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Italy

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Trace elements in full-term neonate hair.

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Proton Induced X-ray Emission (PIXE) was employed to measure simultaneously the concentration of 12 trace elements in the hair of 141 AGA newborn infants at term. Log-normal distributions were measured for all elements. There were no significant differences in trace elements in relation to sex, gestational age and body weight. The geometric means (mg/kg) were: Cr, 1.5 +/- 0.2; Mn, 1.5 +/- 0.1; Fe, 51 +/- 4; Ni, 1.0 +/- 0.2; Cu, 6.1 +/- 0.3; Zn, 133 +/- 3; As, 0.055 +/- 0.005; Se 0.81 +/- 0.05; Br, 1.3 +/- 0.1; Pb, 1.4 +/- 0.2; Rb, 0.22 +/- 0.03; and Sr, 1.5 +/- 0.2. Some direct and inverse partial correlations among elements were found at different levels of significance. Each element was significantly correlated with at least one other. Zinc and copper concentrations were lower compared to data in the literature. The high values of chromium and selenium concentrations were in agreement with previous findings. The manganese level was in accordance with previous data. The Zn/Cu ratio agreed very well with the data in the literature. This indicates that while concentration values may be subjected to large variations due to living conditions, correlations could be more stable and therefore offer insight on the regulatory mechanisms governing trace element metabolism in man.

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141 newborn Italy (Napoli)

mg/kg		PPM	
Cr	1.5	Cr	0.15
Mn	1.5	Mn	0.15
Fe	51	Fe	5.1
Ni	1.0	Ni	0.1
Cu	6.1	Cu	61
Zn	133	Zn	
As	0.055		
Se	0.81		
Pb	1.4		
Rb	0.22		
Sr	1.5		

Hair median Fe 67.3
Some smoker-wine (<5-378)
diet oil Se 0.75
(<0.1-1.83)
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Levels of Some Trace Elements in Selected Autopsy Organs, and in Hair and Blood Samples from Adult Subjects of the Italian Population

Dead?
51-79 years

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ABSTRACT

This study, which is part of a research program for the determination of trace element reference levels in various human tissues for the Italian population, presents the concentrations of Se, Hg, Cr, Cs, Sc, Rb, Zn, Fe, Co, and Sb in lung, liver, spleen, and kidney autopsy samples taken from 14 adult subjects of the Italian population who died from accidental causes. Concentrations of the same trace elements are given also for blood and hair samples taken from subjects of the general Italian population and of a population group living in a small coastal town that has an average annual fish consumption well above the national average. The analytical method used was Instrumental Neutron Activation Analysis. The levels and distribution of the trace elements in the various human organs examined are analyzed and discussed.

Index Entries: Trace elements; human tissues; neutron activation analysis.

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INTRODUCTION

A great number of research studies carried out worldwide during the past few decades have demonstrated the important role of many trace elements in various biochemical processes (*1,2*). It has been shown that the normal growth of living organisms depends on the continuous and adequate supply of certain trace elements. The beneficial biological effect of an element depends on its concentration in the organism, and with increasing concentrations even essential trace elements may give rise to detrimental effects. Knowledge of the normal levels in human tissues and body fluids is indispensable to detect any alteration resulting from inadequate intake of trace elements through the diet, disease conditions, or environmental pollution.

A research program to establish the reference range of trace element concentrations in various human tissues for the Italian general population, and to study the transfer pathways of trace elements from the environment to humans, with particular attention to the food chain, was started in our laboratory in collaboration with the Institute of Legal Medicine of Rome University and the National Institute of Nutrition.

This article presents the levels of Se, Hg, Cr, Cs, Sc, Rb, Zn, Fe, Co, and Sb in lung, liver, spleen, and kidney autopsy samples taken from 14 adult subjects of the Italian population who died from accidental causes. Concentrations of the same trace elements are given also for blood and hair samples taken from subjects of the general Italian population and of a population group living in a small coastal town that has an average annual fish consumption of 27.1 kg, well above the value of 12.5 kg for the Italian population (*3*).

MATERIALS AND METHODS

The human autopsy samples were provided by the Institute of Legal Medicine of Rome University. A standard procedure was followed to remove a representative sample of about 200–300 g from each organ. The samples, after being squeezed and drained to remove most of the blood present in the organs, were enclosed in clean glass containers and kept in a freezer at −40°C until they were freeze-dried. After homogenization, the samples were stored in polyethylene containers. Blood samples were collected with plastic syringes and immediately after enclosed in pure quartz vials. The hair samples were washed, following the IAEA procedure (*4*), with distilled water and acetone.

The analytical method used for the determination of the trace element concentrations was Instrumental Neutron Activation Analysis. The samples and standard reference materials (IAEA and NBS), enclosed in pure quartz vials, were irradiated in the 1 mW TRIGA reactor located in the Casaccia research center for about 14 h in a thermal-neutron flux of approx $2.6 \cdot 10^{12} n \cdot cm^{-2} \cdot sec^{-1}$. The continuous rotation of the irradia-

tion facility ensured a uniform neutron flux for all the samples. After a 2–4 wk cooling time, the samples were measured by γ spectrometry, using a high-purity germanium detector with a relative efficiency of about 20% and a resolution (FWHM) of 1.9 kEv at the 1332 kEv peak. The Camberra computer program Spectran F was used for the analysis of the γ spectra.

RESULTS AND DISCUSSION

Human Autopsy Samples

The samples analyzed in this study were taken from 14 adult subjects, 10 men and four women of the Italian general population, who died from accidental causes. Except for two young subjects, 20 and 25 years old, the ages ranged from 51–79 yr.

Tables 1, 2, 3, and 4 summarize the data of the trace element concentrations in lung, liver, spleen, and kidney, respectively. These organs were selected because, in general, they act as accumulation sites for most of the trace elements. The range of values reported in the literature, when available, is also shown in the same Table for each trace element.

Concise comments on the levels of trace elements measured in the four organs examined are now reported for each element:

Selenium

The intersubject variability is about a factor of 3–4 for all the four organs. This element appears to accumulate more in the kidney, in agreement with the data reported by Iyengar et al. (5). The lung presents the lowest concentration. The ratio between the mean concentrations in the kidney and lung is approx 2.5.

Mercury

The intersubject variability is very high: it is about a factor of 75 for the kidney and 218 for the lung. The analysis of the parameters that generally are considered important in determining the mercury body burden (occupation, dietary habits, age, sex) does not explain the high variability observed. This is probably because of the small number of subjects examined. Also for this element the kidney is the principal organ of accumulation, as reported by other authors (6). The spleen shows the lowest concentrations, the mean value in this organ being a factor 9 less than in the kidney.

Chromium

Chromium concentration is in most samples below the detection limit of 100 ng/g. The high values observed in the lungs of two subjects could be the result of exposure to environmental pollution.

Table 1
Trace Element Concentrations in Lung (Dry Wt)

Element	N[a]	Mean ± SD	Range	Reported range, *5, 7, 9, 10*
Co (ng/g)	12	116 ± 53	25–212	57–3600
Cs (μg/g)	12	0.5 ± 0.4	0.11–1.8	0.12
Fe (mg/g)	12	1.7 ± 1.4	0.4–5.6	0.5–1.3
Hg (ng/g)	12	373 ± 996	16–3532	<300–8800
Rb (μg/g)	12	50 ± 20	16.2–86.6	28.6
Sb (ng/g)	11	82.4 ± 59.6	<2–233	33–749
Sc (ng/g)	12	7.1 ± 5.6	1.2–17	
Se (μg/g)	12	0.7 ± 0.3	0.40–1.22	0.38–1.1
Zn (μg/g)	12	74.2 ± 75.6	38.0–311.6	19–70.5

[a]Number of samples above detection limit.

Table 2
Trace Element Concentrations in Liver (Dry Wt)

Element	N[a]	Mean ± SD	Range	Reported range, *5, 7, 9, 10*
Co (ng/g)	13	129 ± 52	32–215	59–2790
Cs (μg/g)	13	0.6 ± 0.6	0.06–2.3	0.05
Fe (mg/g)	13	1.2 ± 1.0	0.3–3.8	0.03–3.7
Hg (ng/g)	13	319 ± 294	5.9–951	14–1843
Rb (μg/g)	13	55.8 ± 20	12.9–92.1	37.6
Sb (ng/g)	9	32.5 ± 48	<2–155	10–408
Sc (ng/g)	10	2.0 ± 1.2	<1–6.4	
Se (μg/g)	13	1.1 ± 0.3	0.4–1.8	0.3–2.6
Zn (μg/g)	13	174 ± 146	55.8–608	49–770

[a]Number of samples above detection limit.

Table 3
Trace Element Concentrations in Spleen (Dry Wt)

Element	N[a]	Mean ± SD	Range	Reported range, *5, 7, 9, 10*
Co (ng/g)	13	73.8 ± 68.4	14.6–287	
Cs (μg/g)	13	0.5 ± 0.38	0.06–1.6	0.06
Fe (mg/g)	13	1.8 ± 1.5	0.2–6.3	1.4
Hg (ng/g)	13	116 ± 135	3.6–490	
Rb (μg/g)	13	56.8 ± 18.1	23.5–79.6	30.1
Sb (ng/g)	11	52.9 ± 63.3	<2–232	45–73
Sc (ng/g)	10	2.8 ± 5.6	<1–6.4	
Se (μg/g)	13	0.9 ± 0.4	0.60–2.12	0.17–1.2
Zn (μg/g)	13	76.7 ± 38.5	47.3–199	77.5

[a]Number of samples above detection limit.

Table 4
Trace Element Concentrations in Kidney (Dry Wt)

Element	N[a]	Mean ± SD	Range	Reported range, *5, 7, 9, 10*
Co (ng/g)	12	63.1 ± 34.1	25.2–145	26–811
Cs (μg/g)	12	0.5 ± 0.3	0.07–1.1	0.03
Fe (mg/g)	12	0.3 ± 0.1	0.13–0.43	0.24
Hg (ng/g)	12	1062 ± 1453	69–5218	
Rb (μg/g)	12	36.2 ± 18.0	12.9–75.5	19
Sb (ng/g)	8	25.1 ± 28.6	<2–89.6	19–166
Sc (ng/g)	10	1.3 ± 0.7	<1–2.5	
Se (μg/g)	12	1.9 ± 0.6	0.95–3.28	0.3–3.8
Zn (μg/g)	12	108.6 ± 40.4	43.1–180	152

[a]Number of samples above detection limit.

Cesium

Although the intersubject variability is high, going from a factor of 14 in the kidney to a factor of 40 in the liver, the mean concentrations in the four organs are very similar. This result can be explained by the chemical similarities of this element and potassium, which is distributed rather uniformly in the human body. The high variability is probably the result of the fact that, in general, the amount of cesium present in the diet, and consequently absorbed in the body, depends on the geological characteristics of the area where the subject lives.

Scandium

The concentration of this element in all the examined organs is very low, being in the ppb region. The intersubject variability ranges from a factor of 5 in the liver to a factor of 14 in the lung, which appears to be the main accumulation organ for this element. The mean concentration in the lung is in fact a factor of 6 higher than in the kidney, the organ with the lowest mean concentration. As for chromium, the levels measured in the lung should be correlated to the lifetime exposure to air pollution.

