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Serum and urinary selenium levels in obese children: A cross-sectional study

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ABSTRACT

Objective: To determine serum and urinary selenium (Se) levels in children with and without obesity, and to assess if Se influences the risk of obesity.
Subjects and methods: High-resolution-continuum source-atomic absorption spectrometry (HR-CS-AAS) was used to determine the content of Se in 80 children (age 6–17; 40 boys, 40 girls). Correlations between variables were tested with the use of Spearman's correlation coefficient. *U* Mann–Whitney test was applied to assess the difference of Se contents in samples. Measured metabolic risk factors (blood pressure, glucose level, triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol), age, gender, and BMI were correlated. Logistic regression models were fitted to identify predictors of obesity interacting with selenium content in serum and urine, separately.
Results: Obese children, regardless of gender, had lower Se content. Se level in serum ($p = 0.001$, OR 0.74, 95%CI 0.62–0.88) and total cholesterol ($p = 0.001$, OR 1.19, 95%CI 1.08–1.31) were the independent factors significantly influencing the risk of obesity in children. Two separate models were observed for Se in urine: (i) Se level ($p < 0.0001$, OR 0.70, 95%CI 0.58–0.84) and glucose level ($p < 0.0001$, OR 1.22, 95%CI 1.10–1.35), and (ii) Se level ($p = 0.002$, OR 0.60 95%CI 0.43–0.83) and total cholesterol level ($p = 0.003$, OR 1.16, 95%CI 1.05–1.28).
Conclusion: The current study suggests a possible role of Se in obesity. Further research needs to be performed to check if obese children are an at-risk group for Se deficiency.

Introduction

Childhood obesity has become a rapidly growing worldwide problem. It is known that both genetic and environmental factors play a role in the development of obesity. This serious disease process is associated with increased morbidity and mortality in adult life and with several adverse consequences in childhood such as insulin resistance, type 2 diabetes, dyslipidemia, polycystic ovarian syndrome, pulmonary, orthopaedic, and psychological disorders. However, studies of Lloyd et al. [1] do not fully support the view that childhood obesity is an independent risk factor for adult metabolic syndrome or type II diabetes. Moreover, according to the authors, those who are at the lower end of the BMI range in childhood, but continue to be obese during adulthood seem to be at particular risk of metabolic syndrome. Childhood obesity has been linked to obesity in adulthood. It has been reported that 40% of overweight children will continue to be overweight into adolescence and 75–80% will be obese as adults [2]. In a study conducted on 2916 primary school children (1445 girls and 1471 boys) the prevalence of overweight Polish children (including obesity) was found to be 15.4% (15.8% girls and 15.0% boys) while the prevalence of obesity was 3.6% (3.7% girls and 3.6% boys) [3].

Published research reveals that dietary deficiencies for certain trace elements may increase the absorption of toxic metals by certain tissues. Children on low-protein diets, with parenteral nutrition, patients with chronic diseases or with oncologic disorders are especially at risk for the development selenium deficiency [4]. A study of Tascilar et al. suggests no significant difference between groups of obese and non-obese children

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Table 1
Biochemical data and Se levels in the studied and the control groups.

	Average concentration ± SD			
	Controls		Obese	
	Girls $n=20$	Boys $n=20$	Girls $n=20$	Boys $n=20$
Fasting blood sugar (mg/dL)	75.4 ± 2.8	76.1 ± 4.1	89.9 ± 6.7	88.8 ± 2.2
Triglyceride (mg/dL)	80.7 ± 10.3	86.9 ± 18.2	102.8 ± 12.3	125.1 ± 14.1
Low-density lipoprotein (LDL) (mg/dL)	73.0 ± 4.5	88.4 ± 6.9	102.0 ± 4.8	121.3 ± 4.3
High-density lipoprotein (HDL) (mg/dL)	59.3 ± 5.7	53.6 ± 7.9	48.4 ± 10.7	49.7 ± 6.9
Total cholesterol (mg/dL)	148.4 ± 12.9	159.4 ± 10.6	171.0 ± 9.4	196.1 ± 16.1
Blood pressure (systolic/diastolic) (mmHg)	114.4 ± 2.5/75.4 ± 3.4	110.5 ± 1.6/76.4 ± 6.2	126.2 ± 10.5/79.3 ± 9.4	117. ± 10.2/76.3 ± 4.7
Selenium in serum (μg/l)	102.3 ± 7.9	111.1 ± 9.5	80.4 ± 8.2	82.8 ± 10.3
Selenium in urine (μg/l)	55.9 ± 9.4	60.3 ± 11.5	36.0 ± 7.5	36.7 ± 5.6

regarding selenium serum levels [5]. According to Bouglé et al. obese children are not prone to trace element deficiency [6]. Generally, data from Poland concerning trace elements content in obesity is scarce. In our previous study [7] conducted on Polish children, we found significant differences between the content of metals in obese and healthy children: Zn in serum from obese patients of both sexes, and Zn, Co, and Mn in blood, Mn in serum. Negative correlations between body mass index (BMI) and Zn in blood, Cu in serum, and Fe in urine were discovered for girls (control group), while positive correlation between Co content in serum and BMI was discovered for obese boys.

