RESEARCH ARTICLE

Digestive absorption of silicon, supplemented as orthosilicic acid–vanillin complex

Aline Marcowycz[1], Béatrice Housez[2], Corinne Maudet[2], Murielle Cazaubiel[2], Guiseppe Rinaldi[1] and Karine Croizet[1]*

[1] DexSil Labs, Courcelles, Belgium
[2] Biofortis, Saint Herblain, France

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Scope: Silicon (Si) is an abundant element on earth. It is found naturally in water in the form of orthosilicic acid (OSA), however this form is not stable under certain conditions such as in highly concentrated and non-neutral pH solutions, which lead to its polymerization and reduced bioavailability. This study aimed to assess the bioavailability of Si from OSA stabilized by vanillin (OSA-VC).

Methods and results: This was a single-center, double-blind, cross-over randomized controlled trial. Fourteen healthy subjects were recruited and consumed either OSA-VC or a placebo on two separate occasions. Blood and urine samples were collected during 6 h following ingestion and analyzed to determine Si absorption and excretion. Plasma Si area under the curve (0–6 h) was significantly higher after OSA-VC ingestion compared to placebo ingestion ($p = 0.0002$). Significantly higher urinary Si excretion was also reported over the 6-h period after OSA-VC ingestion compared to placebo ($p<0.0001$). Approximately 21% of ingested Si was excreted in urine during this period.

Conclusion: Although many studies have investigated the metabolism and bioavailability of Si supplemented in foods or as a food ingredient, this was the first to investigate and demonstrate the digestibility of OSA administered in a complex form with vanillin.

Keywords:
Absorption / Bioavailability / Metabolism / Silicic acid / Silicon / Vanilin-stabilized orthosilicic acid

1 Introduction

Silicon (Si) is the second most common element in the earth's crust after oxygen, and is prevalent in various human tissues. Although the exact role/function of Si in the human body remains unestablished, evidence is accumulating to suggest that it has a variety of physiological roles. Animal studies and clinical trials indicate functions of Si in skin, bones, and cartilage to support optimal connective tissue formation and maintenance, as well as potential activities to protect against the development of atherosclerosis and Alzheimer's disease [1, 2].

The pharmacokinetics of Si has been clearly described. After ingestion, Si is rapidly absorbed and enters the blood. A majority of Si is excreted in urine and small amounts of absorbed Si can be traced to cells and tissues, where it is involved in metabolism or stored [3]. Urinary excretion of Si is correlated with dietary Si intake, and thus is commonly used as a biomarker for its absorption [4]. However fecal excretion can also reach up to 40% of ingested Si, suggesting an important pathway of Si elimination.

Two decades ago, the human requirement for Si was estimated to be in the range of 5–20 mg/day, on the basis of average daily intake of Si [5]. More recently, Si intake in the United States was estimated to be in the range of 20–50 mg daily, on the basis of the Si content of foods in the US diet [6]. This range was narrowed by Jugdaohsingh et al. [7] who estimated the daily Si intake to be 24–33 mg/day in the Framingham and Framingham Offspring cohorts. However no recommended daily intake for Si has been defined, because it has not been established to be an essential nutrient [8].

Diet is the main source of Si for humans. Among foods, whole grains and cereals products, some vegetables

Correspondence: Karine Croizet, Rue de Liège 2, 6180 Courcelles, Belgium
E-mail: kc@dexsil.be

Abbreviations: **OSA**, orthosilicic acid; **OSA-VC**, orthosilicic acid vanillin complex; **Si**, silicon; **UHP**, ultra-high purity

*This author is no longer is affiliated with DexSil Labs at the time of publication.

including beans, spinach, and root vegetables, and some beverages including water and beer are the major contributors to Si intake [4] by providing 5–10 mg Si/100 g [1, 2, 5–7]. The bioavailability of Si in these foods and beverages depends upon its total content in the products and also the forms in which it is present. Compared to Si-rich food sources, Si-containing beverages have lower Si content but higher Si bioavailability [4]. These characteristics make them essential contributors of Si in human diet [2].

