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Trace metal levels in serum and urine of a population in southern Brazil

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ABSTRACT

This study aimed to evaluate serum and urine concentrations of several trace metals of a non-directly exposed population in southern Brazil and establish reference values. Serum and urine samples were obtained from 240 volunteers (175 males and 65 females, age ranging from 18 to 74 years old). Levels of arsenic, chromium, cobalt, copper, lead, nickel, manganese and zinc were determined by means of dynamic reaction cell inductively coupled plasma mass spectrometry (DRC-ICP-MS). Comparison between genders resulted in no significant difference for all metals but serum copper, as concentrations are higher in females than males. For most metals assessed, a negative correlation between serum concentrations and age was found, but no significant correlation was found between urine concentrations and age.

1. Introduction

Many metals are vital to human health in trace amounts, but can be harmful in greater levels. Other metals, including some metalloids and most heavy metals, have no role in human physiology and can cause intoxications. Everyday exposure to pollution and ingestion of contaminated food/water can cause accumulation of such metals in the body; chronic exposure to metals can lead to impairment of human health, being a serious concern for health agencies [1]. Large scale studies have been conducted in different countries in order to establish reference values for trace metal concentrations of different populations, which serve as a basis for further exposure assessments and other toxicological studies [2,3]. While many such surveys have been conducted worldwide, Brazil falls short on the amount of studies attempting the same.

Surveillance of trace metal concentrations by means of biomonitoring is effective in assessing the exposure profile of a specific population, and different analytical techniques can be used. While flame atomic absorption spectroscopy (FAAS) has been the analytical technique of choice for trace metal determination for quite some time, nowadays more powerful methods are being employed. One such method refers to inductively coupled plasma mass spectrometry (DRC-ICP-MS), a much more robust and sensitive technique than FAAS, which has been extensively used in the last years for analysis of trace metals. DRC-ICP-MS not only has better performance, but it also provides consistent results in different matrices, be them serum, plasma, whole blood or urine [4,5].

This work aimed to evaluate trace concentrations of several metals of a population in southern Brazil by means of a simple "dilute and shoot" procedure using DRC-ICP-MS, in order to determine reference values in different matrices.

2. Material and methods

The studied population was selected from a local blood center in the city of Maringá, located in northwestern Paraná, one of Brazil's southern states. Samples were collected within a 6 month period (January–June, 2013). Blood donors were briefed about the study and those that agreed on participating were further interviewed

Abbreviations: FAAS, flame atomic absorption spectroscopy; DRC-ICP-MS, dynamic reaction cell inductively coupled plasma mass spectrometry.

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Table 1
Serum trace metal reference levels.

Metal analyzed	GM	95% CI GM	P25	P50	P75	P95
Arsenic						
Male	1.153	[1.093, 1.217]	0.981	1.134	1.592	1.904
Female	1.195	[1.099, 1.299]	1.014	1.206	1.598	1.85
Cobalt						
Male	0.1501	[0.1360, 0.1657]	0.1123	0.164	0.239	0.336
Female	0.1584	[0.1348, 0.1861]	0.1115	0.17	0.253	0.496
Copper						
Male	891.4	[855.5, 928.8]	735.7	909.7*	1070	1419
Female	1401	[1270,1545]	1027	1493*	1814	2801
Chromium						
Male	1.951	[1.866, 2.041]	1.702	1.96	2.471	3.005
Female	1.965	[1.831, 2.108]	1.625	1.866	2.555	2.998
Manganese						
Male	0.5295	[0.4759, 0.5892]	0.396	0.584	0.817	1.237
Female	0.5593	[0.4882, 0.6407]	0.3675	0.646	0.837	1.199
Nickel						
Male	0.6993	[0.6084, 0.8037]	0.4075	0.722	1.55	2.069
Female	0.8378	[0.6680, 1.051]	0.3838	0.982	1.75	2.526
Zinc						
Male	738.9	[708.5, 770.7]	630.9	741.7	845.2	1228
Female	700.9	[649.1, 756.7]	600.4	721.5	809.3	1002

Serum values are represented as μg/L.
* Statistically different according to Mann–Whitney *U* test when compared to its gender counter part, assuming $p < 0.05$.

so that data concerning age, sex, occupation and habits that might influence metal concentrations could be gathered. All volunteers signed an informed consent form in accordance with the Brazilian Ethics Committee (registered under the number 0375.0.093.000-11). A total of 240 volunteers participated in this study. The selected blood donors consisted only of adults of age ranging from 18 to 74 years old; 175 were male and 65 were female. A small amount of subjects were smokers and thus were not included in the study, as it is known that cigarette consumption does affect metal levels in body fluids [6]; also, the small number of smokers would not amount a sample group of sufficient size to allow proper analysis.