It is known that people who are overweight or obese commonly experience poorer antioxidant protection, and oxidative stress levels are elevated in obesity [8,9]. Se has antioxidant properties and its protective role against oxidative damage plays an important role e.g. in diabetic complications [10]. Children with excess weight have a poorer selenium status than children of normal weight [11]. Specific correlations have been found between selenium nutritional status and metabolic risk factors in a group of men with visceral obesity [12]. Selenium supplementation in patients with type 2 diabetes may be associated with adverse effects on blood glucose homeostasis, even when plasma Se concentration are raised from deficient status to the optimal concentration of antioxidant activity [13]. Other studies suggest that serum Se level is significantly reduced among morbidly obese female patients seeking bariatric surgery [14]. An association between Se and cardiovascular disease, which is the main outcome of metabolic syndrome, was reported in literature [15]. Activity of enzymes, which prevent the peroxidation of low-density cholesterol, is associated with Se content in the body. The question of whether Se protects against cancer seems to be still open, however, to date, there is no convincing evidence that selenium supplements can prevent cancer [16]. However, epidemiological data has also shown a significant association between obesity and a number of cancers [17,18]. Thus, investigations on the Se content in obesity deserve attention.

There has been considerable interest in studying the content of trace elements in various biological fluids, although it is influenced by many factors (e.g. age, sex, diseases, drugs, diet supplements, nutritional habits, lifestyle factors, etc.). This work was undertaken to estimate Se levels in serum and urine of obese and healthy children, to correlate Se status with BMI, age, sex, lipid profile, glucose, and blood pressure, and to assess if Se influences the risk of obesity.

Methods

Subjects

The studied group of children were admitted to the Department of Paediatric Endocrinology and Diabetology, Medical University of Lublin, Poland for further diagnostic testing related to their obesity. Patients and control group were from the same geographic area (Lublin region, south-east Poland). The non-obese children were present for routine check-ups. Informed consent was obtained from the parents. The study was approved by the Ethics Committee of the Medical University of Lublin. Tests were performed in 80 children who constituted two groups: obese (20 girls and 20 boys, age 6–17 y, the average age: 13.1 y) and the control group (20 girls and 20 boys, age 8–17 y, the average age: 13.5 y). In the obese group of patients, the overall mean height for boys was 162.6 cm, and for girls 154.6 cm; weight was for boys were 82.3 kg, for girls 72.2 kg. Boys had a higher mean BMI (37.7 kg/m^2) than girls (29.7 kg/m^2). In the control group, the mean values for boys were 161.6 cm, 52.8 kg, 20.13 kg/m^2, and for girls: 160.1 cm, 52.9 kg, and 20.4 kg/m^2. BMI was calculated using the formula (weight in kg)/(height in m)^2. A BMI in the 95th percentile or higher was considered obese. According to the interviews with the parents, within 6 months before the study, the children did not take any vitamins, dietary supplements, medication and they were not on any special diets. None of the children reported cigarette smoking. Chronic and acute inflammatory processes were excluded based on physical examination and basic laboratory analysis. Blood and urine samples were drawn after an overnight fast. Biochemical parameters measured using commercial kits are listed in Table 1. An automatic device measured blood pressure in the morning before eating. Blood was collected in EDTA vacutainers. Samples were kept on ice and centrifuged within 3 h of collection. Aliquots of serum were stored at −25 °C until analysis. Casual morning urine samples were collected in clean plastic specimen containers and stored at −25 °C until analysis.

