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Determination of copper, manganese and zinc in human liver

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Autopsied liver tissue samples collected from 42 males and 31 females were analyzed for copper, manganese and zinc using atomic absorption spectrometry (AAS). With the exception of two liver samples for which the copper levels were determined to be 74.8 and 104.0 μg/g (dry weight), hepatic copper concentrations were found to range from 1.7 to 32.4 μg/g with a mean concentration of 14.2 μg/g and standard deviation of 7.0 μg/g. Manganese concentrations (with the exception of one sample having 12.9 μg/g) ranged from 0.22 to 4.6 μg/g with a mean of 2.26 ± 1.00 μg/g. Hepatic zinc levels averaged 118.3 ± 44.4 μg/g and ranged from 38.5 to 231.3 μg/g. There were no apparent trends for the levels of any metals versus age nor were there any differences in average hepatic metal concentrations for males and females.

Keywords: copper, liver, manganese, metals, zinc

Introduction

The role of various elements, including both essential and toxic metals, in human biochemistry has been the subject of a large number of medical and scientific studies in recent years. There is considerable interest in the health effects resulting from both chronic and acute exposure to potentially toxic elements. Exposure to toxic heavy metals such as lead and mercury have received widespread attention from the scientific community and the general public. The role of essential elements in human health, while not as sensationalized as exposure to toxic metals, is nonetheless still highly significant. Many medical studies have been focused on the implications of deficiencies of essential elements such as calcium and iron.

A large proportion of the knowledge concerning human body burdens of various elements has been derived from testing which has been performed on bodily fluids, usually blood or urine. In fact many medical diagnoses rely heavily upon results from the determination of either toxic or trace essential elements in blood or urine. Copper, manganese and zinc may all be considered to be essential trace elements since they are involved in various human enzyme systems, although manganese is frequently classified as being a toxic element. The impact of manganese deficiency in humans has not been established. Manganese poisoning affects the central nervous system with symptoms that may include nausea, vomiting, headaches, disorientation, memory loss, anxiety, and compulsive laughing or crying (Willie 1996). Chronic manganese poisoning may result in symptoms resembling Parkinson's disease with akinesia, rigidity, and tremors.

Unlike manganese, the effects of zinc deficiency from either malabsorption or nutritional inadequacy have been well established. Infants and young children with advanced zinc deficiency may suffer from growth retardation, diarrhea, impaired T-cell immunity, infections and hindered wound healing, and in severe cases even death (Willie 1996). Adolescents and adults with zinc deficiency may exhibit slow growth including delayed puberty or weight loss,

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dwarfism, altered taste, impaired ability to adapt to darkness, central scotomata, alopecia, emotional instability, tremors, cerebellar ataxia, and a rash on extremities.

Copper deficiency results in the reduction of ceruloplasmin levels in blood, anemia due to inhibited iron metabolism, scurvy-like bone disease, depigmentation, growth failure, and neutropenia (Willie 1996). Copper has also been recognized as a factor in several diseases. Some recent examples include the study of hepatic copper levels in patients with Wilson's disease (Yuzbasiyan-Gurkan *et al.* 1991, Ludwig *et al.* 1994, Iancu *et al.* 1996), iron and copper deposition in the liver of patients suffering from chronic active viral hepatitis and posthepatitic liver cirrhosis (Ishida *et al.* 1995), copper accumulation in hepatocellular carcinomas (Kitagawa *et al.* 1991), and elevated hepatic copper concentrations in patients suffering from Indian Childhood Cirrhosis (Baker *et al.* 1995, Prasa *et al.* 1996).

In addition to studies which have been carried out on individuals suffering from specific diseases, there have been several studies which have examined the concentrations of metals in organs collected at autopsy from individuals representative of the general population (Bona *et al.* 1992, Oldereid *et al.* 1993, Bush *et al.* 1995). In the study described herein, autopsied liver samples from the general population were analyzed for copper, manganese and zinc.

Materials and methods

Portions of autopsied human liver specimens from deceased residents of the Province of Saskatchewan (Canada) were collected and stored in a freezer for future analysis. A total of 73 samples, 42 males and 31 females, were subjected to the sample preparation procedure described herein. Approximately 1 to 2 g of wet liver tissue was weighed into a 25 mL round-bottomed tube. The liver was gently dried at 60°C for 90 h and the resulting dry weight was recorded. Randomly selected samples were processed in duplicate to demonstrate the precision afforded by the employed methodology. The specimens were allowed to digest in 5 mL of nitric acid (Ultrex II, ultrapure reagent grade, J.T. Baker Inc., Phillipsburg, NJ, USA) for a period of 72 h at room temperature. The digested/acidified liver samples were diluted as necessary to permit concentration interpolation. All reagent blanks were prepared and treated in exactly the same manner.

As a further quality control check, a standard reference material (SRM 1577a) consisting of lyophilized bovine liver, purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA), was analyzed along with the liver samples. This material has certified values for all of the analytes determined in this study. Two portions of the SRM liver, each weighing approximately 0.5 g, were processed with the human liver samples.

