Atomic Absorption Spectrometry Analysis of Trace Elements in Degenerated Intervertebral Disc Tissue

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Background:
Few studies have investigated trace elements (TE) in human intervertebral disc (IVD) tissue. Trace element presence can have diverse meanings: essential TE show the metabolic modalities of the tissue, while environmentally-related TE indicate pollution and tissue-specific absorption and accumulation. IVD is a highly specific compartment with impaired communication with adjacent bone. Analysis of TE in IVD provides new insights regarding tissue metabolism and IVD communication with other tissues.

Material/Methods:
Thirty intervertebral discs were acquired from 22 patients during surgical treatment for degenerative disease. Atomic absorption spectrometry was used to evaluate the concentrations of Al, Cd, Pb, Cu, Ni, Mo, Mg, and Zn.

Results:
Al, Pb, Cu, Mg, and Zn were detected in all samples. Pb was significantly positively correlated with age, and Ni concentration was weakly correlated with population count in the patient’s place of residence. Only Cu was observed in higher concentrations in IVD compared to in other tissues. Significant positive correlations were observed between the following pairs: Mg/Zn, Mg/Al, Mg/Pb, Zn/Al, Zn/Pb, and Al/Pb. Negative correlations were observed between Mg/Cd, Zn/Cd, Mg/Mo, and Mo/Pb.

Conclusions:
This study is one of few to profile the elements in intervertebral discs in patients with degenerative changes. We report significant differences between trace element concentrations in intervertebral discs compared to in other tissues. Knowledge of the TE accumulation pattern is vital for better understanding intervertebral disc nutrition and metabolism.

MeSH Keywords:
Intervertebral Disc Degeneration • Spine • Trace Elements

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Background

Trace elements (TE) are of growing interest in medical studies, with the majority of publications focused on element deficiencies and related syndromes. Advancements in biochemical knowledge are leading to a better understanding of the roles of various elements in metabolic processes and tissue accumulation.

The definition of TE in biology is somewhat flexible, with the term used to refer to dietary minerals needed in small quantities for proper physiology and development of an organism (including active elements of metabolic processes, such as copper, magnesium, and zinc) as well as elements that accumulate due to environmental pollution (lead and cadmium). There is not yet a comprehensive biological definition or complete list of trace elements found in humans.

TE content is most easily analyzed in bone tissue because it is the trace elements repository, reflecting the turnover for the whole organism [1]. TE content can also be examined in more unstable compartments, such as serum, urine, or cerebrospinal fluid, which show more rapid changes secondary to exposure or specific response of the organism [2]. One of the most highly specific tissues is human intervertebral disc (IVD). In the first decade of life, this tissue becomes avascular, such that all metabolism relies on passive nutrient transport through the end-plates, which are cartilage-remodeling tissue that act as barriers, separating IVD from the bone of the vertebrae. Confined in the center of the IVD, the nucleus pulposus environment is rich in lactic acid and has a lower oxygen concentration and higher pH compared to other tissues. These conditions result in a low cell concentration, with slow metabolism and abundant extracellular matrix (ECM).

Degenerative disc disease (DDD) involves changes related to ECM dysfunction. The causes of DDD are complex and not fully understood, but appear to mainly involve aging, and genetic or environmental factors.

The mainstream concept of degeneration assumes disproportionation levels of anabolic and catabolic activity in the ECM. These processes involve enzymes such as cathepsins, aggrecanases, and matrix metalloproteinases (MMPs) [3]. MMPs are zinc-dependent endopeptidases that are active in protein degradation [4]. Other TE are also involved in enzyme activity – for example, copper is present in cytochrome oxidase or ceruloplasmin – or are involved in collagen synthesis and connective tissue development [5]. Recent studies have analyzed the correlation between DDD and TE concentration in serum [6]. However, few papers have examined the role of TE in human IVD tissue. Tohno et al. [7] evaluated post-mortem Ca, P, S, Mg, and Na concentrations. Minami et al. [8] analyzed platinum levels in bone and IVD from cisplatin-treated ovarian cancer patients, and found that intradiscal metal accumulation after exposure exceeded bone accumulation by up to 4.3-fold. Other observations have also shown that in some cases TE concentrations can be higher in disc or bone tissue than in internal organs, such as liver and kidneys [9].

