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Trace elements in normal and cirrhotic human liver tissue I. Iron, copper, zinc, selenium, manganese, titanium and lead measured by X-ray fluorescence spectrometry

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ABSTRACT – Trace elements (Fe, Cu, Zn, Se, Mn, Ti, Pb) were measured by X-ray fluorescence spectrometry in normal liver tissue obtained at autopsy from 74 subjects (44 ♂, 30 ♀), median age 62 years (range 20–87), and in tissue from 27 cirrhotic livers (14 alcoholic, 13 non-alcoholic cirrhosis). The element content (median and 5–95 percentile interval) in *normal livers* in mmol/kg dry tissue was: *Fe*, 16.51 (7.82–39.03); *Cu*, 0.378 (0.189–0.629); *Zn*, 4.01 (2.59–9.33); *Se*, 0.018 (<0.004–0.035); *Mn*, <0.055 (<0.055–0.237); *Ti*, <0.146 (<0.146–0.919); *Pb*, <0.0005 (<0.0005–0.0154). Only copper content showed a sex difference, being higher in males than in females ($P<0.04$). In both groups of *cirrhotic liver*, *Fe* content was within normal, *Cu* content above normal ($P<0.05$, $P<0.02$), and *Se* content below normal ($P<0.0001$, $P<0.04$). Alcoholic cirrhotic livers had lower *Zn* levels ($P<0.02$), higher *Mn* levels ($P<0.06$), and higher *Pb* levels ($P<0.03$) than normal livers.

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Essential trace elements are necessary for the effective operation of biological systems as components of metalloproteins and/or metalloenzymes (1, 2). The recognition of deficiency or overloading states for such trace elements is important both to clinical medicine and in drawing up guidelines for dietary allowances (1, 2).

The liver occupies a central position in trace element metabolism (1, 3, 4). In the present study a number of essential elements (iron (Fe), copper (Cu), zinc (Zn), selenium (Se) and manganese (Mn)), an "inert" element (titanium (Ti)), and a toxic element (lead (Pb)) have been measured in normal and cirrhotic human liver using X-ray fluorescence spectrometry (XRF analysis).

Material and methods

Material

Normal liver tissue was obtained at autopsy from 32 males and 14 females with a median age of 38 years (range 20–83), all killed in accidents. A previous material comprising 12 males and 16 females who died from cardiovascular or cerebrovascular disease (5), was included in the study. Thus the normal liver material consisted of 44 males with a median age of 52 years (range

20–87) and 30 females with a median age of 71 years (range 20–87). In the entire material median age was 62 years; six females were ≤45 years old.

Cirrhotic liver tissue was obtained from 12 males and two females with alcoholic cirrhosis and a median age of 61 years (range 39–80) and from three males and ten females with non-alcoholic cirrhosis and a median age of 80 years (range 70–86). The latter group comprised one male and one female with posthepatitic cirrhosis, one female with idiopathic haemochromatosis, diagnosed at autopsy, and ten patients with cryptogenic cirrhosis.

Methods

Liver tissue samples weighing approximately 10 g were excised with a plastic knife from beneath the liver capsule on the anterior surface of the right lobe, and kept frozen in polystyrene beakers until preparation. The samples were then freeze-dried to constant weight (mean weight loss 76.1%), homogenized at −196°C, and vanadium was added as internal standard. The tissue was pressed into pellets containing 1 g dry liver tissue and XRF analysis was subsequently performed on these pellets.

In order to evaluate whether the measured elements might be unevenly distributed in different parts of the liver, multiple samples were taken from one normal organ; six samples from both the anterior and lower surface of the right lobe, and three samples from each corresponding surface of the left lobe.

Utensils were stored in citric acid solution and rinsed in demineralised water before use. All reagents were analytical grade (Suprapur®, Merck).

XRF-analysis

The principles and the instrument employed have been described elsewhere (5, 6). Using yttrium as secondary target, the instrument was optimized for analysis of elements with atomic numbers from 16 to 37, determined by the intensities of their K_α spectral lines, displaying increasing sensitivity with rising atomic number (5, 6). Lead (atomic number 82) was determined by the intensity of its L_α spectral line.

At the measuring time of 2 ks employed, the detection limits (DL) in mmol/kg dry liver tissue were as follows; Fe, 0.0430; Cu, 0.0110; Zn, 0.0092; Se, 0.0038; Mn, 0.0546; Ti, 0.1461; Pb, 0.0005.

