A DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF THE EFFECTS OF ALKALINE MAGNESIUM BICARBONATE SOLUTIONS ON ACID/BASE BALANCE, BONE METABOLISM AND CARDIOVASCULAR RISK FACTORS IN POSTMENOPAUSAL WOMEN

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ABSTRACT

Background
A number of health benefits including improvements in acid/base balance, bone metabolism, cardiovascular risk factors and longevity have been associated with the intake of alkaline mineral water. This study was designed to investigate the effects of the regular consumption of magnesium bicarbonate supplemented water compared to non supplemented water on selected biochemical parameters in healthy postmenopausal women.

Methods
In this double-blind, placebo-controlled, parallel-group, study 67 women were randomized to receive between 1500 and 1800 mL daily of magnesium bicarbonate supplemented spring water (650 mg/L bicarbonate, 120 mg/L magnesium, pH 8.3-8.5) (supplemented water group) or spring water without supplements (control water group) over 84 days. Over this period biomarkers of bone turnover (serum parathyroid hormone, 25-dihydroxyvitamin D, osteocalcin; urinary telopeptides and hydroxyproline), inflammation (erythrocyte sedimentation rate and C reactive protein) were measured together with measurements of safety by standard biochemistry, haematology and urine examinations.

Results
This study demonstrated overall that the daily consumption of 1.5 litres of water *per se* resulted in statistically significant increases in serum sodium, potassium and magnesium

concentrations. However the serum magnesium concentrations in subjects consuming the supplemented water were significantly increased at Day 84 over baseline (Day 0) compared to subjects in the control group (95% CI: 0.003 – 0.041 mmol/L; p=0.03). Another notable difference observed was a statistically significant trend for an increase in parathyroid hormone (PTH) concentrations with the consumption of non supplemented water, whereas the PTH concentrations remained stable when the magnesium bicarbonate supplemented water was consumed. These findings highlight the complexities of water and electrolyte balance in the body and suggest that regular ingestion of adequate water and magnesium bicarbonate supplemented water may assist in maintaining sodium and potassium mineral homeostasis, increased serum magnesium and stabilization of PTH. The possible health associated benefits warrant further clinical studies.

TRIAL REGISTRATION

ACTRN12609000863235

BACKGROUND

Water is essential for hydration, yet needs vary according to environmental conditions, physical activity and individual metabolism. Both the World Health Organization (WHO) [1, 2] and the Australian National Health and Medical Research Council (NHMRC) [3] have established an Adequate Intake of fluid (water and other drinks) which for adults be at least two litres per day. There have long been claims related to the positive health benefits of drinking water. In addition, claims have been made for the health benefits of "hard water" (containing mineral salts such as calcium and magnesium) including reduction in the incidence of, and mortality from, cardiovascular disease [4, 5]. However, scientifically rigorous studies are needed to test these claims and to investigate possible mechanisms.

Chronic, low-level, acidosis has been associated with the typical western diet [6-9] and, since the degree of acidosis increases with age, it has been postulated that acidosis may be associated with some of the diseases of aging [7]. In addition, clinical studies have reported that dietary bicarbonate supplementation significantly, positively impacts upon biomarkers of increased bone metabolism [10-12] and decreases urinary nitrogen loss that has been associated with the muscle and tissue wasting of aging [13].

Magnesium is present in the body in abundance in the cationic form and is a cofactor for numerous enzymes, including those involved in the metabolism of fats and carbohydrates and in the synthesis of protein and nucleic acids. About 50% of magnesium in the body resides in bone where it is directly involved in calcium and bone homeostasis [13, 14]. Low magnesium status has been associated with osteoporosis [15] and osteoarthritis [16] and affects the function of the parathyroid glands [17] where magnesium acts as an agonist at the calcium-sensing receptors [18]. Additionally, magnesium deficiency can worsen metabolic acidosis while dietary supplementation with magnesium and other alkaline minerals increases blood pH and can lead to improvements in the buffering

