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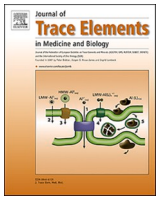

# In utero deposition of trace elements and metals in tissues☆

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ABSTRACT

*Introduction:* All animals, including humans, are exposed to heavy metals which are known to accumulate in different tissues, especially in bone. During pregnancy, the maternal bone turnover is increased and the metals in the mother's body can be mobilized into the bloodstream. Heavy metals in maternal blood are known to pass through the placenta to the fetal blood and finally, deposited to bone tissue. However, there are no studies on the concentration of metals in the fetal solid tissues and until now, the rate of metal transfer from mother to fetus is not exactly known.

*Materials and methods:* Samples of the blood, liver, placenta, and three different bones were collected from 17 pregnant ewes and their 27 fetuses. The animals had no known exposure to heavy metals. The concentrations of Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, Sb, Sn, Sr, Te, Ti, Tl, V, and Zn were analyzed using ICP-MS.

*Results and discussion:* The concentration of Sb, Sn, Te, and Tl were under the detection limit in all the samples. The other metals were found in all maternal and fetal tissues, suggesting that all detectable metals cross the placenta. Blood concentrations were low compared to solid tissue concentrations. The concentrations of essential elements varied between maternal and fetal tissues, which could be explained by biological differences. The differences in concentrations of non-essential elements between the ewe and fetuses were smaller. The most significant differences were between maternal and fetal concentrations of Ba and Sr, which is at least partly explained by the mineralization degree of the bone.

*Conclusion:* Heavy metals accumulate in fetal solid tissues in sheep that are not directly exposed to heavy metals. Because of the differences in anatomy between human and sheep placenta, the accumulation in the tissue of human fetuses should be extrapolated cautiously. However, there might be some clinical relevance for fertile aged women who are exposed to heavy metals, such as women who work in the metal industry or who have undergone joint replacement surgery.

## 1. Introduction

An adequate amount of nutrients and trace elements in pregnancy is essential for the normal development of the fetus. Heavy metals in the blood of pregnant mothers are known to cross the placenta [1–3] and abnormal blood levels of essential and non-essential trace elements can cause adverse pregnancy outcomes [4–6].

Solid tissue concentrations reflect a longer exposure time than blood concentrations, as the half-life for most environmental toxicants is substantially higher in bone and liver than in blood [7]. Bone is one of the main storage sites for metals: over 99% of body burden of Sr [8], 90% of Pb [9] and 60% of Al [10] is located in the bones. In the case of pregnancy, blood concentrations of metals are not only the marker of acute external exposure but can also express the mobilization of metal

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ions from the bone mineral matrix as pregnancy induces bone remodeling [11,12].

In bone, there are two main ways for metals to accumulate [13]. Pb and Sr can substitute for other divalent cations in the body, mainly $Ca^{2+}$, and attach to hydroxyapatite mineral. Transition metals, such as Ti, V, Cr, Mo, and Ni can become attached to phosphoproteins.

Although extensive research has been conducted on the blood concentrations of metals, only very little is known about the accumulation of metals in fetal solid organs. The objectives of this study were to evaluate the exposure of metals in pregnant sheep, the transfer of metals through the placenta and the accumulation of metals in fetal bone tissue and liver.

## 2. Materials and methods

### 2.1. Animals

The pregnant sheep (n = 17) were housed and bred at Lammastila Sikka Talu, University of Turku, Rymättylä, Finland. The animal transport, housing, care, and experimental procedures were conducted according to the national legislation and the EU Directive 2010/63/EU. The study protocol was approved by the National Animal Experiment Board of Finland (ESAVI/1007/04.10.07/ 2014). The animals were primarily used for fetal asphyxia [14] and oxycodone pharmacokinetics [15] studies, and the present study only utilized tissues of anesthetized and euthanized animals. The first group of sheep were operated in the fall of 2017 (n = 7). The second group (n = 10) were operated in the spring of 2018. The first group (the fall experiment) were pasture fed on shore pasture dominated by common reed (*Phragmites australis*) in the spring and early summer and on field pasture dominated by tall fescue (*Festuca arundinacea*) from August. The second group (the spring experiment) were reared on clover fodder and before experiment introduced to oat 250 g/day. The ewes of both groups were fed with same mineral supplements: Lammas Mira Maku mineral supplement (Vilomix, Paimio, Finland); 24–7 Smartrace Adult Sheep bolus (Agrimin, Kirmington, United Kingdom) supplement containing iodine, cobalt, and selenium; and ViloRock Sheep salt block (Swedish Agro, Sweden). Due to complications in catheterization operations, 7 fetuses had already died at the time of the euthanasia and sample collection and the eventual number of fetuses included in the present study was 27. Of the fetuses, 11 were catheterized for a fetal asphyxia experiment and 16 were not. The sheep were euthanized at day 119–120 of single, twin, or triplet gestation (term 145 days).

### 2.2. Sample collection and processing

The whole blood samples were collected from catheterized arteries (the ewe and the catheterized fetus) or postmortem with a needle from the heart (other euthanized fetuses). The blood was allowed to clot and stored at − 20 °C. One placentome including both fetal and maternal side of each fetus was dissected. Pieces from liver were dissected from both ewe and the fetuses. Pieces from tibial bone diaphysis, vertebral body (fetuses) and spinous processes (ewe) and mandibular bone were cut with an electrical saw. From the first (fall) group, the bone pieces were ground with a Retsch CryoMill ball mill (Haan, Germany) for 2 min for fetal samples and 3–4 min for maternal samples, until the bone was a fine powder. Bone marrow was included in the ground samples. From the second group (spring), pieces of cortical bone were dissected with surgical instruments. All samples were stored at − 20 °C until analyses.

### 2.3. Histological imaging

#### 2.3.1. Soft tissue samples

Pieces of liver and placenta were fixed in 4% formaldehyde and embedded in paraffin. 3.5 μm sections were stained with hematoxylin-eosin.

#### 2.3.2. Bone samples

Pieces of bone were dehydrated by increasing ethanol concentrations (70%, 96%, and 100%) and finally immersed in xylene (VWR Chemicals, Fontenay-sous-Bois, France). The samples were cast in methyl-acrylate through a series of first MMA I solution (78 m-% methyl-methacrylate and 22 m-% dibutyl phthalate; Sigma-Aldrich Chemicals, St. Louis, MO, USA) for 3 days and then in MMA II (0.1 m-% benzyl peroxide; Merck, Darmstadt, Germany; in MMA I) for 2 days. The bases of the blocks were prepared by pipetting MMA III solution (0.3 m-% benzyl peroxide in MMA I solution) into gelatin capsules and placing the capsules in a vacuum for 15 min. Then the MMA III was hardened by placing the capsules in an incubator first at + 30 °C for two days and then at + 36 °C for two days. The MMA II-treated samples were then placed into the capsules and the capsules filled with MMA III, which was hardened as described above. Hardened block capsules were opened and allowed to dry under a fume hood. The gelatin was removed by warming the capsules in a water bath and then the gelatin was peeled off. Uneven surfaces were removed with an EXAKT cutter (EXAKT Advanced Technologies GmbH, Norderstedt, Germany). The block was glued with Historesin mounting medium (Leica Biosystems, Nussloch, Germany) into a plastic sample adapter. The samples were cut with a Polycut S heavy duty microtome (Reichert-Jung, Nussloch, Germany) into 5 μm sections and stained with Masson-Trichrome with the following protocol. The plastic was removed with 2-methoxyethylacetate and ethanol (96%). The slides were stained with Weigert's hematoxylin for 5 min and then rinsed with water. The slides were incubated in MFPOG solution (Ponceau xylidine, orange G; Sigma-Aldrich Chemicals, St. Louis, MO, USA; 0.2% acetic acid, Merck KGaA, Darmstadt, Germany; and fuchsin acid, Merck KGaA, Darmstadt, Germany) for 15 min and Light Green (Alfa Aesar GmbH, Karlsruhe, Germany) for 20 min. Between and after the incubations the slides were washed twice with 0.2% acetic acid. Then the slides were dehydrated using 96% ethanol, 100% ethanol twice and xylene twice.

All histological slides were scanned using a Hamamatsu NanoZoomer S60 slide scanner (Hamamatsu City, Japan) and images were captured using QuPath 0.2.3 software [16].

### 2.4. Chemical analyses

The concentrations of elements Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, Sb, Sn, Sr, Te, Ti, Tl, V, and Zn were analyzed in all tissues.

The organic matrix of sheep blood, tissue and bones was digested with nitric acid using the MARS 6 iWave microwave digestion system (CEM Corporation, Matthews, NC, USA) and XPress Teflon vessels. The samples were placed in a digestion vessel; sheep tissue and bone samples (circa 100 mg fresh weight for liver and placenta samples, and circa 100 mg dry weight for bone samples) were weighed with a Mettler Toledo MX5 (readability 0.1 mg) and blood samples were pipetted (0.2–2.0 ml). HNO3 (TraceMetal Grade, Fisher Chemical, A509-P1) was added to the vessel (8.0 ml 67–69% HNO3) and the samples were digested using an Animal Tissue method. After digestion, the samples were diluted to 20 ml with de-ionized water (USF Elga Maxima). The determination of the concentrations of the analytic elements was done with ICP-MS (Perkin Elmer NexION 350D, USA) using the KED mode. The calibration of the instrument was done with multielement standards (TraCERT Periodic table mix 1 for ICP, Sigma-Aldrich, 92091 and TraCERT Periodic table mix 2 for ICP, Fluka, 41135) and single element standard for Hg (CertiPUR, Merck, 170226). Two internal standards, Y-89 and Th-232, were mixed online with the samples to compensate for matrix effects and instrument drift. Triplicate measurements were performed for each sample. Limit of detection values for elements were: Al 0.5, Ca 1.2, Fe 0.2, K 4.1, Mg 0.3, Na 0.11, P 1.3 ppb and As 1.1, Ba 6.8, Cd 5.2, Co 2.1, Cr 11, Cu 6.3, Hg 0.5, Mn 35, Mo 19, Ni 6.3, Pb 1.9, Rb 5.6, Sb 1.3, Sn 10, Sr 3.7, Te 4.8, Ti 5.6, Tl 0.1, V 2.4, Zn 6.3 ppt. Reagent blanks, NIST Standard Reference Material 1577c Bovine Liver and for blood samples

also Seronorm™ Trace Elements Whole Blood L-2 SRM were included in every sample batch to ensure the validity of the methods.