Samples, reagents, and instruments

The studied material consisted of 160 samples of human serum and urine. Samples taken from healthy and obese patients were pre-treated and analyzed in the same way. They were transported and stored in polypropylene containers. 1 mL of each type of sample was divided in two parts (each 0.5 mL) in order to have two independent solutions prior to the mineralization procedure. A microwave-assisted high pressure digestion system (UniClever BM-1, Plazmotronika, Poznań, Poland) was used. Each time an acidic digestion with 65% nitric acid water solution was applied (1 mL of HNO_3: 9 mL of deionized H_2O). The conditions of the mineralization procedure had been previously optimized [19]. The obtained solutions were poured into volumetric flasks (PTFE) and when it was necessary, they were diluted with deionized water (18 MΩ cm) before final analysis. After the mineralization, each sample was analyzed at least in triplicate using high-resolution atomic absorption spectrometer. The measurements were performed with the ContrAA700 high-resolution continuum source graphite tube AAS instrument (Analytik Jena AG, Jena, Germany). A transversely heated graphite furnace was

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used as the atomizer. Method parameters were set as follows: wavelengths was 196.0267 nm, pyrolysis temperature: 950 °C, atomization temperature: 1900 °C, and 5 μL $Pd(NO_3)_2$ (0.1%Pd) as modifier. Concentration of the stock solution (100 μg/L Se in 1% HNO_3) was prepared.

The accuracy of the method was verified by the following certified reference materials: Seronorm™ Trace Elements Serum L-2 (Billingstad, Norway) – human serum and Seronorm™ Trace Elements Urine L-2 (Billingstad, Norway) – human urine. A good correspondence between the certified and the measured concentrations was achieved. The value obtained for Se in serum was 165.6 ± 12.6 μg/L, whereas the certified value was 163 ± 10 μg/L (average recovery for five separate determinations was 97.8%). Analyzed value for Se in urine was 69.7 ± 15.2 μg/L, and certified value was 70.1 ± 14.1 μg/L (average recovery was 98.2%). Limit of detection (3σ) was estimated to be 2.00 μg/L.

All reagents used were at least of analytical grade. Water with a resistivity of 18.2 MΩ cm was deionized in a Milli-Q system (Millipore, Bedford, MA, USA). 65% nitric acid solution and other stock solutions were purchased from Sigma–Aldrich, Germany.

Statistical analysis

In this study, the data analysis was performed in two general steps. The first step involved correlation analysis according to the health status and gender of the children, while the second step involved an assessment of differences between Se concentration in the groups of studied patients according to their health status (obese vs. non-obese children) and body fluids (i.e. serum, urine) taking into account the gender of children. Prior to the statistical testing the data collected were entered into Statistica 10.0 (Statsoft Inc., USA) and checked for accuracy. The distribution of each variable was checked using Shapiro–Wilk's *W* test. In majority of cases the distribution of the variables was not normal, and hence correlation between them were tested with the use of Spearman's correlation coefficient while *U* Mann–Whitney test was applied to assess the differences between Se content in investigated body fluids.

Logistic regression models were fitted to identify predictors of obesity interacting with selenium concentrations in serum and urine, separately. The potential predictor variables were: age (years), systolic and diastolic blood pressure (mmHg), metabolic status: glucose (mg/dL), total cholesterol (mg/dL), HDL (mg/dL), LDL (mg/dL), TG (mg/dL) in serum; these, were tested in separate univariate analyses for their association with obesity in children. Significant univariate predictors ($p < 0.1$) were tested for the inclusion in the multivariate models, and nonsignificant variables were removed sequentially until only those significant at $p < 0.1$ remained. From these models, adjusted odds ratios (OR) and 95% confidence intervals (CI) were derived; corresponding *p*-values were from Wald's test. Goodness of fit was checked using Hosmer and Lemeshow's test. Statistical significance was set at $p < 0.05$.

Results and discussion

The *p*-values of Shapiro–Wilks' *W* test were higher than 0.05 for the two analyzed characteristics, namely for LDL: $p = 0.24$ and for Se content in serum: $p = 0.49$, which proved that results did come from a normally distributed population. In the other cases, it was found that the distribution of variables was not normal. Based on the results of Shapiro–Wilks' test, the Spearman's rank correlation coefficient was applied. For the control group, only in two cases directly proportional correlations occurred both for girls and boys (SBP-AGE and DBP-AGE). In all other cases, the data indicated that significant values of correlation coefficient appeared for one gender. An inverse relationship was found between the content of total cholesterol and DBP. In girls, an inverse correlation was found for HDL and TG levels. In the case of the relationship between DBP and LDL levels, the nature of correlations was associated with the gender of subjects. Such dependence was inversely proportional for boys, while for girls it was directly proportional. A full set of statistically significant coefficients of Spearman's rank correlation between the values of biochemical parameters studied, age, gender, and BMI for a control group (healthy children) and obese children are summarized in Table 2.

At the next stage, the *U* Mann–Whitney test was applied to assess the difference of Se content in urine and serum taking into account the gender of children. Data listed in Table 3 indicate that statistically significant differences in concentration levels exist between the control group and the obese group.

A comparison of the median Se concentrations in plasma and urine (Fig. 1) shows that the group of obese children, regardless of the gender, had lower Se concentrations.