capacity of the blood [19, 20]. Low magnesium levels have been associated with endothelial dysfunction, vascular reactivity, raised C-reactive protein concentrations [21] and decreased insulin sensitivity [22]. Diseases associated with low magnesium status include Type II diabetes [23], hypertension [24], atherosclerosis, coronary heart disease and the metabolic syndrome [22, 24, 25]. Magnesium supplementation has been linked with suppression of bone turnover [26], improvements in lipid profile [27, 28] and reduction in blood pressure in magnesium deficient subjects [29]. Numerous epidemiological studies have reported that magnesium intake in the typical western diet is below the Recommended Daily Allowance (RDA) [17, 20-32]. This clinical study was conducted in order to investigate further some of the metabolic effects of regular ingestion of water supplemented with magnesium bicarbonate over a three month period.

METHODS

This was a randomised, double-blind, placebo-controlled, parallel-arm study conducted from 31 August 2005 to 3 November 2006. The study was approved by the St Vincent's Hospital Human Research Ethics Committee. Ninety-one subjects attended the Clinical Trials Centre for screening for eligibility. All screened subjects were provided both oral and written information in plain language and consent was obtained before screening procedures began.

Only post-menopausal subjects aged 50 -70 years and with BMI 20-35 kg/m^2 were eligible. Subjects were excluded if physical and mental health status, including laboratory abnormalities, indicated serious or chronic illness (LFTs, electrolytes, creatinine clearance <60 ml/min, haemoglobin < 10 g/L), if they were hypersensitive to magnesium, taking certain medications (antacids other than proton pump inhibitors or H_2 agonists, diuretics, calcium or magnesium supplements) or planned medication changes. Subjects using hormone replacement therapy (HRT) were to have been on a stable dose for at least one month prior to screening and continue this dose through the study. Subjects on special diets (including vegan, weight loss or high protein), those with a history of frequent use of magnesium based laxatives and substance abuse (including nicotine) were excluded.

Of the 91 screened, 23 failed to satisfy the entry criteria and one was excluded due to poor venous access. The remaining 67 healthy, post-menopausal women were randomised in a 1:1 ratio to receive between 1500 and 1800 mL daily of magnesium bicarbonate supplemented spring water (650 mg/L bicarbonate, 120 mg/L magnesium, pH 8.3-8.5) (supplemented water group) or spring water without supplements (control water group) over three months (84 days). For those consuming the supplemented water this volume resulted in a daily dose of 975 – 1170 mg bicarbonate and 180 – 216 mg magnesium consistent with the recommended daily intake for these electrolytes. There was no bicarbonate and negligible amounts of magnesium (< 5mg/L) in the spring water given to the control group. Analysis of the spring water (control) was conducted by National Association of Testing Authorities (NATA) accredited (#1884) SONIC HEALTHCARE laboratory NSW.

Subjects were seen in the clinic at Baseline and at Days 14, 42 and 84. Blood and urine samples were collected at every visit to measure markers of bone turnover (PTH, 25-dihydroxyvitamin D and osteocalcin, telopeptides, hydroxyproline) and inflammation (erythrocyte sedimentation rate (ESR), and CRP. Visit examinations included blood pressure (supine and standing), serum fasting lipids, standard serum biochemistry and haematology, urinary and venous blood pH, mid-stream urine and 24 hour urine collection for measurement of concentration and excretion of calcium, phosphate, creatinine and free cortisol. Physical examination was performed and adverse event reports were elicited at each visit.

All biochemical, haematology and urinanalysis testing was performed by NATA accredited (#2115) Institute of Laboratory Medicine (SydPath) St Vincent's Hospital NSW.

There were no directly comparable studies on which to base formal estimates of subject numbers required for this exploratory study. Consequently, broad guidance was obtained from studies examining the metabolic effects of magnesium supplementation [18, 45]. A sample size of 34 subjects in each treatment arm was chosen to ensure that at least 25 subjects in each arm completed the three month study period.