### 2.5. Statistical analyses

All statistical analyses were performed using the IBM SPSS Statistics program version 25.0 (IBM Corp., Armonk, NY, USA). Values that were lower than the absolute values of the lowest negative level of metal were considered lower than the detection limit and were marked as 0. The normality of the data was checked using the Shapiro-Wilk test. Comparison of the median concentrations between the ewe and the fetus and comparison of the concentrations between the two sheep groups were done using the Mann-Whitney U Test. Correlations between the concentrations in maternal and fetal blood were analyzed using Spearman's rho. The graphical presentations were created using OriginPro 9.7 software (OriginLab, Northampton, MA, USA).

## 3. Results

The elements Al, Ba, Ca, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, P, Rb, Sr, and Zn were found in detectable levels in all tissues. As, Cd, Co, Cr, Ni, Pb, Ti, and V could only be detected in some of the samples. The elements Sb, Sn, Te, and Tl were under the detection limit. Each element had a unique tissue distribution. There were no statistically significant differences between the catheterized and non-catheterized fetuses. The mass ratios of the most abundant elements are shown in Fig. 1A. Fig. 1B depicts the mass ratios for Ca and the most abundant non-essential elements in bone. The amounts of the other elements in Fig. 1A and Fig. 1B were so marginal that they could not be visualized.

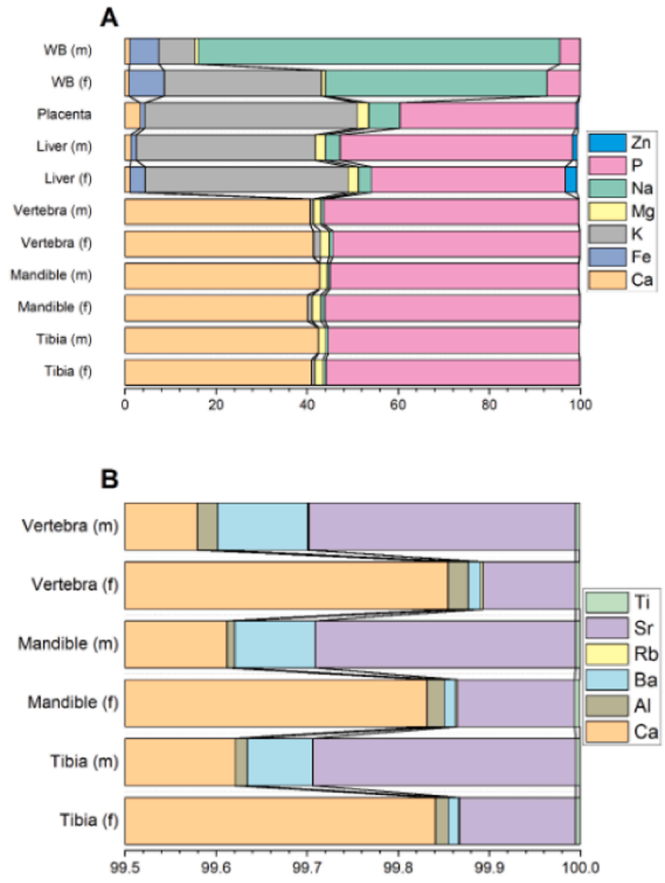


**Fig. 1.** A. The mass ratios of most abundant elements in different tissues. The differences can be explained by anatomical and biochemical differences of the tissues. B. The mass ratios of calcium and the most abundant non-essential element in bone. Over 99.5 per cent of the analyzed elements in bone is calcium. Other analyzed elements are not visualized because of the comparatively low concentrations. (m) =maternal, (f)=fetal.

The medians and interquartile ranges of metal concentrations are listed in Table 1. The levels of essential elements are shown in Fig. 2. The most prevalent elements were Fe, Na, K, P, Ca, and Mg. All essential element concentrations had statistically significant differences between the ewe and the fetus (see below). Na and Fe had the highest levels in blood, whereas the highest K levels were in the liver and the placenta. The highest levels of P, Ca, and Mg were in the bones.

The levels of non-essential and heavy metals are shown in Fig. 3. Levels of non-essential metals were lower than essential metals and minerals. In general, the levels of non-essential metals were lower in blood than in solid organs and higher in the ewe than the fetus. Elements Al, As, Cd, and Hg had roughly equal levels in all solid tissues. Levels of Ni, Hg, and Pb had wide variation. The histological differences between fetal and maternal liver and bone and the histology of the placenta are shown in Fig. 4.

### 3.1. Elemental distribution in blood

The most abundant essential elements found in blood were Na, K and Fe as expected. The levels of other essential elements were low in blood in comparison to other tissues. The levels of trace elements and heavy metals were lower in blood than in solid tissues as well. There were statistically significant differences between ewe and fetus in the levels of Al, Ba, Ca, Co, Cu, Fe, K, Mg, Mn, P, Rb and Sr (Figs. 2 and 3, Table 1.); Ba, Co, Cu, Na, Cu being higher in ewe blood and Al, Ca, Fe, K, Mg, Mn, P, Rb, and Sr being higher in fetal blood. The only non-essential elements that showed strong positive correlation between maternal and fetal blood were Hg ($\rho = 0.676$) and Pb ($\rho = 0.693$).

### 3.2. Elemental distribution in placenta

The most abundant essential elements in placenta were K and P. None of the elements showed specific accumulation in placental tissue, but in general the levels were higher than in maternal or fetal blood and lower than in other solid tissues. Of the non-essential elements, the highest levels were Al, Rb, Ti, and Sr, although the levels were low.

### 3.3. Elemental distribution in liver

The levels of Cd, Cu, K, Mn, Mo, Zn and Rb were higher in liver than in other tissues. Fe level was high in liver, but not as high as in blood. There was no statistical difference between maternal and fetal levels of Cu or K. Fe, Mn and Zn levels were higher in fetal liver and Cd, Mo and Rb higher in maternal liver.

### 3.4. Elemental distribution in bone

In bone, the most prevalent minerals were Ca, K, Mg, Na and P. The highest Ca content was in the tibia in both ewe and fetus. There was a statistically significant difference between maternal and fetal levels of essential elements. Ca, Co, Cu, Fe, Mg, Mn, Mo, Na, P were higher in bones of the ewes showing matrix mineralization. The level of K was higher in fetal bones showing more abundant cellularity in the developing bone.

Ba, Pb, Sr, and Ti were higher in maternal than fetal bones and showed deposition to bone with relatively higher levels in bone than other tissues. Cr levels were higher in bones than in other tissues and had a wider range in the fetal than in the maternal bones, but the difference between them was not statistically significant. Ni and Rb levels were higher in fetal bones than in the maternal bones.

### 3.5. Differences between fall and spring groups

Fetal blood Ca was higher in the fall group (1.2-fold, $P = 0.013$) but

**Table 1**
Median metal levels in different tissues in the ewe and the fetus and P-values of the difference between maternal and fetal concentrations. ppm = parts per million (fresh weight for liver and placenta; dry weight for bones). LOD = limit of detection.

