal diet. The primary objective was to investigate Si handling and the consequential effects on urinary Al excretion.

Different protocols - healthy individuals

Despite widespread evidence signifying a beneficial role of Si in biota, there is little reliable information on Si handling in humans, particularly regarding healthy individuals. In order to determine whether there is any change in Si handling in disease it is first important to explore Si handling in these 'normal' individuals. Thorough investigations concerning healthy individuals were therefore performed, using two different protocols, exploring two different excretory mechanisms, sweat and urine.

As Si is known to be rapidly absorbed and excreted (Popplewell et al. 1998), it was considered that a period of one week between each protocol would be sufficient to minimize any influence on Si excretion between each study. However, it could be thought that Al body burden would have been influenced, if not lowered, with each additional protocol.

Al intake in the diet is approximately 20 mg day^{-1} (Pennington & Jones, 1989); only 0.1% of this is absorbed (Day et al. 1991), leading to around 140 µg of Al possibly contributing to Al body burden from dietary exposure each week. As we bare this in mind, it is unlikely that the weekly time periods between each different protocol were sufficient to replenish the Al body burden removed from the elevated Si dose.

However, as each protocol consisted of a control and treatment period in which the urinary excretions were compared, the overall effect on Al excretion could be explored.

24-hour collections provide the most reliable information on urinary excretions; however, these were not sensible for the longer term studies, especially when these studies concern vulnerable individuals (i.e. MS patients). For this reason creatinine corrected measurements were also documented. The negative correlation between creatinine concentration and sample volume verifies that creatinine concentration provides an acceptable indication of dilution effects. The positive correlations between the urinary excretions of Si and Al when expressed as total amounts and when corrected for creatinine validate that both methods are appropriate for determining urinary excretions.

The mean creatinine concentration at baseline (5.8 ± 1.7 mM) and volume of urine produced (1670 ± 399 mL) correlates well with the literature values (Table 7.1), confirming that the healthy participants had normal kidney function.

Table 7.1: Comparisons of creatinine concentration and sample volume with other studies

Creatinine Concentration (mM)		Sample Volume (mL)	
Range	**Reference**	**Range**	**Reference**
3.22-8.54	This study	650-2350	This study
2.6-11.5	(Rodriguez et al. 2004)	550-1150	(Morie et al. 1996)
4.3-9.4	(Costa et al. 2007)	450-3250	(Rabinowitch 1933)
9.1-15.8	(Powell et al. 1999a)	1020-2740	(Powell et al. 1999a)

Consumption of total beverages was higher in the treatment period compared to the control ($P < 0.001$) for the healthy volunteer population indicating that the participants consumed the mineral water in addition to, rather than in replacement of, their normal intake. This is likely to be due to the short time period over which the mineral water was consumed. From this, it could be argued that the increase in Si excretion is merely a reflection of the higher consumption of beverages, independent of their Si content. However, a positive correlation was only witnessed between the excretion of Si with total beverages consumed during the treatment period, which verifies that the witnessed increase was due to the addition of the mineral water.

Silicon handling was variable between individuals

Urinary excretion of Si was variable between healthy individuals, the range of urinary Si at baseline was 61-231 µg/24h. As urinary Si is a good marker of Si absorbed, dietary differences between individuals would have contributed to these variations (Jugdaohsingh et al. 2002; Sripanyakorn et al. 2009).

Distinctions in Si handling between individuals were also highlighted. Assuming that the dietary intakes of Si, excluding the mineral water, were similar between the control and treatment periods for each individual, it would be expected that the difference in Si excretion equated to the amount of Si ingested as the mineral water. However, in the loading study only 59 ± 31% of Si ingested as the mineral water was excreted in the urine of health individuals; and this was highly variable between individuals. In the dosing study where a steady stream of Si was delivered throughout the day, this difference equated to 95 ± 25% of Si ingested as the mineral water excreted. This supports the variability between Si handling and suggests that a consistent dose of Si exposure throughout the day ensures more of this Si is bioavailable and becomes absorbed into the bloodstream.

The changes in Si excretions (treatment minus control) between the 19 participants who consumed the full 1.5L of mineral water during the dosing protocol were considerably different. Two individuals (M1, F2) appear to have excreted less than 70% of the Si consumed as the mineral water (*Table 3.1.3.2*), suggesting that they have reduced Si handling. Seven individuals (M2, M3, M4, M6, M7, F8, F9) excreted more than the expected 100% Si following the consumption of the mineral water during the dosing protocol. It is possible that these individuals consumed other Si rich sources on this day.

Effect that the mineral water had on urinary excretions

The baseline urinary excretion of Si was 126 ± 55 µg/24 hours. This was significantly correlated (P = 0.015) with Si excretion in both loading treatment period (614 ± 253 µg/24h) and dosing treatment period (951 ± 246 µg/24h), despite the single individual who excreted two fold more Si after

consuming the mineral water, fourteen out of the fifteen patients saw Si excretion increase by more than three-fold of the baseline concentration for the loading period, this value further increases when considering the dosing data. Positive correlations were also seen for Al ($P < 0.001$) which indicates that the amounts of Si and Al excreted in the treatment period were dependent on the baseline levels.

Consumption of the mineral water significantly increased the amount of Si excreted in the urine of females ($P < 0.001$), although for males, this value increased only marginally ($P = 0.41$). Increases were coincident with increased Al excretion ($P < 0.001$). This signifies that the consumption of silicic acid-rich mineral water successfully enhanced the urinary excretion of Al in healthy individuals.

Males excreted more Si and Al than females. These differences were seen throughout the study, highlighting that both genders metabolise the elements similarly. Males excreted slightly more Si compared to females during the treatment periods, however, this was not surprising as males consumed, on average, more of the mineral water ($P = 0.001$*)*

Co-excretion of Si and Al was apparent in the loading Si data; the relationship between Si and Al along with the rapid excretion of Si demonstrates that Al is excreted as a bolus following the consumption of the mineral water. In addition, regular consumption of the mineral water throughout the day was observed to promote lesser Al excretion, although may maintain this at a lower level; 17/19 healthy individuals showed a larger increase in Al excretion which was coincident with a loading effect of Si consumption. An increase in Al excretion was witnessed for 2/19 individuals during the dosing treatment period, indicating that regular Si supplementation could prevent Al accumulation more efficiently in these individuals. It is anticipated that over a longer period of time Al excretion would be reduced in more individuals.

To summarise the healthy individual study; consumption of the mineral water significantly increased the urinary excretions of Si and Al. Al were rapidly excreted as a bolus during Si loading although Si excretion was at a higher level when a steady stream of Si was consumed throughout the day. Treatment over a long term period could result in reduced Al body burden.

Baseline 24h urinary excretions of Si and Al were compared with literature values (*Table 7.2*). With the exceptions of the studies by Roberts *et al*. (1998) and Kazi *et al*. (2008), who used large populations, the group sizes presented in the other studies were much larger. These differences would have contributed to the higher fluctuations in urinary excretions between the literature sources. For instance, the urinary excretion of Al seems to be higher compared to other studies using a similar age range (Morie *et al*. 1996; Reffitt *et al*. 1999), however, these studies had population size of 6 and 5 respectively. In addition, the low excretion of Al in the study by Reffitt *et al*. (1999) may be attributed to the participants fasting. The higher excretion of Al within the healthy volunteer study could also be a reflection of a university student's diet, which is typically high in processed foods and alcohol (Devine *et al*. 2006). Processed foods are generally high in Al (Pennington & Jones, 1989) and alcohol is known to increase gut permeability (Barchfeld & Deamer, 1988) which could augment Al uptake. Other dietary factors, such as citrate (Taylor *et al.* 1998) contributes to the absorption of Al, therefore, an individual who regularly consumes fruit juice may have an increased absorption of Al.

The majority of individuals in the present study were also regular tea drinkers 13/19. Tea is a diuretic which contains high levels of Al (Forster *et al*. 1995). Despite the bioavailability of Al in tea being considered to be low (Powell *et al*. 1993; Gardner & Gunn, 1995), regular consumption is believed to contribute to enhanced absorption (Nieboer *et al*. 1995). However, it was

demonstrated that higher daily consumption of tea results in reduced Al excretion (*see section 3.1.10*). A possible explanation of this is that Al is in too great an excess of Si to allow the complex to form and enable Al to be excreted.

In the study by Reffitt *et al.* (1999), the participants fasted in order to investigate whether silicic acid promotes the urinary excretion of endogenous Al. Results of the study demonstrated no significant change in Al excretion (*Table 7.2)*; however, in view of the young age of the group (and the small population size) it is unlikely that Al accumulation was unusually high and consequently, effect on eradicating Al from body stores would have been minimal. It would be of interest to carry out this study with a larger pool size of elderly participants, furthermore those with susceptibility to an increased bioburden of Al, like individuals with Alzheimer's disease.

Table 7.2: Comparisons in urinary excretion (mean ± SD) of silicon and aluminium with other studies.

Summary of urinary excretions				
Element	**Reference**	**µg/ng/24h**	**µg/ng/L**	**Notes**
Si (µg)	This study	126 ± 55	97±42	n = 19, 18-34y
	(Reffitt et al. 1999)	719±229		n = 5, 24-31y fasted
	(Roberts et al. 1998)		471±322	n = 115, 30-65y healthy control
Al (ng)	This study	967 ± 687	744± 589	n = 19, 18-34y
	(Reffitt et al. 1999)	187±43		n = 5, 24-31y fasted
	(Roberts et al. 1998)		950±820	n = 54, 30-65y healthy control
	(Morie et al. 1996)	667±222		22-23y, n = 6
	(Morie et al. 1996)	2000±444		69.2±4.4y, n = 6

Involvement of HAS

The direct relationship highlighted between Si and Al supports the theory of HAS being involved in Al excretion. In the presence of free Si, Al is considered to exist as a low molecular weight HAS, giving the potential for reabsorption prevention and perhaps promoting its excretion. It is likely that this interaction predominantly occurs in the proximal convoluted tubule (Bellia *et al*. 1996), where Si becomes concentrated, at this point it may be allowed to exceed the critical level (0.1mM) required for the formation of HAS species (Birchall, 1992).

The current literature has not yet demonstrated the formation of HAS in-vivo (Exley, 1998); however, a further study could potentially be measured using atomic force microscopy, which has been used to identify HAS in acidic solutions (Doucet *et al*. 2001). In order to fully uncover and add knowledge to the mechanisms behind the valuable effect of silicic acid-rich mineral waters, more research is needed, focusing on the biochemical interaction between Si and Al in-vivo.

Silicic acid is a small, neutral molecule, composed of a single Si atom, a single O atom and four hydrogen atoms. Silicic acid is freely diffusible within the body and is rapidly absorbed and excreted (Popplewell et al. 1998); the desirable level of Si, to create its protective effect, is 0.1mM and may therefore not be withstanding in the body. Considering this, and also taking into account the effects of consuming Si in different doses in the HV study, in order to remove systemic Al, it may be necessary to regularly consume large quantities of mineral water over short time periods, rather than drinking small quantities throughout the day.

The 24-hour loading study where the mineral water was consumed soon after waking, would have allowed surplus Si for this interaction to occur. Exact amounts consumed (quantities at specified times) for the dosing study were not available, so it was difficult to judge how often this critical level would have been reached, despite the entire volume of water being consumed by participants in the 24

hours, and a suggestion of 150mL per hour was made. This may explain some of the suggestions that Al absorption across the gut may actually be enhanced at lower Si concentrations (Birchall *et al*. 1996).