The change from Baseline (Day 0) to the final study visit at Day 84 was compared between treatment groups for all biochemical, haematological, urinalysis, blood gas, blood lipid and blood pressure measures, and bone turnover and inflammatory biomarkers. Tables present the mean and standard deviation (SD) for each parameter at Baseline and Day 84, while figures present means and standard error of the mean at each visit. Continuous measures were compared across treatments using independent, two-sample, unpaired t-tests if the data were normally distributed, otherwise the Wilcoxon Rank Sum Test was used. Categorical measures were compared using Chi square or Fisher's Exact test. No adjustments were made for multiple testing as this was an exploratory study and it is acknowledged that there is an increased risk of making Type I errors given the number of tests performed.

Also, where results of the initial tests suggested that further analysis was warranted, repeated measures analyses of variance were performed for all outcome variables, incorporating all visits from baseline to Day 84 together with treatment group as factors in the analysis. If the p-value for the comparative change in the variable from Day 0 to Day 84 was less than or equal to 0.10 then a generalised linear mixed model with a random intercept term (for patient) was fitted using the Proc Mixed procedure in SAS to adjust for the lack of independence of the repeated measures on the same patient. The outcome in the model was the measure of interest at Days 0, 14, 42 and 84 and the model included the main effects of group and visit. The p-value from the test of the interaction between group and time was the result of interest.

RESULTS

Sixty-seven eligible subjects received at least one dose of magnesium bicarbonate supplemented spring water {supplemented water group; n=34} or one dose of spring water without supplement {control group; n=33}, had a valid baseline measurement and returned for at least one post-baseline visit. This constituted the protocol defined intention-to-treat (ITT) population which was identical to the safety population and, consequently, all analyses were carried out on this population. Thirty-two and 33 subjects completed the study in the control and supplemented water groups respectively.

Mean (SD) age {57 (4.4) years and 59 (5.5) years in the control and supplemented water groups respectively}, weight {68.1 (11.75) kg and 64.2 (8.15) kg respectively}, height {164.5 (7.90) cm and 163.2 (7.02) cm respectively} and BMI {25.1 (3.68) g/m^2 and 24.2 (3.14) g/m^2} were similar in both groups. The most common pre-existing conditions in both groups were self-reported drug hypersensitivity, osteoarthritis and lipid disorders for which subjects used hypolipidaemics, anti-inflammatory and antirheumatic products and analgesics. Most subjects {28 (84.8%) and 29 (85.3%) in the control and supplemented water groups respectively} were fully compliant according to daily diary records where 100% compliance was defined as daily consumption of between 1500 mL and 1800 mL of water. This volume of water was well tolerated by the subjects.

Outcome analyses

In the control group the mean concentration of serum PTH increased from Day 0 to Day 84 (3.85 to 4.60 pmol/L) compared to the supplemented water group for which the concentration remained relatively constant (4.24 and 4.21 pmol/L, respectively). There was a trend towards a difference in the change in mean PTH concentration between the two groups from Day 0 to Day 84 (0.74 pmol/L; 95% Confidence Interval (CI): -0.03 to 1.52 pmol; p = 0.059). The p-value for the interaction term in the repeated measures analysis of variance was 0.155 (Table 1, Figure 1).

Additional analyses of change including all days showed that there was a statistically significant interaction effect between treatment groups and visit (p = 0.036). The mean concentration of serum PTH increased significantly among subjects in the control group. There was no change in the mean concentration of serum PTH in the group which received supplemented water.

There was no statistically significant difference in the change of venous or urine pH, blood pressure, serum lipids, blood gases, urinalysis, haematology parameters or most biochemistry parameters between the two groups from Day 0 to Day 84.

There were statistically significant differences in change from Day 0 to Day 84 in the mean concentration of serum creatinine (p=0.030) and of serum magnesium (p=0.027) between the two groups using a two-independent sample t-test (Table 2). The mean increase in serum creatinine (which remained within normal range of values) was 3.00 µmol/L (95% CI: 0.30 to 5.69 µmol/L) higher and the mean increase in serum magnesium was 0.022 mmol/L (95% CI: 0.003 to 0.041 mmol/L) higher in the

supplemented water group compared with the control group. When data from all time points were analysed using a repeated measures analysis of variance there was a statistically significant difference between groups for serum creatinine (p=0.030) and for serum magnesium (p=0.015; Figure 2).