| | LOD (ppm) | | Maternal level (ppm) | | | | Fetal level (ppm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Tissue | Median | Percentile 25 | Percentile 75 | n | Median | Percentile 25 | Percentile 75 | n | P-value |
| Al | 0.0005 | Whole blood | 0.35 | 0.28 | 0.61 | 16 | 0.51 | 0.34 | 1,1 | 26 | 0.04 |
| | | Placenta | | | | | 7.6 | 4.5 | 10.0 | 27 | |
| | | Liver | 7.9 | 5.1 | 13.3 | 18 | 7.2 | 6.1 | 12 | 27 | 0.63 |
| | | Vertebra | 13 | 7.3 | 16.2 | 18 | 8.5 | 6.4 | 15 | 27 | 0.13 |
| | | Mandible | 8.0 | 6.1 | 14 | 18 | 7.5 | 5.7 | 12 | 27 | 0.47 |
| | | Tibia | 14 | 8.3 | 17 | 18 | 8.7 | 7.1 | 17 | 27 | 0.49 |
| As | $1.1 \times 10^{-6}$ | Whole blood | 0.0016 | 0.00090 | 0.0020 | 14 | 0.0018 | 0.0011 | 0.0036 | 22 | 0.46 |
| | | Placenta | | | | | 0.019 | 0.0075 | 0.034 | 19 | |
| | | Liver | 0.013 | 0.0072 | 0.023 | 12 | 0.018 | 0.012 | 0.027 | 19 | 0.44 |
| | | Vertebra | 0.026 | 0.012 | 0.038 | 14 | 0.019 | 0.013 | 0.031 | 25 | 0.32 |
| | | Mandible | 0.025 | 0.013 | 0.043 | 13 | 0.019 | 0.016 | 0.029 | 20 | 0.40 |
| | | Tibia | 0.023 | 0.013 | 0.033 | 16 | 0.023 | 0.011 | 0.034 | 23 | 0.82 |
| Ba | $6.8 \times 10^{-6}$ | Whole blood | 0.044 | 0.032 | 0.056 | 16 | 0.011 | 0.0059 | 0.020 | 26 | 0.00 |
| | | Placenta | | | | | 0.15 | 0.094 | 0.23 | 27 | |
| | | Liver | 0.11 | 0.081 | 0.17 | 18 | 0.056 | 0.028 | 0.11 | 27 | 0.00 |
| | | Vertebra | 55 | 49 | 61 | 18 | 5.1 | 4.5 | 6.1 | 27 | 0.00 |
| | | Mandible | 83 | 77 | 87 | 18 | 4.7 | 4.2 | 5.7 | 27 | 0.00 |
| | | Tibia | 71 | 64 | 89 | 18 | 7.0 | 6.3 | 8.7 | 27 | 0.00 |
| Ca | 0.0012 | Whole blood | 32 | 27 | 34 | 16 | 34 | 30 | 38 | 26 | 0.04 |
| | | Placenta | | | | | 172 | 142 | 211 | 27 | |
| | | Liver | 92 | 70 | 119 | 18 | 71 | 55 | 111 | 27 | 0.16 |
| | | Vertebra | 55,614 | 49,045 | 64,715 | 18 | 37,894 | 20,441 | 42,155 | 27 | 0.00 |
| | | Mandible | 93,444 | 80,369 | 102,356 | 18 | 38,722 | 31,340 | 48,572 | 27 | 0.00 |
| | | Tibia | 99,496 | 92,940 | 106,260 | 18 | 60,992 | 57,851 | 68,239 | 27 | 0.00 |
| Cd | $5.2 \times 10^{-6}$ | Whole blood | 0.00043 | 0.00015 | 0.00065 | 16 | 0.00035 | 0.00016 | 0.00092 | 23 | 0.80 |
| | | Placenta | | | | | 0.015 | 0.0057 | 0.032 | 16 | |
| | | Liver | 0.064 | 0.042 | 0.082 | 18 | 0.027 | 0.018 | 0.033 | 14 | 0.00 |
| | | Vertebra | 0.015 | 0.0084 | 0.026 | 13 | 0.0090 | 0.0024 | 0.029 | 20 | 0.59 |
| | | Mandible | 0.017 | 0.0033 | 0.027 | 15 | 0.017 | 0.0038 | 0.027 | 17 | 0.72 |
| | | Tibia | 0.017 | 0.0083 | 0.024 | 14 | 0.016 | 0.0093 | 0.034 | 16 | 0.59 |
| Co | $2.1 \times 10^{-6}$ | Whole blood | 0.0029 | 0.0020 | 0.0082 | 16 | 0.00082 | 0.00044 | 0.0015 | 26 | 0.00 |
| | | Placenta | | | | | 0.024 | 0.016 | 0.036 | 27 | |
| | | Liver | 0.089 | 0.080 | 0.114 | 18 | 0.030 | 0.023 | 0.036 | 27 | 0.00 |
| | | Vertebra | 0.043 | 0.016 | 0.269 | 18 | 0.0076 | 0.0059 | 0.013 | 27 | 0.00 |
| | | Mandible | 0.038 | 0.018 | 0.085 | 18 | 0.0084 | 0.0063 | 0.018 | 27 | 0.00 |
| | | Tibia | 0.029 | 0.012 | 0.103 | 18 | 0.0082 | 0.0061 | 0.016 | 27 | 0.00 |
| Cr | $11 \times 10^{-6}$ | Whole blood | 0.0016 | 0.00079 | 0.0039 | 14 | 0.0013 | 0.00062 | 0.0023 | 18 | 0.57 |
| | | Placenta | | | | | 0.025 | 0.0082 | 0.0362 | 25 | |
| | | Liver | 0.065 | 0.035 | 0.074 | 15 | 0.016 | 0.0016 | 0.041 | 22 | 0.00 |
| | | Vertebra | 0.17 | 0.071 | 0.28 | 18 | 0.049 | 0.025 | 0.80 | 27 | 0.13 |
| | | Mandible | 0.25 | 0.11 | 0.42 | 18 | 0.067 | 0.032 | 2,3 | 27 | 0.13 |
| | | Tibia | 0.096 | 0.061 | 0.19 | 17 | 0.081 | 0.031 | 0.82 | 26 | 0.82 |
| Cu | $6.3 \times 10^{-6}$ | Whole blood | 0.81 | 0.72 | 0.95 | 16 | 0.37 | 0.29 | 0.52 | 26 | 0.00 |
| | | Placenta | | | | | 1,2 | 1,1 | 1,4 | 27 | |
| | | Liver | 24 | 15 | 42 | 18 | 29 | 18 | 43 | 27 | 0.78 |
| | | Vertebra | 0.48 | 0.40 | 0.56 | 18 | 0.92 | 0.83 | 1,1 | 27 | 0.00 |
| | | Mandible | 0.37 | 0.28 | 0.42 | 18 | 0.73 | 0.54 | 0.98 | 27 | 0.00 |
| | | Tibia | 0.31 | 0.23 | 0.41 | 18 | 0.78 | 0.48 | 0.99 | 27 | 0.00 |
| Fe | 0.0002 | Whole blood | 213 | 146 | 239 | 16 | 318 | 270 | 382 | 26 | 0.00 |
| | | Placenta | | | | | 49 | 27 | 74 | 27 | |
| | | Liver | 84 | 75 | 113 | 18 | 234 | 111 | 672 | 27 | 0.00 |
| | | Vertebra | 23 | 16 | 30 | 18 | 46 | 38 | 57 | 27 | 0.00 |
| | | Mandible | 6,4 | 4,9 | 12 | 18 | 17 | 13 | 29 | 27 | 0.00 |
| | | Tibia | 4,4 | 2,5 | 5,8 | 18 | 31 | 23 | 43 | 27 | 0.00 |
| Hg | $0.5 \times 10^{-6}$ | Whole blood | 0.0034 | 0.0020 | 0.010 | 16 | 0.0043 | 0.0027 | 0.0078 | 26 | 0.78 |
| | | Placenta | | | | | 0.017 | 0.012 | 0.069 | 27 | |
| | | Liver | 0.029 | 0.014 | 0.11 | 18 | 0.019 | 0.013 | 0.047 | 27 | 0.31 |
| | | Vertebra | 0.021 | 0.011 | 0.034 | 18 | 0.013 | 0.011 | 0.036 | 27 | 0.63 |
| | | Mandible | 0.027 | 0.013 | 0.16 | 18 | 0.017 | 0.012 | 0.11 | 27 | 0.42 |
| | | Tibia | 0.016 | 0.012 | 0.036 | 18 | 0.016 | 0.013 | 0.036 | 27 | 0.66 |
| K | 0.0041 | Whole blood | 262 | 213 | 646 | 16 | 1417 | 1112 | 1772 | 26 | 0.00 |
| | | Placenta | | | | | 2398 | 2028 | 2610 | 27 | |
| | | Liver | 2872 | 2243 | 3209 | 18 | 3111 | 2744 | 3382 | 27 | 0.16 |
| | | Vertebra | 922 | 781 | 1218 | 18 | 1510 | 1360 | 1653 | 27 | 0.00 |
| | | Mandible | 272 | 216 | 311 | 18 | 1000 | 909 | 1105 | 27 | 0.00 |
| | | Tibia | 237 | 199 | 281 | 18 | 1080 | 932 | 1267 | 27 | 0.00 |
| Mg | 0.0003 | Whole blood | 24 | 20 | 28 | 16 | 36 | 34 | 42 | 26 | 0.00 |
| | | Placenta | | | | | 127 | 117 | 141 | 27 | |
| | | Liver | 164 | 154 | 180 | 18 | 160 | 140 | 175 | 27 | 0.23 |
| | | Vertebra | 2326 | 2028 | 2734 | 18 | 1679 | 1087 | 1951 | 27 | 0.00 |
| | | Mandible | 3594 | 3381 | 3844 | 18 | 1843 | 1377 | 2004 | 27 | 0.00 |
| | | Tibia | 3778 | 3583 | 3990 | 18 | 2815 | 2589 | 3053 | 27 | 0.00 |

(*continued on next page*)