Blood and urine samples were obtained from each subject. Blood samples were collected by standard venous puncture and urine samples were collected by the volunteers themselves while they were urinating. Both types of samples were collected and stored in metal-free glass collection recipients that had been previously decontaminated. Decontaminating was carried out with all recipients washed with running water and bathed in a 10% nitric acid solution for 48 h; later, recipients were again washed in ultrapure MilliQ water and dried out at 40 °C. Finally, all recipients were stored until moment of use without making any contact with metallic surfaces or any kind of dust. Following blood collection, samples were centrifuged at 10,000 rpm for 10 min in order to obtain serum from each sample, which were then stored at −20 °C. Since samples would later be screened for metals in a different laboratory, storage of whole blood was not possible, and therefore serum was preferred as a matrix. Urine samples underwent no treatment after collection, and were stored at −20 °C until moment of analysis as well.

Serum and urine samples were fractioned into three different tubes at a volume of 100 μL and sent to colleagues at the Laboratory of Metal Essentiality and Toxicology at the University of São Paulo (Ribeirão Preto, Brazil). Prior to sample analysis, all samples (urine and serum) were diluted 1 + 19 in a solution containing 0.5% of sub-distilled HNO_3 (DST-100, Savillex, USA) and 0.005% (v/v) of Triton® X-100 (Sigma, USA) and analyzed according to Batista et al. [7] for serum and Batista et al. [8] for urine. The quality control of all analyzed samples was performed by using standard reference materials from the National Institute of Public Health from Canada (ICP04U04 for urine) and from the New York Department of Health from USA (NYSDOH 05S07 for serum). Certified standard reference materials were analyzed in each batch and were in agreement with reference values. The following isotopes were analyzed: ^{75}As (reaction gas H_2/Ar 5/95% used for urine during analysis), ^{60}Ni, ^{59}Co, ^{55}Mn, ^{64}Zn, ^{63}Cu and ^{52}Cr (NH_3 99.999% used for serum during analysis). Rhodium at 10 ng ml^{-1} was used as internal standard. All analyses took place in the period of June–July, 2013.

Data were analyzed using the software GraphPad Prism 5.0®. Mann–Whitney tests were conducted in order to compare metal levels by sex (only creatinine corrected results were analyzed this manner) and Spearman correlation coefficients were determined to assess how metal concentrations correlated with age. Results were considered significant assuming $p < 0.05$.

3. Results

Overall metal values (Tables 1–3) did not follow a normal distribution as determined by D'Agostino and Pearson normality test; proposed reference values for each metal analyzed are presented as percentiles and geometric means with their respective 95% upper and lower confidence intervals.

Results for serum and urine metal levels found for male and female subjects are shown in the following order: arsenic, cobalt, copper, chromium, manganese, nickel and zinc. Male serum concentrations (μg/L): 1.153; 0.1501; 891.4; 1.951; 0.5295; 0.6993 and 738.9. Female serum concentrations (μg/L): 1.195; 0.1584; 1401.0; 1.965; 0.5593; 0.8378 and 700.9. Male urine concentrations (μg/L): 15.17; 0.2563; 29.69; 2.936; 1.160; 1.996; 278.9. Female urine concentrations (μg/L): 11.74; 0.2593; 27.97; 2.143; 1.169; 1.654; 193.6. Male urine concentrations (μg/g creatinine): 12.48; 0.2047; 24.53; 2.217; 0.9352; 1.523 and 228.9 μg/g creatinine. Female urine concentrations (μg/g creatinine): 11.34; 0.2505; 27.01; 1.990; 1.104; 1.475 and 186.9 μg/g creatinine.

Mann–Whitney tests comparing results between genders resulted in no significant statistical difference for all metals but serum copper, as concentrations are higher in females than males.