The present study aimed to analyze the selected TE profile (Al, Cd, Pb, Cu, Ni, Mo, Mg, and Zn) in human degenerated intervertebral disc tissue, and to compare the concentrations with those found in other tissues.

Material and Methods

The study material included 30 intervertebral discs obtained from 22 patients undergoing surgical discectomy or spine fusion due to degenerative disc disease. Twelve specimens were from the cervical spines of 6 patients, and 18 specimens were harvested from the lumbar spines of 16 patients. Preoperative magnetic resonance images were used to evaluate the degeneration status of the operated disc according to Pfirrmann score [10]. Atomic absorption spectrometry (AAS) was used to determine the concentrations of the trace elements Al, Cd, Pb, Cu, Ni, Mo, Mg, and Zn, which were calculated using the dry weight (dw) of the disc. The use of tissue in this study was approved by the appropriate bioethics committee, and written consent was obtained from all patients.

The mean age of the patients at the time of operation was 47.6 (range, 28–64 years). All patients were interviewed using a questionnaire to collect data on demography, health status, and occupational heavy metals exposure. None of the patients had knowledge of being inadvertently exposed to heavy metal pollution.

Based on postal zip codes, we obtained information regarding each patient’s places of residence and work over the last 5 years. Places of residence and employment were stratified according to population count as follows: >500 k, 200–499 k, 100–199 k, 20–99 k, <20 k, and village inhabitants.

Analytical procedure

First, the frozen intervertebral disc samples were freeze-dried using a Lyovac lyophilizer GT2e (Steris, Germany) for 24 hours. The samples were weighed after drying. Then 65% nitric acid using a Lyovac lyophilizer GT2e (Steris, Germany) for 24 hours. First, the frozen intervertebral disc samples were freeze-dried to determine the concentrations of the trace elements Al, Cd, Pb, Cu, Ni, Mo, Mg, and Zn, which were calculated using the dry weight (dw) of the disc. The use of tissue in this study was approved by the appropriate bioethics committee, and written consent was obtained from all patients.

The samples were weighed after drying. Then 65% nitric acid (Merck, Germany) was added to obtain a dilution factor (DF) of 10, with amounts in the ranges of 0.2–0.6 g of sample and 2.0–6.0 ml of nitric acid.

The prepared samples were allowed to stand overnight to slow mineralization. Then the samples were mineralized in a microwave oven (Mars Xpress 5, CEM USA).
Trace element concentrations in the mineralized samples were determined using an AAS 7000 spectrometer (Shimadzu, Japan) with graphite furnace atomization (GF-AAS) for Al, Cu, Cd, Mo, Ni, and Pb, or with flame atomization (F-AAS) for Mg and Zn. All analyses were run in 3 replicates. The percent RSD did not exceed 5% for GF-AAS analysis, and did not exceed 7% for F-AAS analysis. Table 1 presents the basic optimized parameters for the determination of Al, Cu, Cd, Mo, Ni, Pb, Zn, and Mg using AAS.

### Statistical analysis

The results are presented as means and ranges. Where applicable, standard deviation (SD) was also calculated. Spearman’s rank-order correlation analysis was used to determine the relationships among different parameters. P<0.05 was accepted as indicating statistical significance. When an element was not detected in the sample, the ½ limit of detection (LOD) value was left out of calculations. Statistica (Statsoft, Tulsa, USA) software was used for statistical analysis.