**Table 1** (*continued*)

| | LOD (ppm) | | Maternal level (ppm) | | | | Fetal level (ppm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Tissue | Median | Percentile 25 | Percentile 75 | n | Median | Percentile 25 | Percentile 75 | n | P-value |
| Mn | $35 \times 10^{-6}$ | Whole blood | 0.10 | 0.08 | 0.11 | 16 | 0.16 | 0.12 | 0.20 | 26 | 0.00 |
| | | Placenta | | | | | 0.36 | 0.30 | 0.45 | 27 | |
| | | Liver | 2,5 | 2,2 | 3,3 | 18 | 4,3 | 3,4 | 4,8 | 27 | 0.00 |
| | | Vertebra | 0.36 | 0.34 | 0.42 | 18 | 0.97 | 0.80 | 1,3 | 27 | 0.00 |
| | | Mandible | 0.52 | 0.46 | 0.65 | 18 | 0.98 | 0.74 | 1,3 | 27 | 0.00 |
| | | Tibia | 0.59 | 0.51 | 0.68 | 18 | 1,2 | 0.94 | 1,4 | 27 | 0.00 |
| Mo | $19 \times 10^{-6}$ | Whole blood | 0.01 | 0.012 | 0.021 | 16 | 0.021 | 0.015 | 0.040 | 26 | 0.11 |
| | | Placenta | | | | | 0.10 | 0.08 | 0.14 | 27 | |
| | | Liver | 1,2 | 1,0 | 2,1 | 18 | 0.28 | 0.19 | 0.45 | 27 | 0.00 |
| | | Vertebra | 0.66 | 0.49 | 1,2 | 18 | 0.15 | 0.089 | 0.20 | 27 | 0.00 |
| | | Mandible | 1,7 | 1,2 | 1,9 | 18 | 0.19 | 0.11 | 0.28 | 27 | 0.00 |
| | | Tibia | 1,2 | 0.86 | 1,7 | 18 | 0.22 | 0.15 | 0.26 | 27 | 0.00 |
| Na | 0.00011 | Whole blood | 2633 | 2315 | 2767 | 16 | 1995 | 1799 | 2312 | 26 | 0.00 |
| | | Placenta | | | | | 351 | 326 | 387 | 27 | |
| | | Liver | 233 | 205 | 296 | 18 | 201 | 179 | 260 | 27 | 0.25 |
| | | Vertebra | 864 | 779 | 942 | 18 | 737 | 684 | 776 | 27 | 0.00 |
| | | Mandible | 1319 | 1204 | 1359 | 18 | 848 | 723 | 902 | 27 | 0.00 |
| | | Tibia | 1247 | 1165 | 1347 | 18 | 854 | 816 | 888 | 27 | 0.00 |
| Ni | $6.3 \times 10^{-6}$ | Whole blood | 0.0060 | 0.0027 | 0.010 | 15 | 0.0071 | 0.0038 | 0.010 | 23 | 0.69 |
| | | Placenta | | | | | 0.053 | 0.049 | 0.083 | 6 | |
| | | Liver | 0.068 | 0.044 | 0.074 | 5 | 0.035 | 0.026 | 0.050 | 6 | 0.20 |
| | | Vertebra | 0.17 | 0.16 | 0.19 | 2 | 0.24 | 0.16 | 0.68 | 3 | 0.25 |
| | | Mandible | 0.047 | 0.039 | 0.056 | 6 | 0.17 | 0.14 | 0.19 | 10 | 0.00 |
| | | Tibia | 0.19 | 0.17 | 0.26 | 4 | 0.26 | 0.21 | 0.31 | 6 | 0.20 |
| P | 0.00013 | Whole blood | 148 | 130 | 157 | 16 | 298 | 243 | 368 | 26 | 0.00 |
| | | Placenta | | | | | 1993 | 1825 | 2426 | 27 | |
| | | Liver | 3729 | 3528 | 3938 | 18 | 2979 | 2476 | 3295 | 27 | 0.00 |
| | | Vertebra | 76,688 | 63,587 | 89,906 | 18 | 49,813 | 30,257 | 58,154 | 27 | 0.00 |
| | | Mandible | 119,920 | 104,545 | 132,673 | 18 | 54,160 | 41,835 | 64,954 | 27 | 0.00 |
| | | Tibia | 129,469 | 120,395 | 137,102 | 18 | 83,179 | 75,061 | 95,364 | 27 | 0.00 |
| Pb | $1.9 \times 10^{-6}$ | Whole blood | 0.0053 | 0.0007 | 0.029 | 15 | 0.0028 | 0.0012 | 0.010 | 26 | 0.70 |
| | | Placenta | | | | | 0.027 | 0.0076 | 0.043 | 26 | |
| | | Liver | 0.023 | 0.012 | 0.054 | 18 | 0.040 | 0.015 | 0.11 | 27 | 0.36 |
| | | Vertebra | 0.11 | 0.077 | 0.14 | 18 | 0.038 | 0.032 | 0.060 | 27 | 0.00 |
| | | Mandible | 0.16 | 0.14 | 0.23 | 18 | 0.052 | 0.031 | 0.083 | 27 | 0.00 |
| | | Tibia | 0.17 | 0.13 | 0.22 | 18 | 0.063 | 0.038 | 0.088 | 27 | 0.00 |
| Rb | $5.6 \times 10^{-6}$ | Whole blood | 0.33 | 0.28 | 0.67 | 16 | 1,0 | 0.83 | 1,2 | 26 | 0.00 |
| | | Placenta | | | | | 2,0 | 1,6 | 2,1 | 27 | |
| | | Liver | 5.3 | 4.3 | 6.1 | 18 | 2.8 | 2.3 | 3.4 | 27 | 0.00 |
| | | Vertebra | 0.89 | 0.82 | 1,1 | 18 | 0.90 | 0.74 | 1,3 | 27 | 0.89 |
| | | Mandible | 0.23 | 0.14 | 0.26 | 18 | 0.67 | 0.53 | 0.82 | 27 | 0.00 |
| | | Tibia | 0.13 | 0.11 | 0.16 | 18 | 0.60 | 0.45 | 0.86 | 27 | 0.00 |
| Sr | $3.7 \times 10^{-6}$ | Whole blood | 0.039 | 0.0058 | 0.10 | 16 | 0.043 | 0.030 | 0.066 | 26 | 0.74 |
| | | Placenta | | | | | 0.29 | 0.26 | 0.45 | 27 | |
| | | Liver | 0.18 | 0.17 | 0.22 | 18 | 0.13 | 0.10 | 0.17 | 27 | 0.01 |
| | | Vertebra | 163 | 141 | 209 | 18 | 39 | 27 | 49 | 27 | 0.00 |
| | | Mandible | 268 | 249 | 324 | 18 | 50 | 37 | 55 | 27 | 0.00 |
| | | Tibia | 289 | 270 | 333 | 18 | 78 | 67 | 82 | 27 | 0.00 |
| Ti | $5.6 \times 10^{-6}$ | Whole blood | 0.010 | 0.0067 | 0.015 | 16 | 0.022 | 0.016 | 0.036 | 24 | 0.00 |
| | | Placenta | | | | | 0.44 | 0.32 | 0.88 | 26 | |
| | | Liver | 0.34 | 0.26 | 0.70 | 18 | 0.29 | 0.27 | 0.54 | 26 | 0.34 |
| | | Vertebra | 2.5 | 2.1 | 3.6 | 18 | 1.8 | 1.2 | 2.4 | 27 | 0.00 |
| | | Mandible | 4.7 | 4.3 | 5.5 | 18 | 2.2 | 1.7 | 2.9 | 27 | 0.00 |
| | | Tibia | 4.5 | 3.5 | 5.2 | 18 | 2.9 | 2.7 | 3.2 | 27 | 0.00 |
| V | $2.4 \times 10^{-6}$ | Whole blood | 0.00073 | 0.00045 | 0.0010 | 16 | 0.00059 | 0.00048 | 0.0011 | 26 | 0.98 |
| | | Placenta | | | | | 0.0033 | 0.00169 | 0.0052 | 23 | |
| | | Liver | 0.0037 | 0.0018 | 0.0051 | 17 | 0.0051 | 0.0032 | 0.0077 | 19 | 0.35 |
| | | Vertebra | 0.0070 | 0.0030 | 0.012 | 18 | 0.0035 | 0.0022 | 0.0053 | 23 | 0.03 |
| | | Mandible | 0.0112 | 0.0064 | 0.013 | 17 | 0.0035 | 0.0023 | 0.015 | 23 | 0.23 |
| | | Tibia | 0.0085 | 0.0047 | 0.018 | 18 | 0.0037 | 0.0022 | 0.0089 | 24 | 0.02 |
| Zn | $6.3 \times \cdot 10^{-6}$ | Whole blood | 1.7 | 1.3 | 2.0 | 16 | 1.9 | 1.6 | 2.2 | 26 | 0.13 |
| | | Placenta | | | | | 26 | 20 | 33 | 27 | |
| | | Liver | 76 | 53 | 92 | 18 | 176 | 107 | 237 | 27 | 0.00 |
| | | Vertebra | 79 | 66 | 86 | 18 | 83 | 62 | 106 | 27 | 0.46 |
| | | Mandible | 80 | 64 | 103 | 18 | 63 | 51 | 75 | 27 | 0.01 |
| | | Tibia | 92 | 82 | 102 | 18 | 96 | 88 | 105 | 27 | 0.44 |

in maternal blood the difference was not significant. Hg levels were higher in the fall group in both maternal (5.2-fold, $P = 0.001$) and fetal (2.3-fold, $P = 0.004$) blood. Zn was higher in the spring group in fetal blood (1.2-fold, $P = 0.022$), maternal liver (1.8-fold, $P < 0.001$), fetal liver (1.6-fold, $P = 0.035$), and both maternal and fetal vertebra (1.2-fold, $P = 0.034$ and 1.8-fold, $P < 0.001$, respectively) and mandible (1.6-fold, $P = 0.016$ and 1.3-fold, $P = 0.031$, respectively). In placenta, the levels of Co (2.1-fold, $P < 0.001$) and Cr (8.9-fold, $P < 0.001$) were higher in the spring group and Pb (4.6-fold, $P = 0.002$) and Rb (1.5-fold, $P < 0.001$) higher in the fall group. In liver, the levels of Mo, Pb, and Rb were higher in the fall group in both maternal (2.0-fold, $P = 0.027$; 4.1-fold, $P = 0.004$; and 1.3-fold, $P = 0.006$, respectively) and fetal (2.0-

