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Reference values for serum silicon in adults

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Abstract

Silicon is an essential nutrient of fundamental importance to human biology. It has been shown that silicon is required for bone, cartilage, and connective tissue formation. However, the assessment of silicon concentration is difficult as reference values are lacking. The aim of the present study was to establish reference values for apparently healthy individuals. Silicon concentrations were determined in serum of 1325 healthy subjects 18–91 years of age using atomic absorption spectrometry. Medians for serum silicon concentrations showed a statistically significant age and sex dependency. In men 18–59 years of age the median was 9.5 μmol/L and decreased to 8.5 μmol/L at 60–74 years of age. In women there was an increase in the median from age 18–29 years (10.00 μmol/L) to 30–44 years (11.10 μmol/L) followed by a decrease in the age group of 45–59 years (9.23 μmol/L). In subjects aged over 74 years the median serum silicon values were 7.70 μmol/L for men and 8.00 μmol/L for women. The most important findings in this study are the decrease of silicon and the course of the silicon concentrations with age, especially in women. The present study is an important prerequisite for studies that aim to identify the health effects and medical implications of silicon.

Keywords: Silicon; Serum reference values; Atomic absorption spectrometry

After oxygen, silicon is the second most abundant element in the lithosphere, accounting for approximately 28% of the earth's crust [1,2]. It is ubiquitously distributed in nature, occurring mainly in the forms of aluminosilicates and hydrates of silicon dioxide [2]. Appreciable quantities of silicon are found in various foodstuffs, particularly monocotyledons such as grain, and in all natural waters. Silicon has been recognized as an essential element for a variety of primitive plants and animals including sea algae and protozoans [3]. In vertebrates it has been found in marked amounts in hair, feathers, skin, liver, heart, muscle, kidney, and tendon [4–7]. The concentration of silicon in parenchymal tissues has been estimated at 2–10 μg/g. Thus, assuming a similar range of silicon deposition in other tissues, its total body content for a subject with 70 kg body weight could be extrapolated to range from 140 to 700 mg [8]. This would make silicon the third most abundant trace element constituent of the body (after iron and zinc). Several studies have indicated that silicon may be an essential trace element in vertebrates [9,10]. The first evidence for its biological importance was obtained from in vitro bone culture experiments in which a physiological role for this trace element in bone calcification processes was established [11–13]. Experimental of silicon deprivation in growing animals demonstrated marked effects on growth, and observed symptoms indicated aberrant connective tissue and bone metabolism [9,10]. Of potential significance to human biology are the discoveries that the silicon content of the human aorta decreases with age and that the concentration of silicon in the arterial wall decreases with the development of atherosclerosis [4,14]. Moreover, a beneficial effect of silicon administration in human patients and other animals with osteoporosis has

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been reported [14–19]. Despite these findings, neither silicon deficiency nor a silicon-responsive condition have yet been identified. Therefore, reliable data on reference values in healthy individuals are needed to help identify patients displaying a silicon deficiency.

Subjects and methods

Subjects

A total of 1325 apparently healthy volunteers (674 female and 651 male) were included in the study. The median age of the entire group was 42 years with a range of 18–91 years. All subjects gave oral informed consent and the study was approved by the local ethical committee (University Hospital, Albert-Ludwigs Universität Freiburg, Germany). Exclusion criteria included serious diseases, metabolic disorders, particular dietary habits, and the taking of silicon-containing medicines.

Blood collection

Venous blood samples of fasting subjects were collected into disposable serum or EDTA polypropylene tubes (Sarstedt, Nümbrecht, Germany). The tubes were then centrifuged at 3000*g* for 10 min at 4 °C. Afterwards, serum and plasma samples were kept as aliquots in polystyrene tubes (Eppendorf) and stored at −20 °C. Extreme caution was taken to prevent all significant preanalytical contaminations during sample acquisition. Briefly, no glassware was used and the sample preparation was carried out in a dust-free room. Before use all plastic tubes (except for EDTA syringes) were treated by washing with 0.2% trace-element-quality nitric acid in 0.1% Triton X-100 solution and then dried at 50 °C.

Apparatus

A Perkin–Elmer Model 4110 ZL Zeeman atomic absorption spectrometer equipped with a PE AS-72 autosampler (Perkin–Elmer Instruments GmbH, Rodgau-Juegesheim, Germany) was used.