A mixed model analysis of change for absolute difference between groups in the mean concentrations of measurements at Days 14, 42 and 84 also showed statistical significance for serum creatinine (p=0.014) and serum magnesium (p=0.007).

There was a significant trend in the mean change in concentrations of serum sodium, potassium and magnesium between Day 0 and Day 84 in both groups. For the whole study group between Day 0 and Day 84 there was a statistically significant increase in the mean concentration of serum sodium (0.0153 mmol/L/day; $p < 0.0001$), of serum potassium (0.0015 mmol/L/day; p=0.003) and of serum magnesium (0.0002 mmol/L/day; p=0.015; Figures 2, 3, 4).

The mean urinary inorganic phosphate concentration was 12.58 mmol/L at Day 0 and 10.74 mmol/L at Day 84 in the control group and 15.01 mmol/L at Day 0 and 9.86 mmol/L at Day 84 in the supplemented water group. The difference in change between the two groups was 3.3 mmol/L which was statistically significant (95% CI; 0.6 to 6.1 mmol/L: p=0.019). Additional analyses using mixed models analysis of change for absolute difference between groups in the mean concentrations of measurements at Days 14, 42 and 84 showed statistical significance for urinary inorganic phosphate concentration (p=0.043) (Figure 5). There were no other statistically significant differences in change from Day 0 to Day 84 comparing control and supplemented water groups in any of the other 24-hour urinary analytes.

For all visits during the study 65% of the control group and 56% of the supplemented water group reported experiencing some positive benefit of the treatment as a subjective response to a question provided in the evaluation. The percentage of subjects who reported a positive benefit increased during the study and at the final visit approximately 50% of subjects in both groups reported experiencing some benefit. The most common benefits reported at the final visit were improvement in skin appearance so that it looked or felt better (36%) and having more energy (34%) consistent with other reports of the health improvement due to adequate body hydration.

SAFETY EVALUATION

Thirteen (39%) subjects in the control group and 19 (56%) subjects in the supplemented water group had an adverse event that was considered to be possibly, probably or definitely related to the study treatment, most commonly diarrhoea in five (15.2%) and eight (23.5%) subjects in the control and supplemented water groups respectively. No subject suffered a serious adverse event during this study.

DISCUSSION

The consumption of magnesium in alkaline aqueous formulation (magnesium bicarbonate supplemented spring water) provides a bioavailable form of magnesium as evidenced by the increase in serum magnesium concentrations observed within 14 days of starting treatment and which was maintained over the 84 day study period.

In the supplemented water group PTH concentrations remained stable. This finding is consistent with other studies in normal subjects where intake of oral alkaline [7, 12] and magnesium [32] loads do not alter PTH levels in the short term. The trend towards an increase in concentrations of PTH during the study for the control water treated group is also consistent with those of other researchers with respect to the role of magnesium in regulating PTH release. Although calcium is considered to be the primary regulator of PTH secretion, magnesium is also involved [17,] in its capacity as an agonist at the calcium-sensing receptors of the parathyroid glands [18].

PTH strongly limits proximal tubular phosphate reabsorption in the kidneys, resulting in increased urinary phosphate concentration. Therefore, the difference in PTH concentrations between the two groups may explain the significantly lower urinary inorganic phosphate concentrations in the supplemented water group relative to the control group.

The regimens followed also resulted in small, but statistically significant increases in serum sodium, potassium and magnesium concentrations. The consumption of water *per se* appears to enhance renal tubular reabsorption of these electrolytes.

The increase in serum creatinine, the non-enzymatic breakdown product of creatine and phosphocreatine, in the supplemented water group may reflect an increase in the synthesis of creatine in the liver and kidneys or an increase in muscle mass or muscle energy stores.

CONCLUSIONS

The study has provided evidence that magnesium bicarbonate supplemented water increases serum magnesium in healthy postmenopausal women. The study also demonstrated that hydration *per se* resulted in increases in plasma electrolytes. Subjective outcomes recorded by subjects included improvements in skin appearance and energy levels. Whether these changes are of clinical relevance with respect to various processes, such as those associated with aging which have been linked to magnesium insufficiency and/or acidosis, remain to be determined by further research.