The results of the logistic regression analysis are summarized in Table 4.

In univariate analysis, the differences in biochemical parameters of obese and control patients turn out to be statistically significant (Table 4). Obese children had higher level of glucose ($p < 0.0001$), total cholesterol ($p < 0.0001$), LDL ($p = 0.016$) and TG ($p = 0.003$) in serum, but lower level of HDL ($p = 0.011$). It was shown that obese children had significantly lower level of selenium in serum ($p = 0.0001$) and urine ($p = 0.001$) in comparison to the control group. Table 4 includes the predictors of obesity interacting with selenium in serum and urine separately obtained by multivariate analysis. Obesity risk in children increased by 19% with elevated total cholesterol concentration but decreased by 26% with higher selenium level in serum. The similar association was found in relation to interaction of selenium level in urine (40% decline) and total cholesterol (16% growth) with obesity (Model 1, Table 4). In the second fitted regression model (Model 2, Table 4), lower selenium level in urine and higher glucose level in serum were identified as independent factors contributing to obesity in children.

Our findings are consistent with the recent study concerning Se levels in serum of obese Egyptian children [20] suggesting that obese children had lower serum Se levels (63.5 ± 15 vs. 78.3 ± 18 μg/L, $p < 0.01$), and the recent results of Ortega et al. studies, in which lower serum Se levels in obese children than those of normal weight (64.6 ± 16.8 μg/L compared to 75.3 ± 12.2 μg/L; $p < 0.001$) are described [11].

Systematic determinations of Se content in blood of Polish inhabitants, demonstrate low levels of Se in the blood of subjects of almost the entire country (perhaps, mainly due to a deficiency in the diet) [21]. The concentration of Se in plasma of healthy children (cited data comes from 1990 to 1991) were relatively low-about 30 μg/l for 1–3 y old children, and about 40 μg/l for 3–15 y children, with a calculated daily dietary intake of about 30–40 μg/day. The population described in the present study comes from a south-eastern geographical region of Poland (mainly from Lublin region)–unfortunately, not included in reported studies [21]. Therefore, we are not able to compare our data with previous Se status in healthy Polish children. Comparing our findings regarding healthy children, our data on serum Se differs considerably from German studies (a neighbouring country with a similar diet) where the overall means reported for 1–18 y old healthy children in 1997 were assessed to be 0.83 μmol/L (i.e. 65.53 μg/L) [22]. Our findings show that content of Se in serum display an age- and gender dependency. Therefore there is a continuous need for establishing the current region/age/gender -adjusted reference values for Se content in body fluids.

With regard to Se content in urine – a widely used matrix for biomonitoring, variations in urinary Se concentrations (mainly a

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Table 2
Correlation of Se content in serum and urine with biochemical variables of the obese and healthy children (values presented above and below diagonal of the matrix present Spearman's correlation coefficients of obese and healthy children, respectively).