A Varian SpectrAA-300Z atomic absorption spectrophotometer (Victoria, Australia) equipped with a graphite furnace and Zeeman background correction system was used in conjunction with a programmable Varian autosampler. Individual hollow cathode lamps were employed for the determination of manganese and copper. Details regarding the conditions of the radiation source for the analysis of each of these elements are given in Table 1. Pyrolytically-coated partition graphite tubes purchased from Varian were used for all analyses carried out on the graphite furnace atomic absorption spectrometer (GFAAS). The graphite furnace programs utilized for each analysis are shown in Table 2. Extra steps were employed at the termination of the atomization furnace program to allow the graphite tube to cool slowly to 40°C and thus prevent spattering of the subsequent injection. Along with this modification, an extended dispensing rate (with heated injection at 40°C) was used to ensure that all the dispensed liquid would be slowly introduced to be contained within the platform cavity for subsequent temperature programming. The autosampler was programmed to deliver 5 μL of solution to the graphite tube.

A Varian SpectrAA-20 flame atomic absorption spectrophotometer equipped with a deuterium background correction system was employed for the determination of total zinc. The zinc hollow cathode lamp operating information is given in Table 1.

Table 1. Radiation source conditions for AAS analyses

	Cu	Mn	Zn
wavelength (nm)	324.8	279.5	213.9
slit width (nm)	0.5	0.2	1.0
current (mA)	4	5	5

Table 2. Graphite furnace temperature programming profiles

Element	Step no.	Temp. (°C)	Ramp time (s)	Hold time (s)	Argon gas flow (L/min)
Cu	1	120	30	10	3.0
	2	700	30	10	3.0
	3	2600	1	7	0.0
	4	40	13	2	3.0
Mn	1	110	35	15	3.0
	2	650	35	20	3.0
	3	2500	1	67	0.0
	4	40	10	4	3.0

Results

Autopsied liver samples were collected from 42 male and 31 female subjects. The age of the male subjects ranged from 5 months to 88 years with an average age of 44 ± 23 years. Similarly, the female subjects ranged in age from 2 months to 86 years of age with an average of 49 ± 22 years. The average age of all 73 human subjects was 46 ± 23 years. The results of hepatic metal concentration analysis are given in Tables 3 and 4.

Hepatic copper concentrations in the 73 samples which were analyzed ranged 1.7 to 104.0 μg/g, with 71 of the 73 samples containing less than 33 μg/g. If the two results which are outside of three standard deviations of the mean (i.e., 74.8 and 104.0 μg/g) are not included in the statistical analysis, the average hepatic copper concentration was 14.2 ± 7.0 μg/g. The concentrations of copper found in the liver tissue samples of 41 males and 30 females (again not including the two elevated results) were found to average 13.1 ± 7.2 and 14.8 ± 6.7 μg/g, respectively.

Manganese concentrations were found to range from 0.22–12.9 μg/g, with 71 of the 72 results being less than 4.70 μg/g. If one eliminates the result which is greater than three times the standard deviation above the mean, the average manganese concentration becomes 2.26 ± 1.00 μg/g. The mean hepatic manganese concentrations for 41 male and 31 female subjects were determined to be 1.89 ± 1.00 and 2.67 ± 0.83 μg/g, respectively.

Hepatic zinc concentrations in the 72 samples tested ranged from 38.5–231.3 μg/g with a mean of 118.3 ± 44.4 μg/g. The mean zinc concentration in 42 male liver tissue samples was determined to be 121.9 ± 44.3 μg/g. Similarly, the mean zinc concentration for the liver samples collected from 30 female subjects was calculated to be 113.3 ± 43.6 μg/g.

Discussion

The major purpose behind this study was to determine the range of concentrations of copper, manganese and zinc in autopsied liver tissue samples collected from the general population. With the exception of two elevated samples, the mean copper concentration was calculated to be 14.2 ± 7.0 μg/g based on 71 samples. In a similar study performed by Bush *et al.* (1995) on 30 subjects, hepatic copper concentrations ranged from 9–55 μg/g with a mean concentration of 20 ± 12 μg/g. Interestingly the two highest copper concentrations in our study were found in liver samples collected from both of the subjects who were less than one year of age at the time of their deaths.

The results obtained for manganese also compared favorably with those reported by Bush *et al.* (1995). Excluding one elevated result (12.90 μg/g), the mean manganese concentration based on the analysis of 71 samples was calculated to be 2.26 ± 1.00 μg/g. If the elevated result was included in the statistical calculation, the mean would become