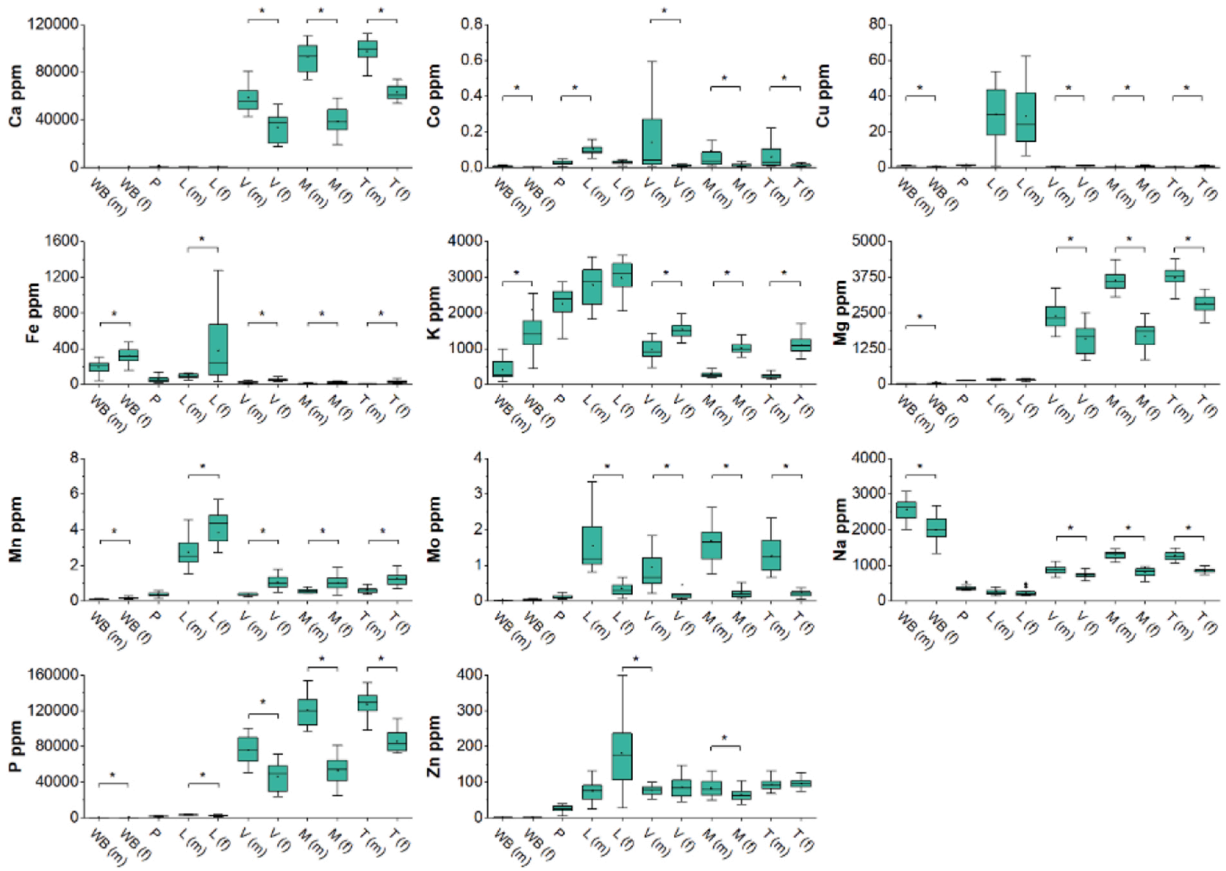


**Fig. 2.** Levels of analyzed essential elements. WB=whole blood, P = placenta, L=liver, V=vertebra, M=mandible, T = tibia. (m)=maternal, (f)=fetal. ppm = parts per million (μg/g; fresh weight for liver and placenta; dry weight for bones).

fold, $P < 0.001$; 7.0-fold, $P = 0.001$; and 1.4-fold, $P < 0.001$, respectively) liver. In addition, Ba (2.29-fold, $P = 0.015$) and Cd (1.70-fold, $P = 0.042$) were higher in the fall group in fetal liver and Sr (1.3-fold, $P = 0.012$) and Ti (3.6-fold, $P = 0.16$) in maternal liver. The differences of Mo, Pb, and Rb in liver and Pb and Rb in placenta remained after adjusting for Ca. Ca levels were higher in the spring group in all maternal bones (in vertebra 1.3-fold, $P = 0.043$; in mandible 1.3-fold, $P < 0.001$, and in tibia 1.2-fold $P < 0.001$) and fetal vertebra (2.0-fold, $P < 0.001$).

## 4. Discussion

To our knowledge, this is the first study on trace elements in fetal bone. In this study we found that Aland sheep tissues with no evident external exposure contain many non-essential metals in detectable concentrations. The blood concentrations of non-essential elements were low in both ewes and fetuses. All detectable elements were able to cross the placenta in considerable amounts and accumulation of non-essential elements in fetal bone was detected. Trace elements were shown to accumulate in bone already during the fetal period.

Levels of the most abundant essential elements have a biological explanation. The levels of K reflect the cellularity of the tissue, since 98% of K in the body is located inside the cells [17]. Na reflects the liquid extracellular matrix and Ca the mineralized extracellular matrix. The role of P in bone in the mineralized matrix is binding with Ca in a 5:6 ratio in hydroxyapatite and in other tissues the main P containing component is ATP [18] ATP binds Mg and binding of a second Mg activates ATP binding to kinases [19]. Fe is a component of heme in many proteins, including hemoglobin, and takes part in many essential cellular functions such as cellular respiration and DNA synthesis [20]. A biological function for Al has not been shown but it is found in all tissues. It binds to transferrin in blood and is distributed in bodily fluids by passing between the cells (reviewed in [21]).

Blood is a carrier of elements and the low levels of trace elements in blood is logical. The only way of storing substances in blood is to bind these to proteins and thus the capacity is limited. The concentrations in the blood reflect the immediate intake of substances and, in the case of Na, K and Fe, the biological function of blood. The differences in elemental distribution in blood between the ewes and the fetuses can be mainly explained by biological variables. A higher Na concentration in maternal than fetal blood is needed for the concentration gradient across the placenta [22]. The higher K level in fetal blood has been previously reported in humans [23]. Fe shows the higher fetal hemoglobin concentration [24–26]. Mn has been connected to cellularity [27]. Higher levels of Ca in fetal than maternal blood are maintained by active transport channels, such as PMCA, in the syncytiotrophoblast layer of the placenta [28], and parathyroid hormone-related peptide (PTHrP) [29]. Lower levels of Cu and Co in fetal than in maternal serum has been previously reported in humans [30]. Lower levels of Cu in fetal blood likely result from highly regulated transport mechanisms in the placenta [31]. Co has been shown to transport through the placenta in humans, possibly via passive diffusion [32]. Physiologically Co functions as a cofactor in vitamin B12. Little is known about the effects on Co in pregnancy. In sheep, Co deficiency has been linked to higher perinatal mortality, likely because of decreased vitamin B12 [33]. Vitamin B12 metabolism differs between humans and ruminants, as ruminants' gastrointestinal tract allows the absorption of vitamin B12 produced by intestinal bacteria [34].

The blood concentrations of non-essential metals in the present study were essentially in agreement with previously reported concentrations in humans (see Table 2). During pregnancy, the absorption of metals in the intestine is increased to ensure sufficient intake of essential metal

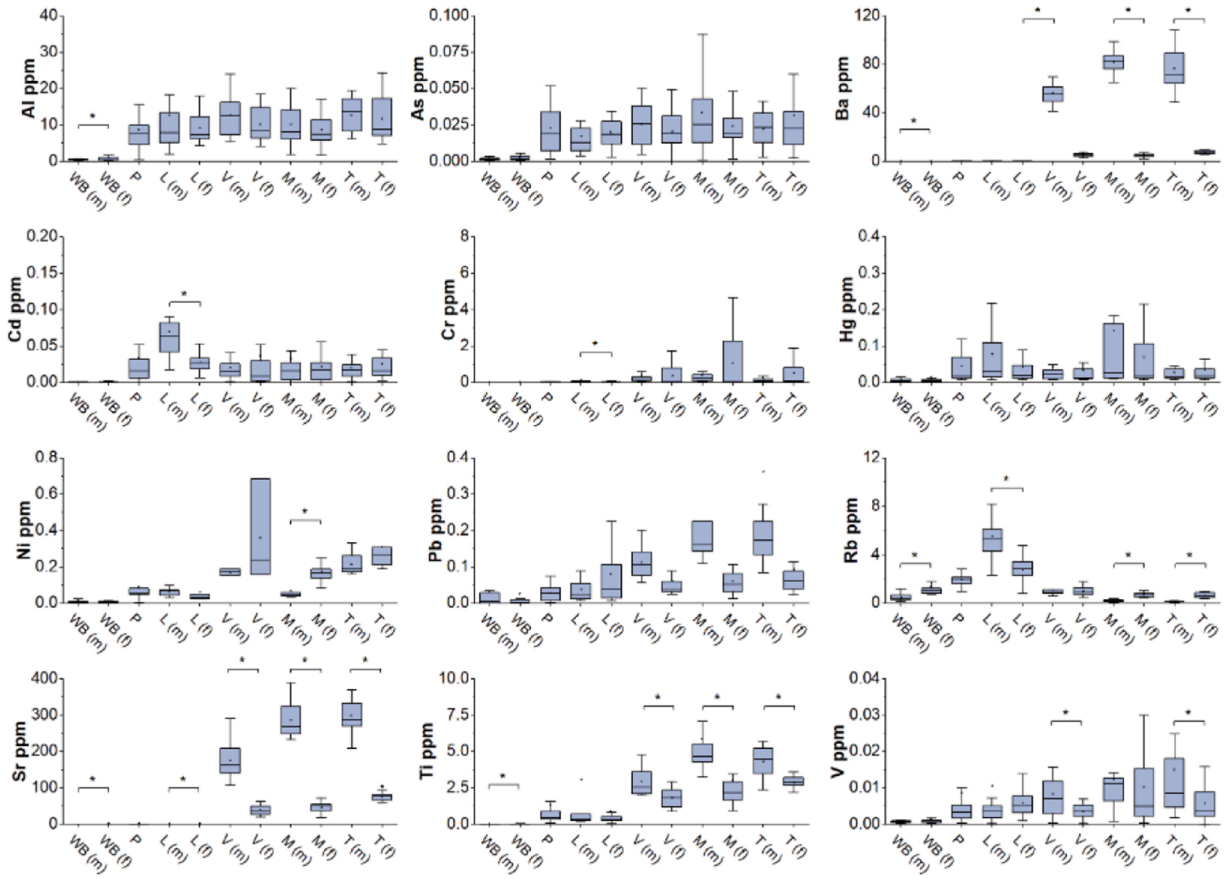


**Fig. 3.** Levels of analyzed non-essential elements. WB=whole blood, P = placenta, L=liver, V=vertebra, M=mandible, T = tibia. (m)=maternal, (f)=fetal. ppm = parts per million (μg/; fresh weight for liver and placenta; dry weight for bones).