COMPETING INTERESTS

Dr Beckett of Unique Global Possibilities Medical Pty Ltd sponsored the trial. All other authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

ROD developed the study design and plan as well as oversaw the drafting of and was a major contributor to the manuscript. WL participated in the study design, provided input in the clinical study protocol and contributed to the writing of the manuscript. LMRT provided input in the clinical study protocol, provided input into and oversight of the Clinical Study Report, drafted, coordinated and finalised the writing of the manuscript. PM performed the statistical data analysis and provided input into the Clinical Study Report. RJB conceived of the study and contributed to the writing of the manuscript. KMW participated in the study design and contributed to the writing of the manuscript. All authors read and approved the manuscript.

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Figures

Figure 1 - Serum Parathyroid Hormone levels in Magnesium Bicarbonate supplemented water and non supplemented water (control) groups

Values are mean ± standard error by treatment group and study day

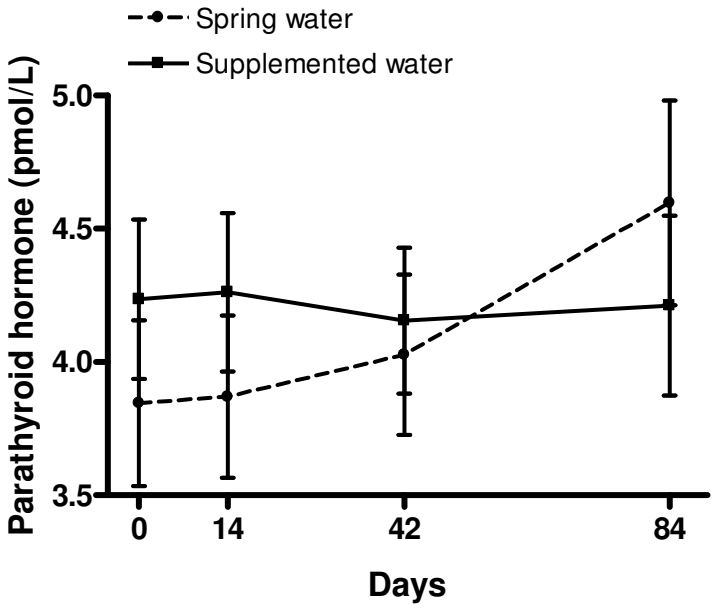


Figure 2 - Serum Magnesium levels in Magnesium Bicarbonate supplemented water and non supplemented water (control) groups.

Values are mean ± standard error by treatment group and study day

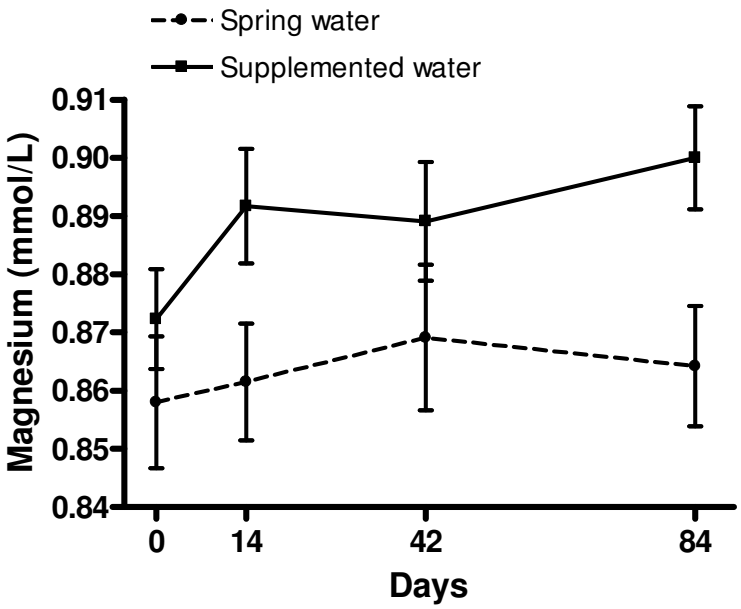


Figure 3 - Serum Potassium levels in Magnesium Bicarbonate supplemented water and non supplemented water (control) groups.