Analysis

Before the determination of silicon was carried out, samples (pool serum and patient samples) were centrifuged (at 3000*g* for 5 min) to remove any particles and diluted 1–4 with trace-element-quality water. The matrix modifier consisted of palladium nitrate (100 mg/L) and magnesium nitrate (10 mg /L). The modifier:sample ratio was 1:2 and the injection volume of the sample was 10 µl. The temperature program used included a drying step (130 °C), an ashing step (600 °C), and an atomizing step (2400 °C). To minimize the matrix effects, addition calibration was used throughout this study. Analysis was performed by the standard addition technique as recommended by the manufacturer. The required amounts of sample and modifier were pipetted automatically into the platform in the graphite tube. A hollow silicon cathode was used and set at 251.6 nm at 40 mA. Argon was used as the purge gas.

Statistical analysis

Statistical analysis was carried out with SPSS for Windows version 11.0 (SPSS, Chicago, USA) The check for normal distribution was performed with the Kolmogorov–Smirnoff test. Group differences were identified with the Mann–Whitney *U* test for non-gaussian-distributed groups or with the two-tailed Student *t* test for normal-distributed groups. Correlation was assessed with regression analysis.

Results

Linearity, recovery, and detection limit

Using the addition method the linearity was assessed by adding different silicon solutions to a pooled serum from healthy persons. As shown in Fig. 1 the calibration curves were linear up to 36 µmol/L with correlation coefficients of 0.999 or better. The detection limit of 0.53 µmol/L and the quantification limit of 1.78 µmol/L were estimated as three times and eight times the standard deviation of 20 blank measurements, respectively.

Precision

Two in-house silicon reference materials (9.61 ± 0.75 and 30.26 ± 2.5 µmol/L) prepared with pooled serum

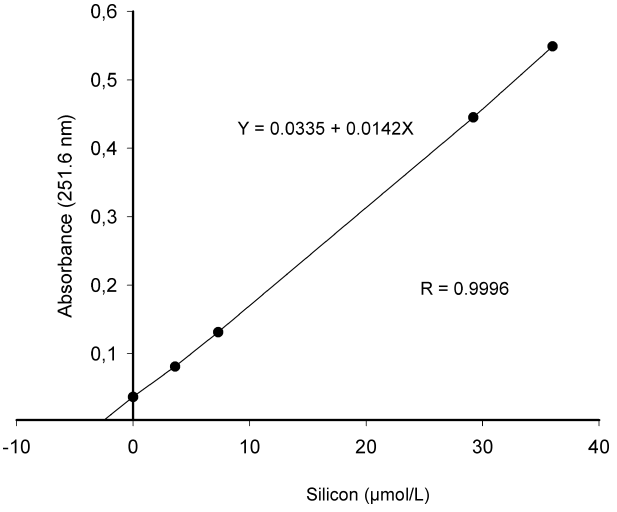

Fig. 1. Linearity of the silicon signal with use of the calibration by standard addition of silicon in serum.

were used to assess the precision of the method. The intraassay CVs were 4.20 and 2.70% ($n = 10$; 9.67 ± 0.40 and 30.63 ± 0.82 μmol/L), respectively. Interassay imprecision was assessed by analyzing two reference materials on 15 different days with duplicate measurements. The interassay CVs were 4.1 and 3.8% ($n = 30$; 9.75 ± 0.40 and 30.26 ± 1.16 μmol/L), respectively.

Accuracy

The accuracy was evaluated by the recovery experiments. Serum samples containing 0.63, 1.25, 2.5, 5, 10, and 20 μmol/L added silicon were prepared, and the silicon content was determined. Analytical recovery of added silicon varied between 96 and 102%.

Serum silicon concentrations in apparently healthy population

A total of 1325 apparently healthy volunteers were included in the study. The serum silicon concentrations determined in the entire group of samples were not normally distributed and the frequency distribution was skewed to the left. The population was divided into five subgroups. These subgroups were then tested for significant differences in their median silicon concentrations. Age groups that did not differ significantly in their median silicon concentrations were combined and recalculated to define age-specific concentrations. The serum silicon concentrations for the different age groups and genders are shown in Figs. 2 and 3. Visual examination of the data supported by the use of the Mann–Whitney U test indicated gender-related differences which are significantly age dependent ($p < 0.012$). In a younger population (18–44 years) the median silicon concentrations were significantly higher in women than in men, and the maximum difference of 21% ($p < 0.003$) was observed in the age group of 30–44 years. There were no significant differences in serum silicon in men and women of 18–29 years of age and in the older population over 60 years of age. Fig. 2A shows box and whisker plots for the serum silicon concentrations according to the age groups in males. In men between the ages of 18 and 59 years there was an increase in serum silicon concentration with age