Provided there is a constant measuring time, the precision of XRF analysis depends on the DL relative to the content of the specific element in the examined sample (5). An acceptable precision is achieved when content $\times DL^{-1} \geq 5$. When content $\times DL^{-1} < 5$, XRF analysis has a semiquantitative character (5). The median content $\times DL^{-1}$ in the analysed liver samples was iron 384, copper 34, zinc 437, selenium 5, manganese 1, titanium 1 and lead 1.

At the median of the measurable concentrations above DL in this study, the precision of XRF analysis, expressed by the coefficient of variation, was Fe 1.8%, Cu 3.2%, Zn 1.3%, Se 26.7%, Mn 35.5%, Ti 28.3%, and Pb 5.4%.

In statistical analysis, differences between groups were evaluated by Mann-Whitney's rank-sum test, and correlation coefficients were calculated according to the method of least squares.

Results

Of the 22 elements with atomic numbers from 16 to 37, 11 elements could be detected in the following fractions of 74 normal liver samples; S, 74/74; Cl 74/74; K, 74/74; Ti, 12/74; Cr, 1/74; Mn, 16/74; Fe, 74/74; Cu, 74/74; Zn, 74/74; Se, 59/74; Br, 74/74. Pb was detected in 13/74 samples. Results concerning S, Cl, K and Br will be reported separately (7).

Homogeneity analysis

There was no difference in element content in tissue taken from the anterior and lower surface of each liver lobe. When comparing element content in the right and left lobe, the distribution of Cu, Zn and Se showed no significant variation, while the Fe content was higher in the right lobe (median 13.82 versus 12.14 mmol/kg) ($P < 0.01$).

Elements in normal liver samples

The content of the various elements in mmol/kg dry liver tissue is shown in Table 1 and Fig. 1; in order to convert into content in wet tissue, multiply by 0.234.

Se content was measurable (i.e. > DL) in 59 livers, mean value 0.024 mmol/kg dry weight (range 0.009–0.076), Mn in 16 livers, mean value 0.176 mmol/kg (0.091–0.364), Ti in 12 livers, mean value 0.936 mmol/kg (0.209–1.983), and Pb in 13 livers, mean value 0.0129 mmol/kg (0.0019–0.0536).

Cu content was higher in males than in females ($P < 0.04$). The other elements showed no significant sex differences, although seven (16%) males had an Fe content above the upper normal limit in females (Fig. 1).

There were positive correlations with age concerning hepatic content of Zn ($r = 0.34$, $P < 0.01$),

Table 1

Content of elements in normal liver samples in mmol/kg dry tissue

Subjects studied		Iron	Copper	Zinc	Selenium	Manganese	Titanium	Lead
	median	16.79	0.425	3.99	0.018	<0.055	<0.146	<0.0005
	mean	20.61	0.409	4.28				
♂	range	5.37–84.92	0.079–0.755	2.14–9.94	<0.004–0.076	<0.055–0.237	<0.146–1.983	<0.0005–0.0536
n=44	5–95 percentile	6.32–40.30	0.189–0.645	2.33–8.23	<0.004–0.043	<0.055–0.182	<0.146–1.002	<0.0005–0.0092
	median	15.49	0.299	4.07	0.022	<0.055	<0.146	<0.0005
	mean	15.92	0.330	4.53				
♀	range	5.01–28.45	0.126–0.629	2.59–9.47	<0.004–0.037	<0.055–0.364	<0.146–0.960	<0.0005–0.0323
n=30	5–95 percentile	7.90–27.18	0.205–0.504	2.94–7.17	<0.004–0.033	<0.055–0.255	<0.146–0.814	<0.0005–0.0154
	median	17.76	0.378	4.01	0.018	<0.055	<0.146	<0.0005
	mean	18.71	0.378	4.38				
♂+♀	range	5.01–84.92	0.079–0.755	2.14–9.94	<0.004–0.076	<0.055–0.364	<0.146–1.983	<0.0005–0.0536
n=74	5–95 percentile	7.82–39.03	0.189–0.629	2.59–9.33	<0.004–0.035	<0.055–0.237	<0.146–0.919	<0.0005–0.0154