	Obese children ($n=40$)											
	AGE	BMI	SBP	DBP	Glucose	T Chol	HDL	LDL	TG	Se (serum)	Se (urine)
Control group ($n=40$)											
AGE	1.00	0.20 (p = 0.403)	**0.62 (p = 0.003)**	0.43 (p = 0.059)	−0.32 (p = 0.166)	**−0.47 (p = 0.036)**	0.10 (p = 0.668)	−0.18 (p = 0.454)	0.09 (p = 0.705)	**−0.52 (p = 0.018)**	−0.26 (p = 0.262)
	1.00	*−0.18 (0.434)*	*0.19 (p = 0.409)*	*0.32 (p = 0.160)*	***0.46 (p = 0.042)***	*0.15 (p = 0.530)*	*−0.19 (p = 0.426)*	***0.47(p = 0.038)***	*−0.16 (p = 0.496)*	*−0.02 (p = 0.901)*	*−0.27 (p = 0.254)*
BMI	0.13 (p = 0.589)	1.00	0.32 (p = 0.161)	**0.50 (p = 0.026)**	−0.19 (p = 0.402)	0.14 (p = 0.551)	−0.01 (p = 0.954)	−0.07 (p = 0.774)	0.33 (p = 0.151)	−0.10 (p = 0.672)	−0.01 (p = 0.969)
	−0.02 (p = 0.945)	*1.00*	*0.23 (p = 0.331)*	*0.34 (p = 0.126)*	*0.07 (p = 0.879)*	*0.35 (p = 0.123)*	*−0.21 (p = 0.383)*	*0.17 (p = 0.476)*	*0.01 (p = 0.954)*	*0.06 (p = 0.801)*	*0.01 (p = 0.954)*
SBP	**0.59 (p = 0.005)**	0.18 (p = 0.440)	1.00	**0.58 (p = 0.008)**	0.13 (p = 0.596)	−0.05 (p = 0.832)	0.06 (p = 0.817)	−0.21 (p = 0.360)	0.26 (p = 0.261)	−0.19 (p = 0.419)	−0.19 (p = 0.427)
	0.49 (p = 0.027)	*−0.22 (p = 0.348)*	*1.00*	***0.72 (p = 0.000)***	***0.54 (p = 0.014)***	*−0.09 (p = 0.711)*	***−0.63 (p = 0.003)***	***0.51 (p = 0.022)***	***0.50 (p = 0.023)***	*0.16 (p = 0.500)*	*−0.31 (p = 0.170)*
DBP	**0.55 (p = 0.012)**	0.30 (p = 0.194)	0.40 (p = 0.078)	1.00	0.20 (p = 0.384)	−0.16 (p = 0.495)	0.11 (p = 0.632)	**−0.60 (p = 0.005)**	0.11 (p = 0.639)	−0.18 (p = 0.423)	0.10 (p = 0.674)
	0.56 (p = 0.009)	*−0.07 (p = 0.781)*	***0.64 (p = 0.002)***	*1.00*	***0.63 (p = 0.003)***	*0.02 (p = 0.913)*	***−0.57 (p = 0.008)***	***0.46 (p = 0.042)***	*0.28 (p = 0.229)*	*0.06 (p = 0.808)*	*−0.26 (p = 0.257)*
Glucose	**0.46 (p = 0.039)**	0.07 (p = 0.776)	**0.45 (p = 0.048)**	0.17 (p = 0.468)	1.00	0.17 (p = 0.465)	0.10 (p = 0.663)	−0.26 (p = 0.262)	−0.25 (p = 0.291)	**0.63 (p = 0.002)**	0.28 (p = 0.236)
	0.32 (p = 0.167)	*−0.11 (p = 0.630)*	*−0.07 (p = 0.779)*	*0.38 (p = 0.098)*	*1.00*	*0.11 (p = 0.627)*	*−0.39 (p = 0.087)*	***0.50 (p = 0.023)***	*0.33 (p = 0.156)*	*−0.13 (p = 0.579)*	*−0.22 (p = 0.352)*
T Chol	0.06 (p = 0.816)	−0.09 (p = 0.685)	−0.03 (p = 0.893)	**−0.51 (p = 0.021)**	−0.18 (p = 0.455)	1.00	−0.29 (p = 0.210)	**0.54 (p = 0.014)**	**0.48 (p = 0.033)**	0.43 (p = 0.054)	0.01 (p = 0.980)
	−0.26 (p = 0.273)	*−0.05 (p = 0.836)*	*−0.12 (p = 0.601)*	*−0.12 (p = 0.612)*	*−0.25 (p = 0.278)*	*1.00*	*0.19 (p = 0.430)*	***0.57 (p = 0.008)***	*−0.17 (p = 0.462)*	*−0.14 (p = 0.539)*	*−0.22 (p = 0.350)*
HDL	0.17 (p = 0.462)	−0.33 (p = 0.160)	−0.14 (p = 0.552)	−0.10 (p = 0.680)	−0.13 (p = 0.577)	0.25 (p = 0.280)	1.00	−0.12 (p = 0.606)	**−0.68 (p = 0.000)**	0.06 (p = 0.802)	−0.12 (p = 0.624)
	−0.38 (p = 0.094)	*0.00 (p = 0.999)*	*−0.41 (p = 0.074)*	*−0.15 (p = 0.537)*	*0.18 (p = 0.436)*	*0.21 (p = 0.372)*	*1.00*	*−0.05 (p = 0.803)*	*−0.32 (p = 0.166)*	*−0.34 (p = 0.147)*	*0.31 (p = 0.175)*
LDL	−0.30 (p = 0.192)	−0.06 (p = 0.811)	−0.17 (p = 0.463)	−0.26 (p = 0.264)	−0.03 (p = 0.895)	0.11 (p = 0.635)	−0.34 (p = 0.144)	1.00	0.29 (p = 0.220)	0.24 (p = 0.303)	−0.31 (p = 0.181)
	−0.20 (p = 0.405)	*−0.05 (p = 0.841)*	*0.09 (p = 0.702)*	*0.05 (p = 0.816)*	*0.01 (p = 0.953)*	***0.59 (p = 0.006)***	*0.17 (p = 0.475)*	*1.00*	*0.13 (p = 0.592)*	*−0.20 (p = 0.394)*	***−0.51 (p = 0.020)***
TG	−0.04 (p = 0.862)	0.03 (p = 0.896)	−0.05 (p = 0.813)	−0.11 (p = 0.627)	0.04 (p = 0.0863)	0.14 (p = 0.555)	0.11 (p = 0.651)	−0.12 (p = 0.599)	1.00	−0.04 (p = 0.857)	−0.16 (p = 0.489)
	0.03 (p = 0.875)	*0.08 (p = 0.721)*	*0.13 (p = 0.594)*	*−0.23 (p = 0.321)*	*−0.10 (p = 0.674)*	*−0.18 (p = 0.450)*	**−0.50 (p = 0.025)**	*0.03 (p = 0.889)*	*1.00*	*−0.20 (p = 0.391)*	*−0.29 (p = 0.210)*
Se	−0.17 (p = 0.470)	−0.10 (p = 0.682)	−0.12 (p = 0.618)	0.05 (p = 0.827)	0.05 (p = 0.814)	−0.12 (p = 0.585)	−0.19 (p = 0.408)	0.10 (p = 0.680)	0.10 (p = 0.682)	1.00	0.06 (p = 0.813)
(Serum)	*−0.27 (p = 0.242)*	*0.13 (p = 0.575)*	*−0.38 (p = 0.102)*	*−0.10 (p = 0.684)*	*0.32 (p = 0.165)*	*0.21 (p = 0.374)*	***0.44 (p = 0.049)***	*0.26 (p = 0.258)*	*1.00*	*1.00*	*−0.21 (p = 0.370)*
Se	−0.02 (p = 0.913)	**0.49 (p = 0.028)**	−0.14 (p = 0.531)	**0.46 (p = 0.038)**	0.04 (p = 0.860)	−0.34 (p = 0.136)	−0.14 (p = 0.560)	−0.01 (p = 0.947)	0.12 (p = 0.594)	−0.19 (p = 0.408)	1.00
(Urine)	*0.11 (p = 0.647)*	*0.09 (p = 0.702)*	*0.01 (p = 0.976)*	*−0.15 (p = 0.527)*	*−0.28 (p = 0.229)*	*−0.23 (p = 0.330)*	*−0.03 (p = 0.887)*	*−0.32 (p = 0.167)*	*−0.34 (p = 0.145)*	*−0.42 (p = 0.067)*	*1.00*

