Department of Medical Chemistry and Clinical Biochemistry

Second Faculty of Medicine, Second Faculty of Medicine, Charles University and University Hospital in Motol

Determination of silicon content in biological materials depending on the consumption of Krondorf mineral water

Investigator:

Prim. MUDr. Jana Čepová, Ph.D., MBA et al.

The final report of the project "Determination of Silicon Content"

Introduction

Silicon plays an irreplaceable role in the human body. It contributes in particular to the formation of connective tissues, as it has an effect on improving their strength and elasticity. Most silicon is found in the bones, skin, nails, hair, and enamel, and it is also present in the aorta, eye lens, muscle mass, pancreas, and thyroid gland. Silicon is also a necessary part of many enzymes. Silicon has a positive effect on lowering cholesterol in the body. It is important in the treatment of aluminium neurotoxicity, which is the hypothetical risk factor for developing Alzheimer's disease. It can be used for the treatment of patients with osteoporosis, as it helps to improve the incorporation of calcium ions into the bone tissue.

No toxicity of silicon has been observed when it is administered orally as a food supplement. However, excessive intake with food can contribute to the formation of urinary stones.

The minimum daily dose is not precisely determined in the available literature. The recommended daily intake of silicon varies among the different literature sources, and the most frequently reported values are from 5 to 20 mg (or up to 35 mg for athletes).

Silicon is mainly found in common horsetail, and it also obtained from this plant for further use (pharmaceutical industry). In a typical diet, silicon occurs in certain mineral waters, beer, chicken skin, pig's liver and whole grain foods. The foods with the highest content of silicon are cereals, such as oats (3400-6300 mg/kg), barley (1400-2900 mg/kg) and wheat (20-190 mg/kg).

According to data published by the WHO in 1996, the silicon concentration in urine ranges from 3 to 12 mg/L, while the serum concentration is only 0.3 mg/L.

Aim of the study

The aim of the study was to determine the amount of silicon in biological materials in healthy subjects after consuming Krondorf mineral spring water enriched with silicon at a dose of 42 mg/L.

Study participants and design

Volunteers

A total of 30 subjects (18 women and 12 men), ranging in age from 20 to 60 years, participated in the study. The mean age of the volunteers was 39.0 ± 18.5 years. All the volunteers were healthy, with no hypertension or drug abuse; they drank no more than three cups of coffee a day and took no food supplements.

Control group

The control group consisted of 10 volunteers (five women and five men) who did not drink the silicon-enriched water, and they ranged within the same age category as the volunteers, i.e. from 20 to 60 years.

Study design

The volunteers were divided into two groups according to the length of the consumption to reveal the potential increase in the amount of silicon in the body during prolonged use of silicon-enriched water at a dose of 42 mg/L. Each group consisted of fifteen volunteers.

The consumption of Krondorf mineral water and collection of biological samples were carried out according to the following schedule:

Group 1

sampling before initiation of water-drinking treatment → 1 week of water-drinking treatment → sampling after 1 week → 1 week of water-drinking treatment → sampling after 2 weeks → 2 weeks' pause → final sampling at the end of week 4

Group 2

sampling before initiation of water-drinking treatment → 1 week of water-drinking treatment → sampling after 1 week → 1 week of water-drinking treatment → sampling after 2 weeks → 2 weeks of water-drinking treatment → sampling after 4 weeks → 4 weeks' pause → final sampling at the end of week 8

The control group of volunteers

only collection of biological samples

Each volunteer provided a biological sample (morning urine and blood) according to the above scheme. This sample was then processed in the laboratory.

The urine was collected into plastic sterile disposable urinary tubes (Monovette, Germany). The whole blood was collected into special plastic tubes intended for the analysis of trace elements (Vacuette, Austria). The whole blood was centrifuged in refrigerated centrifuges (4°C) at 4000 rpm for 10 minutes. The serum obtained was collected, aliquoted and stored at -18°C; the urine samples were handled in the same way.

Methods

Most of the available literature describing the determination of silicon is based on atomic absorption spectrometry (AAS) and applies primarily to the determination of silicon in water (Li et al. Nutrition Journal 2010, 9:44; Barroso et al. Food Additives and Contaminants 2009, 2:2). The only available publication describing the determination of silicon in biological samples is by Davenward in 2013, in which the team of authors monitored the effect of silicon on aluminium toxicity in patients with Alzheimer's disease.