Orthosilicic acid (OSA), the simplest silicic acid, is an oxidized Si form and the main form of Si soluble in water. It is also present in most plants and body fluids of animals [9]. OSA has high bioavailability (approximately 50%) [10]. At neutral pH, it is uncharged and its solubility is limited to 2 mM. In aqueous solution (pH = 7), monosilicic acid remains soluble for long periods at concentrations of approximately 100 ppm [11]. In higher concentrations, OSA polymerizes into polysilicic acid, in a structure that is dependent on the pH of the medium as well as the concentration of the Si [12].

These polycondensed forms are poorly bioavailable (approximately 7%) [7]. To be absorbed in humans, OSA must be effectively stabilized in order to limit polymerization.

The component investigated in this study is a molecule of OSA stabilized by vanillin (OSA-vanillin complex (OSA-VC)). Hydrogen bonds between the free hydroxyl groups of OSA and the phenol group of vanillin stabilize the OSA molecule. After ingestion of this stabilized form, OSA is released from the complex for absorption into the blood.

The objective of the present study was to assess the bioavailability of Si, ingested as OSA-VC in a liquid food supplement by analysis of plasma Si concentration and urinary Si excretion.

2 Materials and methods

2.1 Subjects

Men and women (18–60 years of age) were recruited in the Nantes area of France. Subjects were required to be healthy, with a Body Mass Index (BMI) between 18.5 and 30 kg/m^2. All had normal renal function, normal blood counts, and were within reference values for serum creatinine, urea and glucose, and had levels of gamma glutamyl transferase and liver transaminases (alanine transaminase and alkaline phosphatase) below twice the upper limit of normal (according to the reference values of the laboratory performing the tests). Subjects were excluded if they had any metabolic, functional, or inflammatory disease that had the potential to impact renal or digestive functions, or a history of food allergy or intolerance to any of the study product components. Treatments with the potential to affect gastrointestinal motility or Si absorption or with the potential to interfere with any of the study parameters were forbidden. Subjects were instructed to discontinue consumption of food supplements containing Si at least 2 weeks before the study. Subjects who consumed more than two alcoholic drinks per day, smoked more than ten cigarettes per day, or had food habits considered by the investigator to be incompatible with the study were not eligible.

Signed written informed consent for the study was obtained from all subjects before protocol-specific procedures were carried out and subjects were informed of their right to withdraw from the study at any time.

2.2 Study design

This was a single-center, double-blind, cross-over randomized controlled trial. The study was conducted according to Good Clinical Practice Guidelines and the Declaration of Helsinki and was approved by the regional Ethics Committee Ouest-V (Rennes, France) (ID RCB number: 2012-A00080-43).

Subjects who met the inclusion criteria participated in two experimental sessions and consumed one of the two study products (a liquid food supplement containing Si or a placebo) at each session. The sequence of product consumption (Si-containing supplement/placebo or placebo/Si-containing supplement) was randomly defined. Subjects were instructed to avoid alcohol and Si-rich foods (beer, cereals, green beans, bananas, etc.), not to smoke, and to limit physical activity the day before each session. The day of experimental sessions, subjects collected their urine from the time they got up in the morning and reported to the clinical center in a fasted state. They emptied their bladders again, and the urine was combined with urine already collected in the morning since getting up, to be used as the control for assessing urinary Si excretion. Two blood samples were collected to determine baseline plasma Si value (time 0 min = T0). Subjects then ingested one of the study products and 0.3 L of Ultra-High Purity (UHP) water. Additional blood samples were collected at 30-min intervals for the first 3 h (T30, T60, T90, T120, T150, and T180), and then at 4 h and 6 h after ingestion (T240 and T360). Subjects also collected their urine in two 3-h collections (T0-T180 and T180-T360) in two separate containers. Emptying of the bladder was ordered just after the T180 and T360 blood sampling. At T180, subjects ingested an additional 0.3 L of UHP water (after blood collection and before urine collection).