Note: Normal style – male, italic – female; bold – statistically significant; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; TChol – total cholesterol; HDL – high-density lipoproteins; LDL – low-density lipoproteins; TG – triglycerides.

Table 3
Statistical assessment of the differences of median concentration of selenium according to the type of patient and sex by the use of *U* Mann–Whitney's test.

Analyte (body fluid)	Sex	Rank sum (obese)	Rank sum (control group)	*U*	*Z*	*p*
Se (serum)	Male	216.0	604.0	6.0	*−5.2*[a]	*<0.0001*
Se (urine)		210.0	610.0	0.0	*−5.4*	*<0.0001*
Se (serum)	Female	213.0	607.0	3.0	*−5.3*	*<0.0001*
Se (urine)		221.0	599.0	11.0	*−5.1*	*<0.0001*

[a] Italic style – statistically significantly differences.

reflection of recent exposure) may be also associated with different geographical regions, various diets, and – as it is shown in our studies – with age, gender, and eventually with obesity. According to our knowledge, the data about the relationship between the intensity of exposure and selenium concentration in urine are limited. Data about the mean urinary Se concentrations in individuals in most regions in Europe were found to be lower than 30–40 μg/l [23]. It was reported that the urinary selenium concentration range in healthy German children aged 2–17 ($n=72$) varies between 4 and 39 μg/l [24]. Our studies show higher selenium excretion in healthy children, however cited data are quite similar for obese children. We suggest that obesity results in decreased urinary Se concentrations (lower intake, lower serum content). It was reported that increased muscle activity together with an increased energy consumption may increase the rate of selenium excretion in urine [25]. In spite of this report to the contrary, our findings do not support it. Although increased food consumption correlates obviously with obesity it is difficult to find any associations with an increased muscle activity in obese children.