It would have been interesting to analyse the relationships between Al excretion with the consumption of orange juice, beer and wine, however, the amount of data on these beverages was not sufficient to obtain a reliable outcome.

Sweat

It is interesting to note that in all sets of data, sweat production was slightly higher in the treatment set of data than the control, there is no current literature to suggest that fluid consumption would influence sweat volume production, only that, an appropriate amount of liquid needs to be taken on board to account for fluid loss in sweat.

The volume of sweat collected during the study was consistently larger for males than females, indicating that men produce more sweat than women, although differences in thermoeffector function were largely determined morphologically, rather than being sex dependent (Notley *et al.* 2017). The volume of sweat collected ranged from 0.66 to 1.55 mL and corresponded well with literature values for perspiration rates (Chen *et al.* 2012).

The mean aluminium content in the control period was higher in females (341 ± 245 µg/L) than males (353 ± 141 µg/L) though the data for each group were not found to be significantly different from one another ($P > 0.05$). Contrary to this, the mean aluminium content in the treatment protocol was higher in males (1208 ± 433µg/L) than females (835 ± 334µg/L) and the data for each group were found to be significantly different from one another ($P < 0.05$).

The method blanks demonstrated a very low level of possible contamination by aluminium and were within the range of a previous study (Exley *et al* 2014).The latter demonstrated a high degree of confidence in the concentrations of aluminium measured herein which were towards the higher end of previous literature values (House *et al* 2012).

When the concentrations of aluminium in collected sweat were adjusted to take account of the volume of sweat produced by men (1342 mL/24 h) and women (712 mL/24 h) in this age range (Manz *et al* 2012) and undergoing mild exercise the amount of aluminium excreted over 24 h ranged from 135 to 421 μg/24 h for women to 96–770 μg/24 h for men during the control and from 274 to 875 μg/24 h for women to 857–2250 μg/24 h for men (*Table 4.4.7.2.1 and Table 4.4.7.2.2*) with men excreting significantly more aluminium than women ($P < 0.05$). These data are significantly higher than those that describe the daily excretion of aluminium in urine, up to 100 μg/24 h (Exley, 2013) and therefore they heavily implicate sweating as the major route of excretion of systemic aluminium in humans, especially after the consumption of a Si rich mineral water. In doing so it may be that men, through perspiration, excrete aluminium from the body more effectively than women and it can be suggested that regular exercise might be a way to increase the excretion of aluminium from the body. If sweating is the major route for the removal of systemic aluminium from the body then this observation puts into question the practice of disrupting or blocking perspiration using antiperspirants and aluminium-based antiperspirants specifically.

Multiple sclerosis

The project protocol was divided into two objectives; the primary objective was to measure the urinary excretions of Si and Al over a 24 week period during which patients consumed up to 1.5L of silicon-rich mineral water each day. The first 12 weeks of the study, referred to as the control, required each participant to provide urine samples following their normal diets, thus acting as their own control to determine individual changes. The secondary objective was to observe changes in mobility using EDSS scores at the beginning and end of the study.

Consumption of total beverages was similar in the treatment period compared to the control ($P < 0.001$; *Table 5.4.2.2)* for the MS patient population indicating that the participants consumed the mineral water in replacement of their usual beverages. Urine volume was similar for the control and treatment period in this study population.

The absorption of Si in the MS patient database is relatable to that of the dosing HV study. In this study, Si was incorporated into the diet of MS patients, replacing all normal water sources with the Si rich mineral water, therefore ensuring this constant stream of Si exposure throughout the day. The difference from the control and treatment period equated to a percentage difference of 99 ± 42% of Si ingested as the mineral water was excreted in the urine of the MS patients. Despite this, eight out of the fifteen patients (M1, M2, M3, F1, F2, F6, F7, F8) excreted less than 90% of the additional Si consumed. Si handling may not be sufficient in these individuals, and therefore they may be more susceptible to Al toxicity.

Of course, an individual's dietary Si intake can fluctuate (Widner *et al*. 1998), therefore, this assumption may not be totally reliable. Nevertheless, the amount of bioavailable Si in mineral water is much higher compared to other dietary sources; intake from silicic acid-rich mineral

water significantly increased Si excretion compared to Si excreted from dietary sources, it is therefore reasonable to assume that the change in Si excretion is mainly due to the mineral water (Sripanyakorn *et al*. 2009.

It is apparent that the interactions between Si and Al witnessed within the healthy volunteer study are also viable for patients with multiple sclerosis.

Creatinine concentration was significantly lower (P = 0.003) in MS patients compared to young healthy individuals (Table: 6.1). However, it has been established that creatinine concentration decreases with age (Rodriguez et al. 2004) and the MS study population were also older than those taking part in the HV study.

Regular consumption of silicic acid-rich mineral water successfully enhanced the urinary excretion of Al in individuals with MS.

For two individuals (F006 and F009), an increase in Si excretion in W13 resulted in a decreased excretion of Al. This is comparable to the results seen in the preliminary study conducted by the silicon and aluminium research group at Keele (Exley *et al.* 2006a); where a significant increase in Si excretion (34.3 ± 15.2 to 55.7 ± 14.2 µmol/mmol creatinine) concomitantly reduced the urinary excretion of Al (86.0 ± 24.3 to 62.2 ± 23.2 nmol/mmol creatinine).

The concentration of Al excreted in the final week (W13) of the treatment period was lower than the concentration at baseline (W1) for 8/15 patients (Figure 4.1); indicating a reduced body burden of Al. This is comparable to the preliminary study performed by this research group (Exley et al. 2006a), which showed that increased Si excretion (34.3 ± 15.2 to 55.7 ± 14.2 µmol/mmol creatinine) was concomitant with reduced Al excretion (86.0 ± 24.3 to 62.2 ± 23.2 nmol/mmol creatinine). However,

an enhancement in Al excretion was witnessed for 15/15 patients by the final treatment Wk 24; an observation not seen in the preliminary study.

Multiple sclerosis patients are expected to have a greater Al body burden compared to controls, however, there was no overall difference (P = 0.844) in Al excretion between patients and control groups at baseline. Excretion of Al was higher for the MS patient group during the equivalent dosing treatment period, which may suggest that the mechanism to reduce body Al by enhanced excretion are less effective than in healthy individuals. After 12 weeks of drinking the Si rich mineral water, it may be suggested that some patients had become non-compliant with the protocol, explaining the decrease in Si excretion during this final treatment week. Despite this, all patients were said to be very compliant with following the protocol and consuming the 1.5L of mineral water provided for each day. The average percentage difference was 98.7%, suggesting that the increase in Si consumption through Si rich mineral water was mainly due to the Si rich mineral water added to the diet.

As with many neurological conditions, gender plays a role in occurrence of the disease. In the case of MS, it is women that are more likely to develop the disease. The data surrounding disease severity and link to gender is sparse, but it may prove useful to determine whether those with a higher body bioburden of Al are further along with the progression of the disease. Females showed a significant difference in the overall excretions of Si and Al. Females excreted significantly more Si (P = 0.005) and Al (P = 0.012). This confirms the preliminary study (Exley *et al.* 2006a), where females excreted significantly more Si (P = 0.008) and Al (P = 0.007) compared to males. However, female healthy volunteers excrete less Al than male controls (P <0.001), and male healthy volunteers

excrete more Al (P = 0.058) compared to female healthy volunteers. The higher excretion of Al in female patients may be responsible for the higher incidence of MS in females compared to males.

There was no significant change in EDSS score for either the patient group. However, despite the relatively short treatment period, disability level was improved (EDSS scores ≥0.5 points; Schrag & Schott, 2012) for 2 patients (F1 and F6), which highlights the potential use of silicic acid-rich mineral water in slowing the rate of decline in aged individuals and also suggests the possibility of relieving the symptoms of secondary progressive multiple sclerosis. Longer term studies on larger populations are now needed to fully elucidate these findings and double blind studies should be used to determine any placebo effects.

The baseline Al excretion was variable between these individuals showing a marked improvement in mobility performance, there was also no pattern in the excretion of Al between these individuals. F8 demonstrated an initial increase in Al excretion in Wk 13 then a gradual decrease up until Wk 24 when Al excretion had reduced to a similar value ($P < 0.001$) to the initial baseline period. For patient F001, a large increase in Al excretion was seen during the initial treatment Wk and by Wk 24, this level had decreased to once similar to that of the initial treatment week.

Suitability of Si-rich mineral water as a treatment against Al toxicity

In young, healthy volunteers the application of a silicic acid-rich mineral water would be most effective as a prophylactic measure to prevent the accumulation of Al over time, which could

potentially slow the rate and progression of Al-related disorders. However, in aged individuals, where Al has presumably already accumulated, silicic acid-rich mineral water could be utilised as an Al chelator, removing systemic Al from body stores, which could potentially reduce the symptoms of Al toxicity.

In comparison to other potential 'treatments' for Al toxicity, silicic acid is inexpensive, readily available, non-invasive and does not have any associated side effects (DFO has been connected with several side effects and its clinical application has been questioned in terms of its poor absorption and rapid degradation (reviewed in Liu et al. 2005).

A large daily dose of silicic acid, as opposed to a steady dose, is the most effective way to remove systemic Al from the body.

Could silicon remove brain aluminium?

The improvements in cognitive function following the treatment of the mineral water could be attributed to reduced brain Al. The presence of aluminosilicates in senile plaques (Candy *et al*. 1986) indicates that Si is able to cross the blood brain barrier and enter cerebral circulation. However, it is not certain whether the products of interaction, presumably HAS, will also be able to cross the blood brain barrier, to enable the removal of Al from the brain. Presently, confirmation of this would require brain biopsies both before and following the treatment of the water. Another possibility is that the Si-Al complex remains dormant in the brain, preventing Al from interfering with important biological molecules. This could also provide an explanation as to why aluminosilicates are also found within the brains of aged 'normal' individuals.

Conclusions

This research brings new and exciting evidence to support the highly debated link between Si, Al and neurological disorders. Not only does it consolidate the application of bioavailable Si supplementation as a long-term chelation therapy against Al related neurodegenerative disorders, but it also suggests that reduced Si handling could be another risk factor, resulting in Al toxicity.

As lower Si levels may increase susceptibility to Al toxicity, supplementing Si into the diet, by means of a silicic acid-rich mineral water, may be particularly beneficial for individuals with impaired Si handling, aging individuals (Si intake is estimated to reduce by 0.1mg with each year of a person's age (Jugdaohsingh et al. 2002)) and to those residing in areas with geographically low Si (Taylor et al. 1995).

Epidemiological studies have suggested that municipal waters containing silicic acid at concentrations above 0.2mM can protect against neurodegenerative disease (Rondeau et al. 2009). Given the evidence, within the present study, that promoting Al excretion is most effective following a bolus of silicic acid-rich mineral water, the protective effects would be greater with Si concentrations above 0.5mM, such as those used within the present studies.

Silicic acid-rich mineral water offers an inexpensive, easily available and non-invasive therapy against Al toxicity. Longer term studies are now warranted to determine whether incorporating silicic acid rich mineral water into a regular diet has any influence on the progression and symptoms of neurological disease. It would be of interest to explore the benefits of the loading dose of Si in SPMS patients as a method of promoting an increase in Al excretion in the urine.

