

# LEAD CONTENT IN AUTOPSY LIVER TISSUE IN SAMPLES FROM GREENLANDIC INUIT AND DANES

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## ABSTRACT

**Objectives.** To measure the quantity of lead (Pb) in liver tissue samples from Greenlandic Inuit, and compare the results with those obtained in Caucasian Danes.

**Study design.** Observational, descriptive survey on environmental pathology.

**Methods.** The setting was related to forensic medicine and hospitalised care in Nuuk, Ilulissat and Copenhagen. Participants were 50 Greenlandic Inuit (27 men) with a median age of 61 years (range 23-83) and 74 Danes (44 men) with a median age of 60 years (range 15-87). Liver tissue samples (normal by macroscopic and microscopic examination) were obtained at autopsy. Total liver lead content was measured by X-ray fluorescence spectrometry with a detection limit of 0.05 µmol/kg dry weight.

**Results.** In the entire series, Inuit had higher liver lead contents than Danes ( $p < 0.0001$ ). Inuit men had higher liver lead content than Inuit women ( $p = 0.02$ ). In Danes, men tended to have higher liver lead contents than women, but the difference was insignificant. The median (5-95 percentile) lead content was 14.96 µmol/kg dry liver (4.83-74.80) in Inuit, and  $< 0.05$  µmol/kg dry liver ( $< 0.05$ -29.44) in Danes. All Inuit had liver lead contents above the detection limit, whereas 60 Danes (81%) had liver lead content below the detection limit. There was a positive correlation between liver lead content and age in both Inuit ( $r_s = 0.46$ ,  $p = 0.002$ ) and Danes ( $n = 14$ ;  $r_s = 0.71$ ,  $p = 0.01$ ). Inuit had higher hepatic lead indices (liver lead content divided by age) than Danes ( $p < 0.0001$ ). In Inuit, median hepatic lead index was 0.258, being higher in men than in women ( $p = 0.02$ ). In Danes, the median hepatic lead index was 0.001, again higher in men than in women ( $p = 0.03$ ).

**Conclusions.** Our results show a gender-related difference in hepatic lead content, i.e. Inuit men have higher liver lead contents than women. Furthermore, Inuit men and women have higher

hepatic lead contents than Danish men and women. In both Inuit and Danes, the liver lead content increases with age. One reason for the high lead levels in Inuit may be ingestion of seabirds contaminated by lead shot.

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**Keywords:** Danes, Eskimos, Inuit, lead, liver, men, women, X-ray emission

## INTRODUCTION

Lead (Pb) has no known biological function in man and is classified as nonessential. Lead is toxic to humans (1). Toxic manifestations include developmental effects in children, neurological and neurobehavioral effects, anaemia, acute and chronic nephropathy, and hypertension (1).

Greenlandic Inuit are genetically different from Caucasians, and have disparate environmental and nutritional habits (2-4). In a previous study, we found that Greenlandic Inuit hunters from the Thule and Angmagssalik Districts had high blood lead levels, being even higher than in Danes living in a metropolitan area (5, 6).

The purpose of the present observational, descriptive survey on environmental pathology was to analyse the lead content in liver tissue samples from Inuit and Danes, in order to clarify whether the differences in blood lead levels are also expressed in the lead content of parenchymous organs.

## MATERIAL AND METHODS

### Subjects

#### Inuit

Tissue samples from normal livers (by macroscopic and microscopic examination) were obtained at autopsy, performed 8-12 hours

after death, from 27 Inuit men and 23 Inuit women, with a median age of 61 years. The causes of death are shown in Table I. The autopsies were performed between February 1992 to April 1994 at the District Hospitals in Nuuk and Ilulissat (7).

**Table I.** Causes of death in Greenlandic Inuit in the liver autopsy study.

Cause of death	n	%
Cancer	9	18
Heart disease	7	14
Cerebrovascular disease	2	4
Acute respiratory infection	1	2
Chronic bronchitis	3	6
Other natural causes	11	22
Accidents	7	14
Suicide, homicide	10	20
Total	50	100

#### Danes

Tissue samples from normal livers were obtained at autopsy from 44 ethnic Danish men and 30 ethnic Danish women, with a median age of 60 years (range 15-87). Forty-six persons had died from accidents, and 28 persons from cardiovascular, or cerebrovascular disease. The subjects were residents of Greater Copenhagen. The autopsies were performed between 1982-1985 at the Institute of Forensic Medicine and at Bispebjerg Hospital in Copenhagen (8, 9).