Recently, numerous controversial results have been reported for selenium content in patients with different pathologies (cancer, chronic and alcoholic liver diseases, etc.). Conflicting results including inverse, null and direct associations have been reported for some cancer types in relation to body selenium levels [16,26,27]. The complexity of the issues concerning selenium determination in human studies is made up of many factors. Dietary selenium intake and dietary reference intake varies substantially across populations. Regardless of the sample type used (serum, plasma, toenail, hair, etc.) and method, in studies done across large populations, significant differences arise in the determined selenium levels even for healthy individuals [28]. Caution must be used when talking about Se deficiency with respect to epidemiological, nutritional, toxicological and general public health studies. For example, a few randomized controlled trials in adults have evaluated the influence of Se status on lipid profile. In some studies Se supplementation was associated with modest reductions in total cholesterol, and at higher doses with an increase of HDL cholesterol level, although it has been emphasized that Se supplementation may have other side effects, and that the clinical significance of the changes in lipid profile are still unknown [29]. Some cross-sectional studies report strong, positive associations between Se in serum and lipids in adult populations [30]. A cross-sectional analysis carried out on more than one thousand adults from the National Health and Nutrition Examination Survey (NHANES, 2003–2004) indicated an association between serum Se with increasing HDL cholesterol (only at low Se levels), with LDL cholesterol, and total cholesterol, whereas TG–Se relationship was U-shaped [31]. Taking into account that contents of Se are age-related, the observed associations could be different among children.

Additionally, obesity is related to a number of other serious health conditions, like hypertension, cardiovascular disease, and diabetes. Recent investigations concerning Se status and risk for diabetes prove the complex nature of selenium. Some published findings suggest that Se possess antidiabetic properties [32,33]. Other human studies, on the contrary, suggest that an increased

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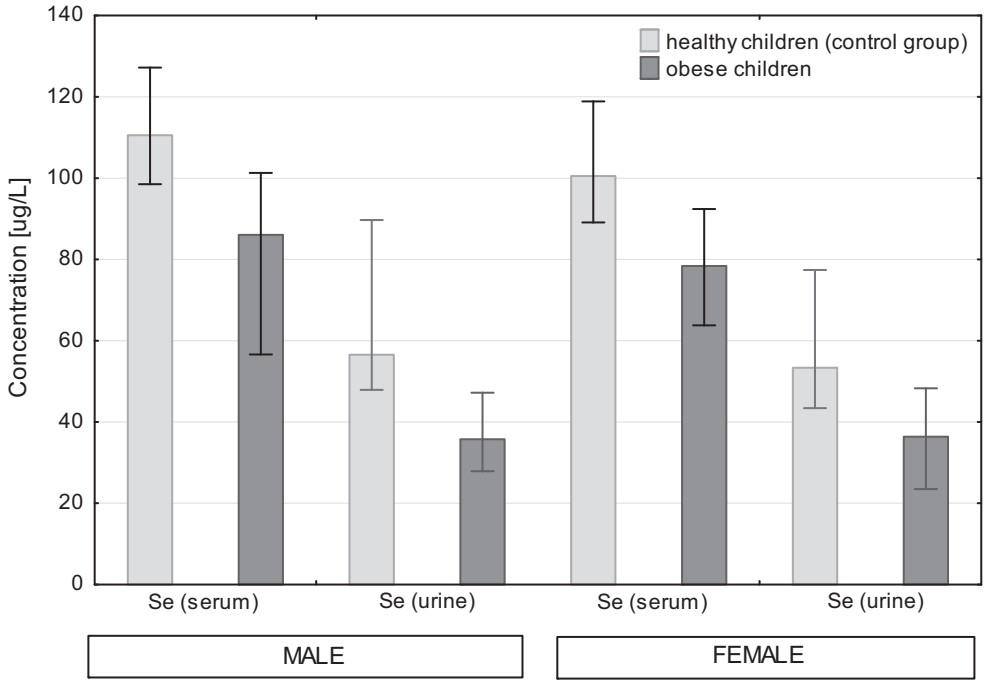

Fig. 1. Median and range of Se concentration in serum and urine according to sex and health status of the patients.

risk of type 2 diabetes can be correlated with high selenium status or intake [34–36]. There is increasing evidence that even mild blood pressure elevation can have adverse effects on vascular structure and function in children [37]. The role of Se in the development of hypertension has been examined both in animals [38], and in humans [39]. While the animal studies cited above [38] indicate a clear association between high dietary Se and lower levels of cardiac oxidative damage and increased antioxidant expression, as well as a reduction in disease severity and mortality in spontaneously hypertensive rats; the human studies conducted on a large adult population [39] report a higher prevalence of hypertension in relation to higher serum selenium. Unfortunately the mechanism explaining these effects is not clear enough. It is further proof that the potential metabolic side effects of Se supplementation must be given more consideration. Arnaud et al. [40] performed a 9-year study in an elderly population to determine possible relationships between cardiovascular-related risk factors and plasma selenium status. Their data indicated that obesity, age and cardiovascular disease significantly increased the longitudinal decline in plasma selenium. Our study indicates the significant correlation between urinary Se and obesity. At present, urine and toenail samples are seeing an increase in use in epidemiological studies due to the ease of obtaining them and their non-invasiveness. Some studies report that trace metals in urine and toenail seems to be poor predictors of BMI [41]. Moreover, no association between toenail Se concentration and age, cholesterol, BMI, hypertension and diabetes was found in a case–control study performed across 10 centres from Europe and Israel in 1991–1992 [42].