Appendices

Appendix 1

Quality assurance - Replicates

Concentration of analyte measured in repeated analytical runs of the same sample. Ref denotes the analysed sample, R denotes analytical run and % Diff denotes the percentage difference of the concentrations determined in each analytical run. Concentration (µg L^{-1})

Silicon				**Aluminium**			
Ref	R1	R2	**% Diff**	Ref	R1	R2	**% Diff**
24M5	150.2	153.5	-2.17	24M3	7.795	7.558	3.09
24M9	98.68	95.42	3.36	24M4	13.36	12.96	3.04
24F6	93.34	95.33	-2.11	24F7	7.334	7.183	2.08
24M8	135.8	129.2	4.98	24F9	11.95	12.42	-3.86
24M2	82.7	79.58	3.85	24M1	11.99	12.57	-4.72
24F10	191.8	193.6	-0.93	24M7	6.229	6.118	1.80
24F2	66.09	66.98	-1.34	24F5	5.104	5.063	0.81

Appendix 2

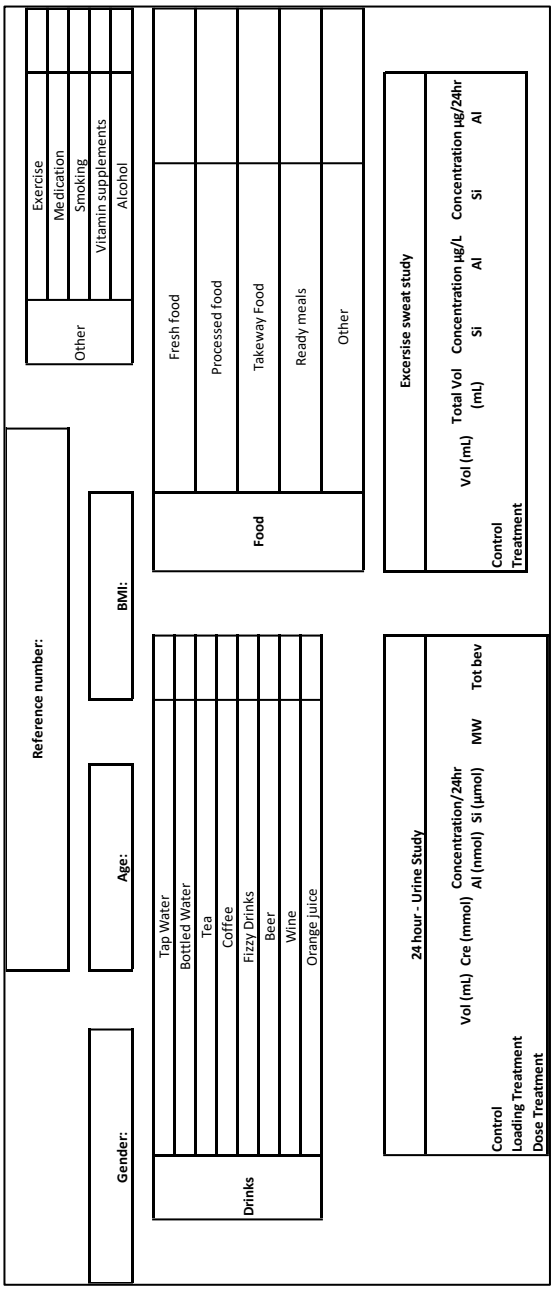
Quality Assurance - Duplicates

Concentration of analyte measured in replicated urine samples (i.e. urine sample was prepared twice). Ref denotes the sample which was duplicated, D denotes the number of duplicate samples and % Diff denotes the percentage difference in concentrations determined in the duplicate samples. Concentration (µg L^{-1}).

Silicon				**Aluminium**			
Ref	D1	D2	**% Diff**	Ref	D1	D2	**% Diff**
24F1	162.5	167.1	**-2.79**	24M3	7.795	7.767	**0.360**
24M1	212.3	218.4	**-2.83**	24F3	11.11	11.18	**-0.628**
24M3	179.4	182.6	**-1.77**	24M5	6.650	6.587	**0.952**
24F6	74.30	71.98	**3.17**	24M9	5.073	5.126	**-1.039**
24M7	157.5	155.7	**1.15**	24M8	13.75	13.99	**-1.730**
24F8	171.1	168.4	**1.59**	24F4	6.801	6.823	**-0.323**
24M2	154.7	161.0	**-3.99**	24F10	6.676	6.742	**-0.984**

Appendix 3

List of healthy individual questionnaires, sweat and urinary excretions (Template shown below)



Reference number F1

Gender: Female

Age: 24

BMI: 22.5

Other	
	Exercise
	Medication
	Smoking
	Vitamin supplements
	Alcohol

Drinks	
	Tap Water
	Bottled Water
	Tea
	Coffee
	Fizzy Drinks
	Beer
	Wine
	Orange juice

Food	
	Fresh food
	Processed food
	Takeway Food
	Ready meals
	Other

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	1300	6.55	1047.27	170.32	0	1680
Loading Treatment	2140	3.42	1531	703.5	1500	2685
Dose Treatment	1790	5.12	1144.41	921.6	1500	2105

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	0.94	5.62	811.9	467.4	584.6	336.5
Treatment	0.98	5.87	1465.8	1183.7	1055.4	852.3

Reference number F2

Gender: Female

Age: 25

BMI: 24.1

Other	
	Exercise
	Medication
	Smoking
	Vitamin supplements
	Alcohol

Drinks	
	Tap Water
	Bottled Water
	Tea
	Coffee
	Fizzy Drinks
	Beer
	Wine
	Orange juice

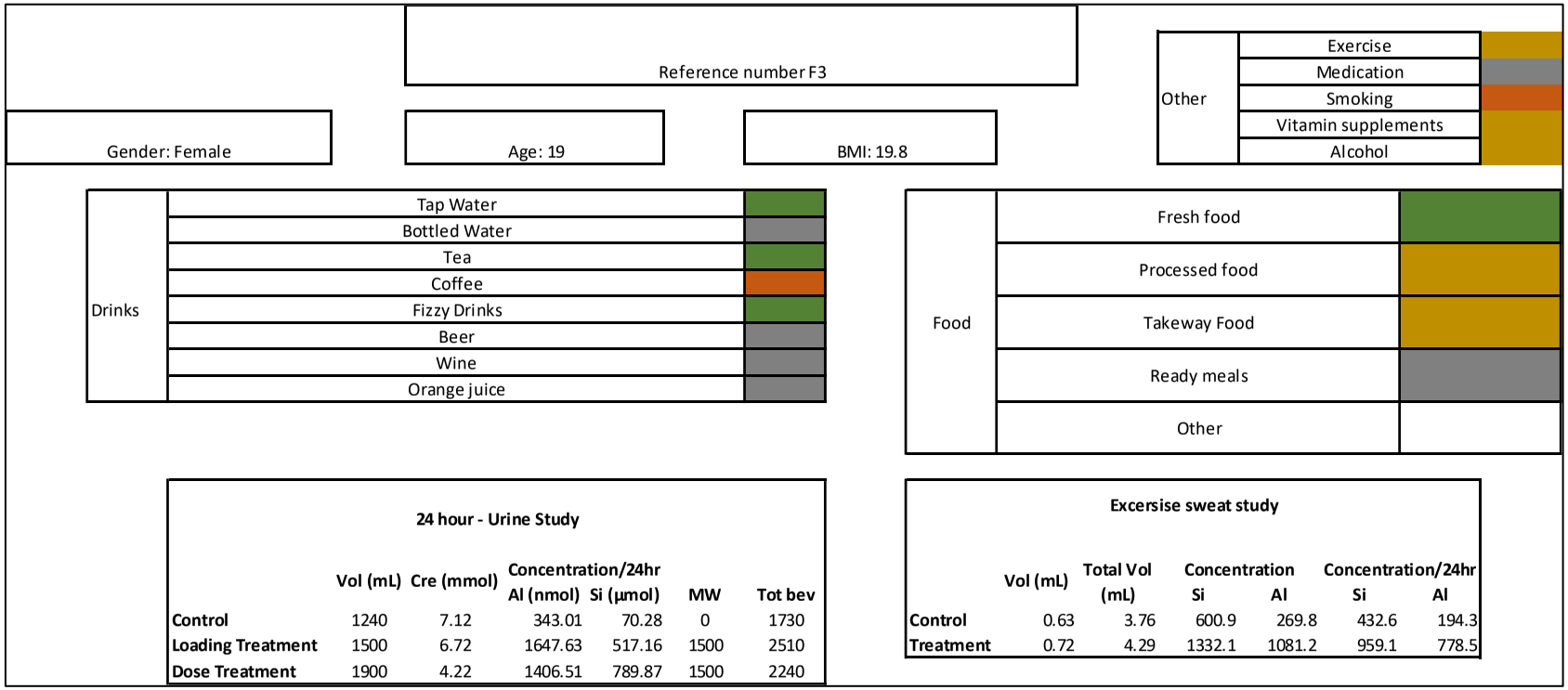
Food	
	Fresh food
	Processed food
	Takeway Food
	Ready meals
	Other

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	550	9.71	661.48	70.03	0	850
Loading Treatment	650	8.54	1605.07	160.72	1500	2460
Dose Treatment	1600	5.54	1271.45	667.13	1500	2025

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	0.44	2.63	646.5	2217.2	465.5	156.4
Treatment	0.5	3.02	1609.2	380.7	1158.6	274.1

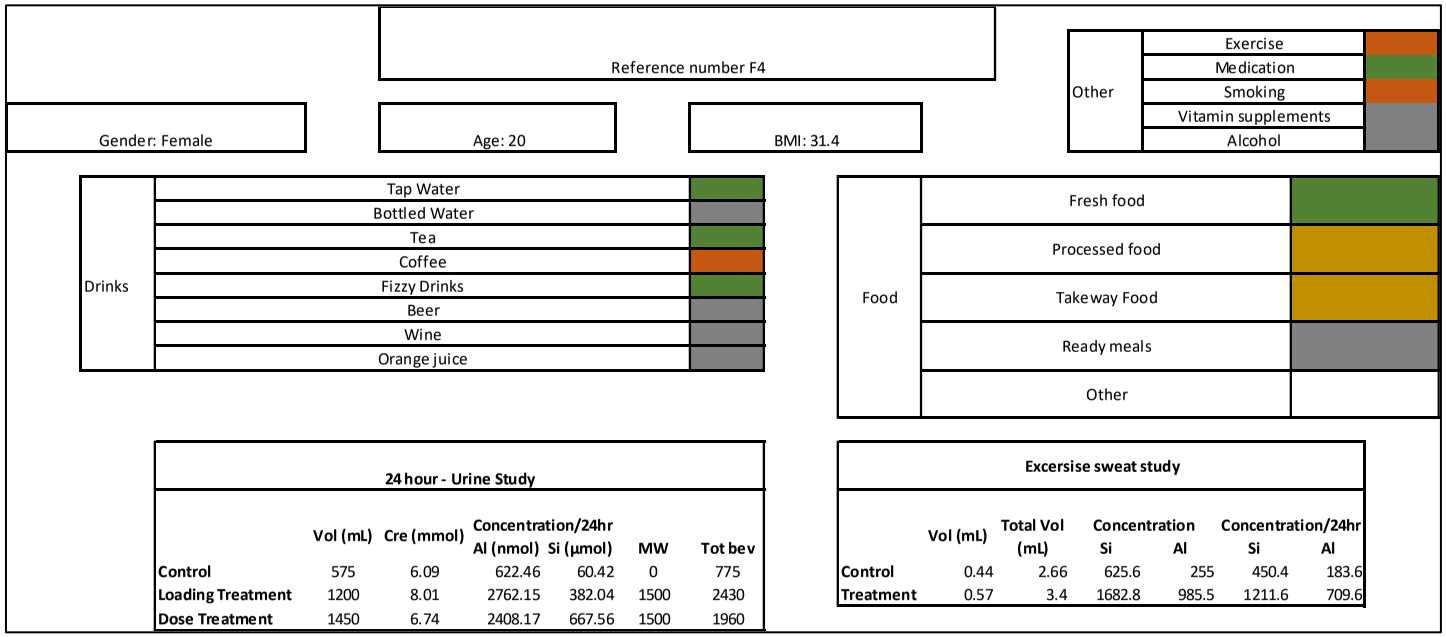


24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	1240	7.12	343.01	70.28	0	1730
Loading Treatment	1500	6.72	1647.63	517.16	1500	2510
Dose Treatment	1900	4.22	1406.51	789.87	1500	2240