2.3 Products

Study products (OSA-VC and placebo) were colorless aqueous solutions. OSA-VC and placebo products also contained small quantities of plant extracts, preservatives, and stabilizing agents. According to the randomization assignment, subjects ingested 15 mL of either the OSA-VC containing supplement or the placebo supplement on the days of the experimental sessions, within 2–5 min.

OSA-VC is a complex of OSA and vanillin (4-hydroxy-3-methoxybenzaldehyde). The ratio of Si and vanillin in the final product was 0.81 (867 and 1070 mg/L for Si and vanillin, respectively). Within the complex, Si was measured by inductively coupled plasma–atomic emission spectroscopy and vanillin was measured by gas chromatography–flame ionization detector. One 15 mL portion of the Si-containing liquid food supplement provided 44 mg of OSA-VC, which is equivalent to 12.8 mg of Si. Overall, Si concentration in the study products was 951 mg/L for the Si-containing liquid food supplement and 0.51 mg/L for the placebo supplement.

UHP water was prepared in the clinical center, using the water purification system OP101 (DiaSys, Condom, France).

2.4 Biological analysis

Blood samples were collected in K2EDTA-containing tubes for trace elements. These tubes were chosen for their absence of Si contamination. Plasma was isolated after centrifugation at 3000 *g* and 4°C. Si analyses were performed by inductively coupled plasma optical emission spectrometry (JY238, Horiba Jobin-Yvon SAS, Longjumeau, France). Samples were diluted at 1/10 using osmosis-treated demineralized water and an internal standard (beryllium) was added according to laboratory procedures. Si is quantified on an emission line at 251.611 nm and beryllium at 313.042 nm. Standards were prepared using a 1 g/L solution. For Si, the limit of quantification was 50 μg/L.

Absence of Si contamination was verified for all materials before the beginning of the study.

2.5 Statistical analysis

The primary endpoint was the digestive absorption of Si, expressed as the area under the curve of plasma Si values between T0 and T360 ($AUC_{T0\text{-}T360}$). Urinary excretion was the secondary endpoint. Incremental area under the curve under the 360-min response was calculated using the trapezoidal rule with the baseline truncated at zero, and considering all time points (T0, T30, T60, T90, T120, T150, T180, T240 and T360).

Sample size was calculated based on the detection of a between treatment difference of 32 577 (12 799.5) μg.min/L in plasma Si $AUC_{T0\text{-}T360}$ (internal data), using a bilateral binomial test with 90% power and alpha risk of 5%. It was determined that 10 subjects would be required, 14 subjects were recruited to take into account the potential for drop-outs.

Data were analysed using SAS® software version 9.3 (SAS Institute Inc., Cary, NC, USA). Results are expressed as Mean (Standard Deviation (SD)). Significance was set at $p<0.05$.

Normal distribution of data was checked before statistical analyses. Because the assumption of normality was violated, a log transformation of values was applied. Plasma concentration of Si was statistically analyzed using a mixed model Analysis of Covariance (ANCOVA) for repeated measurements and urinary excretion of Si was statistically analyzed using Analysis of Variance (ANOVA) model for repeated measurements.

Analyses were performed in both the Intent-To-Treat and Per Protocol populations. This publication presents the Intent-To-Treat results.