Table 2

Content of elements in cirrhotic liver samples in mmol/kg dry tissue

Subjects studied		Iron	Copper	Zinc	Selenium	Manganese	Lead
	median	15.83	0.551	3.14	<0.004	<0.055	0.0019
Alcoholic	mean	23.62	0.787	3.26			
cirrhosis	range	2.54–84.23	0.205–2.156	1.70–5.28	<0.004–0.022	<0.055–0.218	<0.0005–0.0063
n=14	5–95 percentile	7.31–72.52	0.220–1.290	2.59–3.75	<0.004–0.016	<0.055–0.200	<0.0005–0.0058
	median	10.38	0.472	4.28	0.011	<0.055	<0.0005
Non-alcoholic	mean	46.45/13.88*	0.991	4.33			
cirrhosis	range	2.79–437.15/32.12*	0.189–3.179	1.79–8.00	<0.004–0.024	<0.055–0.237	<0.0005–0.0073
n=13	5–95 percentile	3.17–32.12	0.220–2.848	2.00–6.47	<0.004–0.023	<0.055–0.200	<0.0005–0.0034
	median	13.09	0.488	3.47	<0.004	<0.055	<0.0005
Total	mean	34.61/19.12*	0.881	3.78			
n=27	range	2.54–437.15/72.52*	0.189–3.179	1.70–8.00	<0.004–0.024	<0.055–0.237	<0.0005–0.0073
	5–95 percentile	2.79–84.23	0.205–2.848	1.79–6.20	<0.004–0.023	<0.055–0.218	<0.0005–0.0063

*Except value for haemochromatotic sample.

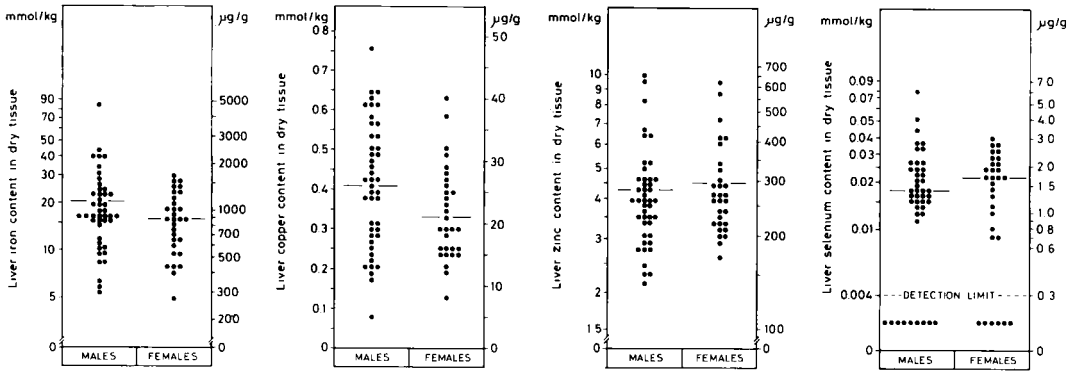

Fig. 1. Content of iron, copper, zinc and selenium in normal human liver samples in mmol/kg and µg/g dry tissue. Arithmetic means and median for selenium are shown.

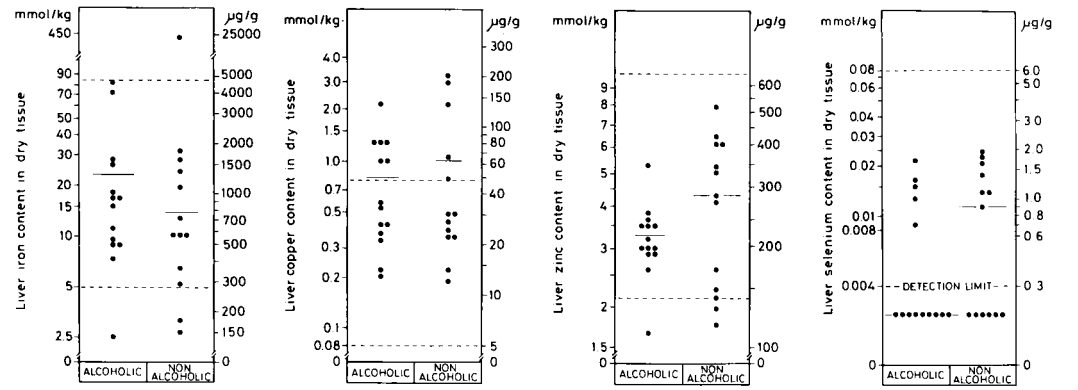

Fig. 2. Content of iron, copper, zinc and selenium in human cirrhotic liver samples in mmol/kg and µg/g dry tissue. Arithmetic means and median for selenium are shown. The upper and lower limits for normal livers are depicted by the dotted lines.