The cross-sectional study design did not allow us to specify the causality of all the observed associations. Nevertheless, we are able to indicate associations that may exist and are therefore useful in understanding aetiology, healthcare planning, and in the

Table 4
Univariate and multivariate analysis of risk factors in obese children.

Predictor	Mediana (range)		OR (95%CI)	*p* value
	Obese	Control		
Univariate analysis				
Age	13 (3–18)	13 (3–18)	1.01 (0.88–1.16)	0.92
Blood pressure (mmHg): systolic	120 (90–153)	115 (90–130)	1.07 (1.02–1.12)	*0.004*
Diastolic	80 (55–95)	76.5 (60–90)	1.03 (0.98–1.08)	0.34
Glucose (mg/dL)	90.05 (71.9–118.6)	75.3 (69.8–96.0)	1.29 (1.16–1.44)	*<0.0001*
Total cholesterol (mg/dL)	174.5 (142–258)	153.0 (118–200.5)	1.08 (1.04–1.12)	*<0.0001*
LDL (mg/dL)	116.5 (71.2–155.3)	95.1 (16.5–186.2)	1.02 (1.00–1.04)	*0.016*
HDL (mg/dL)	45.8 (23.3–90.7)	56.3 (40.1–79.3)	0.95 (0.91–0.99)	*0.011*
TG (mg/dL)	96.0 (45.0–290.0)	81.0 (59.0–131.0)	1.03 (1.01–1.05)	*0.003*
Se in serum (μg/L)	84.1 (56.6–101.3)	104.9 (89.1–127.2)	0.63 (0.49–0.80)	*0.0001*
Se in urine (μg/L)	36.2 (23.5–48.3)	54.4 (43.4–89.7)	0.55 (0.39–0.78)	*0.001*
Multivariate analysis				
Se in serum (μg/L)		Se (serum)	0.74 (0.62–0.88)	*0.001*
		Cholesterol total (mg/dL)	1.19 (1.08–1.31)	*0.001*
Se in urine (μg/L)	Model 1	Se (urine)	0.60 (0.43–0.83)	*0.002*
		Cholesterol total (mg/dL)	1.16 (1.05–1.28)	*0.003*
	Model 2	Se (urine)	0.70 (0.58–0.84)	*<0.0001*
		Glucose (mg/dL)	1.22 (1.10–1.35)	*<0.0001*

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generation of research hypotheses regarding obesity problems. We are also aware of the following limitations: we didn't estimate dietary intake of Se – an indicator of Se exposure. Although, since Se urinary excretion is closely correlated with serum Se, and according to literature [43] urinary Se is approx. 50–60% of the total amount excreted – after appropriate multiplication urinary Se could be used to monitor recent dietary intake. We also realize that there is a need of a speciation analysis in relation to Se determination in biological fluids, since various chemical species can indicate different biological effects and both epidemiological and experimental studies have shown different toxic effects of various inorganic and organic Se compounds [44]. Unfortunately some sample pre-treatment procedures interfere with speciation studies, and therefore the final determination refers to "total" Se concentration in serum or urine. A distinct but equally important limitation is the sample size (80 subjects). However, from the results we can conclude that the concentrations of Se (both in serum and in urine) are connected with the risk of obesity (Table 4). On the other hand, our studies have important strengths, e.g. we present the associations between serum and urinary Se after multivariable (age, gender, BMI, lipid profile, glucose, blood pressure) adjustment, children in our study did not take any dietary supplements and medications (e.g. lipid lowering) that can impact the results, all samples were collected in fasting state, and both groups of children (obese and non-obese) were matched in terms of place of residence, age and sex. Parental smoking, that could have an impact on results, was excluded from our studies.

Conclusions

Our findings indicate that obese children, regardless of gender, had statistically significant lower serum and urine Se concentrations in comparison with healthy children. Since literature supports the notion that proper Se levels in certain disease states provide better outcomes, the findings of lower Se levels in obesity may hold some significance. On the other hand caution must be used since numerous controversial results regarding associations between selenium and different pathologies have not yet been fully explained and studies on the metabolic effects of selenium are still incomplete. Among the host of other complications that are associated with obesity, it appears that children with obesity may be also an at-risk group for Se deficiency. It may prove to be prudent to keep this issue in mind when addressing the needs of obese patients.

Conflict of interest

All authors declare that they have no conflict of interest.

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