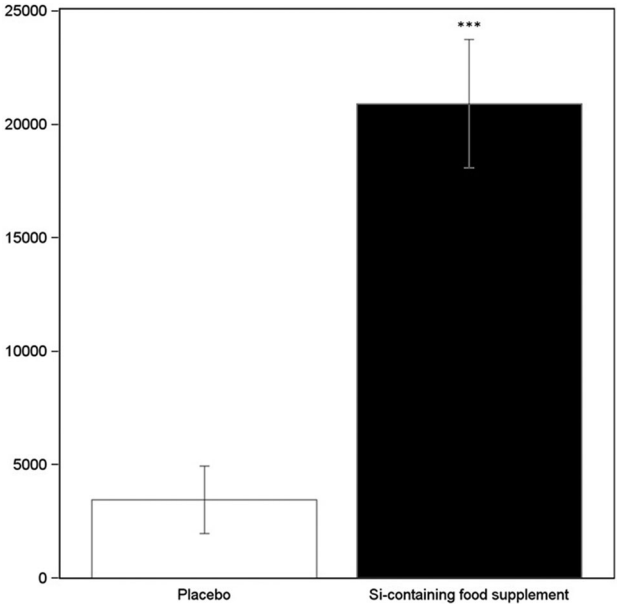

Figure 1. Plasma Si (μg/L) over the 6-h period of experimentation following ingestion of an Si-containing food supplement and placebo. Results are mean, with standard error of the mean represented by vertical bars.

3 Results

3.1 Subjects

Fourteen subjects were recruited and included in the study. Baseline characteristics included: mean age 28.7 (5.8) years, mean BMI 23.5 (3.0) kg/m², and mean plasma creatinine 73.8 (14.9) μmol/L.

3.2 Digestive absorption of silicon

Mean baseline plasma Si concentrations were similar before consumption of placebo or OSA-VC (89.8 (40.1) μg/L and 82.7 (39.8) μg/L, respectively). Si concentration remained stable in plasma after ingestion of the placebo product (Fig. 1), whereas plasma Si level increased markedly after ingestion of OSA-VC, with maximum values observed at T90 (155.9 (27.8) μg/L) and T150 (156.1 (38.5) μg/L, respectively). Si concentration then slightly declined without returning to its baseline value at the end of the measurement period (T360).

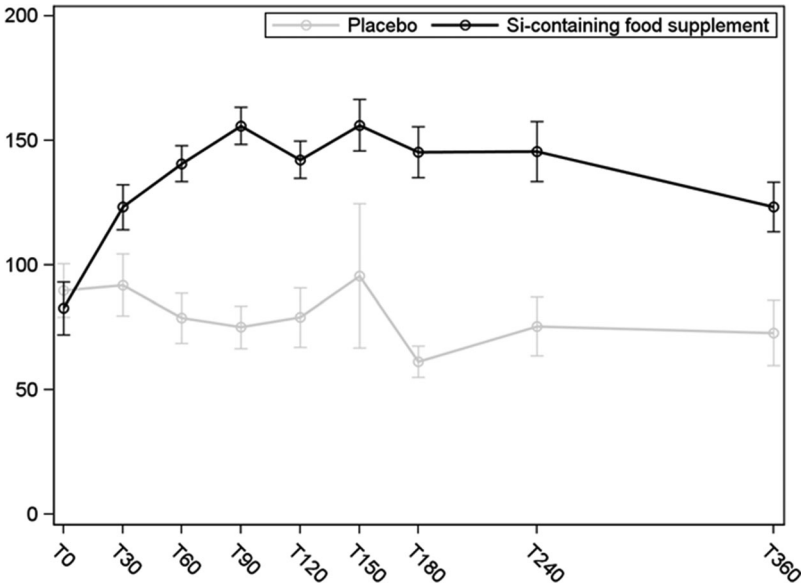

Figure 2. Area under the curve (T0–T360) of plasma Si (μg.min/L) following ingestion of an Si-containing food supplement and placebo. Results are mean, with standard error of the mean represented by vertical bars. Between group comparison: *** $p<0.001$.

Table 1. Urinary Si excretion over 6 h after ingestion of Si-containing food supplement ($n = 14$) and placebo ($n = 14$)

Time period (minutes)	Urinary Si excretion (mg)		*p*-value
	Si-containing food supplement	Placebo	
T0–T180	1.26 (0.51)	0.59 (0.43)	0.0006
T180–T360	1.46 (1.17)	0.38 (0.37)	<0.0001
T0–T360	2.72 (1.12)	0.98 (0.77)	<0.0001

Data are expressed as mean (SD).