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	0.63	3.76	600.9	269.8	432.6	194.3
Treatment	0.72	4.29	1332.1	1081.2	959.1	778.5



24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	575	6.09	622.46	60.42	0	775
Loading Treatment	1200	8.01	2762.15	382.04	1500	2430
Dose Treatment	1450	6.74	2408.17	667.56	1500	1960

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	0.44	2.66	625.6	255	450.4	183.6
Treatment	0.57	3.4	1682.8	985.5	1211.6	709.6

Reference number F5

Gender: Female

Age: 24

BMI: 25.5

Other	
	Exercise
	Medication
	Smoking
	Vitamin supplements
	Alcohol

Drinks	
	Tap Water
	Bottled Water
	Tea
	Coffee
	Fizzy Drinks
	Beer
	Wine
	Orange juice

Food	
	Fresh food
	Processed food
	Takeway Food
	Ready meals
	Other

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	1490	6.27	360.47	80.89	0	1250
Loading Treatment	2100	3.22	2003.14	580.1	1115	2780
Dose Treatment	2150	3.32	1102.43	841.65	1500	1980

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	0.86	5.15	576.3	187.3	414.9	134.9
Treatment	0.97	5.82	1476.2	619.2	1062.9	445.8

Reference number F6

Gender: Female

Age: 23

BMI: 23.8

Other	
	Exercise
	Medication
	Smoking
	Vitamin supplements
	Alcohol

Drinks	
	Tap Water
	Bottled Water
	Tea
	Coffee
	Fizzy Drinks
	Beer
	Wine
	Orange juice

Food	
	Fresh food
	Processed food
	Takeway Food
	Ready meals
	Other

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	1300	6.38	287.54	80.04	0	1180
Loading Treatment	1800	4.04	1684.15	1240.28	1255	2670
Dose Treatment	1760	5.69	551.5	781.92	1500	2025

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	0.55	3.31	667.7	299.7	480.7	215.8
Treatment	0.5	3.32	1612	423.7	1160.6	305.1

Reference number F7

Gender: Female

Age: 18

BMI: 20.7

Other	
	Exercise
	Medication
	Smoking
	Vitamin supplements
	Alcohol

Drinks	
	Tap Water
	Bottled Water
	Tea
	Coffee
	Fizzy Drinks
	Beer
	Wine
	Orange juice

Food	
	Fresh food
	Processed food
	Takeway Food
	Ready meals
	Other

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	610	8.68	567.12	70.12	0	895
Loading Treatment	1470	6.51	3111.44	501.98	1500	2555
Dose Treatment	1700	5.98	2762.4	841.38	1500	2010

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	1.04	6.24	615.8	265.5	443.7	191.2
Treatment	1.41	8.44	1110	425.9	799.2	307.4

Reference number F8

Gender: Female

Age: 25

BMI: 27.1

Other	
	Exercise
	Medication
	Smoking
	Vitamin supplements
	Alcohol

Drinks	
	Tap Water
	Bottled Water
	Tea
	Coffee
	Fizzy Drinks
	Beer
	Wine
	Orange juice

Food	
	Fresh food
	Processed food
	Takeway Food
	Ready meals
	Other

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	520	9.88	467.09	110.95	0	820
Loading Treatment	1980	4.03	2781.73	483.18	1500	2420
Dose Treatment	2050	1.09	2198.51	1307.67	1500	2235

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	0.33	1.99	732.1	520.1	527.1	374.5
Treatmen	0.44	2.64	1634	1034.8	1176.5	745.1

Reference number F9

Gender: Female

Age: 20

BMI: 21.5

Other	
	Exercise
	Medication
	Smoking
	Vitamin supplements
	Alcohol

Drinks	
	Tap Water
	Bottled Water
	Tea
	Coffee
	Fizzy Drinks
	Beer
	Wine
	Orange juice

Food	
	Fresh food
	Processed food
	Takeway Food
	Ready meals
	Other

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	520	10.21	546.94	140.49	0	855
Loading Treatment	1900	4.53	3641.29	522.6	1500	2535
Dose Treatment	2025	1.56	2000.14	1340.67	1500	2270

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	0.56	3.34	786.9	584.9	566.6	421.1
Treatment	0.69	4.42	1777.5	994.3	1279.8	715.9

Reference number F10

Gender: Female

Age: 22

BMI: 26.4

Other	
	Exercise
	Medication
	Smoking
	Vitamin supplements
	Alcohol

Drinks	
	Tap Water
	Bottled Water
	Tea
	Coffee
	Fizzy Drinks
	Beer
	Wine
	Orange juice

Food	
	Fresh food
	Processed food
	Takeway Food
	Ready meals
	Other

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	1575	5.97	342.79	140.73	0	1710
Loading Treatment	2340	3.8	4240.63	628.44	1500	2875
Dose Treatment	1680	4.5	1407.6	797.95	1500	2005

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	0.81	4.85	691	460.5	497.5	331.6
Treatmen	0.9	5.42	1937.8	1214.9	1395.2	874.7

Reference number M1

Gender: Male	Age: 27	BMI: 26.8

Other	Exercise	
	Medication	
	Smoking	
	Vitamin supplements	
	Alcohol	

Drinks	Tap Water	
	Bottled Water	
	Tea	
	Coffee	
	Fizzy Drinks	
	Beer	
	Wine	
	Orange juice	

Food	Fresh food	
	Processed food	
	Takeway Food	
	Ready meals	
	Other	

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	1535	6.14	645.33	150.73	0	1560
Loading Treatment	1550	6.02	1602.61	365.2	1325	2430
Dose Treatment	1150	8.43	741.55	523.16	1500	2275

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	1.32	7.94	944.3	444	1267.3	535.8
Treatmen	1.49	8.96	1660.6	772	2228.5	1036

Reference number M2

Gender: Male	Age: 34	BMI: 25.2

Other	
	Exercise
	Medication
	Smoking
	Vitamin supplements
	Alcohol

Drinks	
	Tap Water
	Bottled Water
	Tea
	Coffee
	Fizzy Drinks
	Beer
	Wine
	Orange juice

Food	
	Fresh food
	Processed food
	Takeway Food
	Ready meals
	Other

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	1850	1.77	2044.43	230.69	0	1700
Loading Treatment	1500	5.85	3147.38	741.39	1500	2525
Dose Treatment	2100	3.49	3042.49	1384.39	1500	2495

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	1.84	11.01	1049.9	71.4	1409	95.8
Treatmen	1.89	11.35	2166.7	1030.2	2907.7	1382.5

Reference number M3

Gender: Male

Age: 26

BMI: 24.8

Other	
	Exercise
	Medication
	Smoking
	Vitamin supplements
	Alcohol

Drinks	
	Tap Water
	Bottled Water
	Tea
	Coffee
	Fizzy Drinks
	Beer
	Wine
	Orange juice

Food	
	Fresh food
	Processed food
	Takeway Food
	Ready meals
	Other

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	2020	2.89	2021.58	170.17	0	2120
Loading Treatment	2150	3.55	4551.6	804.38	1500	2670
Dose Treatment	2300	3.88	3726.44	1109.15	1500	2240

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	1.25	7.5	874	525.5	1172.9	705.2
Treatment	1.37	8.23	1281	1588.9	1719.1	2132.3

Reference number M4

Gender: Male	Age: 24	BMI: 35.6

Other	Exercise
	Medication
	Smoking
	Vitamin supplements
	Alcohol

Drinks	Tap Water
	Bottled Water
	Tea
	Coffee
	Fizzy Drinks
	Beer
	Wine
	Orange juice

Food	Fresh food
	Processed food
	Takeway Food
	Ready meals
	Other

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	1950	5.98	1949.13	140.46	0	2050
Loading Treatment	2150	3.41	4527	761.02	1500	2640
Dose Treatment	2400	2.13	4216.59	1022.64	1500	2375

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	1.01	6.04	743	573.6	997.1	769.8
Treatmen	1.07	6.42	1870.1	1676.6	2509.7	2250

Reference number M5

Gender: Male	Age: 21	BMI: 41.2

Other	Exercise	
	Medication	
	Smoking	
	Vitamin supplements	
	Alcohol	

Drinks	Tap Water	
	Bottled Water	
	Tea	
	Coffee	
	Fizzy Drinks	
	Beer	
	Wine	
	Orange juice	

Food	Fresh food	
	Processed food	
	Takeway Food	
	Ready meals	
	Other	

24 hour - Urine Study

	Vol (mL)	Cre (mmol	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	1835	6.59	1367.64	140.89	0	2015
Loading Treatment	2350	3.75	4652.66	806.1	1500	2515
Dose Treatment	2050	3.49	3144.56	941.12	1500	1950

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	1.12	6.69	809.5	464.2	1086.3	623
Treatmen	1.22	7.31	1347.8	1666.2	1808.7	2236

Reference number M6

Gender: Male	Age: 28	BMI: 31.5

Other	Exercise	
	Medication	
	Smoking	
	Vitamin supplements	
	Alcohol	

Drinks	Tap Water	
	Bottled Water	
	Tea	
	Coffee	
	Fizzy Drinks	
	Beer	
	Wine	
	Orange juice	

Food	Fresh food	
	Processed food	
	Takeway Food	
	Ready meals	
	Other	

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	1870	1.59	1841.95	210.22	0	1980
Loading Treatment	1850	4.88	3702.53	822.62	1500	2345
Dose Treatment	2050	3.76	2961	1202.8	1500	1845

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	1.89	11.36	782.5	183.2	1050.1	245.9
Treatmen	1.93	11.59	2327.2	1275.7	3123.1	1712

Reference number M7

Gender: Male

Age: 21

BMI: 28.7

Other	
	Exercise
	Medication
	Smoking
	Vitamin supplements
	Alcohol

Drinks	
	Tap Water
	Bottled Water
	Tea
	Coffee
	Fizzy Drinks
	Beer
	Wine
	Orange juice

Food	
	Fresh food
	Processed food
	Takeway Food
	Ready meals
	Other

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	1960	1.63	1944.81	210.66	0	2315
Loading Treatment	2300	3.17	5016.88	921.44	1500	2540
Dose Treatment	1900	5.21	2643.54	1203.38	1500	2155

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	1.63	9.79	994.4	151.8	1334.5	203.7
Treatment	1.71	10.23	1941.1	1536.4	2605	2061.8