#### Sample preparation

Liver tissue samples weighing approximately 10 g were excised with a plastic knife from

beneath the capsule on the anterior surface of the right lobe and kept frozen until preparation. The liver tissue samples were subsequently freeze-dried to constant weight, homogenized, and pressed into pellets weighing 0.5-1 g. These pellets were used for X-ray fluorescence spectrometry (XRF) analysis. Utensils were stored in citric acid solution and rinsed in demineralised water before use. All reagents were analytical grade (Suprapur®, Merck).

### XRF-analysis

Using the molybdenum  $K\alpha$ -beam for the excitation, the XRF instrument was optimised for the analysis of elements with atomic numbers ranging from 16 to 37 (8). The measuring time was 2 kilo seconds, yielding a detection limit (DL) for lead of  $0.05 \mu\text{mol}\cdot\text{kg}^{-1}$  dry liver.

Besides the measuring time, the precision of the XRF analysis depends on the DL relative to the content of the specific element in the examined tissue sample. An acceptable precision is achieved when the element content  $\times \text{DL}^{-1}$  is  $\geq 5$  (8). The median value of the lead content  $\times \text{DL}^{-1}$  in all liver tissue samples was 4. The precision of the XRF analysis, expressed as the coefficient of variation at the median content of lead, was 5%.

The hepatic lead index was calculated as the liver lead content in  $\mu\text{mol}\cdot\text{kg}^{-1}$ , dry tissue divided by age in years.

### Statistics

The statistical evaluation of the results was performed using the Statview 5.0™ program. Due to the non-normal distribution of the results, nonparametric statistics were employed. Differences between groups were evaluated by Mann-Whitney's rank sum test,

and correlations by Spearman's rank coefficient of correlation ( $r_s$ ) corrected for ties.

## RESULTS

### Inuit

The dry liver tissue lead contents are shown in Table II and Fig. 1. To convert from  $\text{mmol}\cdot\text{kg}^{-1}$  dry tissue into  $\text{mmol}\cdot\text{kg}^{-1}$  wet tissue, multiply by 0.285. To convert from  $\text{mmol}\cdot\text{kg}^{-1}$  into  $\text{mg}\cdot\text{kg}^{-1}$ , multiply by 207.21. Liver lead contents were significantly higher in men than in women ( $p = 0.02$ ), and there was a positive correlation between the liver lead content and age in both men ( $r_s = 0.46$ ,  $p = 0.02$ ) and women ( $r_s = 0.53$ ,  $p = 0.01$ ), as well as in the entire series ( $r_s = 0.46$ ,  $p = 0.002$ ) (Fig. 2). The median hepatic lead indices were 0.303 in men and 0.197 in women ( $p = 0.02$ ), as shown in Table III and Fig. 3.

### Danes

In livers from 60 subjects (34 men, 26 women), the lead content was below the DL (Table II). To convert from  $\text{mmol}\cdot\text{kg}^{-1}$  dry tissue into  $\text{mmol}\cdot\text{kg}^{-1}$  wet tissue, multiply by 0.239. Although men had higher mean liver lead contents than women, the difference was not statistically significant (Table II). The correlation between the liver lead content and age in the entire series is shown in Fig. 2. The 14 subjects (10 men, 4 women) with liver lead contents above the DL demonstrated positive correlations between the liver lead content and age in both men ( $r_s = 0.73$ ,  $p = 0.03$ ) and women ( $r_s = 0.80$ ,  $p = 0.17$ ), and in the entire series ( $r_s = 0.71$ ,  $p = 0.01$ ). The median hepatic lead index was 0.001 in both men and women, but the male group had significantly higher