Plasma Si $AUC_{T0\text{-}T360}$ was significantly higher after ingestion of OSA-VC, compared to placebo (20 920.0 (10 626.7) μg.min/L versus 3454.8 (5536.0) μg.min/L, respectively; $p = 0.0002$) (Fig. 2).

3.3 Urinary excretion of silicon

In the morning prior to ingestion of placebo or OSA-VC, urinary Si excretion was similar between subjects assigned to either treatment condition (2.9 (1.8) mg and 3.2 (1.8) mg, respectively; not significant). When calculated according to the volume of urine collected in the morning before T0, the excretion was also similar (9.4 mg/L in both cases).

Ingestion of OSA-VC markedly increased the urinary excretion of Si, compared to placebo (Table 1). A significantly higher urinary excretion of Si was reported over the 6-h postsupplementation period, after ingestion of OSA-VC compared to placebo ($p<0.0001$). Significant differences between treatments were observed over the first 3-h period (T0-T180; $p = 0.0006$) and over the second 3-h period of urine collection (T180-T360; $p<0.0001$). Urinary Si excretion following OSA-VC ingestion accounted for 21.1% of the ingested dose of Si (12.8 mg).

4 Discussion

Although there is already a large body of literature reporting the Si content and bioavailability from various sources and dispensed in different forms, to our knowledge, this is the first clinical trial that investigated and reported the bioavailability of Si ingested in a complex form with vanillin. Subjects were supplemented with one dose of OSA-VC (12.8 mg of Si), provided in a liquid food supplement. The digestive absorption of Si was compared to placebo. Blood and urine samples were collected for 6 h following ingestion, and concentrations of Si were measured.

In 2000, Jugdaohsingh *et al.* investigated the bioavailability of Si from silicic acid (monomeric silica), in comparison to oligomeric silica, in five volunteers [13]. Analyses of urine samples showed that 53% of the Si dose was excreted between 0 and 8 h after ingestion of silicic acid, whereas a marginal increase was detected after ingestion of oligomeric silica. The metabolism of Si was also investigated in eight healthy subjects after ingestion of OSA diluted in water at two concentrations (27 and 55 mg/L Si) [10]. Median Si uptake, as evaluated by the urinary Si excretion, was 49%. The authors also showed that after lunch ingestion, a 4-h delay was observed before peak Si in the serum, whereas the peak was visible as soon as 1 h after OSA intake. These data suggest that OSA is readily available for gastrointestinal absorption. In another study, absorption and urinary excretion of Si provided in the OSA form were compared to placebo and to two other forms of Si (standardized herbal silica extract and colloidal silicic acid) among 14 subjects [14]. Only OSA produced a significant increase in serum Si concentration within 2 h

after ingestion, compared to placebo. The authors also reported a significant correlation between individual AUCs of serum Si and urinary Si excretion ($r = 0.43$).

Following an initial study from Bellia *et al.* [15] examining the Si content of beer, Sripanyakorn *et al.* [16] reported that the absorption of Si from beer was 55% within 6 h after consumption of 0.6 L beer containing 22.5 mg Si by nine healthy volunteers. This was close to the absorption of Si from OSA (45%). They also determined that the ultrafilterability of Si from beer was about 80%, suggesting that Si in beer is mainly present in a monomeric form, like OSA, thus explaining its bioavailability. Sripanyakorn *et al.* [17] measured Si absorption from several Si-containing sources, and based upon urinary excretion, reported that 60% of the ingested dose of Si was absorbed from beer over a 6-h collection period (22.9 mg Si per portion ingested), 44% from green beans (6.1 mg Si), 43% from OSA (21.5 mg Si), 17% from choline-stabilized OSA (20 mg Si), 4% from bananas and magnesium trisilicate (13.6 mg and 200 mg Si, respectively), and 1% from colloidal silica (780 mg Si). This confirmed that Si absorption is inversely correlated to the degree of polymerization of Si, demonstrating the importance of Si source when investigating Si bioavailability.