Values are mean ± standard error by treatment group and study day

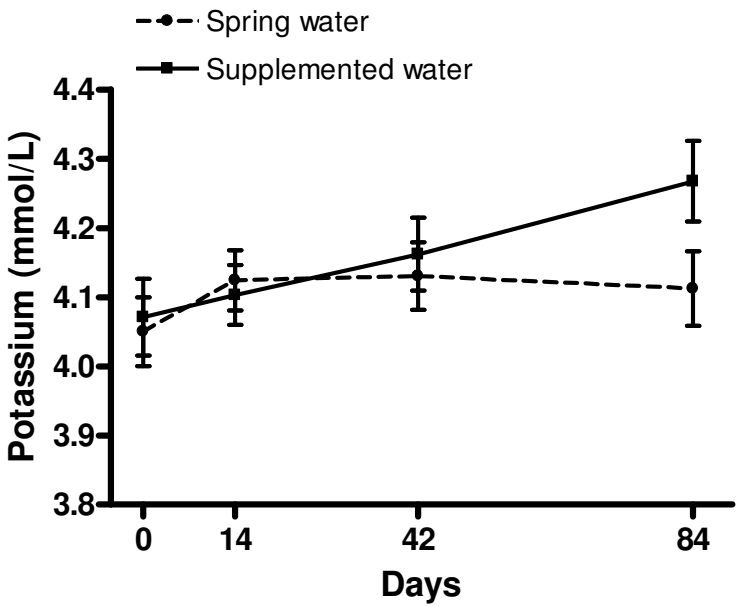


Figure 4 - Serum Sodium levels in Magnesium Bicarbonate supplemented water and non supplemented water (control) groups.

Values are mean ± standard error by treatment group and study day

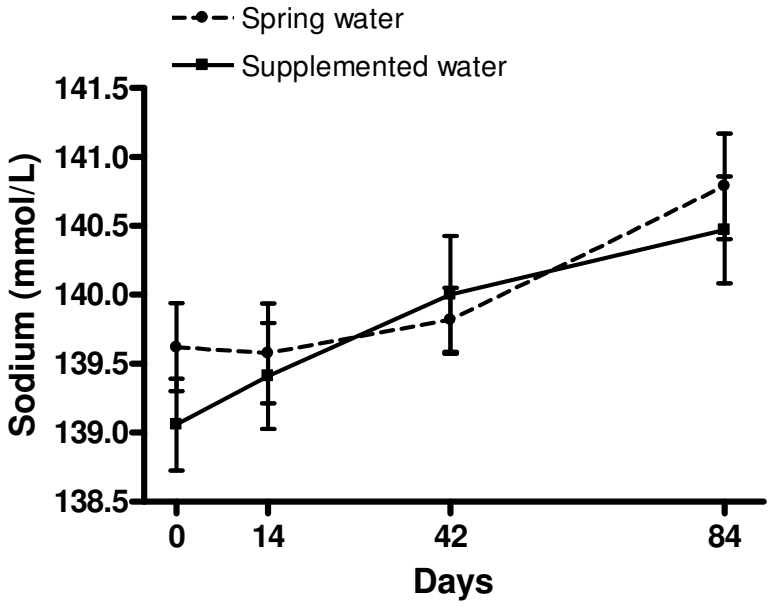
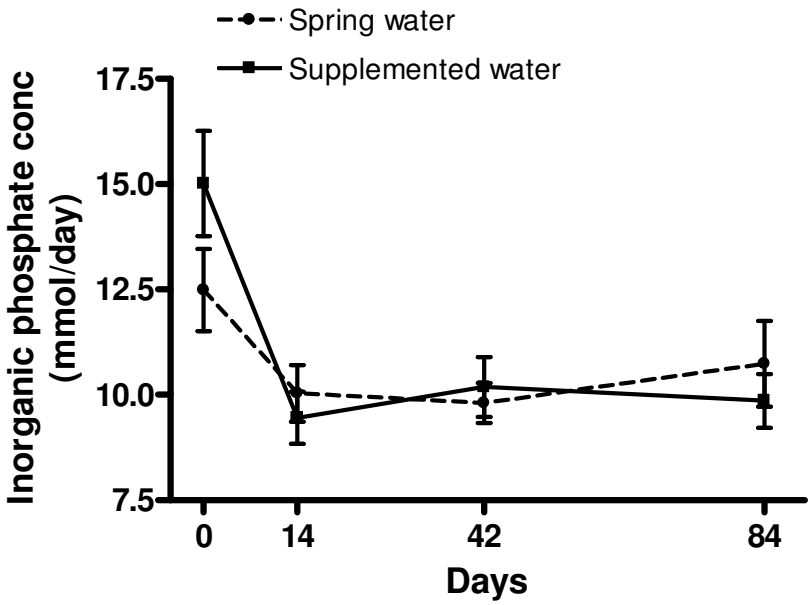