**Table II.** Lead content in liver tissue samples from Greenlandic Inuit and Danes.

		Liver lead ( $\mu\text{mol/kg}$ dry weight)		Danes <sup>#</sup>
		Inuit	p <sup>*</sup>	
<b>Men</b> n = 27/44	median	18.82 <sup>**</sup>	< 0.0001	< 0.05 <sup>***</sup>
	mean	27.47		4.0
	5%-tile	5.16		< 0.05
	95%-tile	92.32		40.06
	range	4.34-109.55		< 0.05-53.57
<b>Women</b> n = 23/30	median	11.58 <sup>**</sup>	< 0.0001	< 0.05 <sup>***</sup>
	mean	14.67		2.3
	5%-tile	4.66		< 0.05
	95%-tile	41.53		17.86
	range	4.34-48.74		< 0.05-32.33
<b>All</b> n = 50/74	median	14.96	< 0.0001	< 0.05
	mean	21.58		3.28
	5%-tile	4.83		< 0.05
	95%-tile	74.80		29.44
	range	4.34-109.55		< 0.05-53.57

\* Mann-Whitney test: \*\* p = 0.02; \*\*\* p = 0.3

# In 60 Danes with liver lead below the detection limit, values were set at 0.05  $\mu\text{mol/kg}$

**Table III.** Hepatic lead indices (lead in  $\mu\text{mol/kg}$  dry liver divided by age in years) in Greenlandic Inuit and Danes.

		Hepatic lead index		Danes <sup>#</sup>
		Inuit	p <sup>*</sup>	
<b>Men</b> n = 27/44	median	0.303 <sup>**</sup>	< 0.0001	0.001 <sup>***</sup>
	mean	0.467		0.060
	5%-tile	0.106		0.0006
	95%-tile	1.210		0.497
	range	0.1-1.739		0.001-0.661
<b>Women</b> n = 23/30	median	0.197 <sup>**</sup>	< 0.0001	0.001 <sup>***</sup>
	mean	0.267		0.028
	5%-tile	0.1		0.0006
	95%-tile	0.693		0.226
	range	0.087-0.886		0.001-0.372
<b>All</b> n = 50/74	median	0.258	< 0.0001	0.001
	mean	0.375		0.047
	5%-tile	0.106		0.0006
	95%-tile	1.076		0.348
	range	0.087-1.739		0.001-0.661

\* Mann-Whitney test: \*\* p = 0.02; \*\*\* p = 0.03

# In 60 Danes with liver lead below the detection limit, values were set at 0.05  $\mu\text{mol/kg}$

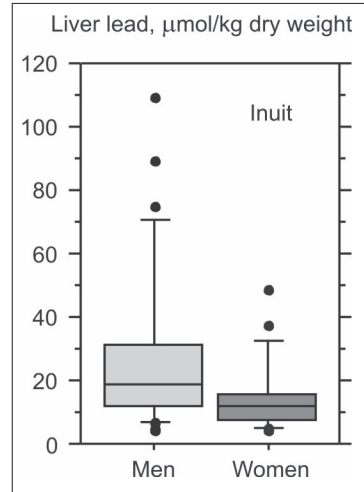
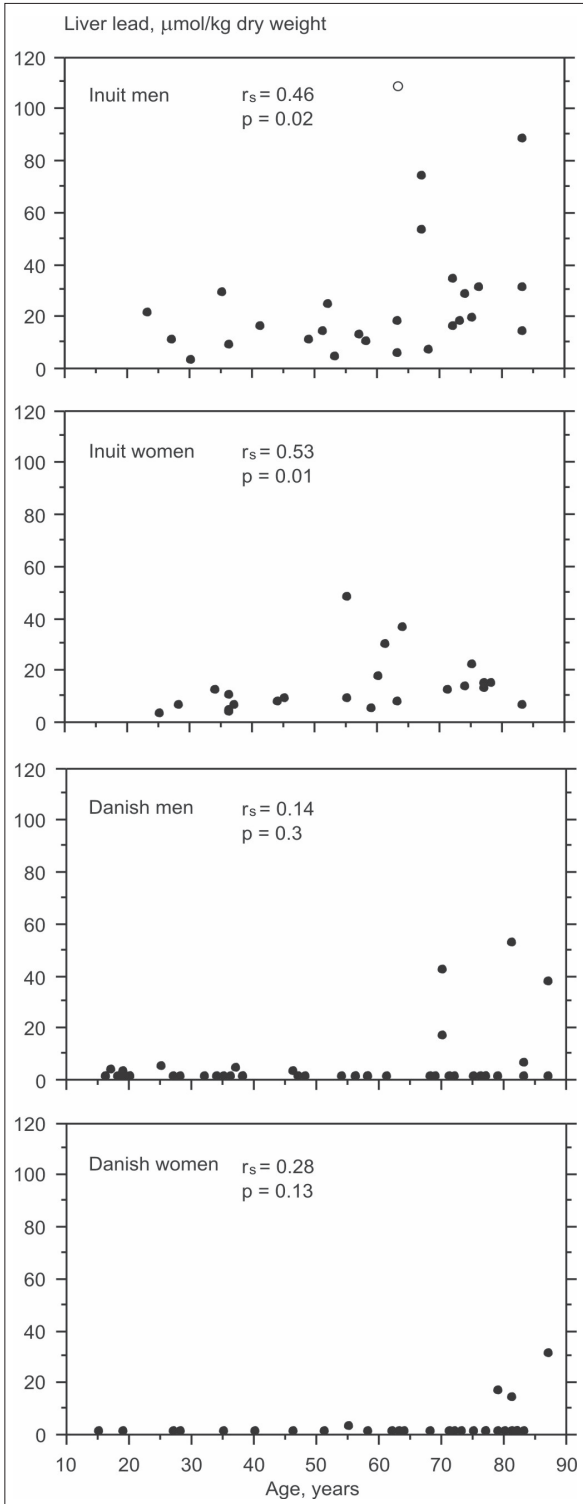