Rubidium

The intersubject variability is contained within a factor of 7. This element, like cesium, although it does not have a definite biological role in the human metabolism, resembles potassium in its metabolic behavior and, as might be expected, its distribution is relatively uniform in the four organs. The body burden generally depends more on the geological characteristics of the area of residence than on dietary habits.

Zinc

The intersubject variability ranges from a factor of 4 in the kidney and spleen to a factor of 8 in the lung. The liver is the main organ of

accumulation, in agreement with the data reported by Iyengar et al. (5). The lung and spleen have mean concentrations that are a factor of 2 lower.

Iron

Although intersubject variability is limited to a factor of 3 in the kidney, it is more than a factor of 10 in the other organs. The spleen and lung, as found in other studies (2), are the organs that present the highest concentration of Fe. The concentration in the kidney is generally a factor of 5 lower. The high levels measured in the lung can be attributed to the substantial contribution from the relatively high amount of residual blood in this organ.

Cobalt

The intersubject variability ranges from a factor of 6 in the kidney to a factor of 20 in the spleen. The observed variability could depend on particular dietary habits of the subjects (like beer consumption). The liver is the main organ of accumulation. A similar mean concentration of Co is present in the lung. The Co levels in the other two organs are only a factor of 2 smaller.

Antimony

The intersubject variability is rather high, ranging from a factor of 7 in the lung to a factor of 90 in the liver. Higher concentrations of this element are observed in the lung. The lowest values are present in the kidney, with a mean concentration value a factor of 3 smaller than in the lung.

In conclusion, for most elements the intersubject variability is rather high, in some cases more than a factor of 10. Hg, Sb, Cr, and Cs are the elements that show the highest intersubject variability, whereas Se is the element with the lowest variability. Nevertheless, the concentration levels of the trace elements in lung, liver, spleen, and kidney found in this work are in general of the same order of magnitude as the values reported in the literature. One exception is cesium, for which higher values were found in this study.

In spite of the intersubject variability observed in the measured concentration levels, it is possible to observe a certain trend in the distribution of some trace elements in the four organs examined. Hg, Sc, and Fe are the elements showing the highest interorgan variability. On the contrary, Cs and Rb are the elements more uniformly distributed in the four organs. The highest concentrations of Cr, Sb, and Sc are observed in the lung, as expected for elements present mostly in air pollution. The liver presents higher concentrations of Zn and Co. Se and Hg accumulate more in the kidney, whereas Fe is more concentrated in the spleen.

Table 5
Trace Element Concentrations in Blood Samples

Element	N[a]	Mean ± SD	Range	Male Subjects Italian population, 12
Co (ng/g)	6	3.3 ± 2.5	1.1–7.8	1.5–4.4
Cs (μg/g)	6	3.4 ± 1.0	1.9–4.4	2.5–56
Fe (mg/g)	6	563 ± 38	506–610	234–616
Hg (ng/g)	6	24 ± 10	15–43	<5–8.6
Rb (μg/g)	6	3.8 ± 0.5	3.0–4.5	1.0–7.0
Sb (ng/g)	3	1.4 ± 1.4	<0.5–3.0	<0.5–1.6
Se (μg/g)	6	178 ± 50	125–267	66–119
Zn (μg/g)	6	6.7 ± 1.6	4.5–8.5	3.9–8.6

[a]Number of samples above detection limit.

Blood and Hair Samples

To verify if dietary habits could explain the high variability of the mercury levels in the autopsy samples, a comparison was made of the concentrations of this and other trace elements in blood and hair samples from subjects of a population group more exposed to the risk of mercury contamination, with the values reported for subjects of the general Italian population. Hair and blood samples were collected from subjects living in a small coastal town where the average annual fish consumption is a factor of 2 higher than the national average.

The mean concentrations and ranges of some trace elements measured in the blood samples are reported in Table 5. The values observed in blood samples collected from other Italian population groups (*12*) are also shown for comparison.

It is clear from the data reported in this Table that the Hg blood levels for the population group examined in this study are much higher than the values found for other Italian population groups. The values measured in this study are of the same order of magnitude as the concentration levels measured in a group of miners exposed occasionally to dust of mercury ore (*8*). Also, the Se concentration is significantly higher than the values measured in other Italian population groups. No significant differences were observed for other elements.

Table 6 shows the mean concentration and range of some trace elements measured in hair samples collected from 90 subjects (57 males and 33 females), with ages ranging from 11–71 yr. The data measured for other Italian population groups are shown for comparison.

The data relative to the subjects for whom both hair and blood samples were collected were analyzed to check if correlations were pre-

Table 6
Trace Element Concentrations in Hair Samples (All Subjects)

Element	N[a]	Mean ± SD	Median	Range	Italian mean values, (*11*)	Reported range, *5, 7, 9, 10*
Co (ng/g)	87	296 ± 320	136.2	<10–1337	130 ± 180	7–2600
Cr (µg/g)	90	2.5 ± 3.2	1.1	0.06–13.6	4.0 ± 12.5	0.02–65
Fe (µg/g)	84	98.2 ± 94	67.3	<5–378	203 ± 530	6–2400
Hg (µg/g)	90	10.4 ± 6.6	8.8	1.4–30	2.4 ± 4.5	0.1–58
Sb (ng/g)	74	155 ± 135	88.1	<10–628	260 ± 680	90–8800
Sc (ng/g)	60	5.4 ± 6.6	1.43	<0.5–28.4	2 ± 1	2–22
Se (µg/g)	86	0.8 ± 0.4	0.75	<0.1–1.83	1.4 ± 3.1	0.64–2.53
Zn (µg/g)	90	185 ± 59	181.3	40.5–376	208 ± 137	26–915

[a]Number of samples above detection limit.

sent between the elemental concentrations in hair and blood. Statistically significant correlations were observed only for Se and Hg, with correlation coefficients $r = 0.91$ and $r = 0.89$, respectively. This result confirms that the subjects studied were exposed to these elements constantly and not just occasionally.

It is evident from an examination of the data reported in Table 6 that the mean Hg hair concentration in the population group having a high fish consumption is much higher than the levels observed in other Italian population groups. Also, for the hair samples, the mercury levels measured in this study are of the same order of magnitude as the values found in the hair of a group of miners occasionally exposed to dust of mercury ore (*8*). The concentrations of the remaining trace elements do not differ significantly from the values measured in other Italian population groups. This result shows that, in subjects not professionally exposed, the hair mercury concentration is strongly correlated to the mercury intake through the diet.

Examining the distribution of the trace element concentrations in all the subjects, it was found that Zn and Se have approximately a normal distribution, the skewness coefficient being 0.6, as expected for essential elements. Hg, Fe, Co, and to a larger extent Cr, Sb, and Sc, present an asymmetrical distribution that is approximately log-normal.

In order to examine if the trace element concentrations vary according to sex, the data have been analyzed separately for male and female subjects. It appears that males have in general higher mean concentrations than women. The difference is statistically significant ($P < 0.05$) for Hg and Cr, although it is not significant for the other elements.

No significant correlations were found between trace elements concentrations and age. The only significant correlation found between elemental pairs was between Cr and Co, with a correlation coefficient $r = 0.85$.

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industrial city

Correlation between blood and hair lead levels in boys and girls of Sardinia (Italy)

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Summary - *The aim of this study was to analyse the correlation between blood lead (PbB) and hair lead (PbH) in Sardinian children. The sample consisted of 330 children (126 boys and 204 girls) from three Sardinian towns with different environmental backgrounds: Portoscuso, Sant'Antioco, and Sestu. The boys of Portoscuso have the highest median value of PbB (10.86 µg/dL), followed by the girls of Portoscuso (7.24 µg/dL); they are followed, but with much lower values, by the boys of Sant'Antioco (4.22 µg/dL) and Sestu (4.06 µg/dL), and lastly by the girls of Sant'Antioco (3.50 µg/dL) and Sestu (3.39 µg/dL). There is a similar pattern for the PbH values: the Portoscuso boys have the highest median value (10.00 µg/g), followed by the Portoscuso girls (7.21 µg/g), Sant'Antioco boys (5.44 µg/g), Sant'Antioco girls (4.69 µg/g) and finally the Sestu boys (3.79 µg/g) and girls (1.56 µg/g). The values of the Bravais-Pearson coefficient of correlation between logPbB and logPbH are statistically significant both for the total sample ($r=0.4351$; $p\leq0.001$) and for the sexes considered separately ($r=0.3989$, $p\leq0.001$, for males; $r=0.3801$, $p\leq0.001$, for females). It should be noted that a high percentage of unexplained variance persists in the total sample (81.07%) and in males (84.09%) and females (85.55%) separately. The pattern among samples with different environmental backgrounds and the significant correlations between the logPbB and logPbH values suggest that hair can be used as a suitable biomarker of lead exposure.*

Keywords - *Lead, Blood, Hair, Boys, Girls, Sardinia.*

Introduction

Hair has been widely used as a biological material for the analysis of metal contents in an organism. Therefore, it is considered to provide information about the degree of exposure to environmental contaminants (Chatt & Katz, 1988; Bergomi *et al.*, 1989; Senofonte *et al.*, 1989; Vienna *et al.*, 1995; Foo & Tan, 1998; Lekouch *et al.*, 1999; Furman & Laleli, 2000; Nowak & Chmielnicka, 2000; Strumylaite *et al.*, 2004).

Nevertheless, there is extensive debate about the limitations of hair as a biomarker of metal exposure (Renshaw, 1976; Ryabukin, 1978; Morton *et al.*, 2002; Barbosa *et al.*, 2005). In fact, the Pb concentration profile has been found to vary significantly among various subpopulations according to age, sex, hair colour and smoking status (Wolfsperger *et al.*, 1994). Moreover, geographical, ethnic and ecological factors can also affect the Pb distribution in hair within a given population. Thus, it is difficult to establish reference ranges because confounding factors impose restrictions on the interpretation of individual results. For example, there is no consensus on the length of the hair specimen

to be collected, or the amount, or the position on the scalp. Variations in Pb content between single hairs from the same individual can be as high as ± 100%, particularly in the distal region (Renshaw, 1976; Barbosa *et al.*, 2005).

Furthermore, it is still debated whether there is good concordance between the levels of metals measured in hair and in biological fluids (Wilhelm & Idel, 1996; Lech, 2002; Sanna *et al.*, 2003; Barbosa *et al.*, 2005).

However, the technique of heavy metals determination in hair has given rise in the last decade to studies in various fields, such as toxicology (Taylor, 1986), medicine (Klevay *et al.*, 1987; Wecker *et al.*, 1985), clinical dietetics (Dorea *et al.*, 1982; Kozielec *et al.*, 1989) and environmental pollution (Wilhelm *et al.*, 1988; Bergomi *et al.*, 1989; Schumacher *et al.*, 1991; Golow & Kwaansa-Ansah, 1994; Revich, 1994; Srivastava & Gupta, 1994; Chlopicka *et al.*, 1995; Olejnik *et al.*, 1997; Nowak, 1998; Sanna *et al.*, 2003; Hasan *et al.*, 2004).

The aim of this study was to evaluate the correlation between lead levels in hair (PbH) and blood (PbB) using data for boys and girls from three Sardinian towns with different environmental backgrounds.

Subjects and methods

Sampling

The investigation was conducted in April and May 1998. The total sample consisted of 330 subjects (126 males and 204 females), 117 from Portoscuso (53 males and 64 females), 108 from Sant'Antioco (47 males and 61 females) and 105 from Sestu (32 males and 73 females). All the children (from 11 to 14 years old) had been resident in their respective municipalities for at least 5 years.