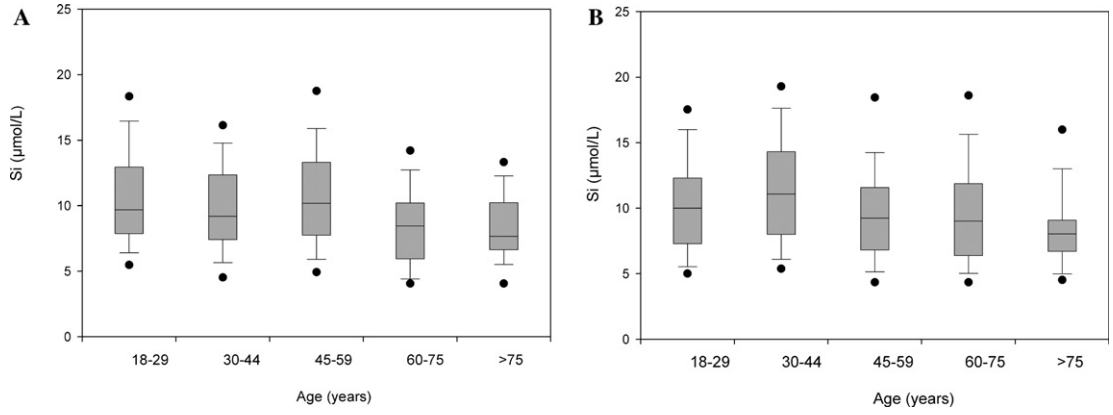

Fig. 2. Box and whisker plots showing median (horizontal black bar) and interquartile range in the box (50th, 25th, 75th, and 95th percentiles) and 2.5th and 97.5th percentiles (whiskers). (A) Males; (B) females.

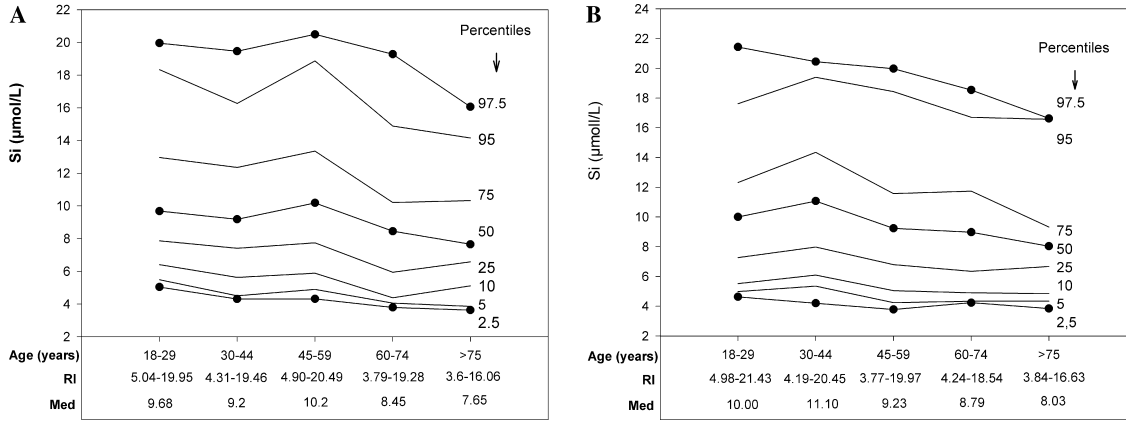

Fig. 3. Age- and sex-related reference intervals (RI) based on the 2.5 and 97.5 percentiles of data derived from 1325 healthy subjects for silicon concentrations in serum. (A) Males; (B) females.