Se ($r=0.24$, $P<0.05$), Ti ($r=0.27$, $P<0.05$) and Pb ($r=0.26$, $P<0.05$). Hepatic content of Fe, Cu and Mn displayed no age correlations.

Elements in cirrhotic liver samples

The results are shown in Table 2 and Fig. 2. *Iron:* Values in cirrhotic livers were not significantly different from normal livers. Only the haemochromatotic liver had an Fe content above normal, whereas the content was below normal in one (7%) liver with alcoholic and in two (15%) livers with non-alcoholic cirrhosis. *Copper:* Values in both alcoholic and non-alcoholic cirrhotic livers were higher than in normal livers ($P<0.05$ and $P<0.02$). *Zinc:* Alcoholic cirrhotic livers had lower values than normal livers ($P<0.02$), while non-alcoholic cirrhotic livers had normal values. *Selenium:* Both groups of cirrhotic livers had lower levels than normal livers ($P<0.0001$ and $P<0.04$). *Manganese:* Alcoholic cirrhotic livers had higher values than normal livers ($P<0.06$), while non-alcoholic cirrhotic livers had normal values. The Mn content was >0.0546 mmol/kg (DL) in seven (50%) alcoholic and in five (38%) non-alcoholic cirrhotic livers. *Titanium:* Values in cirrhotic livers were not significantly different from normal livers. Only one cirrhotic liver had a Ti content >0.1461 mmol/kg (DL). *Lead:* Values in alcoholic cirrhotic

livers were higher than in normal livers ($P < 0.03$), while non-alcoholic cirrhotic livers had normal values. The Pb content was >0.0005 mmol/kg (DL) in eight (57%) alcoholic and in three (23%) non-alcoholic cirrhotic livers.

The haemochromatotic liver sample showed an Fe content of 437.15 mmol/kg, a low Cu content of 0.188 mmol/kg, a low Zn content of 1.79 mmol/kg, an Se, Mn and Ti content <DL, and a Pb content of 0.0072 mmol/kg.

There was a significant correlation between the Fe and Pb content in both alcoholic ($r = 0.82$, $P < 0.001$) and non-alcoholic ($r = 0.88$, $P < 0.001$) cirrhotic livers.

Discussion

The cirrhotic liver samples contained an increased and variable amount of fibrous connective tissue, probably with a low content of trace elements (8). The results were not corrected for the presence of connective tissue. Consequently, in cirrhotic livers, subnormal values (Zn and Se) in element content should be interpreted with some reservation, while supranormal values (Cu, Mn and Pb) most likely indicate a real increase in element content in the hepatocytes.

Iron. The liver is the principal iron storage organ of the body, and assessment of hepatic iron content provides valuable information about iron status (3, 9). XRF analysis does not discriminate between non-haem storage iron and haemoglobin iron. The hepatic haemoglobin iron content may vary from one sample to another, depending both on the haematological status and on the degree of hepatic congestion prior to death (10). The blood content of the liver can therefore contribute significantly to total liver iron, especially at low parenchymal iron concentrations (5, 10).

The content of copper, zinc and selenium was identical in the two liver lobes, while the iron concentration was higher in the right lobe. It remains unclarified whether this finding indicates a functional difference in iron storage pattern, or could be explained by a higher blood content in the right lobe.

In normal livers, total iron content displayed considerable variation, the highest value being more than 13 times the lowest value. Although the highest hepatic iron levels were found in the male group, the difference between the two sexes was insignificant. In the fertile age, females have a lower liver iron content than males (11). This discrepancy disappears in the senium (12, 13), as confirmed in the present material, of which the majority (80%) of the females were postmenopausal.

The present findings are close to data from Australia reporting a normal range for total liver iron of 5–40 mmol/kg dry weight (14). Owing to methodological differences, the results will be somewhat higher compared to studies estimating non-haem liver iron (11, 12, 13, 15). Nevertheless there is reasonably good agreement between the values obtained and the non-haem liver iron content in elderly English and Swedish males (median 11.98 and 12.53 mmol/kg dry weight) and females (12.93 and 10.24 mmol/kg) (12, 15).