The subjects were children attending the middle schools of the respective towns whose parents consented to the data collection and who did not present characteristics that can alter the blood lead concentration (e.g. smokers, drinkers of alcoholic beverages, etc.). The three subsamples represented 52% of the 11-14-year-old children of Portoscuso, 24% of those of Sant'Antioco and 14% of those of Sestu in 1998.

The calendar age of each subject was converted to decimal age according to Eveleth & Tanner (1990).

Blood lead

Blood samples were obtained by venipuncture; each specimen was heparinized immediately and stored at 6° C. At the appropriate time, the material was analysed on a Perkin-Elmer absorption spectrophotometer, Model 1200 (AAS), equipped with a chart recorder, graphite furnace HGA 400, autosampler A40, and deuterium arc lamp background corrector. Qualitative control of the blood analyses was performed with BCR-194 and BCR-195 standards (certified values of 126 and 416 µg/L, respectively, in bovine reconstituted blood, European Union Community Bureau of References).

Hair lead

Hair samples (total sample weight per individual of about 1 g) were taken from the occipital region (nape). The hair was cut near the scalp and the first 2-5 cm of recent growth were used. Each sample was preserved in a carefully sealed plastic bag, labelled with a progressive number, date of birth, sex and place of residence of the individual. All specimens were stored in a cool, dry, ventilated environment until delivered to the laboratory, where they were kept in desiccators until the analysis (Senofonte *et al.*, 1989). The samples were then treated according to the following procedure (Caroli *et al.*, 1992): 0.5 g of hair was cut into pieces no longer than 1 cm, thoroughly washed with a mixture of ethyl ether and acetone (3:1, v/v) under continuous stirring for 10 min, dried at 85°C for 1 h, and treated with a dilute (5%) aqueous solution of EDTA for 1 h. The pieces were repeatedly rinsed with double-distilled water and then dried at 85°C for 12 h in an oven to determine the dry weight of the sample just before the subsequent step. Hair digestion was based on irradiation with a microwave field at 2.45 GHz (Senofonte *et al.*, 1989; Caroli *et al.*, 1992). The treatment

steps were: overnight predigestion with 10 mL of high-purity concentrated (68%) HNO_3; 30 min stage at microwave power of ca. 180 W; 30 min stage at microwave power of ca. 240 W; 10 min cooling; addition of 1 mL H_2O_2 (30%) and 1 mL HF (50%); a further 30 min stage at microwave power 300 W; a 1 h stage at microwave power 360 W; quantitative transfer into polypropylene tubes; dilution up to 20 mL with double-distilled water. It should be emphasized that standardization of the methods of hair lead determination remains an unresolved problem (Furman & Laleli, 2000) and the washing method adopted to remove surface contaminants may produce large differences in PbH values (Lekouch *et al.*, 1999; Sen & Chaudhuri, 2001). The method used for quantification of Pb consists of inductively coupled plasma atomic emission spectrometry with a Jobin-Yvon 38+ Spectrometer. Qualitative control of the analysis of trace lead levels in hair was performed with the IMS 102 multielement standard of 10 µg/mL, certified for the calibration of the instrument by the Ultra Scientific Society, Bologna, Italy, and with BCR-397 (certified lead value of 33 mg/kg in 10 g of human hair, European Union Community Bureau of Reference).

From time to time, AAS measurements were also performed to check the reliability of the data.

Statistical analyses

The results of the Shapiro-Wilk W test indicated that all samples, with the sexes combined or separate, had a significantly non-normal distribution for PbB and PbH, except for the Portoscuso girls and the Sestu boys whose W test values for PbB were at the limit of significance (respectively: W=0.9559, p=0.0538; W=0.9359, p=0.0694).

Since the PbB and PbH distributions for both the combined and separate samples were non-normal, we calculated the values of the medians by interpolation of the cumulative distribution (Must *et al.*, 1991; Marascuilo & Serlin, 1998; Sanna *et al.*, 2000).

The correlation between PbB and PbH levels was tested with the Bravais-Pearson correlation coefficient using log-transformed values. Statistical analyses were performed with Statistica-Statsoft 6.0.

Portoscuso

The town of Portoscuso in south-western Sardinia (Sulcis-Iglesiente area), with 5,560 residents as of December 31, 1998, has been classified as urban (ISTAT, 1986). Since the 1960s, one of the most important industrial complexes on the island has developed in the area of Portovesme about 2 km from Portoscuso. It includes some factories for the refining and processing of aluminium, lead and zinc (Bodano & Dentoni, 1988; Cardia *et al.*, 1989; Melis & Senette, 1990; Giordano *et al.*, 1993).

The main industrial plants include:

- Euroallumina: production of aluminium oxide for electrolytic uses; average annual production 800,000 tons;
- Alluminia: primary and secondary aluminium products; average annual production 125,000 tons of primary product and 20,000 tons of secondary product;
- Comsal: production of aluminium bars and sheets; average annual production 22,000 tons;
- Portovesme srl (ex Enirisorse): production and marketing of non-ferrous metals and alloys (Pb, Zn, Cd and K_2SO_4);
- a thermoelectric plant with a 240 MW coal-fired group.

Among these plants, Portovesme srl produces the highest amount of lead emissions. This plant was constructed in the early 1970s to process Sardinian lead and zinc minerals (sulphides and oxides). In 1997, 115,490 tons of lead were produced.

Investigations carried out in Portoscuso in different periods have revealed environmental lead pollution: in soil and vegetables (Contu *et al.*, 1986); in wine (Melis & Senette, 1990); in wine, soil, grass, vegetables, milk and cheese

(Giordano *et al.*, 1993). A study conducted in 1997-1998 by the Department of Public Health, University of Cagliari, found that levels of metals (Al, As, Cn, Cu, Ni, Pb, Sb, Se, Zn) in the water supply of Portoscuso and of the Sulcis-Iglesiente area were below detectable levels (DISP, 1999). However, lead levels higher than those of control groups have been reported both in blood (Bodano & Dentoni, 1988; Cardia *et al.*, 1989; Floris *et al.*, 1995; Sanna *et al.*, 1995, 1999, 2000, 2002, 2003) and in hair (Sanna *et al.*, 2003).

Sant'Antioco

Sant'Antioco, a coastal centre on the homonymous island in south-western Sardinia, had 11,868 residents as of December 31, 1998. It has been classified as rural (ISTAT, 1986). Its traditional economy is based on agriculture and fishing. At present, tourism and especially the tertiary sector are also important.

As a result of industrial activities in the nearby industrial area of Portovesme, the towns of Portoscuso, Carbonia, Gonnesa, San Giovanni Suergiu and Sant'Antioco have been included in the zone of Sulcis-Iglesiente, defined as an "area of high risk of environmental crisis" on the basis of the Decree of the Italian Council of Ministers dated November 30, 1990.

Sestu

The town of Sestu, about 10 km from Cagliari (the capital of Sardinia), is situated in the area of the Campidano plain (southern Sardinia). It had 13,998 residents as of December 31, 1998, and has been classified as semi-urban (ISTAT, 1986). Owing to the characteristics of its economy and location, Sestu can be considered unexposed to lead pollution (Floris *et al.*, 1995; Sanna *et al.*, 1995, 1999, 2002, 2003, 2005).

Results

ANOVA showed that the differences in decimal age between the groups are not significant, even when they are subdivided by town and sex ($p \geq 0.05$).

Table 1 reports the mean and median for PbB and PbH in children grouped by town and sex. The boys of Portoscuso have the highest median value of PbB (10.86 µg/dL), followed by the girls of Portoscuso (7.24 µg/dL); they are followed, but with much lower values, by the boys of Sant'Antioco (4.22 µg/dL) and Sestu (4.06 µg/dL), and lastly by the girls of Sant'Antioco (3.50 µg/dL) and Sestu (3.39 µg/dL). There is a similar pattern for the PbH values: the Portoscuso boys have the highest median value (10.00 µg/g), followed by the Portoscuso girls (7.21 µg/g), Sant'Antioco boys (5.44 µg/g), Sant'Antioco girls (4.69 µg/g) and finally the Sestu boys (3.79 µg/g) and girls (1.56 µg/g).

Tab. 1 - Mean, and median of PbB (µg/dL) and PbH (µg/g) of the total sample and the sample divided by sex and town.

		Portoscuso				S. Antioco				Sestu			
Sex	Variable	N	Mean	SD	Median	N	Mean	SD	Median	N	Mean	SD	Median
M	PbB	53	11.30	4.01	10.86	41	4.51	1.72	4.22	32	4.09	1.25	4.06
	PbH		15.51	14.83	10.00		6.71	3.86	5.44		4.03	3.00	3.79
F	PbB	64	7.39	2.17	7.24	67	3.57	1.23	3.50	73	3.34	1.10	3.39
	PbH		8.82	7.01	7.21		4.99	2.80	4.69		2.83	2.24	1.56
M + F	PbB	117	9.16	3.68	8.41	108	3.93	1.50	3.70	105	3.57	1.19	3.57
	PbH		11.85	11.68	8.45		5.64	3.33	5.04		3.19	2.54	2.91

Table 2 reports the values of the Bravais-Pearson coefficient of correlation between decimal age, logPbB and logPbH for the children of the three Sardinian towns, with the sexes combined and separate. The values indicate a significant positive correlation between logPbB and logPbH when the sexes are combined ($r=0.4351$; $p\leq0.001$) and considered separately ($r=0.3989$, $p\leq0.001$, for males; $r=0.3801$, $p\leq0.001$, for females). Instead, the correlations between blood lead and age and between hair lead and age are not significant either with the sexes combined or separate.

Discussion

The Bravais-Pearson coefficient of correlation for the total sample, with the sexes combined and separate, indicates a significant correlation between logPbB and logPbH. These results illustrate the significant degree of correlation between the two variables, although a high percentage of unexplained variance persists in the total sample (81.07%) and in males (84.09%) and females (85.55%) separately (Figures 1-3). The amount of unexplained variance could be due either to the limitations of hair as a biomarker of metal exposure (Renshaw , 1976; Ryabukin, 1978; Seidel *et al.*, 2001; Morton *et al.*, 2002; Barbosa *et al.*, 2005) or to the different sensitivities of the analytical techniques used to determine the lead levels in blood and hair.

It should be noted that when the data for Sestu, the town with the lowest PbB and PbH levels (Table 1), are removed from the sample, the values of the correlation coefficient between logPbB and logPbH are slightly lower for the males and for the sexes combined, with respect to the values for the total sample, whereas they remain virtually the same for the females ($r=0.3305$, $p\leq0.001$, for males; $r=0.3802$, $p\leq0.001$, for females; $r=0.4135$, $p\leq0.001$ for sexes combined). This indicates that the degree of exposure does not affect the correlation between PbB and PbH.

Relatively low, but statistically significant, levels of correlation between PbB and PbH have been reported in Italian children living in Sassuolo (north-central Italy), a town with a high density of potteries. In a sample of 210 Sassuolo children aged 7 years, a significant correlation was found between lead in the blood and lead in the hair: $r=0.125$, $p=0.005$ (Bergomi *et al.*, 1989). Significant correlations, albeit with rather low r values, were also found between PbB and PbH in a sample of 158 Polish children (98 males and 60 females) of Miasteczko Šlaskia in Upper Silesia, Katowice province: total sample, $r=0.270$, $p\leq0.001$; male sample: $r=0.254$, $p\leq0.05$ (Chlopicka *et al.*, 1998). A significant correlation was also found between PbB and PbH in a sample of 189 children of the Russian city of Saratov: $r_s=0.45$, $p\leq0.05$ (Esteban *et al.*, 1999).