Reference number M8

Gender: Male	Age: 29	BMI: 25.4

Other	Exercise	
	Medication	
	Smoking	
	Vitamin supplements	
	Alcohol	

Drinks	Tap Water	
	Bottled Water	
	Tea	
	Coffee	
	Fizzy Drinks	
	Beer	
	Wine	
	Orange juice	

Food	Fresh food	
	Processed food	
	Takeway Food	
	Ready meals	
	Other	

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	1145	8.02	300.03	80.32	0	1870
Loading Treatment	1280	7.54	960.41	302.84	1375	2660
Dose Treatment	1870	2.64	1004.77	883.31	1500	2090

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration Si	Al	Concentration/24hr Si	Al
Control	1.45	8.69	578.4	399.8	776.2	536.5
Treatment	1.46	8.77	1587.9	689.8	2131	925.7

Reference number M9

Gender: Male

Age: 25

BMI: 22.9

Other	
	Exercise
	Medication
	Smoking
	Vitamin supplements
	Alcohol

Drinks	
	Tap Water
	Bottled Water
	Tea
	Coffee
	Fizzy Drinks
	Beer
	Wine
	Orange juice

Food	
	Fresh food
	Processed food
	Takeway Food
	Ready meals
	Other

24 hour - Urine Study

	Vol (mL)	Cre (mmol)	Concentration/24hr Al (nmol)	Si (µmol)	MW	Tot bev
Control	1180	7.87	392.91	60.96	0	1650
Loading Treatment	1570	6.01	1723.62	424.01	1320	2750
Dose Treatment	1850	3.09	1208.58	842.78	1500	1850

Excersise sweat study

	Vol (mL)	Total Vol (mL)	Concentration µg/L Si	Al	Concentration µg/24hr Si	Al
Control	1.72	10.33	674.4	255.2	905	342.5
Treatment	1.79	10.71	1726.2	638.7	2316.6	857.1

Appendix 4

Method blank data for aluminium (n=114)

Amount of Al in digest (ng)					
44	71	62	45	85	91
39	86	43	39	92	89
57	58	17	26	98	75
54	95	92	74	86	34
50	45	99	73	51	10
50	66	66	36	49	12
97	91	56	90	74	158
87	80	46	79	54	132
65	64	74	33	35	117
34	38	58	41	73	80
56	81	83	42	38	66
45	108	45	56	55	56
74	100	51	102	75	75
59	66	66	124	94	59
35	46	67	149	45	73
47	59	57	36	25	56
56	54	82	58	37	62
38	90	99	50	66	90
53	106	90	46	65	118
Mean Al in MD digest = 57ng					

Appendix 5

EDSS Score Scale:

9 EXPANDED DISABILITY STATUS SCALE

0 normal neurological exam (all FS grade 0)
1.0 no disability, minimal signs in one FS (one FS grade 1)
1.5 no disability, minimal signs in more than one FS (more than one FS grade 1)
2.0 minimal disability in one FS (one FS grade 2, others 0 or 1)
2.5 minimal disability in two FS (two FS grade 2, others 0 or 1)
3.0 moderate disability in one FS (one FS grade 3, others 0 or 1) though fully ambulatory; or mild disability in three or four FS (three/four FS grade 2, others 0 or 1) though fully ambulatory
3.5 fully ambulatory but with moderate disability in one FS (one FS grade 3) and mild disability in one or two FS (one/two FS grade 2) and others 0 or 1; or fully ambulatory with two FS grade 3 (others 0 or 1);or fully ambulatory with five FS grade 2 (others 0 or 1)
4.0 ambulatory without aid or rest for ≥500 meters; up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps
4.5 ambulatory without aid or rest for ≥300 meters; up and about much of the day, characterised by relatively severe disability usually consisting of one FS grade 4 and combination of lesser grades exceeding limits of previous steps
5.0 ambulatory without aid or rest for ≥200 meters (usual FS equivalents include at least one FS grade 5, or combinations of lesser grades usually exceeding specifications for step 4.5)
5.5 ambulatory without aid or rest for ≥100 meters
6.0 unilateral assistance (cane or crutch) required to walk at least 100 meters with or without resting (see chapter 8, Ambulation)
6.5 constant bilateral assistance (canes or crutches) required to walk at least 20 meters without resting (see chapter 8, Ambulation)
7.0 unable to walk 5 meters even with aid, essentially restricted to wheelchair; wheels self and transfers alone; up and about in wheelchair some 12 hours a day
7.5 unable to take more than a few steps; restricted to wheelchair; may need some help in transferring and in wheeling self
8.0 essentially restricted to bed or chair or perambulated in wheelchair, but out of bed most of day; retains many self-care functions; generally has effective use of arms
8.5 essentially restricted to bed much of the day; has some effective use of arm(s); retains some self-care functions
9.0 helpless bed patient; can communicate and eat
9.5 totally helpless bed patient; unable to communicate effectively or eat/swallow
10 death due to MS

Annex 1

NREC Ref: 14/YH/1115 Approval letter

NHS
Health Research Authority

NRES Committee Yorkshire & The Humber - South Yorkshire

Unit 001
Jarrow Business Centre
Rolling Mill Road
Jarrow
Tyne and Wear
NE32 3DT

Tel: 0191 428 3561

23 December 2014

Professor Christopher Exley
Professor of Bioinorganic Chemistry
Keele University
Birchall Centre
Keele University
Staffordshire
ST5 5BG

Dear Professor Exley

Study title:	**Urinary excretion of aluminium and silicon in secondary progressive multiple sclerosis.**
REC reference:	**14/YH/1115**
Amendment number:	**Substantial Amendment 1**
Amendment date:	**28 November 2014**
IRAS project ID:	**144340**

The above amendment was reviewed the Sub-Committee in correspondence.

This amendment was to include the clarification of the urine collection procedure as well as an initial urine test for current urinary infections in patients entering the study. Poor renal function exclusion criteria had been removed and also clinician Seema Kalra had been removed from documents.

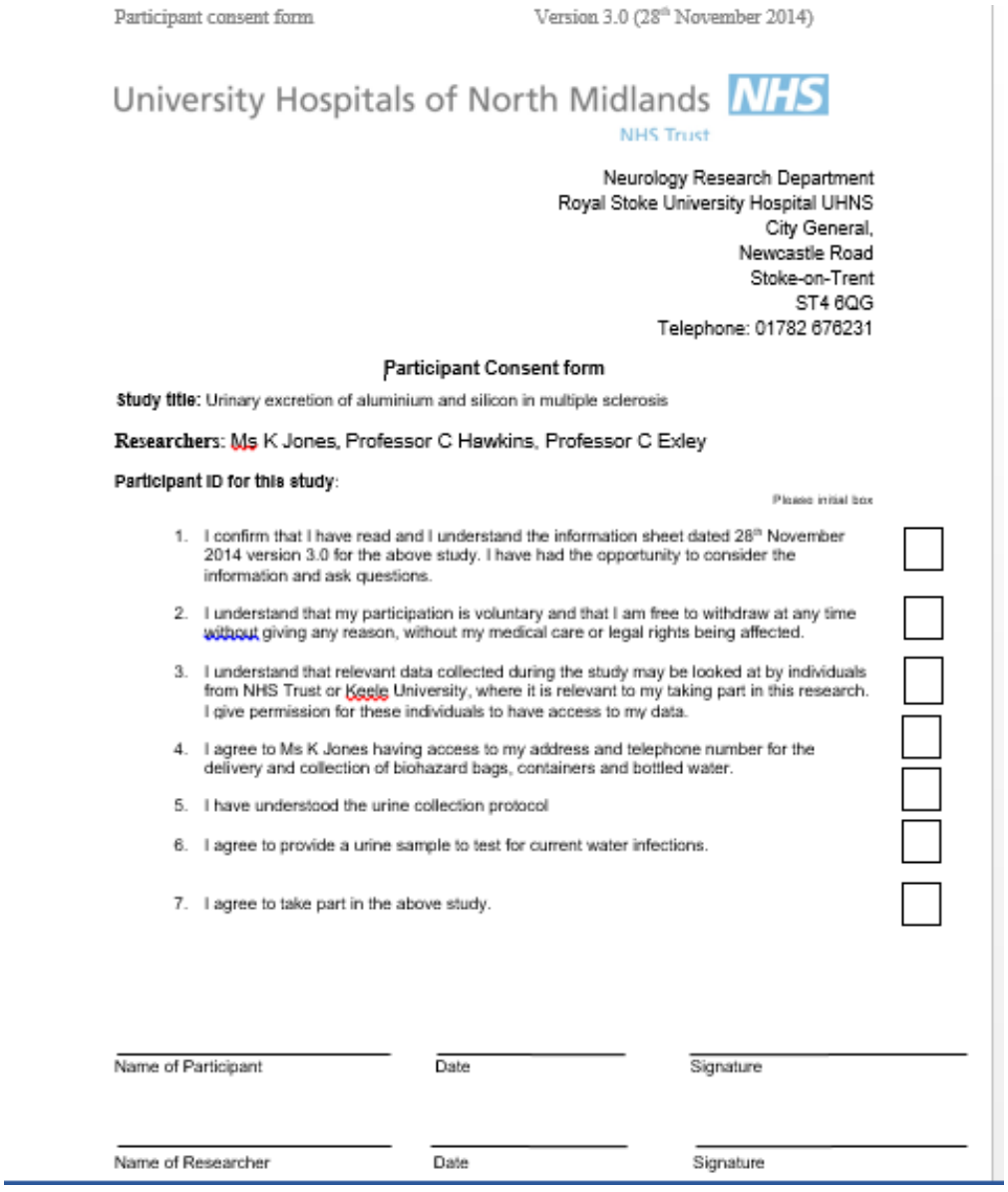
Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

The sub committee did not raise any ethical issues.

Annex 2

Supporting documents – MS Study



Participant consent form Version 3.0 (28th November 2014)

University Hospitals of North Midlands NHS
NHS Trust

Neurology Research Department
Royal Stoke University Hospital UHNS
City General,
Newcastle Road
Stoke-on-Trent
ST4 6QG
Telephone: 01782 676231

Participant Consent form

Study title: Urinary excretion of aluminium and silicon in multiple sclerosis

Researchers: Ms K Jones, Professor C Hawkins, Professor C Exley

Participant ID for this study:

Please initial box

1. I confirm that I have read and I understand the information sheet dated 28th November 2014 version 3.0 for the above study. I have had the opportunity to consider the information and ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant data collected during the study may be looked at by individuals from NHS Trust or Keele University, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my data. ☐
4. I agree to Ms K Jones having access to my address and telephone number for the delivery and collection of biohazard bags, containers and bottled water. ☐
5. I have understood the urine collection protocol ☐
6. I agree to provide a urine sample to test for current water infections. ☐
7. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Researcher	Date	Signature

What will happen to me if I take part?

The study will last for 26 weeks in total and is split into two 13 week periods. Recruited patients will participate in the study, which will last for 26 weeks in total split into two 13 week periods. Patients are not asked to do anything out of the ordinary with respect to their diet except for the inclusion of silicon rich mineral water during the second half of the study. You will either be collecting the whole days (24 hours) urine into a provided container or just the first urine of the day in a provided container, as detailed below. The clinician will ensure that you are happy with the logistics of this before commencing the investigation.

First Part (13 weeks)

(i) Week 1 – You will be asked to provide 5 consecutive 24h urine samples, Monday to Friday inclusive of this week. Biohazard bags will be provided.

(ii) Weeks 2-13 – You will be asked to provide a single spot urine sample, the first urine of the day, on each Wednesday of each of these weeks.