ions, mainly Fe. One reason for increased absorption is the increase in divalent metal transporter 1 (DMT-1) expression in enterocytes [35], which can also transport other metal ions and thus increase levels of heavy metals in the blood. The transfer of Hg, Cd, and Pb through the human placenta are well studied [36], but for other elements the data are insufficient. The similar levels of Pb in maternal and fetal blood are in line with previous studies and suggest that these elements cross the placenta rather freely [37]. In the present study, there were no statistically significant differences in the concentration of Hg between the ewe and the fetus. In a previous study Hg has, on the contrary, been found in higher concentrations in umbilical cord blood than in maternal blood [38]. Inconsistent findings on the transport of Hg can be caused by different transport mechanisms of methyl-Hg and inorganic Hg: methyl-Hg can pass the placenta via passive diffusion or amino acid transporters and inorganic Hg accumulates in the placenta [37]. The sheep placenta is epithelio-chorial in contrast to the human hemochorial placenta. Although both species share similar vascular structure [39], the histological structure is different. The human term placental barrier consists of only two cell layers, syncytiotrophoblasts and fetal endothelial cells, and in sheep there are additionally maternal endothelial cells and uterine epithelial cells [40,41]. Thus, the placental transfer of metals and trace elements might be somewhat different in human and sheep.

The median levels of Cd, Hg, and Pb in placenta were slightly higher than previously reported concentrations in humans (see Table 2). This can result from a species-specific reason or the small sample size of the present study [42]. It has been proposed that placental metallothioneins (MTs) bind to metals, mainly Zn, Cd, and Cu, and form complexes that prevent transfer of these metal ions through the placenta [36]. In our study, Al, Ti, V, As, Rb, Cd, and Hg had higher levels in placenta than in both maternal and fetal blood. The aforementioned binding by MTs could partly explain the accumulation of metals in the placenta.

The metal levels in liver were essentially in agreement to previously reported concentrations in humans and other mammals. The levels of Cd and Pb in the liver were slightly lower than previously reported in humans and bovine (see Table 2). Adult liver gets its blood supply from the portal vein and the hepatic artery. Fetal liver receives most of its blood supply from the umbilical vein, and a great amount of that blood passes the liver in the ductus venosus [43]. On the microanatomical scale, fetal liver contains Fe-rich erythroblastic islets, that are lacking from adult liver [44]. In our study, the levels of non-essential elements in fetal liver were in general either similar (Al, As, Hg, Ni, Pb, Ti, V) or lower (Ba, Cd, Cr, Rb, Sr) compared to maternal liver. Fe was expectedly higher in fetal than maternal liver. Were the lower accumulation into fetal than maternal liver solely due to the ductus venosus, the effect would likely be seen in all non-essential elements. Metals can enter cells by different mechanisms, e.g., via DMT-1, various Zn transporters, Ca channels, sulfate transporters, glutathione (GSH) transporterscarriers and multidrug resistance proteins (MDR) [45]. Most of these transporters are essential for cellular metabolism and after differentiation the fetal hepatocytes function similar to adult hepatocytes [46], thus differences between adult and fetal hepatocytes are unlikely. Data on GSH and MDR expression in fetal liver are sparse, but in vitro and animal experiments suggest, that their expression in fetal liver is high [47,48]. The difference observed in the present study might simply be explained by substitution of Ca, Mg, and Na by other elements.

Our results show slightly higher levels of Al, Ba, and Sr and lower levels of Cd, Hg, and Pb than in previous studies in adult human bone (see Table 2). For fetal bones, references for metal concentrations could not be found. The levels of essential elements in bone reflect low

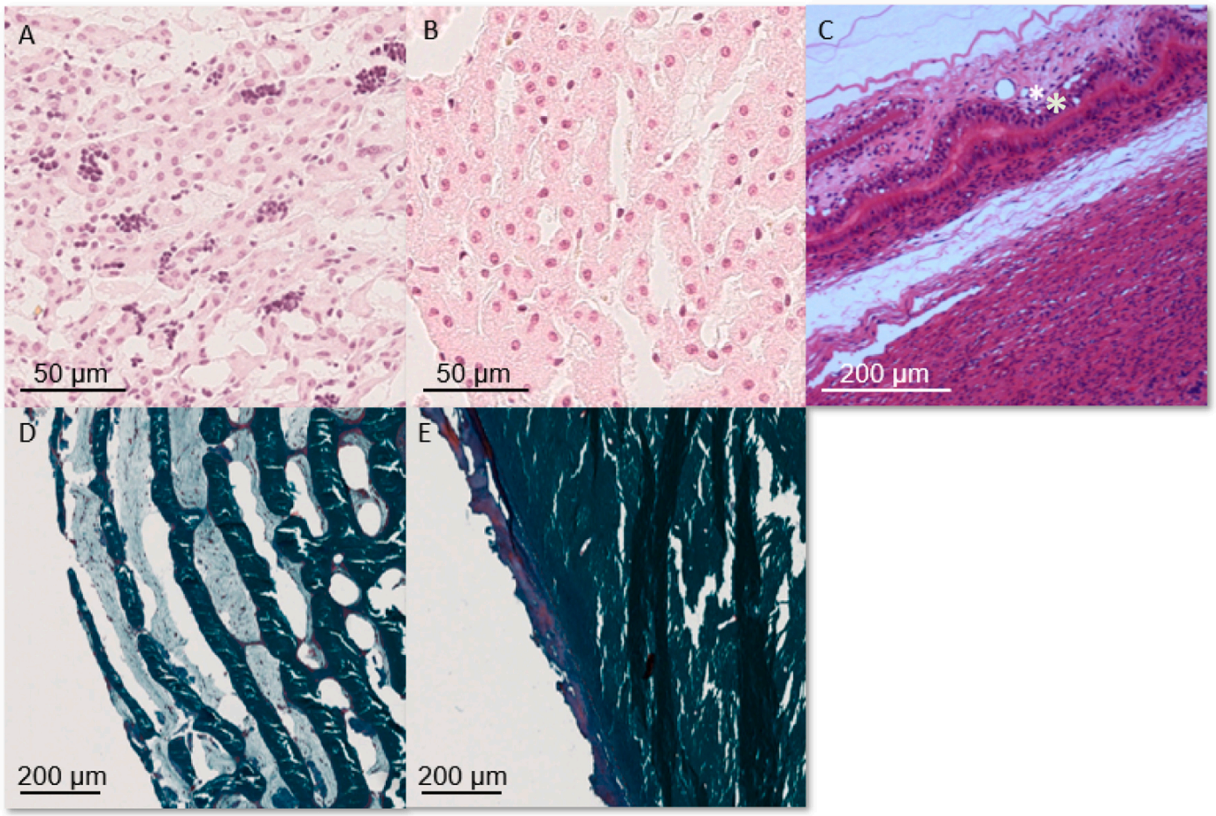


**Fig. 4.** A. Fetal liver. Smaller, darker erythropoietic cells in clusters. Hematoxylin and eosin (HE) stain. B. Maternal liver. Hepatocytes are larger, organized linearly and erythropoietic cells are absent. HE stain. C. Placental barrier. In sheep, there are three cell layers (uterine epithelium, trophoblast epithelium and fetal endothelium) between maternal and fetal bloodstream. Fetal side and fetal vessel marked with asterisk. HE stain. D. Fetal bone. Chondrocyte is the dominant cell type (light blue). Masson trichrome stain. E. Maternal bone. Bone consists mainly of mineralized matrix (green).

cellularity (K) and high extracellular mineralization (Ca, P, Mg). The differences in mineralization of the bones in ewes and fetuses is well shown in the distribution of these elements. It is estimated that 80% of the fetal bones' Ca content is accumulated in the third trimester [49]. A higher concentration of Cu in fetal bones compared to those of the ewe could be explained by the higher metabolic rate and the amount of Cu containing enzymes, such as lysyl oxidase [50] and hematopoiesis-associated enzymes. Al can antagonize $Mg^{2+}$ and competitively replace $Ca^{2+}$, $Fe^{2+}$ or $Fe^{3+}$ and thus act as an inhibitor and accumulate to bone mineral [21].

The lower levels of Ba and Sr in fetal bones are partly explained by mineralization degree. In our study, bone levels of Ba, Cr, Ni, Pb, Sr, and Ti were higher than liver levels, suggesting that bone is more important in terms of long-term low exposure accumulation of metals. On the other hand, Al, As, and Hg showed similar levels in bone and liver. The differences between metals can be partly explained by their half-life in the body. For example, the half-life of methyl-Hg is 70 days and 90% of Hg is excreted from the body [51] in comparison to Cd that has half-life of 10–30 years [52].

The higher blood Ca in the fall group can be explained by seasonal variation of vitamin D and parathyroid hormone (PTH). Vitamin D and PTH stimulate renal absorption of Ca and bone remodeling, and it has been shown that serum levels of Ca are slightly higher in humans in the fall than in the spring [53,54]. The higher physical activity during summer in the fall group could also explain some of the observed differences between the fall and the spring group. Mechanical loading increases bone formation and in large animals, probably also bone resorption [55]. In addition to mechanical loading, there are also other reactions to physical activity, such as an increase in the PTH concentration. Higher PTH concentration enhances both osteoblast and osteoclasts activity [56]. Increased physical activity could enhance the accumulation of metals in the bone, and the release of the captured metal ions from the bone matrix into the bloodstream could increase the fetal exposure to metals. Higher levels of Pb, Rb, Sr, and Ti in the fall group could be explained by lower levels of Zn, as it has been previously shown that Zn deficiency increases the accumulation of Cd in liver [57].