Tab. 2 - Bravais-Pearson coefficient of correlation (r) between logPbB, logPbH and age for the children of the three Sardinian towns with the sexes combined and separate.

Sex	Variables	r
M	logPbB vs. logPbH	0.3989**
	logPbB vs. age	0.0678
	logPbH vs. age	-0.1197
F	logPbB vs. logPbH	0.3801**
	logPbB vs. age	-0.0178
	logPbH vs. age	0.0681
M+F	logPbB vs. logPbH	0.4351**
	logPbB vs. age	0.0168
	logPbH vs. age	-0.0111

** = $p\leq0.001$

Conclusion

The results of the present study do not completely support the hypothesis that PbH values can be used as a substitute for blood lead levels. However, we can generally state that: 1)

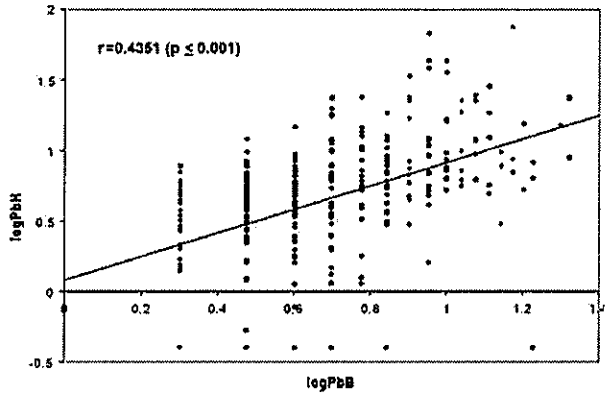

Fig. 1 - Scatterplot of logPbB vs. logPbH for the total sample of Sardinian children.

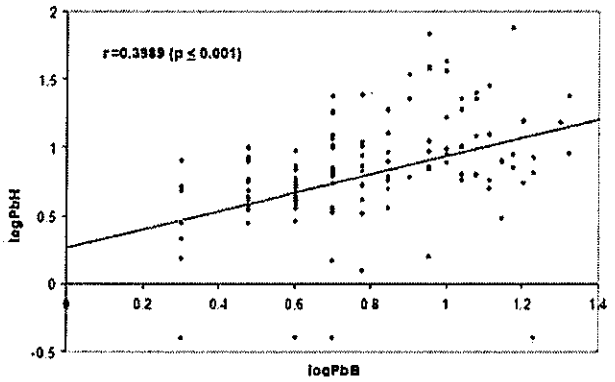

Fig. 2 - Scatterplot of logPbB vs. logPbH for the Sardinian boys.

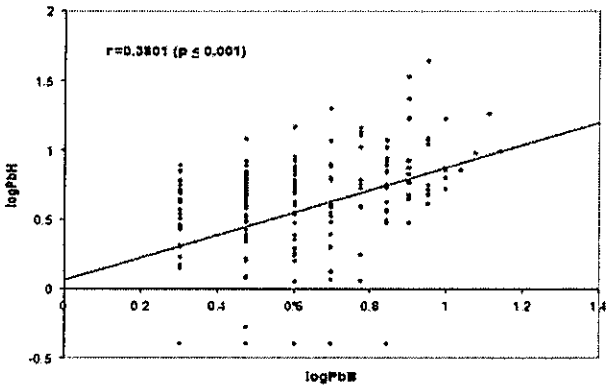

Fig. 3 - Scatterplot of logPbB vs. logPbH for the Sardinian girls.

the Portoscuso samples show higher PbB and PbH levels than the coeval samples of the other two Sardinian towns; 2) the boys present higher values of lead in the blood and hair than the girls of the same town. It is noteworthy that the correlations between logPbB and logPbH values, although statistically significant, present a high percentage of unexplained variance. Therefore, the determination of PbH can be used as a support for classical measurements of lead exposure but it cannot replace them. In fact, it can reveal a general pattern among samples with different environmental backgrounds but it cannot provide consistent results in the study of single individuals. Nevertheless, the pattern among samples with different environmental backgrounds and the significant correlations between the logPbB and logPbH values suggest that hair can be used as a suitable biomarker of lead exposure.

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Assessment of reference values for elements in human hair of urban schoolboys.

Senofonte, Violante N, Caroli S.

Istituto Superiore di Sanità, Rome, Italy.

Hair samples of youngsters (3-15 years of age) from several urban areas of Rome were analyzed to determine the content of 19 minor and trace elements with the aim of assessing Reference Values (RVs). Thirteen essential elements were taken into account, Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, Se, V and Zn. On the other hand, Al, As, Cd, Pb, Sr and Ti were also evaluated on the basis of their potential toxicity. Procedures were developed for the collection, storage and pre-analytical treatment of samples. Measurements were performed by inductively coupled plasma atomic emission spectrometry. Subgroups were formed according to age and sex. Significant differences were found for certain elements depending on age and sex. This was the case, e.g., for Ca which showed a mean value of 336 mg/kg for males and of 537 mg/kg for females. The sex-dependent pattern for this element was also apparent when the three age subgroups of 3-6, 7-10 and 11-15 years were compared. The overall RVs obtained (mg/kg) are as follows Al, 10.2; As, 0.09; Ca, 450; Cd, 0.23; Co, 0.67; Cr, 0.99; Cu, 22.1; Fe, 19.0; Mg, 28.0; Mn, 0.35; Mo, 0.43; Ni, 1.49; P, 195; Pb, 7.11; Se, 0.77; Sr, 1.20; Ti, 0.79; V, 1.22; and Zn 150.

PMID: 10836528 [PubMed - indexed for MEDLINE]

MeSH Terms, Substances

LinkOut - more resources

3 - 5 years italy

Cu 22
Fe 19

Pb 7.11 ↑ wine??

Se 0.77
Mn 0.35
Zn 150

italy - ايطاليا

اروبا

Determination of copper and zinc levels in human hair

Influence of sex, age, and hair pigmentation

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Abstract The Cu and Zn levels of both 607 men (1–85 y old) and 649 women (1–92 y old) were determined by atomic absorption spectrometry. Sex does not influence Cu (14.89±0.89 μg/g and 15.26±0.79 μg/g hair for males and females, respectively) and Zn contents (200.97±9.68 μg/g for men and 209.81±9.49 μg/g hair for women). Age influences Cu and Zn concentrations, but only significantly in females: Cu levels decrease over 60 y of age; whereas Zn levels increase significantly from age groups 2–5 to 20–40 years. Hair color influences Cu concentrations in both males and females. In males, white hair containes less Cu than black hair; in females, white hair's Cu levels are significantly lower than those of dark blond, red, light brown, and brown hair. There are no significant differences in Zn concentrations with respect to different hair colors, in either males or females.

Index Entries Human hair - zinc - copper - sex - age - hair pigmentation

Fulltext Preview (Small, Large)

Cu < 14.89 Male
15.26 Female

Zn < 201 Male
210 Female

Cu decrease over 60 years

Referenced by

9 newer articles

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Trace Elements in Hair of Healthy Children Sampled by Age and Sex

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ABSTRACT

Hair trace element (TE) (Cr, Mn, Fe, Zn, Cu, Br, Rb, Sr, Pb) levels from 336 healthy subjects were measured by the Proton-Induced X-ray Emission (PIXE) method. The subjects were divided in three groups: 157 full-term neonates (75 male and 82 female), 86 children (41 male and 45 female) ages 6 to 11 yr, and 93 adolescents (51 male and 42 female) 11 to 16 yr old. Cu, Zn, Cr, and Br show an increase from birth to 8 yr and then decrease. Fe, Mn, and Sr strongly decrease up to 8 yr and then remain almost stable. Sex differences are present in Fe, Zn, and Br of children and in Cu, Cr, and Br of adolescents.

Index Entries: Hair; trace elements; children; age; sex.

INTRODUCTION

Hair is of potential value as an indicator of the trace element (TE) status in an individual *(1,2)*. Some difficulties interpretative of the status arise from the dependence of hair TE content on several factors, e.g., age, sex, race, diet, and hair color, and contamination problems like environmental exposure and beauty care *(3–5)*.

Because of these problems, the literature has long reported a wide range of TE contents in hair. However, when selected groups of subjects are studied, hair can be very useful to assess marginal zinc deficiency. In fact, hair TE content does not reflect recent changes in the TE status like serum does, but gives a response integrated over several months. An example of this can be seen in children with chronic allergic disorders where reduced Zn hair level is reported *(6)* in the presence of normal Zn

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serum content. The detected marginal Zn deficiency has later been confirmed in a larger group of patients with a reduced erythrocyte Zn *(7)*.

To carry out similar studies, each investigator has to obtain his own reference values from a large number of normal subjects matched for the influencing factors with experimental subjects. This is because the differences are in many cases more relevant than the actual content values.

In addition, normal ranges of hair TE concentrations in children have not been established and, although there are some indications of TE level dependence on age and sex mainly for Zn and Cu *(8,9)*, data are scarce.

Finally, the simultaneous multielemental measurements of several TE are of interest because the correlations between some TE are probably more stable than the single concentration *(10)*.

Following this rationale, we determined Cr, Mn, Fe, Cu, Zn, Br, Rb, Sr, and Pb, in hair from healthy children, by the Proton-Induced X-ray Emission (PIXE) multielemental technique, at birth and primary and secondary school ages. This selection corresponds to three different periods of great physiological development. Findings from neonates have already been published *(10)* and are included in the present paper for comparison and completeness.

MATERIALS AND METHODS

A total of 336 healthy subjects (167 boys and 169 girls) living in urban areas of South Italy were studied. The population under study included 157 full-term neonates (75 male and 82 female) with weight appropriate for gestational age, born at the University hospital of Naples during a 4-mo period. Children (41 male and 45 female) 6–11 yr old and adolescents (51 male and 42 female) 11–16 yr old were coming from primary and secondary schools, respectively, sited in the Puglia region.

In neonates, the hair samples were collected within a week of delivery. Several different hairy spots along the scalp served as collection sites. Hair was cut close to the scalp. Samples from older children were cut from the nape of the neck as close to the scalp as possible.

The preanalytical treatment of the sample and the analytical technique used have been extensively described elsewhere *(10)*. In brief, about 50 mg of hair were carefully washed in nonionic detergent and then acid digested with 0.3 mL of HNO_3 at 70°C. After internal standard addition (200 µg of Pd), a 40 µL drop of the solution onto a kapton backing was dried and irradiated with a proton beam of 2 MeV for measurement with the multielemental analytical technique PIXE. The minimum detection limit (MDL) was of the order of tenths of µg/g depending on the element. The reliability of our method was assessed by measuring the certified elements in human hair reference material *(11)*.

Frequency histograms showed a log-normal distribution for all measured elements. From this, median value and SD of the median were cal-

culated. The SD of the median is given by the quantity $0.926\ IR/n^{1/2}$, where IR is the interquartile range and n is the number of observations. Half of the MDL value was attributed to the concentration whenever a significant concentration was not detectable. The differences between groups were checked by the nonparametric Wilcoxon test at 5% significance level.