### Results

The elements Al, Pb, Cu, Mg, and Zn were detected in every tested sample. Ni was detected in 97% of samples, Mo in 83%, and Cd in 57% (Table 2). The concentrations of Mg, Zn, and Cu were 1 order of magnitude higher than the concentrations of the other examined elements (mg·kg⁻¹ dw vs. µg·kg⁻¹ dw). The measured concentrations were as follows (range values with mean value and standard deviation, respectively): Al, mean of 663.71, range of 165.7–1271, SD of 288.84 (in µg·kg⁻¹ dw); Cu, mean of 3.41, range of 0.97–23.64, SD of 4.045 (in mg·kg⁻¹ dw); Mg, mean of 800.1, range of 182.6–2132, SD of 525.5 (in mg·kg⁻¹ dw); Zn, mean of 39.60, range of 10.56–184.5, SD of 35.95 (in µg·kg⁻¹ dw); Pb, mean of 8.435, range of 0.562–24.76, SD of 5.596 (in µg·kg⁻¹ dw); Ni mean of 251.38, range of 25.48–444.2 (in µg·kg⁻¹ dw); Cd, mean of 8.435, range of 0.562–24.76 (in µg·kg⁻¹ dw); Cd, mean of 54.33, range of 20.02–143.2 (in µg·kg⁻¹ dw) (Table 2). Among the elements that were found in only some of the tested samples, the LOD values were: 0.2 µg·kg⁻¹ for Cd and 1 µg·kg⁻¹ for Ni and Mo.

Correlation analysis showed significant positive correlation of Pb concentration with age (Table 3). Ni concentration showed a weak positive correlation with the population size of the place of residence. A strong positive correlation was observed between pair Mg/Zn, both of which also showed a positive correlation with the non-essential element Al and the toxic element Pb. There was also a significant positive correlation between Al and Pb. Mg and Zn each also showed a weak negative correlation with Cd. Mo showed negative weak correlations with both Mg and Pb.

### Discussion

Our present analysis detected Al, Pb, Cu, Mg, and Zn in all tested samples. Of these 5 elements, Cu, Mg, and Zn are considered...
essential in human metabolism, Pb is considered non-essential and toxic, and the role of Al is not yet fully understood. The other studied elements were only found in some of the tested samples. Of these, Cd is not essential and is considered toxic, but Mo and Ni are considered potentially essential. The present study had possible methodological flaws; however, considering the differences between LOD and mean levels in the rest of the samples, we can likely regard Cd, Mo, and Ni toxic."

Table 2. The contents of elements in intervertebral disc.