Figure 5 - Urinary Inorganic Phosphate levels in Magnesium Bicarbonate supplemented water and non supplemented water (control) groups.

Values are mean ± standard error by treatment group and study day



Tables

Table 1 - Serum parathyroid hormone (pmol/L) over time by treatment group (ITT Population)

Treatment Group	Visit	Summary Statistics				Change from Baseline			
		n	Mean	SD	Med	n	Mean	SD	Med
Control	Baseline	33	3.85	1.79	3.40				
	Day 84	32	4.60	2.17	4.35	32	0.73	1.54	0.70
Supplemented Water	Baseline	34	4.24	1.74	3.85				
	Day 84	34	4.21	1.97	3.95	34	-0.02	1.65	-0.25
p-value (t-test) comparing groups for change from Baseline to Day 84 only							0.059		

Table 2 - Serum biochemistry values by treatment group and change from baseline (ITT Population)

	Treatment Group	Visit	Summary Statistics				Change from Baseline			
			n	Mean	SD	Med	n	Mean	SD	Med
Creatinine (µmol/L)	Control	Baseline	33	67.1	9.48	67.0				
		Day 84	33	66.1	9.37	66.0	33	-0.9	6.21	-2.0
	Supplemented Water	Baseline	34	62.8	9.24	63.0				
		Day 84	34	64.8	9.55	65.5	34	2.1	4.77	2.0
p value (t test) comparing groups for change from Baseline to Day 84 only *								0.030		

Table 2 (continued) - Serum biochemistry values by treatment group and change from baseline (ITT Population)

	Treatment Group	Visit	Summary Statistics				Change from Baseline			
			n	Mean	SD	Med	n	Mean	SD	Med
Magnesium (mmol/L)	Control	Baseline	33	0.858	0.065	0.86				
		Day 84	33	0.864	0.060	0.87	33	0.006	0.043	0.000
	Supplemented Water	Baseline	34	0.872	0.050	0.87				
		Day 84	34	0.900	0.052	0.89	34	0.028	0.035	0.025
p value (t test) comparing groups for change from Baseline to Day 84 only *								0.027		
Potassium (mmol/L)	Control	Baseline	33	4.05	0.30	4.00				
		Day 84	33	4.11	0.31	4.10	33	0.07	0.29	0.10
	Supplemented Water	Baseline	34	4.07	0.32	4.00				
		Day 84	34	4.27	0.34	4.20	34	0.20	0.41	0.20
p value (non parametric) comparing groups for change from Baseline to Day 84 only *										0.136
Sodium (mmol/L)	Control	Baseline	33	139.7	1.77	140.0				
		Day 84	33	140.8	2.20	141.0	33	1.1	1.43	1.0
	Supplemented Water	Baseline	34	139.1	1.94	139.0				
		Day 84	34	140.5	2.26	141.0	34	1.4	2.61	1.0
p-value (t-test) comparing groups for change from Baseline to Day 84 only								0.496		