RESULTS AND DISCUSSION

The results are reported in Table 1. In the last column, the adult values taken from the recent study by Di Pietro et al. *(3)* are reported. The changes of TE in hair with age are presented in Fig. 1. Males and females are kept separately. Arrows indicate statistically significant differences between the sexes.

Some elements, like Cu, Zn, Cr, and Br, behave similarly. Apart from some differences between sexes, they increase from birth to 8 yr and then decrease. Both Cu and Zn increase in the male subjects until higher than in the adults. They later decrease below this level. The same age dependence, although less pronounced, is present in females. The Cu content is significantly higher in hair of female adolescents than in male adolescents. This probably could be attributed to the different hormonal balance between sexes. Cr levels are one order of magnitude higher than the adult value of ref. *(3)*. Cr values similar to ours have also been measured in the first months of life *(12)* and in children 6–16 yr old *(11)*. They suggest that Cr could decrease from birth on. Br shows a significant difference between sexes both at 8 and 13 yr, with female levels being lower.

Another group of elements, such as Fe, Mn, and Sr, exhibits a strong decrease up to 8 yr and then they slightly change. In particular, by 13 yr old Fe reaches adult level. Mn levels are always higher than the adult level, and independent of the sex. High Mn values similar to ours have also been observed in neonates by Saner et al. *(13)*.

Finally, Rb levels are very low, close to our detection limit without any difference between sexes, and Pb increases with age more quickly in males than in females.

CONCLUSIONS

Our findings have shown how age and sex change some TE in the hair of three growth periods. Zn, Cu, Cr, and Br behave similarly, while iron levels are clearly different.

When a comparison is made with Cu, Zn, and Cr in hair of Spanish adolescents 6–15 yr old *(9)*, differences are found in the behavior with age and especially with sex. This is not surprising and motivates the caution that should be exerted when one tries to draw general conclusions

Table 1
Trace Element Contents in Hair from Three Groups of Children[a]

	Neonates		6–11 yr[b]		12–16 yr[c]		
	M	F	M	F	M	F	Adult (ref. 3)
Cr	1.9 ± 0.3	1.3 ± 0.4	5.4 ± 3.0	2.7 ± 0.7	1.3 ± 0.2	1.1 ± 0.1	< 0.15
Mn	1.3 ± 0.1	1.3 ± 0.1	0.5 ± 0.3	0.5 ± 0.3	1.0 ± 0.1	1.0 ± 0.1	0.22
Fe	48.7 ± 7.0	51.6 ± 8.2	15.8 ± 5.0	4.8 ± 2.6	10.2 ± 1.3	8.8 ± 0.9	8.1
Zn	131 ± 5	129 ± 4.3	157 ± 7	139 ± 8.0	109 ± 4	122 ± 6	147
Cu	5.6 ± 0.4	5.6 ± 0.3	10.0 ± 0.9	9.7 ± 0.6	6.2 ± 0.3	8.4 ± 0.6	12.7
Br	1.5 ± 0.1	1.3 ± 0.1	4.7 ± 1.2	2.8 ± 0.3	1.3 ± 0.2	1.0 ± 0.1	—
Pb	1.6 ± 0.2	1.4 ± 0.2	1.7 ± 0.5	3.4 ± 0.6	3.0 ± 0.4	3.1 ± 0.4	2.1
Rb	0.39 ± 0.06	0.35 ± 0.04	< 0.25		0.44 ± 0.05	0.41 ± 0.05	—
Sr	1.6 ± 0.2	1.5 ± 0.2	< 0.5		0.94 ± 0.06	1.5 ± 0.3	3.3

[a]Values are given in μg/g and as median ± SD.
[b]Age ± SE was 8.1 ± 0.3.
[c]Age ± SE was 13 ± 0.3.

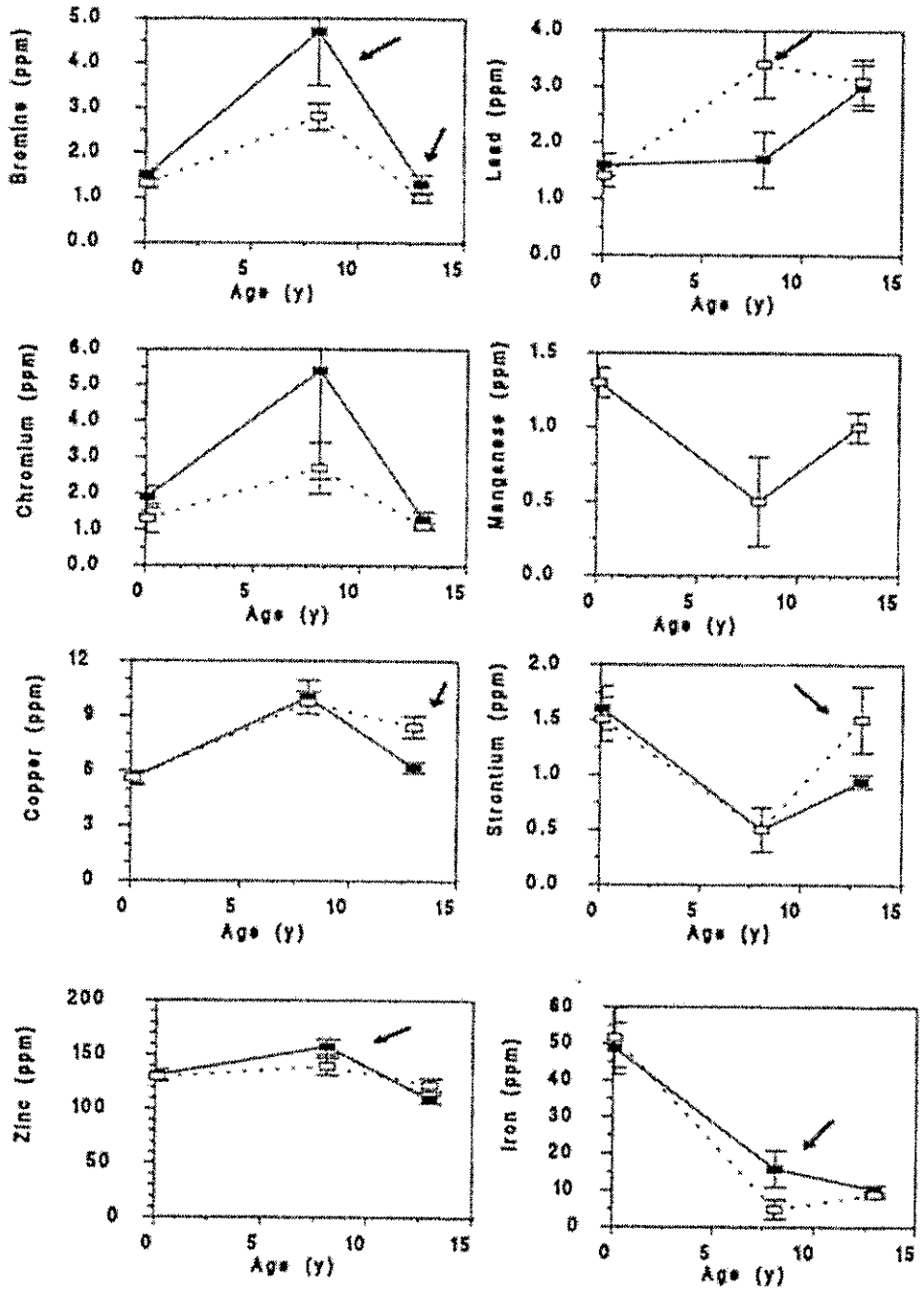

Fig. 1. TE concentrations of healthy children at three ages. -■- male subjects; -⊡-, female subjects. The arrows show significant differences ($p < 0.05$) between sexes.

from TE hair analysis. This indicates the need for a well-matched control group when hair TE content is used to assess nutritional or pathological status.

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A Case-Control Study on Selenium, Zinc, and Copper in Plasma and Hair of Subjects Affected by Breast and Lung Cancer

1996

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66 Patients (38 women Breast Cancer)
(25 Men + 3 women) Lung Cancer

ABSTRACT

The purpose of our study was to investigate the relationship between plasma and hair levels of Se, Zn, and Cu, and cancer. We selected a total of 66 patients affected by either breast (38) or lung (28) cancer. They entered into the study at the onset of disease, and before any chemical or radiotherapy. Controls were randomly selected among healthy people and were matched for sex, age, smoking habits, and residence. In the group of breast cancer, a significant decrease in hair Se was found compared to controls ($p < 0.01$), whereas plasma Se was only slightly decreased. No difference between cases and controls was detected in both hair and plasma levels of Zn and Cu. Subjects who developed lung cancer were significantly lower in hair Zn ($p < 0.05$) and Cu ($p < 0.01$) than controls, whereas there was no difference with regard to Se. In addition, plasma Cu of these patients was increased as compared to controls.

Index Entries: Selenium; zinc; copper; breast cancer; lung cancer; plasma; hair.

INTRODUCTION

The trace elements selenium (Se), zinc (Zn), and copper (Cu) play an essential role in humans. They are important structural and functional

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cofactors of various enzymes crucial for the various biochemical cell activities and the integrity of various apparatus. In particular, Se is a constituent of the glutathione peroxidase, and Zn and Cu are cofactors of superoxide desmutase. These enzymes act as scavangers against free radicals of oxygen that form in various modes in the body and protect from different cellular damaging events. Indeed, derangements of the cell membrane and of DNA, exchange of chromatids, and genetic mutations can be caused by oxidative stress, and they are correlated with carcinogenesis *(1–3)*.

In experimental studies, Se has also been shown to have an inhibitory effect on chemical *(4,5)* and viral carcinogenesis *(6)*; it modulates cellular proliferation (G1 phase), both in normal and neoplastic cells *(7)* and regulates the expression of oncogenes c-*fos* and c-*myc* *(8)*. Zn is a factor required to activate thymus gland hormones, and thus for T-cell mediated response, important for the defence against tumors *(9)*. On the other hand, Zn is a pivotal element in all rapidly growing tissues since it is a component of DNA and RNA polymerase, and seems to have a modulatory and protective action for the growh of both normal and cancer cells *(10)*. Cu, in addition to its physiological activity, might, in some cases, by means of an interaction with free radicals of oxygen, also augment the consequent cellular damages.

Accordingly, in recent years several studies have focused on the relationships between trace elements and cancer in humans. Several of the first studies reported low serum levels of Se *(11–13)* and increased serum levels of Cu *(14–16)* in patients with malignant tumors of various histotype and in different phases of the disease. Subsequently, quite varied behaviors were found for Zn levels *(17,18)*. In any case, definitive data are still lacking. No conclusive data were obtained in prospective studies on cancer risk factors, also when trace element levels were investigated either in plasma or in other biological matrix, such as nails, that represent the Se, Zn, and Cu status over several months *(19–21)*.

Considering the discordant reports on these elements, we thought it would be of interest to simultaneously analyze the levels of Se, Zn, and Cu in plasma and in hair samples of patients with breast and lung cancer at diagnosis and in healthy controls. In this work, we stress the importance to evaluate trace element concentrations in both plasma and hair in different types of cancer. Indeed, plasma levels can be influenced by a variety of different factors including diet composition, circadian variations, acute pathologies, whereas hair is a highly stable material and has a potential for revealing and reconstructing past episodes relevant to health even after their action has ceased *(22–24)*. Hair reflects total body burden of certain elements better than blood and urine and the accumulation over extended periods of time leads to metal concentrations much higher than those in biological fluids, as proven in supplementation studies *(25)*.