There are several limitations in the present study. The differences in feeding during pregnancy, mainly pasture in the fall group and dry food in the spring group, could affect the results. In addition, only one sample from each animal was taken and that could affect the reliability of our results as the elemental composition can vary in the tissue. However, the sizes of the samples were macroscopic and the variation of the concentrations between individual animals in given elements within specific tissues was quite small, thus samples likely contained a representative sample of the tissue.

Moreover, metal contamination of the bone samples cannot be completely ruled out; however, all bone samples within the same group were treated with the same methods and thus a possible contamination would be consistent throughout the samples series and would not have affected the comparison between samples. This possibility should be considered when interpreting the results. Finally, other than acting as toxicants, metals have physiological importance as cofactors in metalloproteins and thus the concentrations of selected metals can also reflect the concentrations metalloproteins in the tissues. In the future tissue metal analyses, it would be interesting to relate the metal concentrations to respective protein concentrations or if feasible, even to the amount of metalloproteins in the tissue.

Metals in bone can have various effects on bone homeostasis, either via direct toxic effects on bone cells or by accumulating in the mineralized matrix and thus predisposing to osteoporosis [58]. In the present

**Table 2**
Levels of selected metals in previous studies. <LOD = below the limit of detection; ppm = parts per million (μg/g); ppb = parts per million (ng/g).

| | Tissue | Species, sex | Median [range] | Mean (SD) | Country | Reference |
|---|---|---|---|---|---|---|
| Al | Umbilical cord blood | Human | 8.6 ppb | 11 (9.2) ppb | Jamaica | [3] |
| | Placenta | Human | [0.46–26] ppm | 4.8 (5.7) ppm | Spain | [61] |
| | Liver | Human, F | 0.61 [0.27–3.1] ppm | | Czech Republic | [62] |
| | Liver | European bison, F | 1.0 ppm | | Poland | [63] |
| | Bone | Human | [0.058–13] ppm | 1.1 (1.7) ppm | Sweden | [64] |
| | Bone | Human, F | 1.3 [0.18–3.5] ppm | | Czech Republic | [62] |
| As | Maternal blood | Human | 1.8 [0.3–7.3] ppb | | Spain | [38] |
| | Umbilical cord blood | Human | 1.2 [0.3–5.9] ppb | | Spain | [38] |
| | Maternal blood | Human | 4.1 [<LOD–17.6] ppb | | Japan | [65] |
| | Umbilical cord blood | Human | 3.6 [<LOD–22.4] ppb | | Japan | [65] |
| | Placenta | Human | 4.4 [1.2–19.6] ppb | | Japan | [65] |
| | Umbilical cord blood | Human | 2.2 [<LOD–11.0] ppb | 2.9 (2.1) ppb | China | [1] |
| | Maternal blood | Human | 1.5 [<LOD–8.9] ppb | 2.0 (1.4) | China | [1] |
| | Liver | Camel, F | 0.19 [0.16–0.21] ppm | | Iran | [66] |
| | Liver | Human, F | 0.027 [0.003–0.27] ppm | | Czech Republic | [62] |
| | Liver | European bison, F | 6 [3.7–16 ppb] ppb | | Poland | [63] |
| | Liver | Bovine | [<LOD–0.36] ppm | 0.016 ppm | Jamaica | [67] |
| Ba | Placenta | Human | [0.12–1.1] ppm | 0.39 (0.22) ppm | Spain | [61] |
| | Liver | European bison, F | 0.053 [0.018–0.21] ppm | | Poland | [63] |
| | Bone | Human, F | 4.8 [2.2–12] ppm | | Czech Republic | [62] |
| | Bone | Human, F | 2.34 [1.1–7.7] ppm | 2.9 (1.8) ppm | Russia | [68] |
| Cd | Maternal blood | Human | 0.4 [0.3–2.5] ppb | | Spain | [38] |
| | Umbilical cord blood | Human | 0.5 [0.2–0.9] ppb | | Spain | [38] |
| | Maternal blood | Human | 1.18 [<LOD–11] ppb | | Japan | [65] |
| | Umbilical blood | Human | 0.53 [<LOD–10.5] ppb | | Japan | [65] |
| | Placenta | Human | 17 [3.5–51] ppb | | Japan | [65] |
| | Maternal blood | Human | 0.52 [<LOD–2.7] ppb | 0.72 (0.51) ppb | China | [1] |
| | Umbilical cord blood | Human | 0.12 [<LOD–0.46] ppb | 0.22 (0.2) ppb | China | [1] |
| | Maternal blood | Human | 0.40 ppb | 0.42 (0.15) ppb | Croatia | [42] |
| | Umbilical cord blood | Human | 0.05 ppb | 0.05 (0.03) ppb | Croatia | [42] |
| | Placenta | Human | 7.6 ppb | 8.3 (7.6) ppb | Croatia | [42] |
| | Umbilical cord blood | Human | 0.007 ppb | 0.07 (0.01) ppb | Jamaica | [3] |
| | Liver | Camel, F | <LOD [<LOD–0.022] ppm | | Iran | [66] |
| | Liver | Human, F | 1.4 ppm [0.24–4.4] ppm | | Czech Republic | [62] |
| | Liver | European bison, F | 0.025 [0.0085–0.56] ppm | | Poland | [63] |
| | Liver | Bovine | [<LOD–82] ppm | 3.2 ppm | Jamaica | [67] |
| | Bone | Human, F | 0.040 [0.004–0.235] ppm | | Czech Republic | [62] |
| Co | Maternal blood | Human | 0.3 [0.1–0.9] ppb | | Spain | [38] |
| | Umbilical blood | Human | 0.3 [0.1–0.6] ppb | | Spain | [38] |
| | Liver | Camel, F | 0.54 [0.014–0.99] ppm | | Iran | [66] |
| | Liver | Human, F | 44[21–89] ppb | | Czech Republic | [62] |
| | Liver | European bison, F | 42 [5.4–5.9] ppb | | Poland | [63] |
| Cr | Maternal blood | Human | 0.5 [0.2–1.2] ppb | | Spain | [38] |
| | Umbilical cord blood | Human | 0.6 [0.1–2.5] ppb | | Spain | [38] |
| | Maternal blood | Human | 3.4 [<LOD–38] ppb | 6.4 (8.1) ppb | China | [1] |
| | Umbilical cord blood | Human | 7.4 [<LOD–238] ppb | 13 (26) ppb | China | [1] |
| | Liver | Human | [60–140 ppb] | 83 (28) ppb | Spain | [61] |
| | Bone | Camel, F | 7.1 [4.7–9.9] ppm | | Iran | [66] |
| | Bone | Human, F | 0.0072 [0.003–0.35] ppm | | Czech Republic | [62] |
| | Bone | European bison, F | 0.037 [0.013–0.71] ppm | | Poland | [63] |
| Cu | Maternal blood | Human | 1.7 [0.89–2.6] ppm | | Spain | [38] |
| | Umbilical cord blood | Human | 0.62 [0.39–0.81] ppm | | Spain | [38] |
| | Maternal blood | Human | 1.3 [0.50–2.7] ppm | | Japan | [65] |
| | Umbilical cord blood | Human | 0.52 [0.46–0.57] ppm | | Japan | [65] |
| | Placenta | Human | 0.71 [0.44–1.4] ppm | | Japan | [65] |
| | Placenta | Human | [0.43–1.5 ppm] | 0.97 (0.24) | Spain | [61] |
| | Maternal blood | Human | 1.5 ppm | 1.5 (0.30) ppm | Croatia | [42] |
| | Umbilical cord blood | Human | 0.56 ppm | 0.56 (0.08) ppm | Croatia | [42] |
| | Placenta | Human | 0.76 ppm | 0.77 (0.18) ppm | Croatia | [42] |
| | Liver | Camel, F | 3.5 [1.6–4.3] ppm | | Iran | [66] |
| | Liver | Human, F | 5.5 [2.0–17] ppm | | Czech Republic | [62] |
| | Liver | European bison, F | 3.7 [0.87–29] ppm | | Poland | [63] |
| | Liver | Wild boar | 3.3 (2.1–5.9 ppm) ppm | | Slovakia | [69] |
| | Liver | Bovine | [<LOD–99.8] ppm | 20.4 ppm | Jamaica | [67] |
| | Bone | Human, F | 0.66 [0.23–2.2] ppm | | Czech Republic | [62] |
| | Bone | Human, F | 0.85 [0.48–12] ppm | 1.3 (1.8) ppm | Russia | [68] |
| Fe | Maternal blood | Human | 420 ppm | 420 (57 ppm) | Croatia | [42] |
| | Umbilical cord blood | Human | 540 ppm | 540 (61) ppm | Croatia | [42] |
| | Placenta | Human | 78 ppm | 81 (22) ppm | Croatia | [42] |
| | Placenta | Human | [25–81] ppm | 51 (14) ppm | Spain | [61] |
| | Liver | Camel, F | 78 [57–100] ppm | | Iran | [66] |
| Hg | Maternal blood | Human | 1.8 [0.5–9.0] ppb | | Spain | [38] |
| | Umbilical cord blood | Human | 2.8 [0.7–8.7] ppb | | Spain | [38] |
| | Maternal blood | Human | 5.4 [0.61–25] ppb | | Japan | [65] |

(*continued on next page*)