Second Part (13 weeks)

(iii) Week 13 – On the first day of this week and each day thereafter for 13 weeks you will be asked to include up to 1.5L of a Si-rich mineral water in your everyday diet. Similarly to Week 1, during Week 13 you will be asked to provide 5 consecutive 24h urine samples, Monday to Friday inclusive of this week. In weeks 14-26 you will be asked to provide a single spot urine sample, the first urine of the day, on each Wednesday of each of these weeks.

If you have agreed to allow the researcher to have your contact details, you will liaise with them to arrange a suitable time; the investigator will come and collect samples at your convenience as well as deliver weekly batches of the silicon rich mineral water. If not then you can transfer the samples to the hospital from where the researcher will collect them and drop off water for you to collect. You will be asked to keep the urine samples in containers and biohazard bags provided in a cool dark place, such as a refrigerator. You will also be given a telephone number to call if you have questions about the study. To see if there are any clinical effects of the above, we will perform neurological examination to measure disease severity including disability score, brief memory testing and quality of life questionnaire during week 1 and week 26 of the study in the form of a questionnaire which will take 20 minutes.

Are there any benefits from taking part?

In previous studies it has been seen that individuals show improvements in cognitive function following silicon mineral water 'therapy' in other neurological conditions (Davenward et al., 2013). We do not know about any benefits in MS. It is not expected that drinking the mineral water will not affect your memory or thinking abilities in a significant way.

Are there any disadvantages from taking part?

Apart from the need to drink up to 1.5 litre of mineral water a day when required and to the need to collect urine samples there should be no disadvantage in taking part in the study. The mineral water may be used to make other drinks, but it is preferred that you drink it as mineral water.

University Hospital of North Staffordshire NHS
NHS Trust

Neurology Research Department
University Hospital of North Staffordshire NHS Trust
City General,
Newcastle Road
Stoke-on-Trent
ST4 6QG
Telephone: 01782 676231

Participant Information Sheet
Urinary excretion of aluminium and silicon in multiple sclerosis

Sponsor: Keele University

Investigators: Dr S Kalra, Ms K Jones, Professor C Hawkins, Professor C Exley

We would like to invite you to take part in a new research study. Before you decide to participate, it is important that you understand why the research is being done and what it involves. Please take time to read the following information and discuss it with your friends and family if you wish. We will go through the information sheet and answer any questions you may have. If you are happy we will ask you to sign a consent form. Thank you for reading this.

What is the purpose of the study?
Multiple sclerosis (MS) is associated with inflammation and nerve damage. Progressive MS has greater degree of nerve damage. Previous research by our group has shown that individuals with many neurological conditions have a high burden of aluminium. Aluminium can contribute to the nerve damage. Also, there is evidence that drinking mineral water containing silicon can remove excess body aluminium in the urine. Whether this is a case in MS we do not know. We therefore, want to study the role of aluminium in MS. We want to know if aluminium could be removed by drinking mineral water in MS and if it would result in any clinical benefit.

Why have I been chosen?
You are being invited as you have a diagnosis of secondary progressive multiple sclerosis (SPMS). We aim to include 20 SPMS patients in this study.

Do I have to take part?
No, it is entirely up to you whether you decide to take part or not. If you decide to take part you will be given this information sheet to keep and you will be asked to sign a consent form. If you decide to take part you are free to withdraw at any time and for any reason. If you withdraw from the study then this will have no effect on the standard of care you receive either now or in future.

There are no risks associated with this study, however, if you do begin to feel unwell during any part of the study you will be asked to stop and seek medical attention.

Will my taking part in the study be kept confidential?
The clinical team will collect certain information about you such as you date of birth, sex, occupation and health. Such information will be kept confidential.

What will happen to the results of the study?
Following the completion of the study the researchers intend to publish the results in scientific journals. The results of the study will be available on request.

Who is organising and funding the research?
The study is being organised and funded by doctors and scientists working at Keele University and University Hospital of North Staffordshire NHS Trust.

Who supplies and pays for the mineral water?
The mineral water has been supplied by the Spritzer. The study team from Keele University will provide it for you.

What happens to the urine samples at the end of the study?
Any urine that is left over after the analyses have been done will be destroyed safely.

Contact for further information?
You may contact Dr Seema Kalra, the study doctor for further information. Contact details are below.

What if I have any concerns?
the study team/PI will ensure that the adult safeguarding procedures are in place and followed throughout the study. If you have concerns or questions about the study or the way it has been carried out you may contact Ms Krista Jones or Chief Investigator Professor Christopher Exley, details below:

Professor Christopher Exley
Contact Address: The Birchall Centre, Lennard-Jones Laboratories,
Keele University, Staffordshire, ST5 5BG, UK
Telephone number/Mobile number: 44 1782 734080
E-mail: c.exley@keele.ac.uk

Miss Krista Jones
Contact Address: The Birchall Centre, Lennard-Jones Laboratories,
Keele University, Staffordshire, ST5 5BG, UK
Telephone number/Mobile number: 44 1782 734080
E-mail: k.l.jones@keele.ac.uk

Annex 3 -

Patient diary template

Silicon water study

Participant lifestyle diary

Participant Number:		
Date:		
Amount of Exercise (minutes):		
Level of Exercise: Gentle Moderate Strenuous		
Consumption of beverages:		
Beverages Consumed	Time Consumed	Amount consumed
Consumption of food:		
Food Consumed	Time Consumed	

One of these forms will be filled in for each day of the study where urine is being collected.

Annex 4

Research passport

CONFIDENTIAL

Keele University

Research Passport: Evidence of Occupational Health Clearance

To be completed by the Occupational Health Service

Name of Researcher:	Krista Jones
Employer or place of study:	The Birchall Centre

This document will be made available to human resources in the employing organisation and in those NHS organisations where the applicant will be undertaking research. It should not contain confidential information without the consent of the applicant.

Occupational Health Clearance given for a Research Passport YES ☑ NO ☐

The following assessments have been carried out in respect of the applicant:-

- Occupational Health self-assessment health questionnaire including physical conditions, psychological conditions and workplace adjustments YES ☑ NO ☐
- Interview with occupational health staff YES ☑ NO ☐
- Medical examination by Occupational Health Staff YES ☐ NO ☑

Has an occupational health assessment confirmed that there are no health related matters that could affect the health and safety of the applicant or others within the NHS? YES ☑ NO ☐

Is the applicant cleared for exposure-prone procedures? YES ☐ NO ☐ NA ☑

If No to either of the above, it may be necessary for occupational health staff in those NHS organisations where the applicant will be undertaking research to discuss health-related information with the occupational health staff of the substantive employer.

Details of Occupational Health Staff

Name:	[signature]	Job Title:	OHN
Signed:	[signature]	Date:	2/7/14.

Department Stamp

OCCUPATIONAL HEALTH
SERVICE
KEELE UNIVERSITY
ST5 5BG
01782 733733

Annex 5

Ethical Approval documents – Healthy Volunteer Study

Keele University

RESEARCH AND ENTERPRISE SERVICES

1st August 2013

Krista Jones
LJ0.04B
Leonard Jones Laboratories
Keele University

Dear Krista,

Re: 'Renal Handling of Silicon'

Thank you for submitting your revised application for review.

I am pleased to inform you that your application has been approved by the Ethics Review Panel. We would also like to thank you for the additional information in your email and the clarity of your revisions, and we wish you every success with your project.

The following documents have been reviewed and approved by the panel as follows:

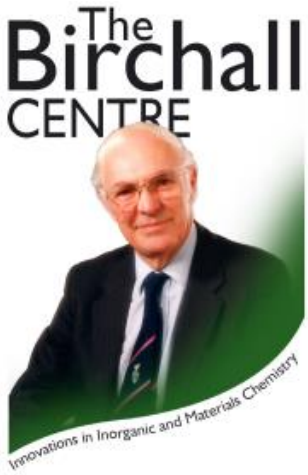
Document(s)	Version Number	Date
Summary Proposal	3	24 July 2013
Letter of Invitation(s)	3	24 July 2013
Information Sheet(s)	3	24 July 2013
Consent Form(s)	3	24 July 2013
Consent Form(s) for use of quotes	-	-
Questionnaire(s)	3	24 July 2013
Interview Topic Guide(s)	-	-
Record Card	3	24 July 2013

If the fieldwork goes beyond the date stated in your application, you must notify the Ethical Review Panel via the ERP administrator at uso.erps@keele.ac.uk stating ERP1 in the subject line of the e-mail.

If there are any other amendments to your study you must submit an 'application to amend study' form to the ERP administrator stating ERP1 in the subject line of the e-mail. This form is available via http://www.keele.ac.uk/researchsupport/researchethics/

Keele University

Ethical Application Document



Project Title: Renal Handling of Silicon

Project Leader: Krista Louise Jones

07006754

Project Supervisor: Professor Christopher Exley

Information for participants

Outlined below is the relevant information about the study and the details of your involvement.

Title of research: Renal Handling of Silicon in Humans.

Overview of research: This PhD research project will investigate the renal handling of silicon in humans. The primary aim of the study is to understand the absorption, retention and urinary excretion of silicon consumed as silicic acid rich mineral waters. The secondary aim is to understand how drinking silicic acid rich mineral water influences the urinary excretion of aluminium and the essential metals, iron, copper and zinc.

Invitation

You are being invited to consider taking part in the research study, Renal Handling of Silicon in Humans. This project is being undertaken by Miss Krista Jones, a PhD student of Professor Chris Exley.

Before you decide whether or not you wish to take part, it is important for you to understand why this research is being done and what it will involve. Please take time to read this information carefully and discuss it with friends and relatives if you wish. Ask us if there is anything that is unclear or if you would like more information.

Why have I been chosen?

You have been chosen because you have shown enthusiasm towards the project through our emails and posters. You pass our inclusion criteria, as shown below, and are able to give your time to take part in the study. There will be around 20 others taking part.

Inclusion/ Exclusion Criteria:

The inclusion criteria are as follows:

- Individuals who are capable of giving informed consent.
- Individuals who are capable of ingesting up to 1.5L of mineral water in a time period of up to 1.5 hours (time between primary and secondary urinary excretion) without causing any practical difficulties.

- Individuals within the age range of 18 – 35 years.

The exclusion criteria are as follows:

- Individuals who are not capable of giving informed consent.
- Individuals who are not capable of ingesting up to 1.5L of mineral water in a time period of up to 1.5 hours (time between primary and secondary urinary excretion) without causing any practical difficulties.
- Individuals outside the age range of 18 – 35 years.

Do I have to take part?

You are free to decide whether you wish to take part or not. If you do decide to take part you will be asked to sign two consent forms, one is for you to keep and the other is for our records. You are free to withdraw from this study at any time and without giving reasons.

Recruitment: Healthy volunteers (see inclusion criteria above) will be recruited by emails and posters, as seen in Appendix 2. The interested individuals will then converse with Miss Jones by email or in person, and given more information about the study. Once the health screening and consent form are completed the individuals will be given the questionnaire to complete. The recruited participants will then come to the Birchall centre to collect their water, labelled sample collection tubes, biohazard bags and food/drink consumption diary sheets

What will happen if I take part?

You will be given a questionnaire to complete and upon completion of this you will receive all of the components required to carry out the study.

If I take part, what do I have to do?

The first 24 hours of urine collection will be following your regular diet; for the second 24 hours of urine collection you will be required to incorporate up to 1.5L of the mineral water provided between your first and second urinary excretion.