The organs involved, the initial clinical picture before starting specific therapies, and the absence of underlying metabolic disorders, were

all elements that contributed to eliminate the multiple interacting factors inherent to this type of investigation.

MATERIALS AND METHODS

We selected 66 patients affected by either breast cancer (38 women) and lung cancer (25 men and 3 women). They entered into the study at the onset of disease, and before any chemio- or radiotherapy. Diagnosis of primary carcinoma was confirmed histologically and all our patients were stage 0–III for breast cancer and stage II–III for lung cancer according to UICC tumor classification. Controls (22 for breast cancer and 20 for lung cancer) were randomly selected among healthy people and were matched for sex, age, smoking habits, and residence (province of Modena). In the case of breast cancer, ages ranged between 41 and 79 yr, and did not differ significantly from controls (58.6 ± 10.3 vs 56.8 ± 11 yr), whereas for lung cancer the range was between 44 and 76, and the mean slightly higher than in controls (61.7 ± 7.0 vs 57.3 ± 6.3 yr). All results are expressed as mean ± SD.

Blood samples were collected from the antecubital vein carefully avoiding external metal contamination and haemolysis. Plasma samples were obtained by centrifuging, and frozen before analysis. Hair samples were cut from the neck close to the scalp and stored in plastic bags at room temperature until analysis. Only the proximal 1–4 cm portions were excised, and washed with a nonionic detergent solution (7x-0 Matic) before analysis.

Se in both plasma and hair was determined by a fluorimetric method following the procedure of Olson et al. *(26)* with slight modifications. Briefly, 1 mL plasma and about 0.4 g hair were digested with concentrated nitric and perchloric acid. After boiling digested samples with hydrochloric acid, 5 mL of 25 mmol/L EDTA were added and the pH of the solution was adjusted to 1.8 with NH_4OH. Each solution was incubated at 50°C for 20 min with 5 mL of 2,3-diaminonaphtalene (1% in 0.1*N* HCl), cooled, and extracted in cyclohexane. Blancks and Se standard solutions were included with each set of determinations. Fluorescence of piazselenol was measured at the 520 nm by using a fluorescence spectrometer (P.E. mod.204).

Sensitivity of the method for 1 mL plasma sample was 10 ng (sd 3.5% in the range 0–100 ng). Sample reproducibility was tested by analysis of the National Bureau of Standards (Gaithersburg, MD) reference material bovine liver which gave a value of 0.72 ± 0.02 µg Se/g (certified value 0.71 ± 0.07 µg/g).

Zn and Cu in plasma were measured by flame atomic absorption spectrometry (Perkin Elmer, mod.5000), using a direct diluition method. A portion of the digested hair sample was used for Zn and Cu determination by flame AAS. Further details are described in previous papers *(27)*.

Statistical analyses were carried out by SPSS/PC program. Differences between groups were tested by the Student *t*-test. Hair values showed a slight non-normal distribution, and log trasformation of data was applied before statistical elaborations.

RESULTS

In the group of patients with breast cancer, we have found, as relevant datum, a significant reduction of Se hair concentration compared to controls (215.2 vs. 338.3 ng/g; $p < 0.01$); the Se plasma concentration was only slightly reduced. We have not found significant differences between patients and controls, neither in hair nor in plasma, for the other two trace elements (Table 1).

In the group of lung carcinoma, we have relevated significantly reduced levels in hair for Zn (147.7 vs 174.6 µg/g; $p = 0.01$) and for Cu (10.1 vs 15.4 µg/g; $p < 0.01$). In addition, only plasma Cu and not Zn of these patients was increased compared to controls (Table 2). No significant differences were observed for Se levels in any relevation.

Indeed, in nothing of the two groups of patients studied could we observe differences related to the hystologic subtype, perhaps because of the low dimensions of the casistic.

DISCUSSION

The simultaneous evaluation of trace element levels in plasma and hair seemed to hold particular promise. Indeed, in our study, modifications of Se, Zn, and Cu were appreciated in hair of patients with cancer, but not in plasma. Moreover, trace elements in hair tended to decrease in patients compared to their controls, whereas plasma levels showed a tendency to increase, and the modifications were specific with the type of tumor. All these data are in favor of a different biological significance of hair analysis as compared to the most commonly used fluids like plasma.

Se deficiency seems to be a consistent observation in breast cancer, although in our study only hair Se was found significantly reduced. In a recent study on a group of patients with early stage, the serum concentrations of Se were found to be reduced, and this datum, very significant, has been proposed as an additional noninvasive parameter for the clinical assessment of this malignant disease *(28)*. A similar finding had already been pointed out only in patients with advanced breast cancer *(29)*.

Only limited increases in both Zn and Cu in plasma were found in our group of breast cancer. From literature data, the behavior of serum Cu appears to have a great variability. This fact is supported by the contrasting findings of a joint case-control study on serum Cu levels in

Table 1
Trace Elements and Breast Cancer

	Patients		Controls		
	Mean	SD	Mean	SD	*p*
Hair					
Selenium, ng/g	215.20	112.50	338.30	151.95	0.001
Zinc, µg/g	162.30	27.20	156.32	30.07	ns
Copper, µg/g	13.50	8.39	13.83	4.77	ns
Plasma					
Selenium, µg/L	71.81	18.12	75.06	16.58	ns
Zinc, µg/dL	86.43	23.46	74.03	24.22	ns
Copper, µg/dL	119.15	19.76	110.39	15.60	ns

Table 2
Trace Elements and Lung Cancer

	Patients		Controls		
	Mean	SD	Mean	SD	*p*
Hair					
Selenium, ng/g	351.17	107.88	360.98	126.82	ns
Zinc, µg/g	147.70	30.35	174.60	31.66	0.01
Copper, µg/g	10.10	2.34	15.40	5.13	<0.01
Plasma					
Selenium, µg/L	69.59	23.77	81.52	22.89	ns
Zinc, µg/dL	80.13	18.31	77.03	20.15	ns
Copper, µg/dL	118.84	41.84	96.80	18.37	<0.05

Southern France and Northern Italy *(30)*. That study found higher concentrations of trace elements in the patients first diagnosed for primary breast cancer, as compared to healthy controls, in Southern France. Instead, in the study performed in Italy, the levels were higher in controls than in patients.

The values of serum Zn seem equally conflicting. In the study cited above, the Zn mean values were significantly higher in cases than in controls in both samples; in other works, including our investigation, neither serum Zn nor the Zn/Cu ratio were able to discriminate between controls or patients with breast cancer *(31)*.

Concerning lung cancer, we could not confirm the existence of a Se deficiency either from plasma or hair analysis, whereas significant diseases were observed in hair Zn and Cu and a parallel increase of both in plasma although only for Cu it reached a statistical significance. The absence of a drop in Se content in patients with lung cancer with respect to controls may at first seem contradictory also because low intake of Se

and vitamins A and E are considered risk factors for this disease (*32*). However, cigarets provide an exogenous source of trace elements and in smokers even normal levels of body Se could be not sufficient to protect the subjects from the intense exposure to cancerogens of tobacco.

In previous works on lung cancer, a rise in serum Cu levels and a fall in Se and Zn levels are more consistently observed in patients than in controls (*33*). An inverse association between Se status and the risk of lung cancer was reported in prospective studies (*34,35*) and a direct correlation between Se status and good prognosis in clinical studies (*36*). Moreover, a tendency toward normalization of Zn levels was found in patients who respond better to therapy with a shift of trace elements from the pathological tissue to the blood compartment as confirmed by studies using labeled Zn^{65} (*37*). Lastly, an association has recently been reported between low Zn levels and various type of immune deficiencies in subjects with lung cancer (*38,39*).

The process underlying tumor development can lead to an uptake of trace elements by the neoplastic cells. Indeed, increased levels of Se, Zn, and Cu and gluthatione-peroxidase have been found in neoplastic tissue from the human breast, lung, and colon (*40–43*). These factors might be useful for the tumor cells cooperating in their growth assuming a protective role against the deleterious effects of free radicals.

These considerations are back by experimental data on animals, where an inibition of tumor growth was found in animals fed Zn-poor diets (*44–46*). Lastly, the possible modifications in the relationships between trace elements and their carrier molecules must be kept in mind. Thus, for example, increases in the serum concentration of Cu may be associated with reduced catabolism or increased neoplastic synthesis of ceruloplasmin (*47*). The high levels of Cu might then trigger competitive events with the absorption of other trace elements, such as Zn.

In conclusion, the available literature points out the interest and further need for both experimental, epidemiological and clinical studies on these trace elements. Lastly, it is necessary to further define the relationship between trace elements and cancerogenesis, the profiles in various districts of the body under normal conditions, and their variations in the presence of neoplastic disease in light of the complex relationship between host and tumor.

Only after these points are elucidated will we be able to compile guidelines for the prevention, clinical monitoring, and evaluation of the therapeutic efficacy of trace elements in the field of medical oncology.

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Original Paper

Assessment of reference values for elements in human hair of urban schoolboys

Abstract

The concentrations of copper, iron, magnesium, and zinc in the hair of eighteen different adult male subjects have been determined by atomic absorption spectroscopy over periods ranging from 4 to 10 months. The mean for each of the four analysis elements is shown for each subject. A mean, median, and range are given for each subject. A mean, median, and range are given for each element taking all subjects into consideration. A study was made of the

Healthy italian (3–15 years)

School boys

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Summary

Hair samples of youngsters (3–15 years of age) from several urban areas of Rome were analyzed to determine the content of 19 minor and trace elements with the aim of assessing Reference Values (RVs). Thirteen essential elements were taken into account, Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, Se, V and Zn. On the other hand, Al, As, Cd, Pb, Sr and Ti were also evaluated on the basis of their potential toxicity. Procedures were developed for the collection, storage and pre-analytical treatment of samples. Measurements were performed by inductively coupled plasma atomic emission spectrometry. Subgroups were formed according to age and sex. Significant differences were found for certain elements depending on age and sex. This was the case, e.g., for Ca which showed a mean value of 336 mg/kg for males and of 537 mg/kg for females. The sex-dependent pattern for this element was also apparent when the three age subgroups of 3–6, 7–10 and 11–15 years were compared. The overall RVs obtained (mg/kg) are as follows Al, 10.2; As, 0.09; Ca, 450; Cd, 0.23; Co, 0.67; Cr, 0.99; Cu, 22.1; Fe, 19.0; Mg, 28.0; Mn, 0.35; Mo, 0.43; Ni, 1.49; P, 195; Pb, 7.11; Se, 0.77; Sr, 1.20; Ti, 0.79; V, 1.22; and Zn 150.

Keywords: Hair analysis; trace elements in human hair; reference values assessment

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Al 10.2 Ca 450 Cu 22.1
As 0.09 Co 0.67 Fe 19.0
Cd 0.23 Cr 0.99 Mn 0.35
Ni 1.49 Mg 28 Se 0.77
Pb 7.11
V 1.22 Mo 0.43 Sr 1.20
P 195 Ti 0.79

pre-treatment of hair, including a comparison of detergent washed and organic solvent washed hair and an investigation of other wash parameters. Recovery studies were made for each analysis element.