Figure 1. Lead content in autopsy liver tissue samples from Greenlandic Inuit.

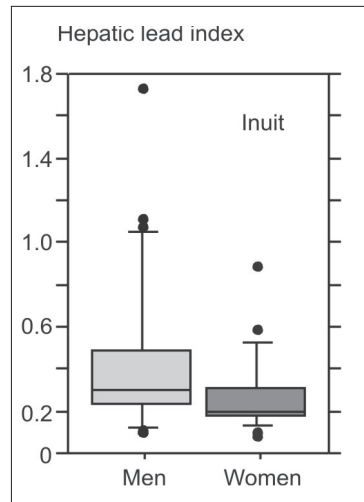


Figure 3. Hepatic lead indices (lead in  $\mu\text{mol kg}^{-1}$  dry liver divided by age) in autopsy liver tissue samples from Greenlandic Inuit.

Figure 2. Correlation (Spearman's  $r_s$ ) between age and liver lead content in autopsy liver tissue samples from Greenlandic Inuit and Danes. In 60 Danes with liver lead contents below the detection limit, values were set at  $0.05 \mu\text{mol kg}^{-1}$ . An out-lier (open circle) was excluded from the correlation analysis.

hepatic lead indices than the female group ( $p = 0.03$ ) (Table III).

### **Inuit vs. Danes**

There was no significant difference between the age distribution in Inuit and Danes. Inuit men and women had significantly higher liver lead contents than Danes ( $p < 0.0001$ ) (Table II). Likewise, hepatic lead indices were significantly higher in Inuit than in Danes ( $p < 0.0001$ ) (Table III).

## **DISCUSSION**

XRF analysis measures the total lead content in the liver and does not discriminate between lead in various subcellular fractions. The blood content of the liver varies from one sample to another, depending on the degree of hepatic congestion prior to death, but this does not contribute significantly to the lead content of the liver parenchyma (8). Removing the blood by perfusion of the liver samples with demineralised water has no influence on the hepatic lead content (8).

Hepatic lead content has not been previously assessed in Greenlandic Inuit. In livers from both Inuit and Danes, the lead content displayed considerable variation. The highest hepatic lead levels were found in Inuit men, who had significantly higher levels than Inuit women and Danes.

In the present series, there is a positive correlation between the liver lead content and age, in both Inuit and Danes. Accordingly, blood lead levels in Inuit hunters display a positive correlation with age (5). These findings suggest that the age-related

increases in blood and liver lead are due to either a continuous body lead accumulation and/or a release of lead from the bones in association with the physiological, age-dependent reduction of the total bone mass.