| Sample No. | Case No. | Al [µg/kg dw] | Cd [mg/kg dw] | Pb [µg/kg dw] | Ni [mg/kg dw] | Mo [µg/kg dw] | Cu [mg/kg dw] | Mg [µg/kg dw] | Zn [mg/kg dw] |
|------------|----------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| 1          | 1        | 539.5         | 4.921         | 170.1         | 222.9         | 111.2         | 5.602         | 413.9         | 18.29         |
| 2          | 2        | 1203          | –             | 1264          | 134.8         | 54.35         | 2.492         | 1195          | 61.26         |
| 3          | 2        | 915.1         | 9.171         | 209.7         | 387.2         | 62.67         | 2.793         | 489.5         | 16.88         |
| 4          | 3        | 992.1         | –             | 1252          | 369.9         | 29.38         | 2.092         | 1599          | 71.22         |
| 5          | 3        | 1084          | –             | 1343          | 62.23         | 35.55         | 1.844         | 854.7         | 36.12         |
| 6          | 3        | 1021          | –             | 1417          | 444.2         | 33.41         | 1.889         | 2132          | 121.75        |
| 7          | 4        | 572.7         | 16.02         | 867.3         | 143.4         | 77.23         | 1.459         | 672.1         | 25.72         |
| 8          | 5        | 1003          | –             | 1825          | 43.22         | –             | 0.9701        | 1622          | 29.38         |
| 9          | 6        | 1271          | –             | 1599          | 373.4         | 38.89         | 1.079         | 371.4         | 14.06         |
| 10         | 7        | 451.2         | 4.751         | 191.2         | 312.9         | 35.89         | 1.542         | 1008          | 38.97         |
| 11         | 8        | 539.5         | –             | 61.47         | 53.58         | –             | 1.326         | 634.8         | 12.65         |
| 12         | 9        | 552.2         | –             | 566.9         | 245.4         | 42.56         | 1.542         | 1008          | 38.97         |
| 13         | 10       | 470.1         | 6.444         | 167.3         | 213.4         | 69.82         | 1.616         | 371.4         | 14.06         |
| 14         | 11       | 520.6         | 0.562         | 2333          | 165.9         | 21.24         | 1.664         | 1032          | 55.76         |
| 15         | 11       | 697.3         | 9.292         | 314.2         | 312.9         | 35.89         | 1.799         | 516.7         | 34.35         |
| 16         | 12       | 490.6         | 7.640         | 665.9         | 421.2         | 43.58         | 2.046         | 977.9         | 43.84         |
| 17         | 13       | 545.9         | –             | 525.9         | 117.5         | –             | 2.519         | 999.4         | 18.53         |
| 18         | 14       | 440.1         | 1.625         | 266.3         | 1625          | 55.21         | 1.417         | 482.8         | 36.78         |
| 19         | 15       | 444.7         | –             | 589.2         | 48.15         | 73.16         | 6.089         | 856.2         | 41.18         |
| 20         | 16       | 240.1         | 5.634         | 495.9         | 1812          | 62.38         | 2.715         | 368.3         | 48.51         |
| 21         | 17       | 165.7         | 7.595         | 1983          | 1033          | 53.91         | 4.562         | 246.5         | 11.35         |
| 22         | 18       | 192.6         | 8.520         | 186.6         | 1699          | 34.65         | 4.101         | 182.6         | 10.56         |
| 23         | 19       | 546.6         | 13.209        | 478.4         | 106.2         | 35.42         | 3.827         | 371.5         | 22.19         |
| 24         | 20       | 748.9         | 24.764        | 274.2         | 308.6         | 143.2         | 3.073         | 291.1         | 15.44         |
| 25         | 20       | 389.2         | 6.218         | 338.3         | 427.6         | 61.61         | 3.356         | 276.4         | 16.11         |
| 26         | 21       | 461.1         | 8.026         | 204.2         | 400.7         | 43.13         | 3.695         | 299.4         | 17.56         |
| 27         | 21       | 743.2         | –             | 1050          | 311.5         | –             | 2.517         | 512.5         | 37.32         |
| 28         | 22       | 632.9         | –             | 773.2         | –             | –             | 2.517         | 512.5         | 37.32         |
| 29         | 22       | 776.8         | –             | 717.6         | 158.5         | 47.75         | 4.968         | 614.3         | 35.46         |
| 30         | 22       | 360.6         | 9.016         | 355.8         | 25.48         | 20.02         | 3.356         | 884.3         | 29.62         |
as non-essential in IVD metabolism and as unrelated to degenerative changes.

Except for Pb, the majority of non-essential elements tend to concentrate in the tissue up to some critical point, and once this threshold is exceeded, the element is excreted through a variety of methods. For some TE, the accumulation capacity has only been determined for bone tissue; thus, complete analysis regarding bioaccumulation was not possible in our present study. The trace elements present in intervertebral disc tissue have not yet been extensively studied. Tohno et al. [7] investigated the elements abundantly present in IVD tissue, but their report did not include degeneration criteria. Due to the lack of relevant data from this particular biological compartment, here, we primarily compare our present findings to previous results from other tissues – the most relevant being cartilage and tendons, due to their metabolism, morphology, and biomechanical role [11,12].

**Mg**

Magnesium content has been reported for a variety of tissues, including intervertebral disc and temporomandibular joint disc (TMJD) [13–15]. Tohno et al. [7] reported the average Mg content of IVD to be 1.196 mg·g⁻¹ (range, 0.6–2.2 mg·g⁻¹) from a study of 9 specimens that were acquired post-mortem with no definition of degeneration stage. Our present results showed a lower mean Mg concentration of 800.1 mg·kg⁻¹, with a range of 182.6–2132 mg·kg⁻¹. This discrepancy could be due to the different methods of tissue acquisition between the 2 studies, and our present results may be grounds to question the use of cadavers as a control group. In another study of cadavers, Takano et al. [15] reported the average Mg concentration in TMJD, which was substantially lower than our present results (mean value 524.74 mg·kg⁻¹ vs. 800.1 mg·kg⁻¹ dw), and showed a narrower concentration range (393.4–764.9 mg·kg⁻¹ dw).