Spearman's test correlating age and metal concentrations showed a negative correlation between serum concentrations and age for most metals assessed, with the exception of nickel and zinc

Table 2
Urine trace metal reference levels (as observed).

Metal analyzed	GM	95% CI GM	P25	P50	P75	P95
Arsenic						
Male	15.17	[13.90, 16.55]	11.80	16.02	20.93	40.20
Female	11.74	[10.13, 13.62]	9.530	12.00	16.02	25.73
Cobalt						
Male	0.2563	[0.2273, 0.2890]	0.1800	0.2800	0.4000	0.7700
Female	0.2593	[0.2022, 0.3327]	0.1300	0.2850	0.4725	1.531
Copper						
Male	29.69	[28.41, 31.04]	26.51	31.38	34.87	44.56
Female	27.97	[25.63, 30.53]	21.51	29.90	36.18	48.02
Chromium						
Male	2.936	[2.569, 3.355]	1.860	3.200	5.150	9.757
Female	2.143	[1.683, 2.728]	1.570	2.990	3.990	6.224
Manganese						
Male	1.160	[1.082, 1.244]	0.8400	1.080	1.585	2.573
Female	1.169	[1.031, 1.327]	0.7850	1.060	1.790	3.095
Nickel						
Male	1.996	[1.682, 2.369]	1.198	2.280	4.095	8.165
Female	1.654	[1.172, 2.336]	0.5100	2.120	4.510	7.096
Zinc						
Male	278.9	[251.5, 309.4]	171.6	291.8	466.6	827.8
Female	193.6	[161.1, 232.7]	125.0	195.7	291.8	701.6

Urine values are represented as μg/L.

Table 3
Urine trace metal reference levels (creatinine corrected).

Metal analyzed	GM	95% CI GM	P25	P50	P75	P95
Arsenic						
Male	12.48	[11.39, 13.67]	8.492	13.57	19.18	29.74
Female	11.34	[9.716, 13.23]	8.176	11.58	17.46	27.05
Cobalt						
Male	0.2047	[0.1806, 0.2321]	0.1452	0.2151	0.3454	0.6318
Female	0.2505	[0.2000, 0.3137]	0.1583	0.2438	0.4011	1.196
Copper						
Male	24.53	[22.80, 26.39]	17.45	25.32	35.78	53.79
Female	27.01	[24.13, 30.23]	19.55	27.54	36.53	59.29
Chromium						
Male	2.217	[1.971, 2.494]	1.549	2.399	3.482	6.109
Female	1.99	[1.606, 2.466]	1.783	2.347	3.431	5.19
Manganese						
Male	0.9352	[0.8524, 1.026]	0.5994	0.9028	1.37	3.15
Female	1.104	[0.9626, 1.267]	0.7666	1.009	1.618	3.611
Nickel						
Male	1.523	[1.294, 1.793]	0.8131	1.757	2.928	5.313
Female	1.475	[1.049, 2.074]	0.5784	1.967	3.876	6.632
Zinc						
Male	228.9	[207.1, 253.0]	152.1	239.2	367.6	702.1
Female	186.9	[154.9, 225.5]	113	224.5	307.7	521.5

Urine values are represented as μg/g creatinine.

in females. No significant correlation between urine concentrations and age for most metals assessed was observed, cobalt and manganese being exceptions (Table 4).

4. Discussion

The values found in the present study corroborate to some extension with the values other authors report in similar exposure assessment studies. It must be noted, however, that populations in different areas worldwide show different environmental exposure profiles and different dietary patterns, which should explain the discrepancies of values reported between different areas. This is evidenced in several different studies conducted worldwide, as metal levels in serum and urine are similar to the values presented in this study in some cases, but can also be lower or higher, depending on the area assessed. For example, studies report a very wide range for arsenic in urine, which goes from values as low as 1.29 μg/g creatinine found in healthy Spanish individuals to 19.0 μg/g creatinine in Canadian subjects [9,10]. In other cases, the discrepancy is much more evident; a study performed in the United Kingdom reported a chromium blood concentration of 0.19 μg/L, almost 10-fold lower than the values found in the present study [11]. It must be noted, however, that DRC-ICP-MS has limitations when used to analyze some trace metals such as chromium; a Japanese study carried out with inductively coupled plasma sector field mass spectrometry (ICP-SF-MS), a technique

Table 4
Serum and urine correlation coefficients between age and gender.