Animal studies show that lead absorption takes place in the duodenum and jejunum (10). In humans, iron absorption also takes place in the duodenum and in the proximal part of the jejunum (11). The absorption of lead in humans is probably dependent on transportation into the intestinal cells by the divalent metal transporter 1 (DMT1). This protein plays a crucial role in the absorption of other divalent metal ions, e.g. iron, zinc, manganese, copper and cadmium (12, 13). The resulting competition for absorption-facilitated DMT1 protein binding between the divalent metal ions may explain the relationship between the uptake of iron and lead.

In addition, there is a positive correlation between food iron absorption and lead absorption in humans, i.e. high iron absorption due to low iron status is accompanied by high lead absorption (14). Iron absorption is impaired by a concomitant intake of calcium (11). Intestinal absorption of lead is also reduced by ingestion of calcium, including calcium in cow's milk (15).

The age ranges were similar in Inuit and Danes. With a median age of 61 years, the majority of the Inuit belonged to the elderly generation, who consume a traditional Greenlandic diet, rich in meat and pluck from Arctic sea mammals, fish and sea birds (2, 3). The traditional diet is rich in iron with a high bio-availability, and Inuit hunters have a high iron status (2, 3). When body iron stores reach a stable high level, this will tend

to reduce iron and thus lead absorption. On the other hand, the intake of milk (calcium) is low in the traditional diet, which tends to favour the absorption of iron and lead.

The content of lead in marine fish, seabirds and marine mammals, depends on the level of lead pollution in the area where the animals live and feed (16, 17). Lead concentrations in Arctic marine fish, seabirds and sea mammals are low, rarely exceeding 1 mg/kg wet weight in any tissue (16). However, lead shot is widely used in bird hunting in Greenland. Lead shot crippling and lead shot ingestion can induce lead poisoning of waterfowl (18). Twenty-four percent of the common eiders in Greenland carry imbedded lead shot (18) and they have high tissue lead contents (19), which tend to increase with age. The mean lead concentration in the breast muscles of eider of more than three years of age, and killed by lead shot, is 7.9 mg/kg wet weight. In contrast, eider that have succumbed to accidental drowning in fishing nets have a mean breast muscle lead concentration of 0.2 mg/kg wet weight (19). Blood lead levels in Inuit are significantly associated with the consumption of seabirds (20).

Lead levels in the Greenland environment are low (20). Therefore, lead shot contamination of consumed seabirds, especially eider and murre, is probably the main source of lead pollution for most people in Greenland (19, 20).

Inuit men have higher blood lead levels than Inuit women of similar ages (5). We hypothesised that this difference was related to the higher haemoglobin concentration in men compared to women. However, the present results show that men have significantly higher liver lead levels compared to

women, suggesting that total body lead is higher in men than in women. This could be due to differences in environmental exposure to lead, dietary lead intake, or lead absorption.

Similarly, Danish men have higher blood lead levels than Danish women of similar ages (21). Hepatic lead concentrations in Danish men also tend to be higher than in Danish women. Although the difference is not statistically significant (probably because many Danes have lead levels below the detection limit), it suggests that Danish men have higher total body lead than women.

The lead pollution in Denmark originates mainly from leaded gasoline. Concomitantly with the abolishment of the lead content in gasoline, there has been a decline in blood lead levels in Danes (5, 21). Coincidentally, the use of lead shot has been banned since 1996 in Denmark, but in Greenland there are no restrictions.

In conclusion, the present results show the existence of gender- and age-related differences in hepatic lead content. Greenlandic Inuit men have higher liver lead contents than women. Furthermore, Inuit men and women have significantly higher liver lead contents than Danish men and women living in a metropolitan area. The reason for the high lead levels in Inuit appears to be complex. As the Arctic environmental lead contamination is low (16, 17, 20, 22), the main reason is probably "local lead pollution" by the ingestion of seabirds contaminated by lead shot, causing a high dietary lead intake (20). Restrictions on the use of lead shot in Greenland may reduce the regional lead pollution and dietary lead exposure.

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