Other methods enabling the determination of silicon are photometric methods. Their sensitivity is lower, but they are very easy to perform and regular laboratories have the necessary equipment for their performance.

The most widespread methods are based on the reaction of silicates with ammonium molybdate in an acidic environment, where molybdate reacts with silicates to form yellow silicomolybdic acids. A range of wavelengths from 200 to 500 nm was tested and the wavelength of 350 nm was selected as optimal for the photometric determination.

Solutions

Molybdenum reagent: 7.5 g of ammonium molybdate was dissolved in 50 mL distilled water and added to 50 mL of diluted nitric acid solution (1: 1)

Formate buffer: 56.1 g of potassium hydroxide was dissolved in 250 mL of distilled water, 50 mL of anhydrous formic acid was added and the solution was completed to 500 mL

Calibration solutions of silicon: were prepared from a stock solution of the analytical silicon standard for AAS (Sigma Aldrich) at a concentration of 1000 mg/L

The calibration curve was constructed from four calibration points with concentrations of 1 mg/L, 2 mg/L, 5 mg/L and 10 mg/L. The silicon concentrations in the biological samples were calculated from the regression equation that was obtained. The reliability value (R) was always greater than 0.94.

Analysis of samples

0.5 mL of molybdenum reagent was added to 5 mL of formate buffer and completed with distilled water to the volume of 12 mL. Finally, 0.5 mL of sample was added. Then, the sample was briefly vortexed and measured on a Helios (Thermo Fisher Scientific) spectrophotometer at a wavelength of 350 nm. Laboratory plasticware was used for handling all the samples to prevent the leaching of silicon from the glass.

Results

Validation of the method was performed and the validation parameters were set at two concentration levels.

The coefficient of variation of repeatability and reproducibility of both levels did not exceed 7.3%. The repeatability bias at both levels did not exceed 12.0%. The reproducibility bias at both levels did not exceed 15.4%.

The results in both groups were presented in the tables. Table 1 shows the values of the volunteers who took Krondorf water for two weeks. Table 2 shows the values of the volunteers who took Krondorf water for four weeks. In this study, a control group of volunteers was also measured, in whom only one sampling was performed (Table 3).

Discussion

To determine the positive and negative effects of the consumption of Krondorf mineral water on the human body, the volunteers who drank the mineral water were asked to complete a short anonymous questionnaire.

- 71% of the volunteers had no digestive problems (constipation, diarrhoea) during or shortly after the end of consumption, while 29% had mild gastrointestinal problems (increased peristalsis and flatulence)
- 24% of the volunteers observed a positive effect on their skin, hair and nails (reduced hair loss, increased strength of nails)
- 95% of the volunteers did not mind the gentle sparkling of the water during its consumption.
- The average score (1=best, 5=worst) by the participants evaluating the taste of the water was 2.19.

Other effects observed during the given water-drinking regimen, as reported by the volunteers, were weight loss and feeling thirsty after drinking a whole bottle.

Conclusion

Because of the time-consuming development of the method, sample preparation and measurement, we managed to develop only a spectrophotometric method for the determination of silicon in the urine. The development of a method for the determination of silicon in serum would be far more time-consuming, and this task was found unfeasible in the available time span.

In the control group of volunteers, the silicon level did not exceed the detection limit (<1 mg/L).

In the attached tables, the volunteers are divided according to the length of time for which they drank the Krondorf water and by gender (F=female M=male).

The data was compared between the respective weeks of sampling in both groups of volunteers by a t-test. In the group of volunteers drinking Krondorf water for two weeks (Table 1), a statistically significant difference ($p < 0.01$) was found between the levels of silicon before the beginning of the experiment and after two weeks of drinking the mineral water (end of experiment). The silicon levels were not statistically different ($p > 0.01$) when the levels before the experiment and two weeks after completion of the water-drinking treatment were compared.