The iron content in cirrhotic livers was lower than in normal livers, and, with the exception of the haemochromatotic liver, none of the samples showed iron concentrations in the haemochromatosis range (>100 mmol/kg dry weight) (16, 17). Normal amounts of liver iron were present in the majority of alcoholic cirrhotic livers. Apparently there is a lower frequency of hepatic iron overload in alcoholics in Scandinavia (18) than in Australia or other European countries (14, 16, 19), possibly due to differences in dietary and/or drinking habits.

Copper. The liver is the major storage and excretory organ for copper, and estimation of hepatic copper content yields definitive information on copper balance (4). As for iron, the copper content in normal livers displayed marked variation, with a tenfold difference between the lowest and highest value. The results obtained in normal livers showed good agreement with studies in Caucasians from various countries (4, 20–22). Copper was the only element in which a significant difference existed between the two sexes, an observation not previously reported (4, 20–22).

Hepatic copper content was moderately elevated in 41% of cirrhotic livers, equally distributed between alcoholic and non-alcoholic cirrhosis. None of the cirrhotic livers displayed copper concentrations in the Wilson's disease range (>3.93 mmol/kg dry weight). The haemochromatotic liv-

er, showing no evidence of cholestasis, had a low copper content, in contrast to the high values reported by Sheldon (23).

Zinc values in normal livers showed a fivefold variation from the lowest to the highest value, and were practically identical to those reported in Norwegian subjects (21). The low hepatic zinc content found in alcoholic cirrhotic livers confirms the conclusions of previous studies (24, 25). However, the relation between clinical zinc deficiency and liver zinc in man has not been evaluated (26). Patients with alcoholic cirrhosis have an abnormal zinc metabolism with low serum zinc, increased urinary zinc excretion and low hepatic zinc levels, while the absorption of zinc seems unaffected (24, 25, 27). It is unclear whether zinc depletion plays a pathogenic role in the development and modification of the cirrhotic process, or is just a secondary phenomenon of the disease.

Selenium is the active site of glutathione peroxidase (28), and, like zinc and manganese, this element probably has profound effects in protecting against membrane lipid peroxidation (29). Selenium is to some degree accumulated in the liver (30), but it is not known to what extent hepatic selenium content reflects body balance. Scandinavia is recognised as a low selenium area (31), suggesting that selenium intake might be suboptimal in Scandinavians. The selenium levels in normal livers were comparable with the results in Danish and Norwegian studies using neutron activation (32) and fluorimetric analysis (21).

In cirrhotic livers the selenium content was markedly reduced, most pronounced in alcoholic cirrhosis, of which 64% had levels <0.0038 mmol/kg, compared to 46% of non-alcoholic cirrhosis and 20% of normal livers. Other investigators have likewise reported low serum and liver selenium values in patients with alcoholic cirrhosis, and suggested that selenium depletion might accelerate the progression of alcoholic liver disease by decreasing the protective activity of glutathione peroxidase (33).

Manganese plays an essential role in several metalloenzymes (34). Manganese homeostasis seems to be regulated at the excretory level, mainly through elimination via the liver and biliary tract (1, 34), and this element is found in highest concentrations in liver and pancreatic tissue (22, 32, 35). The manganese content of normal livers was lower than previously reported in USA and Denmark (22, 32); part of this difference could be due to the low sensitivity of the XRF analysis. Alcoholic cirrhotic livers showed slightly higher manganese levels than normal livers, possibly due to impaired biliary excretion.

Titanium. The biological role of titanium is unclear, and so far there is no evidence that this element is essential to man (1). The reported Ti concentrations of human tissues are extremely variable (1). Normal livers had a low titanium content, and only 16% contained this element in detectable quantities. In USA residents, titanium was detectable in 18% of normal livers, with an upper limit of 0.012 mmol/kg dry weight (22).

Lead is toxic to man (36) and was shown to accumulate in tissues with age, as reported in USA residents (1). It was detectable in 18% of normal livers, and the levels were lower than reported in USA (median 0.0232 mmol/kg dry weight, range 0.0106–0.0449) (22).

Owing to the lead content in wine, alcohol consumption is an important indicator for lead exposure in adults (37), and generally alcoholics have high blood lead levels (38). Accordingly alcoholic cirrhotic livers contained significantly more lead than normal livers. The positive correlations between hepatic iron and lead content in cirrhotic livers suggest a relationship between these two elements on the consumptive and/or the absorptive level (39).

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