Table 3. Metals in female human liver tissue

No.	Age (yr)	Cu (μg/g)	Mn (μg/g)	Zn (μg/g)	No.	Age (yr)	Cu (μg/g)	Mn (μg/g)	Zn (μg/g)
1	0.17	104	1.86	58.2	17	54	14.4	1.78	131.0
2	2	22.9	4.61	93.1	18	56	8.2	2.79	76.8
3	14	14.9	2.01	122.7	19	57	27.1	4.27	147.0
4	27	21.5	2.17	111.6	20	58	14.4	2.14	138.2
5	29	10.9	3.80	92.5	21	62	15.1	2.29	126.9
6	31	15.4	2.45	139.3	22	63	7.6	1.12	53.4
7	33	29.7	3.96	195.3	23	63	27.7	2.56	100.6
8	33	9.1	2.29	47.1	24	66	21.4	1.99	200.4
9	37	25.4	3.36	128.7	25	69	6.3	2.01	123.5
10	39	16.5	3.23	52.3	26	72	12.4	1.46	117.6
11	40	6.8	2.00	NR	27	76	9.9	3.96	185.9
12	40	14.7	2.98	192.8	28	78	13.4	2.93	122.2
13	41	14.3	3.03	121.8	29	80	11.7	3.09	128.3
14	42	17.8	2.10	47.5	30	81	12.6	2.75	118.4
15	48	5.5	2.37	66.1	31	86	12.8	3.13	109.4
16	51	5.1	1.86	51.8					

NR = no result.

Table 4. Metals in male human liver tissue

No.	Age (yr)	Cu (μg/g)	Mn (μg/g)	Zn (μg/g)	No.	Age (yr)	Cu (μg/g)	Mn (μg/g)	Zn (μg/g)
1	0.42	74.8	1.37	91.1	22	43	9.0	1.82	107.7
2	1	11.7	3.52	110.4	23	44	6.0	NR	95.4
3	5	20.3	3.91	107.8	24	45	6.7	0.84	98.0
4	16	13.0	3.90	108.1	25	49	10.2	1.27	119.0
5	16	26.6	2.62	194.1	26	50	1.70	12.9	47.0
6	17	21.1	2.01	120.9	27	51	32.4	3.30	83.5
7	19	15.1	1.13	81.8	28	56	4.0	0.22	72.9
8	20	17.4	0.93	123.0	29	57	6.3	3.20	159.6
9	24	17.5	1.72	115.2	30	58	19.7	1.08	141.4
10	26	10.8	0.78	110.3	31	62	7.1	0.38	113.6
11	26	18.2	1.74	130.2	32	65	18.5	1.28	231.3
12	28	5.0	1.92	38.5	33	66	9.8	1.30	186.9
13	29	17.2	2.01	201.0	34	68	8.0	0.85	80.4
14	32	6.5	1.61	79.0	35	71	5.1	1.69	61.1
15	33	12.1	3.70	116.5	36	73	13.8	2.85	157.7
16	33	15.1	2.08	149.5	37	74	17.9	4.07	180.8
17	35	22.5	1.23	154.1	38	75	9.9	1.72	155.7
18	37	22.5	1.22	122.1	39	77	8.6	1.52	170.9
19	39	3.4	2.18	55.2	40	80	9.3	2.71	193.0
20	39	30.3	1.14	81.6	41	85	12.5	2.28	171.1
21	41	6.5	0.65	96.6	42	88	9.7	1.90	107.0

NR = no result.

2.40 ± 1.59 μg/g. Either way this data compares favorably to the mean manganese concentration 4.04 ± 1.59 μg/g reported by Bush *et al.* (1995). Excluding the liver sample containing 12.9 μg/g of manganese, the hepatic manganese concentration of the 71 samples was found to range from 0.22–4.61 μg/g in our study versus a range of 1.48–6.85 μg/g in the work performed by Bush and colleagues.

The concentration of zinc found in 72 liver samples was found to range from 38.5–231.3 μg/g with a mean of 118.3 ± 44.4 μg/g. Bush *et al.* (1995) analyzed 30 liver samples and reported zinc concentrations which ranged from 109–368 μg/g with a mean concentration of 191 ± 56 μg/g.

As part of intralaboratory quality control, duplicate samples which consisted of separate portions of tissue were processed along with all of the samples. In general, the relative per cent difference (i.e., the difference in the two results relative to the average concentration) was found to be typically less than 10%. This is consistent with previously reported data which indicates that the distribution of both essential and toxic elements is relatively homogeneous in human liver (Bush *et al.* 1995). It should be noted, however, that hepatic copper concentrations have been reported to vary two- to three-fold throughout liver tissue subsamples taken from a patient who died of Wilson's disease (Faa *et al.* 1995).

The use of a standard reference material for which a certified analyte concentration has been established provides an excellent means of verifying the accuracy of the analytical procedure which has been employed. Based upon the duplicate analyses which were performed on the bovine liver SRM (see Table 5) processed along with the human liver samples, the analytical procedure appears to provide results which are both accurate and precise. Analysis of 2 portions of the SRM liver yielded results which were within the acceptable range of the certified trace metal concentrations.

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Table 5. Analysis of trace metals in SRM 1577a lyophilized bovine liver

Element	Certified concentration (μg/g)*	GFAAS & flame AAS results (μg/g)
Cu	158 ± 7	162
		164
Mn	9.9 ± 0.8	9.81
		9.43
Zn	123 ± 8	118
		117

*National Institute of Standards and Technology, Standard Reference Materials Program, SRM 1577a certificate of analysis for bovine liver.

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