Another study shows Mg concentrations of 445 mg·kg⁻¹ in cervical spine posterior longitudinal ligaments (PLL) [14], which are lower than those presently found in IVD and comparable to concentrations reported in TMJD. The standard deviation reported for cervical spine PLL Mg concentrations (161 mg·kg⁻¹) was smaller than that in our study (523.5 mg·kg⁻¹). The Mg concentration in bone has been reported as 1792.9 mg·kg⁻¹ [13], which is more than 2 times higher than that found for IVD.

Magnesium has many biological functions, and is the fourth most abundant cation in the body and the second most abundant intracellular cation [16]. Soft tissue accumulates only 19.3% of Mg ions. The majority of Mg ions are found in bone and muscle, with up to 60% of magnesium located within the bone, where it most probably forms the hydroxyapatite surface. In cases of deficiency, Mg from bone can be readily exchanged with serum, although this exchangeable form can decline with increasing age [17].

Our results showed that Mg concentrations in IVD of living patients with DDD were higher than those in ligamentous tissue and TMJD, and smaller than the concentrations found in cadaver IVD. The difference in Mg concentration between bone and IVD tissues was less pronounced than we expected based on the cellularity of each tissue. Since the cellularity of bone is substantially higher than that of IVD, we can conclude that Mg in IVD is mainly extracellular. The intracellular range of Mg

### Table 3. Spearman’s rank correlation between elements, place of living, work and age.

| Pfirrmann grade | Place of living | Place of work | Pb | Ni | Mo | Cu | Mg | Zn | Age |
|----------------|----------------|---------------|----|----|----|----|----|----|-----|
| Place of living | −0.17          | 0.18          | −0.16 | 0.36* | −0.03 | 0.29 | −0.18 | −0.14 | −0.27 |
| Place of work   | 0.18           | 0.03          | 0.24 | −0.07 | 0.26 | 0.05 | 0.21 | −0.15 | −0.16 | −0.12 |
| Pfirrmann grade | −0.03          | 0.32          | 0.03 | −0.29 | 0.16 | −0.07 | 0.00 | −0.19 | 0.20 |
| Al              | −0.21          | 0.61*         | 0.17 | −0.12 | −0.21 | 0.60* | 0.44* | 0.26 |
| Cd              | −0.36          | 0.00          | 0.24 | 0.28 | −0.48* | −0.58* | −0.14 |
| Pb              | 0.02           | −0.43*        | −0.15 | 0.76* | 0.79* | 0.50* |
| Ni              | −0.02          | 0.05          | −0.40* | −0.38 | 0.18 |
| Mo              | −0.31          | −0.08         | −0.02 | 0.19 | 0.10 |
| Cu              | 0.75*          | 0.21          | 0.28 |

* Significant p<0.05.
concentration is maintained within strict limits, except under severe hypoxic stress or magnesium depletion. Up to 5% of intracellular magnesium is present in the form of free ions, with the rest bound to ionic compounds, such as ATP, ADP, citrate, proteins, RNA, and DNA, or stored in the mitochondria and endoplasmic reticulum.

It has been shown that Mg can decrease MMP secretion to the extracellular matrix after stimulation with growth factors [18]. Migration of cells, such as macrophages and fibroblasts, in the early stages of skin wound repair is associated with Mg²⁺ increase [19]. Mg is connected with migratory phenotype activation and maintenance through modifications of integrins and E-cadherin. It has further been observed that magnesium deficiency results in loss of replicative capacity, and increases the expression of senescence-associated biomarkers in human fibroblasts [20]. Overall, Mg is predominantly located in ECM and its activity is related to repair process, which is supported by the presently observed higher concentration in DDD tissue compared to ligaments.

Cu

Cu concentration is best documented in bone tissue, with average reported concentrations of 0.62 mg·kg⁻¹ dw [13] and 0.8 mg·kg⁻¹ dw [21]. Similar Cu concentrations have also been found in cartilage, with an average value of 0.79 mg·kg⁻¹ dw and range of 0.20–1.78 mg·kg⁻¹. Our results showed that the copper concentration in IVD (mean 3.41, range 0.97–23.64 mg·kg⁻¹ dw) was more than 3 times higher than those previously observed in bone and cartilage.