In the second group of volunteers (Table 2), who consumed mineral water for four weeks, similar differences were found between the data as in the first group of volunteers. The levels

of silicon were statistically significantly ($p<0.01$) different between the samples before the experiment and after two weeks of drinking the water. The same applies to the silicon levels between the samples taken before the experiment and after four weeks of drinking the water ($p<0.01$). The silicon levels after two weeks of treatment and after four weeks of treatment showed no significant differences ($p>0.01$). A non-significant difference ($p>0.01$) was recorded for the levels of silicon between the samples taken before the experiment and four weeks after stopping the water-drinking treatment.

Further, we compared the two groups of volunteers with each other. When the levels of silicon in the samples taken before the experiment were compared, we demonstrated statistically non-significant differences between the data ($p>0.01$). The same applies for comparisons of the data from samples taken after two weeks of drinking the mineral water, and for comparisons of the data from samples taken after absence of mineral water. A statistically non-significant difference between the data was also demonstrated in the silicon levels from the samples taken after four weeks of drinking vs. after two weeks of drinking the mineral water ($p>0.01$).

When comparing the differences between the data for the samples before the initiation of the experiment in both groups of subjects and the control group of volunteers (Table 3), we demonstrated a statistically non-significant difference in the data obtained ($p>0.01$).

In the available literature (Davenward et al. Journal of Alzheimer's Disease 2013, 33) describing the use of mineral water with a high content of silicon, volunteers consumed mineral water at a dose of one litre a day for up to 12 weeks. This study demonstrated that after two weeks of drinking silicon-enriched water, we can observe increased levels of silicon in urine. After the completion of the drinking treatment, silicon levels returned to their normal values.

Acknowledgment

This study was financially supported by a grant from the Karlovy Vary Region.

Appendices

Table 1 – Two weeks of drinking Krondorf mineral water

Si concentration in urine (mg/L) / Volunteer No.	before starting use	after one week of use	after two weeks of use (end of use)	after two weeks of abstinence
2/F	2.69	2.84	5.92	<1.00
6/F	3.48	8.63	8.88	1.39
9/F	7.20	9.36	10.24	9.03
10/F	4.09	6.92	8.70	2.75
11/F	<1.00	1.27	5.38	<1.00
23/F	<1.00	6.55	7.32	3.65
25/F	<1.00	4.04	6.72	4.21
26/F	2.25	4.91	6.45	3.59
27/F	<1.00	<1.00	1.37	<1.00
28/F	<1.00	<1.00	1.05	<1.00
12/M	1.07	2.81	5.04	2.99
24/M	<1.00	6.62	8.13	3.02
30/M	3.28	6.99	8.58	4.51
31/M	<1.00	5.73	6.88	4.70
33/M	1.82	2.16	5.28	4.19
Mean	**2.19**	**4.72**	**6.40**	**3.20**
SD	**1.76**	**2.79**	**2.58**	**2.13**

Table 2 – Four weeks of drinking Krondorf mineral water

Si concentration in urine (mg/L) / Volunteer No.	before starting use	after one week of use	after two weeks of use	after four weeks of use (end of use)	after four weeks of abstinence
5/F	3.47	4.59	7.04	9.27	1.99
13/F	<1.00	7.88	8.48	8.48	<1.00
15/F	<1.00	4.14	7.66	2.62	<1.00
18/F	<1.00	7.69	9.20	9.42	<1.00
19/F	1.05	1.55	4.12	6.94	1.54
20/F	<1.00	1.10	1.71	2.19	<1.00
21/F	<1.00	8.42	8.52	9.25	2.68
22/F	4.07	6.74	9.03	9.19	7.32
1/M	1.02	3.62	3.89	5.39	1.52
3/M	<1.00	<1.00	<1.00	4.80	2.39
4/M	<1.00	<1.00	<1.00	5.15	3.34
7/M	6.27	6.83	7.26	12.33	7.35
8/M	5.10	5.33	7.32	9.38	3.16
14/M	<1.00	1.63	1.82	<1.00	<1.00
17/M	<1.00	1.10	3.05	9.36	<1.00
Mean	**2.00**	**4.17**	**5.41**	**6.99**	**2.49**
SD	**1.80**	**2.83**	**3.12**	**3.29**	**2.13**

Table 3 – Control group

Control group	Si concentration in urine (mg/L)
M	1.27
M	<1.00
M	<1.00
M	<1.00
M	1.02
F	<1.00
F	<1.00
F	1.36
F	2.45
F	<1.00
Mean	**1.21%**
SD	**0.46**