Adsorption and elution of trace elements on human hair
The International Journal of Applied Radiation and Isotopes, Volume 17, Issue 7, July 1966, Pages 417-423
L.C. Bate

Abstract
Since 1962 the trace element content of human hair has been studied as a possible means of comparing physical-evidence hair samples. It is recognized that for hair to be of wide value in this application, its trace element content must not change rapidly with time. Limited studies have indicated, however, that changes do occur over relatively long periods of time. These changes may be due to natural growth processes and/or environmental contamination.

This study was made to see how extensively the trace element content of hair might be modified by the adsorption of environmental trace elements and to see if solvents could be found that would remove the adsorbed elements. The procedure used was to place a sample of hair in a solution containing measured quantities of an element and a radiotracer. After 16 hr, the sample was removed from the solution, rinsed, and then washed. The

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PATHOBIOCHEMISTRY

Calcium, copper, iron, magnesium, silicon and zinc content of hair in Parkinson's disease

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Abstract

The aetiology of Parkinson's disease (PD) is still unknown, but some hypotheses have focused on the imbalances in body levels of metals as co-factors of risk. To assess whether hair could be a reliable marker of possible changes, calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), silicon (Si) and zinc (Zn) were determined in hair from 81 patients affected by PD and 17 age-matched controls.

Care was taken to eliminate external contamination of the hair by thorough washing. Digestion of the matrix was achieved by an acid-assisted microwave procedure. Quantification of the elements was performed by inductively coupled plasma atomic emission spectrometry.

Results indicated significantly lower levels of Fe in the hair of patients ($p = 0.018$) compared with controls. Ca and Mg levels were slightly lower while Zn levels were higher in patients, although these differences were not significant; neither were variations in Cu and Si. Ca and Mg were at least 1.5 times higher in females than in males in both controls and patients. In addition, Ca correlated positively with Mg in both groups and in both sexes (p-value always less than 0.03), and negatively with age in patients ($p < 0.01$). Finally, element levels did not correlate with either the duration or the severity of the disease or with anti-Parkinson treatment.

Keywords: Parkinson's disease; Human hair; Inductively coupled plasma atomic emission spectrometry; Major and trace elements

Introduction

Parkinson's disease (PD) is characterized by damage to dopaminergic neurons in the *Substantia Nigra* (SN), with a reduction in striatal dopamine. This causes loss of control in the nerve cells, with both physical (e.g., slow body movements, muscular rigidity, tremor in resting limbs and loss of postural balance) and mental (e.g., memory impairment and cognitive dysfunction) consequences.

The aetiology of this disease is still not fully clear, but it is now widely accepted that many factors are involved in the development of this neuropathology. Exposure to toxicants (metals, pesticides and other neurotoxic substances), diet (sugar, vitamins, animal fats, etc.) and age may act as co-determinant agents in the disease, while other important factors implicated in neurodegeneration are the enhanced production of oxidant species, such as lipo-and hydroperoxides and/or weakened anti-oxidant defences [1,2]. As regards metals,

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their involvement in PD has been indicated by several neurochemical observations of abnormal deposits in the brain of certain metals and of imbalances in their natural levels [3].

In particular, it is well known that iron (Fe) is accumulated in different brain regions in presence of neuropathologies. In this context, increased levels of Fe were found in the SN of patients with PD. At this level, Fe creates complexes with neuromelanin inducing oxidative stress and death of the dopaminergic neurons. For this reason, Fe is considered one of the most important metals involved in PD [4–8]. With reference to copper (Cu), although involved as a cofactor in detoxifying enzymes and proteins, its altered brain homeostasis may enhance the production of oxidative molecules with the consequent cell death and PD development [9,10]. In addition to this, Cu and Fe facilitated the aggregation of the α-synuclein brain protein producing the characteristic intracellular inclusions of PD, i.e., Lewy bodies [11].

Zinc (Zn) plays a key role in several brain functions. In fact, it participates in the superoxide dismutase and Zn-thioneine enzymes to reduce oxidative stress as well as in the modulation of the response of both excitatory and inhibitory receptors. At present, the Zn involvement in the onset of PD is not directly evidenced even if some hypotheses have been suggested [10,12,13].

The neurotoxicity of calcium (Ca) is due to its increased levels in the cytosol, promoting free radicals generation. In normal condition this phenomenon is limited by a voltage-dependent magnesium (Mg) blockade [14,15]. In addition, an unbalanced metabolism of Ca and Mg in the central nervous system and an increased oxidative damage due to the deficiency of Mg have been observed in PD [10,16]. Mg seems also to avoid the spontaneous and Fe-induced aggregation of the α-synuclein [17].

Silicon (Si), in its turn, appears to have the effect, as silicic acid, of reducing the bio-availability of aluminium (Al), avoiding its deposit in neurofibrillary tangles [18].

Most of the literature on metals as causative factors in PD is based on *post-mortem* analyses of brain tissues taken from patients. Knowledge of the levels of elements in the body fluids and tissues of living persons, especially during the early stages of disease, could provide useful information.

In recent decades, hair has attracted considerable attention as a marker of basic levels of metals in the human body, of professional exposure and of experimental medicine with potentially noxious elements [19–23]. The advantages of using hair are manifold. One is its ability to reflect the total body intake of certain elements better than more frequently used markers such as blood, serum and urine; another is the phenomenon of accumulation, which implies a higher concentration of metals in hair than in fluids [19]. The affinity of metals for hair is primarily due to the relatively high presence of cystine in the keratin structure, as well as to follicular melanin, which is able to bind cations by ionic interactions. Moreover, whilst the level of metals in biological fluids represents in most cases a current status, the distribution of elements in hair reflects more extended exposure, on account of growth. In addition, the sampling of hair is easy, painless and non-invasive. Samples can be collected in plastic bags and stored at room temperature in the dark and their composition does not change with time. Although much research has been done into the distribution of elements in the hair of healthy individuals, there is still a lack of data regarding the presence of elements in the hair of patients affected by neurodegenerative diseases [24–28].

For these reasons, the purpose of this study was to determine the levels of Ca, Cu, Fe, Mg, Si and Zn in the hair of PD patients and compare them with those of control subjects in order to evaluate their possible implication in the pathology. Quantification was performed by inductively coupled plasma atomic emission spectrometry (ICP-AES).

Material and methods

Subjects

81 Patients

Eighty-one patients (62 males, 19 females) affected by PD as diagnosed in accordance with the London Brain Bank Criteria, were selected for the present study [29]. The patients' mean age was 65.5±9.6 years, with a mean duration of disease of 5.0±4.2 years. Approximately 25% of the patients were not undergoing treatment with pharmacological drugs, while the others were being treated with dopaminergic agonists, or L-dopa, or both. The Hoehn and Yahr phase range was 1–4 [30,31]. The control group consisted of 17 age-matched individuals (9 males and 8 females, with a mean age of 64.8±12.6 years), who had not reported any symptoms of PD or of other motor neurological disorders.

Exclusion criteria for both population groups were: cardiological, respiratory, kidney or liver disorders; intestinal absorption abnormalities; active infections; intake of thyroid hormones or lithium; any other psychoactive drug intake except for anti-Parkinson medication; consumption of vitamins or mineral supplements. All subjects known to have metallic body parts such as prostheses or surgical screws were also excluded.

The study was approved by the Ethics Committee of the Neuroscience Department of "Tor Vergata" University of Rome and all subjects signed an informed consent form.

Table 1. ICP-AES settings and conditions for the quantification of metals in hair

Instrument	Optima 3100 XL (Perkin Elmer, Norwalk, CT, USA)
RF generator	Frequency, 40 MHz; power output, 1300 W
Induction coil	Four turns; o.d., 32 mm; height, 20 mm
Nebulizer	Cross-flow type with Ryton Scott condensation chamber
Argon flows (L/min)	Plasma, 13; auxiliary, 0.50; nebulizer, 0.70
Polychromator	Echelle grating (ruling density, 79 lines/mm) combined with a Schmidt cross disperser; the detection was achieved by a simultaneous solid-state Segmented-array Charged-coupled device Detector (SCD); maximum resolution, 0.006 nm at 200 nm
Spectral lines (nm)	Ca (II), 393.3; Cu (I), 324.7; Fe (II), 259.9; Mg (II), 279.5; Si (I), 251.6; Zn (I), 213.8
Internal standard (nm)	Y (II), 371.0

Sample collection and treatment

Hair samples were cut from the sub-occipital zone of the head at ca. 1 cm from the scalp, collected in individual plastic bags and stored in a desiccator in the dark until required.

The endogenous level of metal content in hair is significantly affected by external contamination due to environmental dust and dirt, sweat and desquamation of the epidermis, as well as detergents and cosmetic treatments. For these reasons, it is mandatory to submit hair samples to adequate external washing in order to quantify only internally bound analytes [19,32,33]. This consisted in three steps: (i) three washes under continuous stirring for 10 min each in a mixture of 3:1 (v/v) ethyl ether-acetone (Riedel-de Haën, Seelze, Germany) to remove the sebaceous film covering the hair; (ii) soaking under stirring for 1 h in 5% sodium ethylenediamine tetracetic acid (EDTA) (Merck, Darmstadt, Germany) to bind the chemical elements present on the hair surface and, (iii) repeated rinsing with high purity deionised water (EASY-pure UV, PBI, Milan, Italy).

After drying, approximately 0.25 g of hair from each sample was then weighed and transferred into polytetrafluoroethylene (PTFE) vessels and a 3:1 mixture of HNO_3 and H_2O_2 (both of Suprapur grade, Merck, Germany) was added. After overnight pre-digestion, samples were mineralized in an MLS 1200 Mega microwave oven (FKV, Bergamo, Italy) as follows: 3 min at 250 W; 6 min at 0 W; 5 min at 250 W; 5 min at 0 W; 5 min 450 W and 5 min at 500 W. After cooling, the samples were made up to 10 mL with water. In each cycle a blank sample of the reagent was included to assess the degree of contamination during digestion, together with the certified reference material BCR CRM 397, Human Hair, to check the accuracy of the analytical procedure. All the preparatory steps were performed in a Class 100 (maximum 100 particles of 0.5 μm per cubic foot of air) clean room (Tamco, Rome, Italy) to minimise the risk of metal contamination.

Determination of metals in hair

The quantification of Ca, Cu, Fe, Mg, Si and Zn was performed using the ICP-AES technique after a further 1 + 3 dilution of the digests with water. Instrumental working conditions and settings are reported in Table 1.

The addition-calibration approach was adopted and yttrium (Y) was used as internal standard (IS) at a concentration of 500 ng/mL to provide adequate means for dealing with matrix-induced variations and instrumental drifts. Calibrants and IS solutions were prepared daily by diluting 1 mg/mL single-element standard solutions (SPEX, Edison, NJ, USA). The accuracy of the whole procedure was tested by using the above-mentioned BCR CRM 397 and the precision of the method was checked by 10 replicated measurements of a sample.

Statistical evaluations

Element concentrations in the hair of Parkinson patients and control subjects were described in terms of mean, standard deviation (SD), median and 25th–75th percentiles. The normality of the data relating to the population groups was checked by Kolmogorov–Smirnov's test. Values were statistically compared using one-way analysis of variance (ANOVA), also taking into account sex and age as grouping variables. Correlation analysis between metal concentrations in hair and age, duration and severity of the disease and drug therapy was performed using Pearson's test. SPSS version 11.0 was used as statistical package (SPSS, Chicago, IL, USA).