and the median silicon lies within the ranges of 9.7–10.17 µmol/L. As shown in Figs. 3 and 4 the maximum increase of about 5% achieved by 45–59 years was not statistically significant for males 18–59 years of age. The multiple comparison of age groups showed no statistically significant differences in median silicon values. These subgroups were then combined and the median was 9.5 µmol/L (Table 1). However, a statistically significant decrease of serum silicon concentrations of 25% ($p < 0.002$) occurred up to 60 years of age (Fig. 4). Serum silicon concentrations in females according to age are illustrated in Fig. 3. As shown in Fig. 4 there was an increase in silicon concentration with age in females between the ages of 18 and 44 years ($p < 0.014$). The median serum silicon concentration ranged from 10 to 11.1 µmol/L. The course of the concentrations (Fig. 4) shows the highest serum silicon concentration (11.1 µmol/L) in females of the age group 30–44 years. This age group and the age group over 74 years showed a statistically significant difference in their median silicon concentrations (11.1 and 8.0 µmol/L, respectively, $p < 0.000$) and were statistically significantly different from other age group in both women and men ($p < 0.000$ and $p < 0.012$, respectively). The decrease of the silicon concentrations was approximately 19% in women of the age group of 45–59 years and it was about 28% in those over 60 years of age. A significant decrease in silicon concentrations occurred in women between the fourth and the fifth decades, consistent with the average age of menopause. Percentiles for serum silicon concentrations and the resulting reference intervals (RI) for the different age groups and sex were determined by the nonparametric method and are illustrated in Fig. 3, respectively. The reference intervals are given as the central 95% confidence interval bounded by the 2.5 and 97.5 percentiles [20].

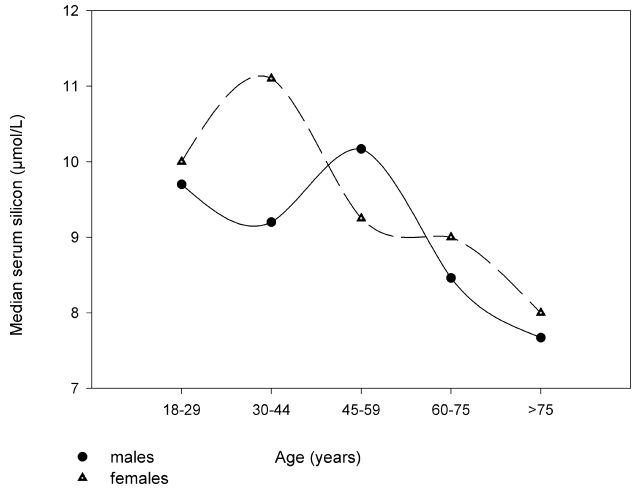

Fig. 4. Course of the median silicon concentration in serum of healthy subjects grouped according to age and sex.

Discussion

In the past 30 years silicon has been recognized as an essential trace element involved in the normal metabolism of higher animals. It is required for growth and bone cartilage formation [8,21,22] and may act as a biological cross-linking agent in the formation of connective tissue [7]. It is surprising, however, that the detection and accurate measurement of this element in a biological medium has received scant attention in clinical chemistry, probably due to the intractable analytical problems. Indeed up to now few or no serum reference values have been available for the assessment of silicon status in different age groups. Therefore, in the present study we performed an extensive evaluation of the analytical performance of a new method for the determination of silicon in serum. We found that this method exhibits good analytical performance with a high degree of reproducibility and accuracy (within-run CV of 4.2% and between-run CV of 4.1%). It showed excellent linearity for silicon calibration curves, often with a correlation coefficient of 0.999.

Previous reference values [23–36] (see Table 2) showed great variability and silicon concentrations in serum different from those of normal subjects estimated only by statistical means and simple standard deviation.

Table 1
Cross tabulation of p values for group differences

	Men				Women			
	18–59 (M1)	60–74 (M2)	>75 (M3)	18–29 (F1)	30–44 (F2)	45–59 (F3)	60–74 (F4)	>75 (F5)
n	508	108	35	199	155	150	136	34
Median (µmol/L)	9.5	8.5	7.7	10.0	11.1	9.2	8.8	8.0
M1		0.000	0.003	0.660	0.020	0.024	0.008	0.003
M2			0.944	0.000	0.000	0.020	0.070	0.900
M3					0.000	0.122	0.230	0.93
F1					0.014	0.1000	0.153	0.012
F2						0.000	0.001	
F3							0.940	0.12
F4								0.140

Statistical analysis of group differences by median silicon concentration. Statistically significant at $p < 0.055$.