Copper and zinc are both predominantly associated with animal proteins, and are known for their antagonism, in that they tend to neutralize each other’s actions. Our results did not confirm a negative correlation between these 2 elements. Copper is a redox-active metal that is predominantly used by organisms living in oxygen-rich environments, and that fluctuates between the oxidized (Cu²⁺) and reduced (Cu⁰) states [22]. It is also a compound of the XIAP protein, which acts as an apoptosis inhibitor. Cu ions are a key element of cytochrome oxidase, which is responsible for mitochondrial phosphorylation and ATP production. Thus, Cu deficiency may lead to respiratory disturbance. The integral membrane protein Ctr1 functions as a major copper importer at the plasma membrane. Cu²⁺ is the only known physiological substrate for Ctr1. Recent findings in yeast and mammals strongly suggest that Ctr1 facilitates uptake of the metal-based anti-neoplastic drug cisplatin. This may explain the higher Pt concentration in cisplatin-treated patients reported by Minami et al. [8]. On the other hand, Ctr1 knock-out mice exhibit growth retardation.

There may be a connection between high Cu concentration, low oxygen environment, and the Warburg effect [22]. The Warburg effect includes a down-regulation of cell respiratory capacity that is observed in many cancer cell types, with a corresponding shift to glycolysis for cell energy generation. This process can be related to p53 protein mutation, which is a common genetic change found in a broad range of cancer cells. p53-inactive mutation cells show significantly reduced oxygen consumption, while generating increased levels of lactate. It has been proposed that a link between p53 and SCO2 gene expression levels may hamper copper acquisition and promotion of the Warburg effect.

The high lactic acid concentration due to glycolysis is the natural environment for IVD tissue. It is possible that glycolysis may be related to Cu concentration other than in the Warburg effect, which, in turn, may also be related to the p53 gene. However, IVD tissue is virtually free from mutagenesis, except for chordoma that arises from the remnants of the notochord, suggesting a low risk of p53 mutations in IVD cells. Another hypothesis is that higher Cu concentration may be a tissue-specific countermeasure to primary oxygen deficiency or promotion of oxygen-based energy acquisition. A possible third explanation of higher Cu concentration in disc tissue may relate to the element’s role in the repair process. Higher copper concentrations have been observed in wounds during the healing process, and the element has been observed to induce vascular endothelial growth factor expression [23]. However, this explanation is less likely because the process is bound with vascular ingrowth, which is not observed in disc tissue except for outer parts of the annulus fibrosus.

Zn

The presently determined intervertebral disc zinc concentration (39.9 mg·kg⁻¹ dw) was similar to the levels in PLL (36 mg·kg⁻¹ dw) reported by Kumai et al. [24]. On the other hand, the presently reported IVD concentrations are less than half that of the average concentrations reported in cartilage (88.3 mg·kg⁻¹; range, 54.3–163.8 mg·kg⁻¹ dw) [13] and bone (84.58 mg·kg⁻¹; SD 17.68 mg·kg⁻¹) [21].

Zn plays a well-recognized role in the immune system, is an antioxidant, and has anti-inflammatory actions [25]. Zinc-deficient cells display decreased gene expressions of interleukin-2 and IL-2 receptor alpha [26], suggesting that immune reaction would be less likely during the disc degeneration process. Zinc supplementation has been shown to decrease oxidative stress markers and inflammatory cytokine generation.

It also stabilizes the molecular structure of cellular membranes and organelles, contributing to cell and organ integrity. Zinc also plays an essential role in DNA transcription and genetic expression [27]. MMPs are Zn-dependent compounds involved...
Compared to the presently determined Cd concentration in bone tissue of the Cracow, Poland population (correlation, 0.68) by Jurkiewicz et al. [13]. Further research is required to link metabolic background with TE content.

Al

It was very interesting that our results showed aluminum in all tested samples. Aluminum is the third most common element on earth, but is not considered essential in humans. Exogenic sources of aluminum may include oral antacids and anti-perpirants applied to the skin. Aluminum cookware is considered safe, since only uncoated cookware can lead to leaching of particles into food. Aluminum can be part of highly reactive biomolecules. Although its role is not entirely known, several studies have shown its influence on neurodegenerative disorders, including Alzheimer's disease, and brain tissue is considered the sink for systemic aluminum. The average concentration in the human tissue is at the level of 2.6 mg·kg⁻¹ [28].