For the first 24 hours, collect all urine into the 3L container provided, ultrapure water will be provided to rinse the jug between excretions.

Once you have woken on the second day, flush away your first urinary excretion, then begin to consume up to 1.5L of the silicon rich mineral water provided, in the time between your first and second urinary excretion, up to 1.5 hours. Then continue to collect all subsequent urinations throughout the day into the second 3L container. The provided food diary and time of urination must be maintained throughout each day.

Duration of participation: Total of 2 days.

Amount to be consumed: Up to 1.5L of Silicon rich mineral water provided by Mis Jones, in a time period of up to 1.5 hours (time between primary and secondary urinary excretion), allowing for 1L/hour filtration rate. The bottle with any remaining water should be returned with the samples in order for the amount, which is actually consumed, to be measured.

Retrieval: Samples will be collected at the end of the 48 hour period. Volunteers are required to liaise with Miss Jones to arrange a time for collection that suits both the participant and Miss Jones. This can be at any time point over the week.

Storage/ Disposal of samples: Each volunteer will be responsible for their samples. Samples are to be kept within the biohazard bags, provided by Miss Krista Jones, and retained in the participant's fridge until collection. It is crucial to ensure that any individuals sharing amenities with the participant are aware of the use of their fridge for this purpose; this is to ensure that the sample isn't accidentally ingested and that no offence is caused by its presence in the fridge. Upon receipt at the Birchall Centre samples will be acidified to ca 20% v/v 15.8M HNO_3 to render them acellular for longer-term keeping prior to analyses of Si and metals. Samples will then be kept in a designated fridge until use, after which, they will be disposed of appropriately. Since all samples will be converted to acid digests upon receipt then no samples of urine will be stored. Volunteers are advised to wash their hands after each urinary collection.

Access to data and samples: Only Krista Jones and supervisor Prof. Chris Exley will have access to data and samples. All data will be kept confidential and anonymous.

Benefits/Risks: There are no known risk to the individual taking part of this study, the consumption of up to 1.5L water in a time period of up to 1.5 hours (time between primary and secondary urinary excretion) poses no risk to a healthy individual. Aprevious investigation, using the same protocol, by Samantha Ward saw no negative effects on participants. The standard clinical test, the water loading test, used by the NHS is a consistent method and apart from feeling slightly bloated, there are no risks reported with this test, which involves a considerable amount more than is being asked to be consumed in this study. Benefits of drinking a silicon rich mineral water have been seen in many scientific investigations. Silicon, as well as playing a vital role in bone growth, is believed to influence the excretion of aluminium, the lower the bioburden of aluminium on the body the lower the risk of developing aluminium related diseases (Exley, 2006).

Funding: The study is being funded by Keele Acorn and additional support from Spritzer.

What if there is a problem? It has been reported that in rare cases some people experience some dizziness under water loading, so if you begin to feel unwell then stop drinking the water.

Contact the below for any queries:

Nicola Leighton

Research Governance Officer

Research & Enterprise Services

Dorothy Hodgkin Building

Keele University

ST5 5BG

E-mail: n.leighton@uso.keele.ac.uk

Tel: 01782 733306

Krista Jones

Email: k.l.jones@keele.ac.uk

Prof. Chris Exley

Email: c.exley@chem.keele.ac.uk

CONSENT FORM

Title of Project: *Renal Handling of Silicon*

Name and contact details of Principal Investigator: *Krista Jones, k.l.jones@keele.ac.uk,*

Please tick box if you
agree with the statement

1	I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions.	□
2	I understand that my participation is voluntary and that I am free to withdraw at any time.	□
3	I agree to take part in this study.	□
4	I understand that data collected about me during this study will be anonymised before it is submitted for publication.	□

_____	_____	_____
Name of participant	Date	Signature

_____	_____	_____
Researcher	Date	Signature

Healthy Volunteer Questionnaire

Gender: Male □ Female □

Age: ……..

Height: ……..

Weight:……..

For the following questions, please indicate the most suitable answer.

- In an average week how often do you eat the following types of food?

	Never	Once a week	A few times a week	Every day
Fresh foods	□	□	□	□
Processed foods	□	□	□	□
Ready meals	□	□	□	□
Take Outs	□	□	□	□
Other (please state)	□	□	□	□

……..

- In an average week how often do you consume the following soft beverages?

	Never	Once a week	A few times a week	Every day
Water (Tap)	□	□	□	□
Water (Bottle)	□	□	□	□
Milk/Milkshake	□	□	□	□
Fresh fruit Juice	□	□	□	□
Long life juice	□	□	□	□
Cordial	□	□	□	□
Fizzy drinks (Cans)	□	□	□	□
Fizzy drinks (bottles)	□	□	□	□
Tea	□	□	□	□
Coffee	□	□	□	□
Other (please state)	□	□	□	□

……..

- If you consume bottle water, which brand do you prefer?

……..

- In an average week how often do you consume the following alcoholic beverages?

	Never	Once a week	A few times a week	Every day
Beer (can)	□	□	□	□
Beer (bottle/draft)	□	□	□	□
Cider (can)	□	□	□	□
Cider (bottle/draft)	□	□	□	□
Wine	□	□	□	□

Spirits	□	□	□	□
Other (please state)	□	□	□	□

…….

- How often a week do you use the following products?

	Never	Once a week	A few times a week	Every day
Deodorant	□	□	□	□
Hairspray	□	□	□	□
Cosmetics	□	□	□	□
Soap/Shower gel	□	□	□	□

- Which best describes your smoking habits?

Regular Smoker □

Social Smoker □

Ex-smoker □

Non-smoker □

- How often do you exercise in an average week?

Every day □

5-6 days □

3-4 days □

1-2 days □

Never □

- How would you best describe your activity level?

Strenuous □

Moderate □

Gentle □

N/A □

- Do you suffer from any medical conditions: Yes □ No □

If yes, please specify……..

- Are you currently taking any pharmaceuticals: Yes□ No □

If yes, please select which type:

Paracetemols □

Aspirins □

Ibuprofen □

Antacids □

Other □ (please specify……..)

- How often are you taking pharmaceuticals?

Daily □

A few times a week □

Once a week □

Less than once a week □

- Are you currently taking any vitamin supplements: Yes □ No □

If yes, please specify……..

- How often are you taking vitamin supplements?

Daily □

A few times a week □

Once a week □

Less than once a week □

Thank you for your time and participation.

Record Card - 24hour sample Day 1

Start Date:	Start Time:	
Finish Date:	Finish Time:	
Amount of Exercise (m):	Level of Exercise: Gentle Moderate Strenuous	
Consumption of beverages:		
Beverages Consumed	Time Consumed	Amount Consumed

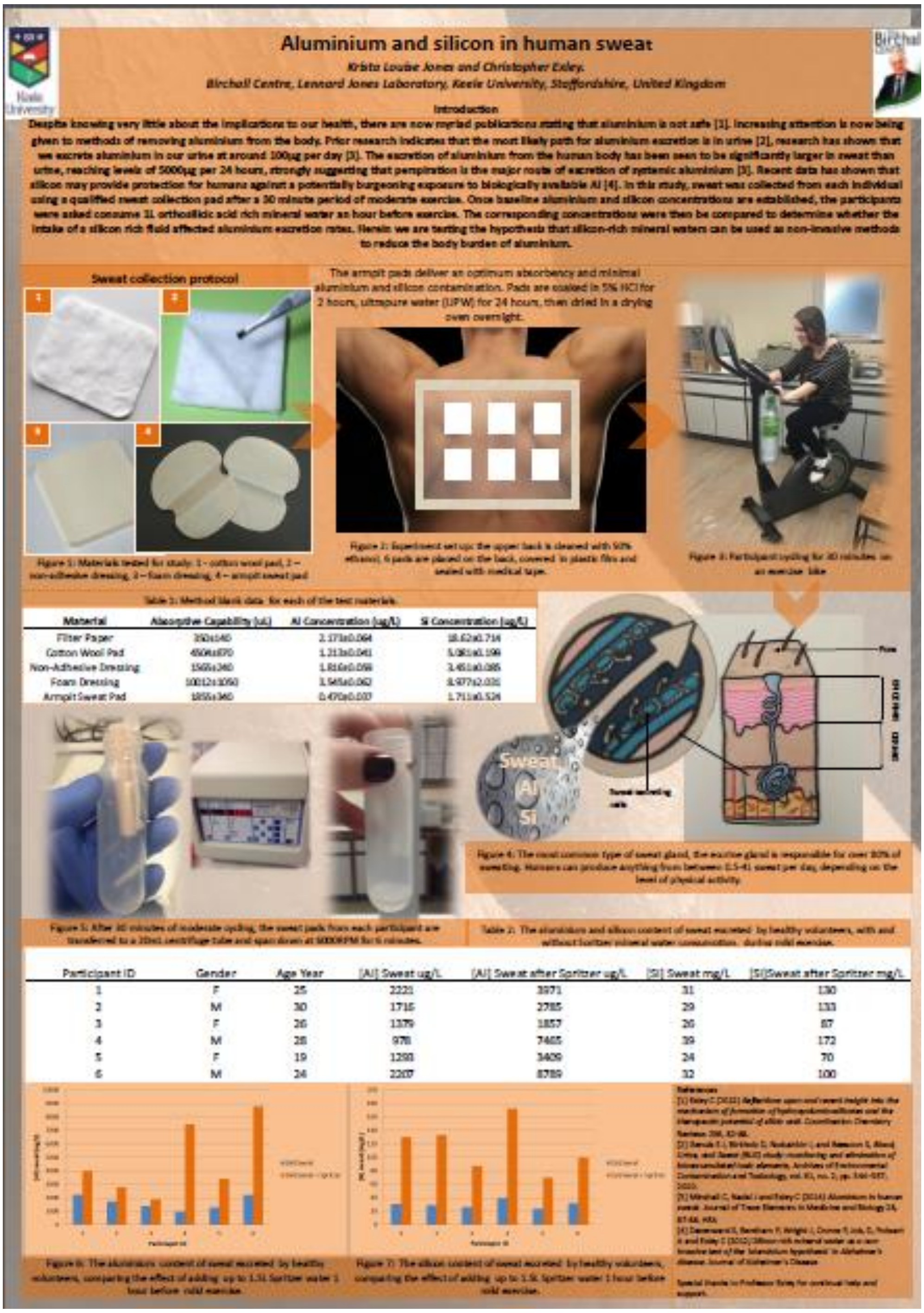
Record Card - 24hour sample Day 2 (addition of Spritzer)

Start Date:	Start Time:	
Finish Date:	Finish Time:	
Amount of Exercise (m):	Level of Exercise: Gentle Moderate Strenuous	
Consumption of beverages:		
Beverages Consumed	Time Consumed	Amount Consumed

Poster for advertisement of the study.