The Si balance, determined by comparing Si dietary intake with Si urinary and fecal excretions, was recently investigated by Pruska *et al.* [18]. Twelve subjects received OSA, in a liquid form (28.9 mg Si), or UHP water on two different occasions. Meals were controlled and biological samples (serum, urine, and feces) were collected at baseline and for 48 h following supplementation. The total Si excretion (urine + feces) reported by the authors during the 24 h following ingestion was 96.3%: 57.0% of ingested Si was excreted in the urine and 39.3% was excreted in the feces.

When comparing the data observed in our clinical trial to previous publications, we can note that plasma Si concentration at baseline (T0) was in the range of baseline values commonly observed in the literature [14, 17, 18]. Ingestion of Si from OSA-VC led to a marked increase in plasma Si concentration – almost twice the baseline value and the concentration observed after placebo ingestion. The plasma Si concentration reached its maximum at approximately 120 min after ingestion of OSA-VC, with a first peak at 90 min. This is similar to the time reported in other publications for maximal serum Si concentration, which was typically 1–2 h after supplementation [16–18]. Interestingly, in our study, plasma Si concentration did not return to its baseline value at the end of the 6-h follow-up, but rather after a slight decline remained 1.5 times higher than the baseline value 360 min after Si supplementation. Other studies have reported a similar pattern of serum Si values after ingestion of other stabilized forms of OSA [17] whereas serum Si concentrations appeared to drop more quickly following the ingestion of nonstabilized OSA forms [18].

At the same time, urinary excretion of Si increased as soon as the first hours after OSA-VC ingestion. Considering the Si content of urine following placebo ingestion, we can assume that it is representative of the basal Si urinary excretion. These values are consistent with those observed by authors during baseline collection [16, 17], corroborating our hypothesis. In future studies, a baseline collection would be necessary to confirm this assumption and would also be of interest for before–after comparisons. In our study, after supplementation with 12.8 mg of Si, the Si urinary excretion was 2.7 mg over the 6-h period of follow-up. This accounted for 21.1% of the ingested Si dose. This value is lower than the 43–50% excretion commonly observed after OSA ingestion [10, 16, 17]. One hypothesis to explain this difference is that the bond between OSA and vanillin leads to a delay in Si delivery following OSA-VC ingestion, because the complex has to be first broken to release OSA. The rate of Si absorption and then excretion would therefore be decreased, and the 6-h excretion of Si would be reduced compared to other OSA molecules. Similar lower rates of urinary excretion have been previously reported for choline-stabilized OSA [17]. A longer follow-up urine collection timeframe would be of use to evaluate the urinary excretion of Si beyond the first 6 h after supplementation.

In conclusion, our study is the first to investigate and demonstrate the absorption of OSA when administered in a complex form with vanillin (OSA-VC). Si levels in serum and urine were significantly increased after supplementation of OSA-VC compared with placebo, thus confirming the absorption of Si provided in OSA-VC. The relatively low urinary excretion of Si observed in this trial and the delay before returning to baseline plasma values may be explained by the complex of OSA with vanillin. Further research is needed to provide additional information on this complex molecule, including its pharmacokinetic properties and the potential impact of chronic consumption.

AM substantially contributed to the conception and design of the study and revised critically all versions during the process of writing of the manuscript. KC substantially contributed to the conception and design of the study and revised critically all versions during the process of writing the manuscript. BH participated in the manuscript writing and revision. CM performed the analysis and interpretation of statistical data. MC designed and conducted the study. GR revised the final version of the manuscript and gave final approval of the last version to be submitted. All authors have contributed to the revision of the submitted manuscript and agreed with this version.

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Potential conflict of interest statement: BH, CM and MC are employees of Biofortis, KC is an employee of DexSil Labs and GR is one of the two managing directors of DexSil Labs.

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