**Table 2** (*continued*)

| | Tissue | Species, sex | Median [range] | Mean (SD) | Country | Reference |
|---|---|---|---|---|---|---|
| | Umbilical cord blood | Human | 9.97 [1.6–44] ppb | | Japan | [65] |
| | Placenta | Human | 13[2–52] ppb | | Japan | [65] |
| | Umbilical cord blood | Human | 4.0 ppb | 4.4 (2.4) ppb | Jamaica | [3] |
| | Maternal blood | Human | 1.3 ppb | 2.4 (3.6) ppb | Croatia | [42] |
| | Umbilical cord blood | Human | 1.5 ppb | 3.5 (6.4) ppb | Croatia | [42] |
| | Placenta | Human | 3.0 ppb | 9.1 (3.0) ppb | Croatia | [42] |
| | Liver | Human, F | 0.33 [0.035–1.2] ppm | | Czech Republic | [62] |
| | Liver | European bison, F | 2.7 [<LOD–5.0] ppb | | Poland | [63] |
| | Liver | Wild boar | 0.032 [0.003–0.11] ppm | | Slovakia | [69] |
| | Bone | Human, F | 0.061 [0.005–0.25] ppm | | Czech Republic | [62] |
| Mg | Placenta | Human | [54–140] ppm | 78 (18) ppm | Spain | [61] |
| | Liver | European bison, F | 140 [100–180] ppm | | Poland | [63] |
| | Bone | Human, F | 2300 [1400–2900] ppm | 2200 (340) ppm | Russia | [68] |
| Mn | Maternal blood | Human | 16 [5.6–33] ppb | | Spain | [38] |
| | Umbilical cord blood | Human | 28 [7.7–77] ppb | | Spain | [38] |
| | Maternal blood | Human | [<LOD-19] ppb | 24 (20) ppb | China | [1] |
| | Umbilical cord blood | Human | [11–110] ppb | 49 (18) ppb | China | [1] |
| | Umbilical cord blood | Human | 43 ppb | 44 (18) ppb | Jamaica | [3] |
| | Placenta | Human | [0.07–58] ppm | 1.7 (9.6) ppm | Spain | [61] |
| | Liver | Camel, F | 0.90 [0.50–1.3] ppm | | Iran | [66] |
| | Liver | Human, F | 1.3 [0.82–2.7] ppm | | Czech Republic | [1] |
| | Liver | European bison, F | 3.2 [0.24–4.2] ppm | | Poland | [63] |
| | Bone | Human, F | 0.22 [0.02–0.58] ppm | | Czech Republic | [62] |
| Mo | Placenta | Human | [7.0–15] ppb | 9.6 (2.3) ppb | Spain | [61] |
| | Liver | Human, F | 0.82 [0.025–1.9] ppm | | Czech Republic | [62] |
| | Bone | Human, F | 0.090 [0.05–0.17] ppm | | Czech Republic | [62] |
| Ni | Maternal blood | Human | 0.6 [0.4–1.9] ppb | | Spain | [38] |
| | Umbilical cord blood | Human | 0.7 [0.5–1.7] ppb | | Spain | [38] |
| | Maternal blood | Human | 13 [<LOD–47] ppb | 15 (12) ppb | China | [1] |
| | Umbilical cord blood | Human | 4.5 [<LOD–21] ppb | 6.1 (3.8) | China | [1] |
| | Placenta | Human | [23–110] ppb | 39 (23) ppb | Spain | [61] |
| | Liver | Human, F | 0.080 [0.002–1.1] ppm | | Czech Republic | [62] |
| | Liver | European bison, F | 0.15 [0.009–3.8] ppm | | Poland | [63] |
| Pb | Maternal blood | Human | 12 [5.2–41] ppb | | Spain | [38] |
| | Umbilical cord blood | Human | 7.9 [2.8–32] ppb | | Spain | [38] |
| | Maternal blood | Human | 11 [3.1–70] ppb | | Japan | [65] |
| | Umbilical cord blood | Human | 9.9 [3.7–62] ppb | | Japan | [65] |
| | Placenta | Human | 11 [2.1–130] ppb | | Japan | [65] |
| | Maternal blood | Human | 18 [3.2–150] ppb | 23 (21) ppb | China | [1] |
| | Umbilical cord blood | Human | 13 [3.5–44] ppb | 14 (7.6) ppb | China | [1] |
| | Maternal blood | Human | 11 ppb | 13 (6.9) ppb | Croatia | [42] |
| | Umbilical cord blood | Human | 6.5 ppb | 7.7 (5.5) ppb | Croatia | [42] |
| | Placenta | Human | 5.4 ppb | 6.3 (4.2) ppb | Croatia | [42] |
| | Umbilical cord blood | Human | 6 ppb | 8 (13) ppb | Jamaica | [3] |
| | Placenta | Human | [24–85] ppb | 42 (15) ppb | Spain | [61] |
| | Liver | Camel, F | 0.69 [0.11–1.5] ppm | | Iran | [66] |
| | Liver | Human, F | 0.19 [0.048–1.8] ppm | | Czech Republic | [62] |
| | Liver | European bison, F | 0.040 [0.011–0.13] ppm | | Poland | [63] |
| | Liver | Wild boar | 0.19 [0.040–1.3] ppm | | Slovakia | [69] |
| | Liver | Bovine | [<LOD–1.3] ppm | 0.052 ppm | Jamaica | [67] |
| | Bone | Human, F | 4.7 [0.43–16] ppm | | Czech Republic | [62] |
| Rb | Liver | Human, F | 3.2 [1.8–8.5] ppm | | Czech Republic | [62] |
| | Bone | Human, F | 0.29 [0.09–1.4] ppm | | Czech Republic | [62] |
| Sr | Placenta | Human | [0.09–1.1] ppm | 0.31 (0.25) ppm | Spain | [61] |
| | Liver | Human, F | 0.045 [0.024–0.21] ppm | | Czech Republic | [62] |
| | Bone | Human, F | 69 [23–120] ppm | | Czech Republic | [62] |
| Ti | Maternal blood | Human | 15 [<LOD–15] ppb | 18 (12) ppb | China | [1] |
| | Umbilical cord blood | Human | 16 [<LOD–63] ppb | 18 (9.6) ppb | China | [1] |
| | Liver | Human, F | 0.93 [0.55–1.5] ppb | | Czech Republic | [62] |
| V | Maternal blood | Human | 0.53 [<LOD–8.4] ppb | 1.8 (1.9) ppb | China | [1] |
| | Umbilical cord blood | Human | 0.37 [<LOD–6.2] ppb | 1.0 (1.5) ppb | China | [1] |
| | Placenta | Human | [40–120] ppb | 72 (22) ppb | Spain | [61] |
| Zn | Maternal blood | Human | 6.7 [4.0–9.1] ppm | | Spain | [38] |
| | Umbilical cord blood | Human | 0.23 [1.5–3.0] ppm | | Spain | [38] |
| | Maternal blood | Human | 4.8 [2.7–14] ppm | | Japan | [65] |
| | Umbilical cord blood | Human | 2.0 [1.1–22] ppm | | Japan | [65] |
| | Placenta | Human | 9.1 [6.8–17] ppm | | Japan | [65] |
| | Maternal blood | Human | 5.6 ppm | 5.5 (0.92) ppm | Croatia | [42] |
| | Umbilical cord blood | Human | 2.7 ppm | 2.7 (0.46) ppm | Croatia | [42] |
| | Placenta | Human | 13 ppm | 13 (2.6) ppm | Croatia | [42] |
| | Placenta | Human | [6.4–11] ppm | 8.4 (0.97) ppm | Spain | [61] |
| | Liver | Camel, F | 100 [71–150] ppm | | Iran | [66] |
| | Liver | Human, F | 69 [28–130] ppm | | Czech Republic | [62] |
| | Liver | European bison, F | 40 [34–100] ppm | | Poland | [63] |
| | Liver | Wild boar | 26[20–53] ppm | | Slovakia | [69] |
| | Liver | Bovine | [5.3–74] ppm | 92 (30) ppm | Jamaica | [67] |

(*continued on next page*)

**Table 2** (*continued*)

| Tissue | Species, sex | Median [range] | Mean (SD) | Country | Reference |
|---|---|---|---|---|---|
| Bone | Human, F | 92 [58–130] ppm | | Czech Republic | [62] |
| Bone | Human, F | 94 [54–130] ppm | 93 (14) ppm | Russia | [68] |

study we did not measure the strength of the bones and as the levels were low, toxic effects were unlikely. However, our findings suggest that in some pregnancies the accumulation in fetal bones can be substantial: in women who work in the metal industry [59] and in women who have undergone joint replace surgery, as abundant placental transfer of Co and Cr has been detected in pregnancies of women with a metal-on-metal hip device [32]. In a case report, an uneventful pregnancy occurred with high levels of Co and Cr [60] but our study suggests that the accumulation of metals in fetal bone occurs already at low blood levels.

## 5. Conclusion

In conclusion, we found that even in externally unexposed sheep detectable amounts of trace and heavy metals can be found already in fetal tissues. Heavy metals accumulate in hard tissues already in utero, but concentrations are lower than in maternal bones.

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## CRediT authorship contribution statement

**Ella Vuoti:** Methodology, Investigation, Data curation, Visualization, Writing – original draft preparation. **Sanna Palosaari:** Visualization, Writing – original draft preparation, Project administration, Supervision. **Sirpa Peräniemi:** Investigation, Resources. **Arja Tervahauta:** Investigation, Resources. **Hannu Kokki:** Resources, Writing – review and editing. **Merja Kokki:** Resources, Writing – review & editing. **Juha Tuukkanen:** Methodology, Investigation, Resources. **Petri Lehenkari:** Conceptualization, Resources, Supervision.

## Conflict of interest

Authors declare no conflict of interest through any financial engagements.

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