Table 2
Previously publications reporting silicon concentrations in normal human serum and plasma (p)

Authors and year	References	Method	*N*	Si (μmol/L)
Lo and Christian, 1978	[23]	ET-AAS[a]	5	27.0
Mauras et al., 1980	[24]	ET-AAS[a]	33	3.9 ± 1.2
Dobbie and Smith, 1982	[25]	F-AAS[b]	50	21.5 ± 4.5
Berlyne and Caruso, 1983	[26]	ET-AAS[a]	21	11.0 ± 2.9
Berlyne et al., 1985	[27]	ET-AAS[a]	17	9.8 ± 0.78 (p)
Tanaka and Hayashi 1986	[28]	ICP-AES[c]	30	8.5 ± 3.0
Berlyne et al., 1986	[29]	ET-AAS[a]	23	9.4 ± 2.9
Gittelman, 1990	[30]	ET-AAS[a]	8	0.60 ± 0.36
Roberts and Williams, 1990	[31]	DCP-AES[d]	20	5.0 ± 0.5 (p)
Gittelman et al., 1992	[32]	ET-AAS[a]	14	6.0 ± 1.1 (p)
Bercowy et al., 1994	[33]	DCP-AES[d]	43 385	14.2–142 (p) 7.1–2421
Teuber et al., 1995	[34]	ICP-AES[c]	55	4.6 ± 2.5
Leung and Edmond, 1997	[35]	ETAAS[a]	60	1.1–7.4
Jugdaohsingh et al., 2002	[36]	ICP-OES[e]	8	7.5 ± 3.1

[a] Electrothermal atomic absorption spectrometry.
[b] Flame atomic absorption spectrometry.
[c] Inductively coupled plasma atomic emission spectrometry.
[d] Direct current plasma atomic emission spectrometry.
[e] Inductively coupled plasma opticalvemission spectrometry.

In one study of 385 healthy subjects estimated reference ranges for total silicon in serum were from less than 7.12 to 2421 μmol/L [33]. This puts serious obstacles in the way of identifying patients who might display a silicon deficiency or silicon-related maladies. Presumably the reasons for this broad reference interval are due to the matrix disturbances and loss of absorption resulting from the formation of silicon carbide. Neither sex nor age dependency has been reported. A statistical comparison of published data with the results of the present study is not viable because of different experimental designs and small cohorts. In the present study, the silicon concentrations determined in a large number of apparently healthy subjects allow a more specific definition of reference intervals. Due to the uniform age distribution in the population examined between 18 and 90 years it was possible to investigate age-related reference values and sex dependency (Fig. 3, Table 1). Subsequent multiple comparisons of groups indicated that serum silicon values significantly increased in women of 30–44 years of age. The median silicon concentration in this age group was statistically different from that of other age categories in both women and men. This effect was also observed when the population was segregated into premenopausal (20–49 years) and postmenopausal (50–80 years) groups. We present evidence that the serum silicon concentrations decrease in both men and women with age. In the older population (45–59 years), however, the decrease of the silicon concentrations was faster in women than in men ($p < 0.02$). There is a long period of steady state serum silicon concentration in males throughout the age range of 18–59 years in which no significant differences in serum silicon concentrations were observed. A statistically significant overall decrease in serum silicon concentrations with age occurred in men over 60 years of age. The differences in dietary silicon intakes in women and men reported by Jugdaohsingh et al. [36] is in contrast with the sex dependency for serum silicon observed in this study. Some studies assessing the concentration of silicon in humans have demonstrated a decrease of silicon concentration with age. The silicon concentration of the skin dermis has been reported to decline with age [8,22,37]. Loeper et al. [4,14] showed a marked decrease of the silicon concentration of the normal human aorta with increasing age. They observed also a significant positive relationship between the decrease of the silicon concentration in the arterial wall and the development of atherosclerosis. The physiological mechanism responsible for these effects is unknown at this time. However, Charnot and Pérès [38] reported an association between silicon, age, and endocrine balance. They assumed that the decline in hormonal activity may be responsible for the changes in silicon concentrations in senescence. This assumption is supported by the course of the serum silicon concentrations in both women and men observed in this study (Fig. 4). It has been reported that silicon intakes of both sexes decrease with increasing age. This may also contribute to the changes in serum silicon concentration in senescence. However, considering our data in this study, we share the assumption of Charnot and Pérès [38] that the hormonal activity may be the major factor influencing the changes of silicon concentrations. Indeed, the highest serum silicon concentration was found in women in the age category with the highest hormonal activity.

There is currently little available information on the silicon concentrations in blood, in other body fluids, and in tissues. Further studies are in progress in our laboratory to provide additional information on silicon concentrations in humans.

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