Our present data cannot exclude a metabolic role of the element, or the possibility that it plays a role in degeneration induction or the process itself. The levels observed in our study were smaller than reference values, suggesting that Al content is environment-related and depends on end-plate permeability. Answering these questions will require further analysis of Al compounds in IVD.

Pb

Lead accumulates in bone, and is thereafter difficult to excrete and is retained by the tissue, such that its concentration tends to increase over time. The Pb accumulation presently observed in IVD resembles that in bone, as Pb was the only element that was positively correlated with age. Compared to that in IVD, the Pb concentration in bone is up to 2 times higher, with an average of 1.35 mg·kg⁻¹ dw [13]. The cartilage Pb concentration is lower, with an average of 0.41 mg·kg⁻¹ and range of 0.21–0.65 mg·kg⁻¹ [21], which is comparable to our results in IVD tissue. Viewing cartilage as a reference, we may conclude that Pb accumulation is not related to end-plate permeability, in contrast to Al.

Cd

Compared to the presently determined Cd concentration in IVD (8.4 µg·kg⁻¹ dw), the concentration in bone is reportedly up to 3 times higher (30 µg·kg⁻¹ dw) [13]. Łanocha et al. [29] reported a slightly lower Cd concentration in bone tissue (average, 22 µg·kg⁻¹; range, 13–34 µg·kg⁻¹). In a population that is occupationally exposed to Cd, the bone concentration can be higher, ranging from 0.11–1.2 mg·kg⁻¹ dw. Cartilage Cd concentrations are congruous with levels observed in bone (average, 0.031 mg·kg⁻¹; range, 0.001–0.151 mg·kg⁻¹ dw) [21]. Interestingly, in our study of IVD, cadmium was detected in only 57% of the samples. The lower concentration of this element in IVD compared to cartilage, and its detection in only some of the tested samples, suggest that the end-plate acts as a selective barrier in Cd transport.

Ni

Nickel is predominantly associated with vegetable food sources, and has no potential biological activity in human metabolism [30]. Brodziak et al. [31] reported a mean Ni concentration in bone of 4.82 mg·kg⁻¹ (SD, 10.74 mg·kg⁻¹; range, 0.03–71.49 mg·kg⁻¹). The same study found a similar cartilage content (mean, 4.40 mg·kg⁻¹; SD, 7.38 mg·kg⁻¹), and a lower concentration in the joint capsule (mean, 1.38 mg·kg⁻¹; SD, 2.47 mg·kg⁻¹). The Ni concentration in IVD found in our study (mean, 251.38 µg·kg⁻¹; range, 25.48–444.2 µg·kg⁻¹) was only 18% of the previously reported concentration in joint capsule and only 5% of the bone concentration. Although Ni presence was confirmed in 97% of tested samples, the results suggest impaired transport of this element into the disc.

Mo

Like zinc and copper, molybdenum is predominantly bound with animal protein, and is related to animal food intake. Mo concentration in human tissues ranges from 1 to 400 µg·kg⁻¹, with the skeletal concentration being in the upper part of this range [30]. In our study, the mean Mo concentration in IVD was 54.33 µg·kg⁻¹ dw, which is more than half of the reference value from the literature (75 µg·kg⁻¹).

Conclusions

The present analysis is one of the few to address trace element concentrations in IVD tissue. The results add to a preliminary picture of the IVD chemical environment, and possible dependencies between TE in IVD tissue. This study showed significant differences between the trace element concentrations in intervertebral disc and in other tissues, especially bone.

TE concentration may be influenced by dietary components rich in particular elements, as well as by biogenic factors, such as pregnancy and lactation. The major consideration in IVD tissue is selective end-plate permeability, which can be suspected in...
the cases of Ni, Cd, and Al, but not Pb. Biochemical, environmental, and metabolic functions could also favor the accumulation of some elements in higher quantities, as is the case for Cu. Further cross-referencing against other tissues and a better understanding of the biochemistry of the IVD are essential to answer the questions arising in this study.

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