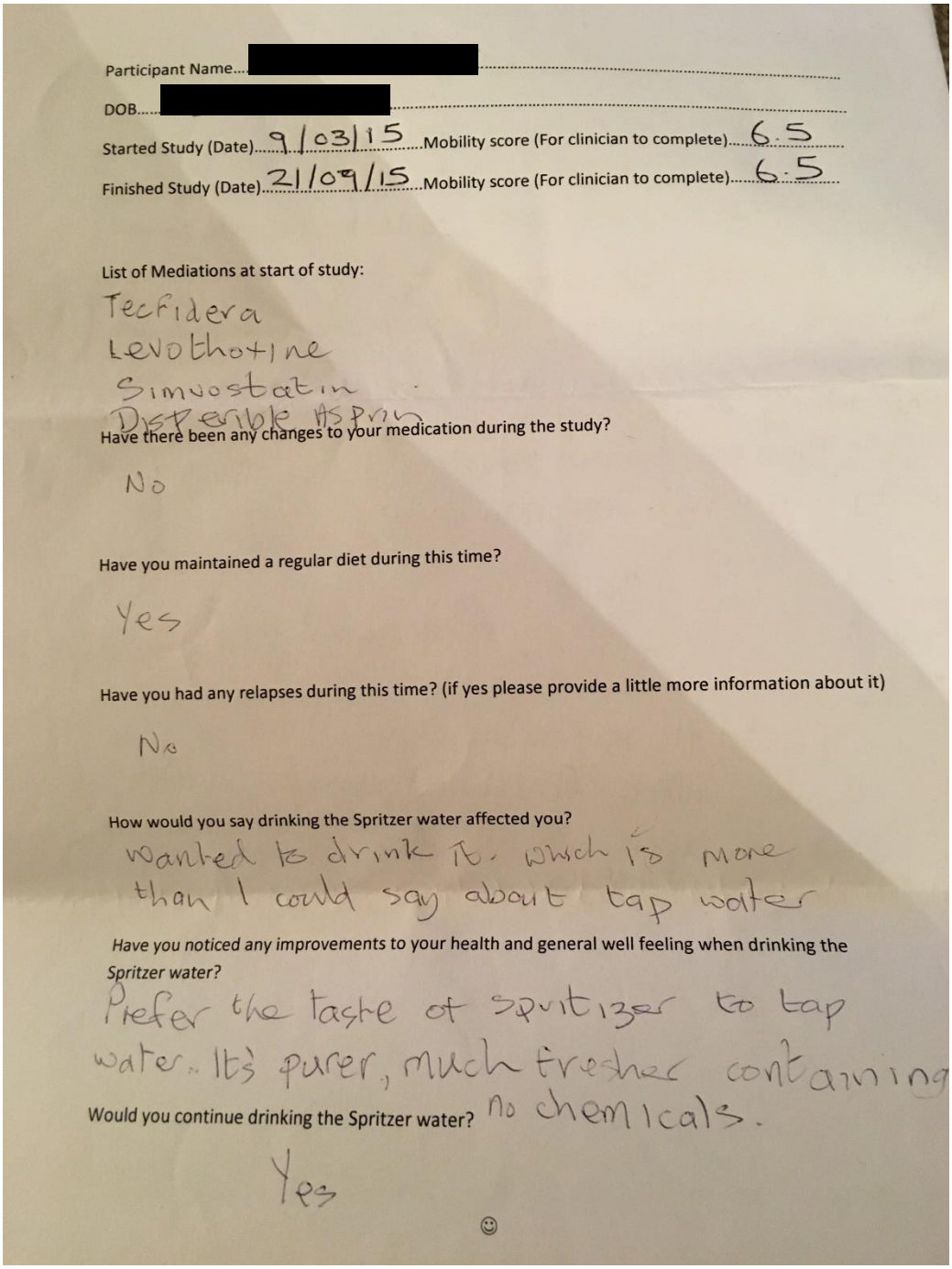
Annex 6

Sweat poster presentation given at the 11th Keele Meeting on Aluminium on 3th March 2015 at the Le Couvent Des Minimes Hotel & Spa in Lille, France.



Annex 7

MS Patient feedback



Participant Name....[redacted]

DOB....[redacted]

Started Study (Date)....9/03/15....Mobility score (For clinician to complete)....6.5

Finished Study (Date)....21/09/15....Mobility score (For clinician to complete)....6.5

List of Mediations at start of study:

Tecfidera
Levothoxine
Simvostatin
Dispersible Aspirin

Have there been any changes to your medication during the study?

No

Have you maintained a regular diet during this time?

Yes

Have you had any relapses during this time? (if yes please provide a little more information about it)

No

How would you say drinking the Spritzer water affected you?

Wanted to drink it. Which is more than I could say about tap water

Have you noticed any improvements to your health and general well feeling when drinking the *Spritzer water?*

Prefer the taste of spritzer to tap water. It's purer, much fresher containing no chemicals.

Would you continue drinking the Spritzer water?

Yes

☺

Participant Name [redacted]

DOB [redacted]

Started Study (Date) 9/3/2015 Mobility score (For clinician to complete) 6.0

Finished Study (Date) 19/9/2015 Mobility score (For clinician to complete) 5.5

List of Mediations at start of study:

Pregablin 600mg.
Amantadine hydrochloride 100mg.
Cleni / Modulite 400mgx2 daily
Ventolin - as required

Have there been any changes to your medication during the study?

Bisoprolol 2.5mg. Antibiotics June chest infection
Antibiotics - July for calculus
" September UTI
" October chest infection

Have you maintained a regular diet during this time?

YES - usual diet

Have you had any relapses during this time? (if yes please provide a little more information about it)

No relapses

How would you say drinking the Spritzer water affected you?

I felt my head to be cleared

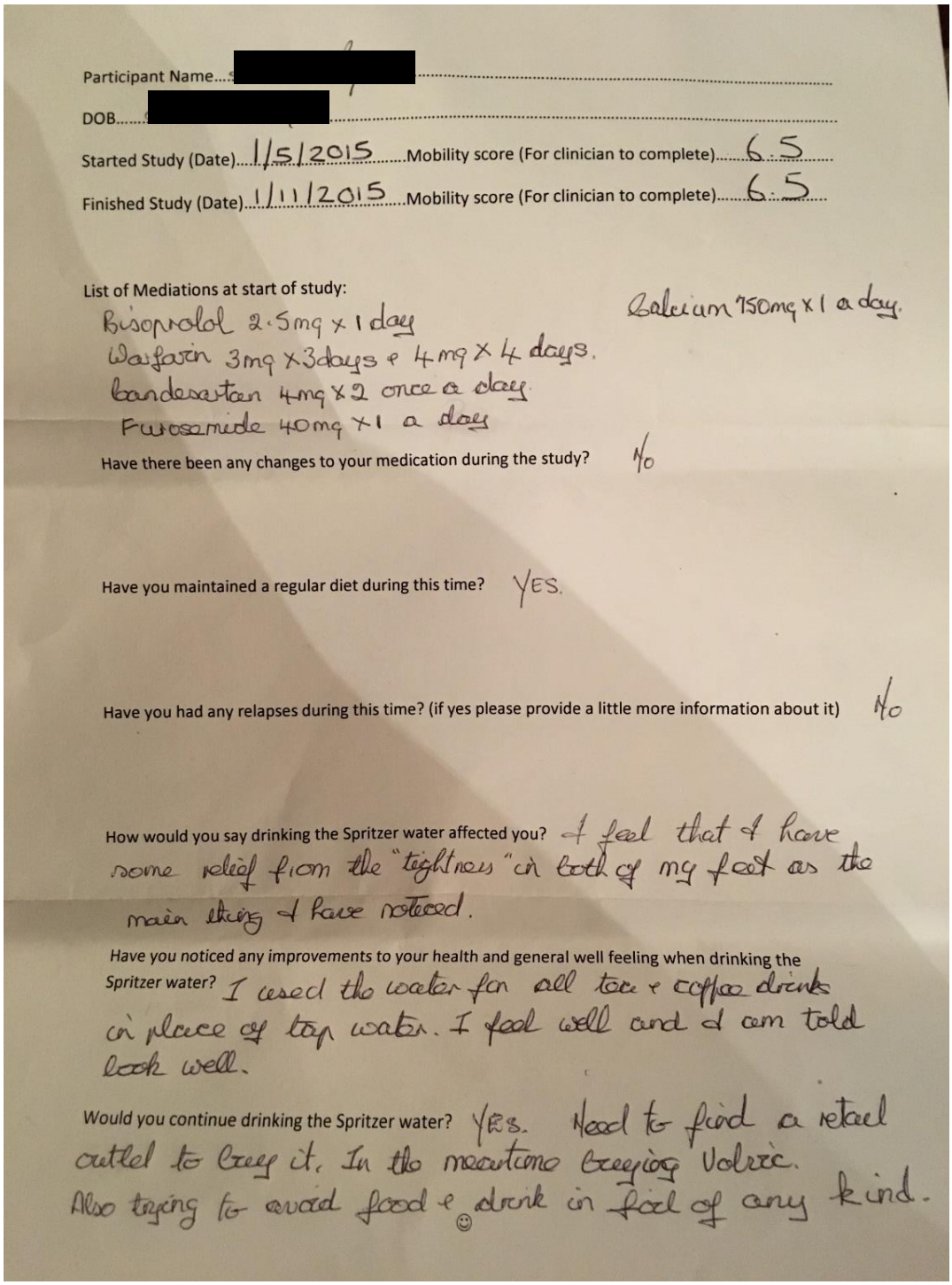
Have you noticed any improvements to your health and general well feeling when drinking the Spritzer water?

I have felt well, however I have had calculus, a chest infection and a UTI during the time I was drinking Spritzer. In between did feel well. (I have never had such an unhealthy year - 7 lots of A.B)

Would you continue drinking the Spritzer water?

I would continue to drink Spritzer as a cold drink but not in my tea. (I am still drinking the Spritzer I have left) ☺

(My writing has deteriorated during last 12 months.)



Participant Name... [redacted]

DOB... [redacted]

Started Study (Date) 1/5/2015 Mobility score (For clinician to complete) 6.5

Finished Study (Date) 1/11/2015 Mobility score (For clinician to complete) 6.5

List of Mediations at start of study:

Bisoprolol 2.5mg x 1 day
Warfarin 3mg x 3days & 4mg x 4 days.
Candesartan 4mg x 2 once a day.
Furosemide 40mg x 1 a day
Calcium 750mg x 1 a day.

Have there been any changes to your medication during the study? No

Have you maintained a regular diet during this time? YES.

Have you had any relapses during this time? (if yes please provide a little more information about it) No

How would you say drinking the Spritzer water affected you? I feel that I have some relief from the "tightness" in both of my feet as the main thing I have noticed.

Have you noticed any improvements to your health and general well feeling when drinking the Spritzer water? I used the water for all tea & coffee drinks in place of tap water. I feel well and I am told look well.

Would you continue drinking the Spritzer water? YES. Need to find a retail outlet to keep it. In the meantime keeping Volvic. Also trying to avoid food & drink in foil of any kind. ☺

Annex 8

Urinary Excretion of Aluminium and Silicon in Secondary Progressive Multiple Sclerosis

EBioMedicine 26 (2017) 60–67

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Urinary Excretion of Aluminium and Silicon in Secondary Progressive Multiple Sclerosis

Krista Jones [a], Caroline Linhart [b], Clive Hawkins [c], Christopher Exley [a,*]

[a] The Birchall Centre, Lennard-Jones Laboratories, Keele University, United Kingdom
[b] Department of Medical Statistics, Informatics and Health Economics, Medical University of Innsbruck, Austria
[c] Institute of Science and Technology in Medicine, Keele University, United Kingdom

ARTICLE INFO

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Keywords:
Secondary progressive multiple sclerosis
Aluminium and human health
Silicon-rich mineral water
Urinary aluminium excretion
Urinary silicon excretion, non-invasive therapy

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 Crt (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 Crt (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 (μmol/24 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.

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 Tarara, 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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