Metal analyzed	Spearman coefficient	
	Serum	Urine
Arsenic		
Male	−0.2246*	−0.07339
Female	−0.3024*	−0.07771
Cobalt		
Male	−0.2374*	−0.2126*
Female	−0.2658*	−0.08943
Copper		
Male	−0.1994*	0.03993
Female	−0.4582*	−0.02219
Chromium		
Male	−0.2332*	−0.1462
Female	−0.2593*	−0.04163
Manganese		
Male	−0.3199*	0.1919*
Female	−0.248*	0.2062
Nickel		
Male	−0.1808*	−0.1307
Female	−0.2503	−0.0786
Zinc		
Male	−0.3135*	0.01896
Female	−0.00254	−0.02116

Spearman's coefficient was determined assuming age as the independent variable and metal concentration as the dependent variable.
* Statistically significant correlation, assuming $p < 0.05$.

with higher sensitivity, reported urine levels of chromium of a non-exposed population of 0.55 μg/L, almost 4-fold lower than the values reported in this study, showing that low concentrations of chromium can be overestimated when assessed by DRC-ICP-MS [12,13]. Finally, Brazil unfortunately lacks other exposure assessment studies, making it difficult to compare the present results with a local population. While there are a few studies attempting to measure lead in Brazil (due to more urgent toxicological concerns rather than population profiling), there are only a couple that report on other metals [14,15].

Serum and urine metal levels were found to be similar for all metals assessed when genders were compared, save serum copper. When comparing both genders there could be found a significant difference, female serum copper concentrations being higher than those found in males. Similar patterns are often reported in different studies conducted in different areas of the world; in the present study, women show copper serum levels almost 2-fold higher than men, and while other studies show that such difference can be as low as 1.2-fold higher, copper serum levels tend to always be higher in women than in men [16–19]. Such difference is expected, however, as it is well-known that women, especially those in the age range of 20–60, have increased copper absorption and also higher levels of serum ceruloplasmin, a copper-binding protein. Estrogens, even more so when contraceptives are taken, also directly influence copper metabolism, contributing to increased plasma levels of this metal. The effects estrogens have on copper levels are also more evident in pregnant women, as they tend to show even higher copper levels in comparison to non-pregnant women [17,20–22].

As for metals and age, there is a trend for metal levels to decrease with aging, as their concentrations diminish in a significant manner in most cases, and even when there is no statistical significance, there is still a trend for decrease. The sole exception to this pattern is seen in manganese in urine for both male and female subjects and in zinc in urine only for male subjects, and even then, there is only significance for manganese in male subjects.

It could be possible that exposure to metals in food and environment decreases as people age. It is known that metal availability does reduce in aging men and women, as nourishment usually tends to decrease in quality, and also due to decreased intestinal absorption. This is especially true for functional metals such as copper, manganese and zinc [23]. A study performed with more than 10,000 participants concluded that arsenic correlates negatively with aging, and the authors also verified a decreased ability of older subjects to methylate arsenic; arsenic methylation is a known means for detoxification and excretion, and these results corroborate the findings shown in this study [24]. A recent study performed in Pakistan reported decreased concentrations of copper, nickel, chromium and cobalt in younger subjects in both blood and urine, and the authors also attributed the results to dietary patterns and gastrointestinal absorption [25]. Other authors also report a decrease of metal levels in aging populations in comparison with middle-aged adults [13,26,27], but the opposite is also reported by other authors working with different populations, which implies that the increase in manganese levels in urine in male subjects found in the present study is nothing more than a characteristic of the studied population [18,28,29].

5. Conclusion

In the present study, samples from healthy volunteers in southern Brazil were screened for different metals. It could be detected that with the exception for serum copper, sex poses no influence on the reference values for the studied population. It could also be inferred that as the population ages, overall serum and urine levels for all metals tend to decrease, with the exception of manganese in male subjects. The present study aims to fill a gap in the scientific literature regarding reference values for trace metals, as not many studies of similar scope have been performed in Brazil in the past few years, which could aid future researchers attempting to perform any sort of metal related study.

Conflicts of interest

The authors declare that they have no conflict of interest.

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