Results and discussion

Method performance

The multielement capacity, wide dynamic range and relative freedom from matrix interferences make the

Table 2. Analytical performances of the ICP-AES element determination in human hair (Concentrations and SD are in μg/g)

Element	LoD	Precision (%)	Accuracy	
			BCR CRM 397	
			Found	Certified
Ca	0.004	3.13	1500 ± 139	1560 ± 40[a]
Cu	0.01	1.92	108 ± 10	110 ± 5[b]
Fe	0.01	2.14	576 ± 41	580 ± 10[a]
Mg	0.004	3.02	192 ± 17	200 ± 5[a]
Si	0.01	2.49	289 ± 22	nc
Zn	0.01	2.83	198 ± 16	199 ± 5[c]

LoD, limit of detection; nc, not certified.
[a]Informative value.
[b]Indicative value.
[c]Certified value.

Table 3. Element concentrations (μg/g) in the hair of the control group and PD patients

	Ca	Cu	Fe	Mg	Si	Zn
Control group						
Mean	419	7.49	13.1	43.3	29.4	131
SD	228	3.41	7.11	29.1	18.2	47
Median	465	7.21	11.4	36.5	21.7	136
25th	155	5.51	7.71	20.1	15.6	106
75th	578	8.10	17.0	69.4	38.3	163
PD patients						
Mean	376	7.91	9.51	36.4	28.2	142
SD	240	2.91	4.90	23.6	19.2	48
Median	310	7.76	8.35	28.7	22.3	143
25th	219	6.24	6.41	19.1	13.6	98
75th	441	9.43	10.5	53.4	37.0	178

ICP-AES technique one of the most widely used for this type of analysis. Table 2 reports the ICP-AES limit of detection (LoD), precision and accuracy. The LoD – calculated as three times the SD of 10 replicates of a digestion blank–were satisfactory and suitable for the intended purpose. The accuracy, tested on BCR CRM 397, varied from 95.8% for Mg to 99.4% for Fe. In the case of Si, the only element not certified, a recovery test was applied. To the raw sample of hair 10 μg/g of Si was added and the recovery, after the whole procedure, was found to be 98.7%. The precision of the method covered the interval from 1.92% for Cu to 3.13% for Ca.

Metal contents in hair

Table 3 shows the full picture of the distribution of metals in the hair of patients and controls. Table 4 reports the element levels as a function of sex and age groups. Outliers were removed from the raw data; they were identified by the box-and-whisker plot approach, i.e., values higher and lower than 1.5-times the inter-quartile difference were considered as outliers. Data were then checked for normality, after which they were treated by parametric tests without need for further transformation and in each test a p-value of $\leqslant 0.05$ was considered significant.

In the case of Ca, mean values were slightly lower in patients than in controls, although the difference was not significant. When comparing females and males, the mean concentration of Ca was ca. 1.5-fold higher in females than in males both in PD patients and in healthy controls (at $p < 0.05$). No significant difference was observed between the Ca levels of controls and patients in the three age groups. In this context, the Ca content in the hair of female PD patients were significantly higher than in males in the $\leqslant 60$ year and > 70 year age groups ($p < 0.01$ for both), while no difference was found in the case of the 61–70 year age group. A lower Ca content in the hair of Parkinson patients was also found in a Japanese study [24]. This trend is also supported by other studies involving hair of patients affected by other neuropathologies, namely Alzheimer's disease, Down syndrome and schizophrenia [26,34,35]. However, in another study of epileptic and Alzheimer subjects, the differences were found to be not significant [27,28,36].

The levels of Cu in the hair of PD patients were virtually superimposable on those of the controls, with no statistical difference. The same result was obtained when females and males were matched, although the content of Cu in females was slightly higher than in males for both groups. No variation in Cu levels was found between controls and patients in the different age groups. Normal values of Cu have been found in patients with Alzheimer's disease [27,28] and schizophrenia [34]. Moreover, even in the case of the Wilson's disease, where a genetic problem causes the accumulation of Cu in various organs, the level of this element did not differ between hair samples of affected and healthy individuals [37]. In contrast to this, Cu levels were lower in Down syndrome [35] and higher in epilepsy patients [36].

The values for Fe in the hair of PD patients were significantly ($p = 0.018$) lower than in controls and in the 61–70 year age group this decrease appeared to be strongly marked ($p < 0.001$). This decrease was also confirmed when males of both groups were compared ($p = 0.01$), but it was not present in females. Moreover, lower Fe levels in PD hair of the elder patients have been observed (see Table 4), even if without statistical significance. In the elder patients groups, as expected, the PD severity passed from 1.40 ($\leqslant 60$ years) to 1.81

Table 4. Mean element concentrations and SD (μg/g) in the hair of females (F) and males (M) and of subjects grouped by age

	Ca	Cu	Fe	Mg	Si	Zn
Control group						
F	512±212	8.71±4.53	9.45±4.36	59.3±29.9	33.4±21.8	130±37
M	357±227	6.53±2.02	15.9±7.73	30.5±22.4	24.8±13.1	131±56
⩽60 years	485±216	8.54±4.02	13.6±5.08	42.1±27.9	35.2±22.8	137±52
61–70 years	254±200	6.75±1.30	21.3±10.5	31.7±9.73	20.3±5.75	162±17
>70 years	435±245	6.62±3.48	8.38±2.94	49.3±36.5	27.0±16.2	108±45
PD patients						
F	536±278	8.73±2.81	10.8±5.85	51.3±23.6	28.4±17.0	131±47
M	333±211	7.65±2.92	9.11±4.55	32.1±22.0	28.1±20.0	145±47
⩽60 years	453±254	7.76±3.88	10.7±6.26	39.0±29.5	23.6±16.8	136±55
61–70 years	345±245	8.25±2.33	9.25±3.47	31.1±20.6	36.6±40.4	145±48
>70 years	295±180	7.79±2.51	8.96±4.88	35.2±19.1	29.5±20.1	148±42

(>70 years) as mean values of the Hoehn and Yahr staging. This trend of reduced Fe concentrations in hair with age and illness severity confirmed analogous pattern found for peripheral fluids by other authors [38]. In general, the findings for Fe in PD were in contrast to those in patients affected by schizophrenia and epileptic convulsions, where Fe levels in hair were significantly higher than those in healthy people [34,36].

Mg concentrations in hair were lower in the PD group, but the difference was not significant. Other authors have found slightly lower levels of Mg in patients with Alzheimer's disease and schizophrenia [25,28,34] and higher levels in patients with epilepsy [36]. Moreover, the Mg levels in females were ca. twice as high as those in males. This statistically significant difference was more pronounced in the control group ($p = 0.003$) than in PD patients ($p > 0.05$). When the subjects were grouped by age, Mg levels were similar for both. In addition to this and as for Ca, in the ⩽60 year and >70 year age groups, females with PD had higher contents of Mg being statistically different from those of PD males with a p level of 0.047 and 0.005, respectively. This difference was not observed between PD males and females of the middle age group.

Data for Si did not show any significant difference when PD patients and healthy subjects were compared. Nor were any differences found in hair concentrations between the sexes. Among the different age ranges, the 61–70 year group of patients appeared to have slightly higher levels, whilst the ⩽60 year group showed higher levels in controls.

Zn content in hair showed a slight though not statistically significant increase in PD patients. After dividing the two population groups according to sex, this variation was found to persist only in males. In subjects over 71 years of age, Zn was significantly ($p = 0.047$) higher in PD subjects than in controls. In other neurodegenerative pathologies no difference in Zn values has been found between normal subjects and patients [26,28,34]. On the contrary, epileptic patients have been found to have lower than normal values of Zn in hair [36].

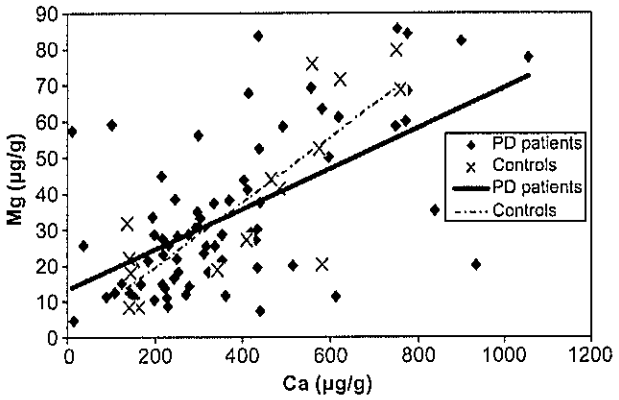

Fig. 1. Correlation between Ca and Mg in the hair of controls and PD patients.

Regarding correlations among metal levels, Fe correlated negatively with Zn at $p = 0.029$ only in PD patients. A positive correlation between Ca and Mg was also found in both healthy subjects and PD patients ($p < 0.001$ for both), as shown in Fig. 1. This correlation persisted when the subjects were divided by sex ($p = 0.026$). Shore et al. [28] also found a significant positive correlation between Ca and Mg in both controls and Alzheimer's disease patients.

The absence of any correlation in the hair of controls and patients as a function of age was observed for Cu, Fe, Mg, Si and Zn. A strong relationship was noted in the case of Ca in PD patients, with a noticeable decrease in older subjects ($p = 0.003$), also after grouping by sex ($p < 0.018$ for both females and males). However, this negative correlation was not found in controls. The lower values for Ca in the hair of PD patients with age are shown in Fig. 2.

Finally, there was no significant correlation between hair metal concentrations and the duration of the disease, Hoehn and Yahr staging or anti-Parkinson

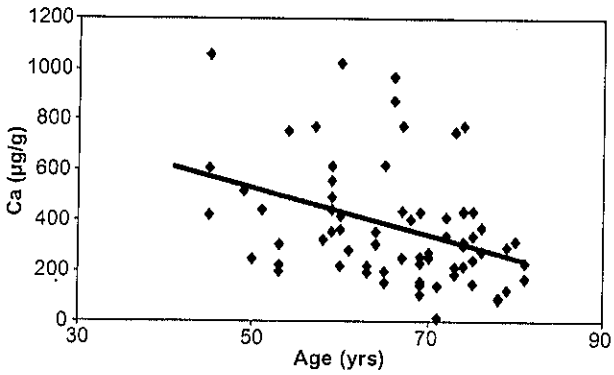

Fig. 2. Correlation between hair Ca concentration and age of PD patients.

treatment. Moreover, no statistical difference in metal absorption – neither for Mg nor for the other elements – was revealed comparing patients with and without levodopa treatment. Similar observations were also made by Jiménez-Jiménez et al. [39] in serum and cerebrospinal fluid of Parkinson subjects.

Conclusions

Our findings in PD patients suggest a probable relationship between the pathology and a deficiency of Fe in the hair of patients. Ca deficiency was less important, although Ca levels in the PD group decreased significantly with age. Variations in values of Cu, Si and Zn were insignificant. Differences were found for Ca and Mg depending on sex but they were not useful as causative determinants for PD, as they were found equally in patients and controls. Finally, the levels of metals in hair were not affected by the duration or severity of the disease or by the type of pharmacological therapy.

In summary, our findings allow us to conclude that hair is only a partially useful biomarker of imbalances in the studied metals in PD, the main observation remaining a marked decrease in Fe levels in male patients. Further studies including other elements as well as larger subgroups of controls are needed to clarify further whether hair can effectively supply information on the body burden of metals to complement data derived from biological fluids.

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