# Metal accumulation in the human uterus varies by pathology and smoking status

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**Objective:** To evaluate the content of Al, Cd, Cr, Cu, Mn, Ni, Pb, and Zn in human endometrium (END), endocervix (ECX), and endometrial (END-P) and endocervical (ECX-P) polyps in relation to histologic image and female demographic characteristics and habits.
**Design:** Tissues were collected during curettage of the uterine cavity, subjected to histopathologic examination, digested, and analyzed with the use of a microwave induced nitrogen plasma atomic emission spectrometer. Demographic/lifestyle characteristics were assessed with the use of a questionnaire.
**Setting:** University hospital and research laboratory.
**Patient(s):** One hundred nine white Polish women undergoing curettage of the END (n = 60) or ECX (n = 23) or dissection of END-P (n = 16) or ECX-P (n = 10).
**Interventions(s):** None.
**Main Outcome Measure(s):** Trace element concentrations in collected tissues.
**Result(s):** Histologic states of analyzed END included: normal (n = 22), irregularity (n = 3), polypoid (n = 12), simple hyperplasia (n = 10), leiomyoma (n = 5), and cancer (n = 8); whereas for ECX: normal (n = 10), inflammation (n = 8), irregularity (n = 2), and cervical intraepithelial neoplasia (CIN; n = 3). All elements were identified in the sampled material. Compared with histologically normal tissues, endometrial cancer, hyperplasia, and CIN revealed significantly increased levels of toxic metals (Cd and Pb), altered status of Cu and Mn, and an elevated Cu/Zn ratio. Current and former smoking was associated with significantly higher Cd and Pb levels in investigated tissues. Polyps represented significant accumulators of Al, Cd, Ni, and Pb (END-P) or Al, Cd, and Cu (ECX-P).
**Conclusion(s):** The findings of this study are important in understanding the presence and role of metals in the female reproductive system and its pathology. (Fertil Steril® 2016;105:1511–8. ©2016 by American Society for Reproductive Medicine.)
**Key Words:** Trace metals, uterine, polyps, bioaccumulation, cigarette smoking

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The role of trace metals in human pathology has been subject to a great deal of attention over recent decades. As natural constituents of the earth's crust they are ubiquitously found, at varying levels, both in the ambient environment and in a wide range of human goods, including food, beverages, drugs, dietary supplements, cosmetics, and tobacco (1–4). Despite the significant effort to decrease global exposures to harmful elements such as Cd and Pb, certain human activities and locations still pose a relevant hazard to human health. At the same time, the status of biologically essential metals, though toxic at elevated levels or particular valance states (e.g., Cu, Cr, and Zn), may also be altered by various environmental, dietary, or lifestyle factors. Therefore, numerous studies have addressed the relationship between ambient air pollution, food intake, or hazardous lifestyle habits and metal content in human blood, urine, feces, hair, or nails. Beyond any doubt, these sample types are generally easy to collect and process, but to what extent they reflect the elementary burden at the tissue level remains controversial (5, 6). The assessment of the latter is, however,

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important because a deficiency of particular metals and/or accumulation of others may lead to molecular and biochemical impairments at the cellular level and histologic alterations, including malignancy (5).

The evaluation of metal status in female reproductive tissues and determination of factors potentially influencing this status are of particular interest given that some elements support normal reproductive function whereas others are recognized to induce its serious impairment (7). Particular attention is drawn to an emerging class of so-called metalloestrogens that includes Al, Cd, Ni, and Pb. There is growing evidence that they possess the ability to bind estrogen receptors and subsequently give rise to estrogen agonist responses (8, 9). Epidemiologically, they have been potentially implicated in estrogen-dependent diseases such as breast and endometrial cancer as well as endometriosis (10–13).

It has been initially found that the functional layer of human endometrium contains detectable levels of certain metals and that their content, particularly of Cd and Pb, may be increased in women with a history of smoking. Interestingly, histologically altered endometrium revealed generally greater concentrations of the investigated metals than normal tissue, but owing to low sample size data, it was not possible to compare it in groups with particular abnormalities (12). Nevertheless, these results and a few other previous pilot investigations indicate, with the use of different analytical techniques, that glandular tissues of human uterus can be a potential target of metal accumulation within the human body but also highlight the need to explore this issue cross-sectionally (14, 15). To date, no studies have aimed at investigating the presence of metals in endocervix or uterine polyps, and no factors that may potentially be associated with such a presence have been identified.

To extend the body of knowledge on metal status in female reproductive tissues, the present study recruited more than 100 subjects and compared the status of metals in different histologic images of endometrium and endocervix as well as uterine polyps of both origins. The influence of various factors (age, menopausal status, place of living, history of cigarette smoking) possibly altering the status of the studied elements was taken into consideration. Our study adds to the ongoing discussion on the relationship between environmental agents and female reproduction function.

## MATERIALS AND METHODS

### Studied Group

The research project was conducted from late 2013 to early 2015. Inclusion criteria were age >18 years and normal weight (body mass index 18.5–24.9 kg/m$^2$), pathologic bleeding, or endometrial hypertrophy. Exclusion criteria included: occupational risks of metal exposure, history of metal poisoning, history of drug or alcohol addiction, uptake of any dietary supplement within the year before study, anemia (verified by hemoglobin concentration), and early pregnancy (verified by $\beta$-hCG serum concentration), chronic hepatic, renal, gastrointestinal, pulmonary, endocrine, or cardiac disorders, osteoporosis, history of antiestrogenic, GnRH analogues, or cancer treatment. A total of 172 patients meeting the above criteria were contacted, of whom 109 agreed to take part in this study. Tissue samples were collected from white Polish women undergoing diagnostic or therapeutic curettage of the uterine cavity under general anesthesia in the Gynecologic and Obstetrical University Hospital in Poznań, Poland. Sixty women contributed samples of endometrium, 23 contributed endocervical tissue, 16 contributed endometrial polyps, and 10 contributed endocervical polyps. One sample per women was collected. Owing to ethical considerations, the samples were not obtained from subjects having no medical indication for this procedure. An indirect "control" group was therefore composed of tissue samples which were obtained from women having an indication for curettage but which were found to be histologically unaltered (hereafter referred to as "normal"). All procedures were performed as a routine medical treatment. To maintain diagnostic quality, samples were divided with the use of a plastic instrument for metal determination (one sample per woman owing to limited tissue material) and the rest of the material was sent for routine pathologic examination. The samples used for metal determination were profusely flushed (to remove blood), immersed in sterile distilled water, and placed in cryogenic tubes at −40°C before metal determination. Based on a short questionnaire, the menopausal status and smoking habits of the investigated women were specified. The studied individuals were also divided according to their self-reported place of residence into those living in urban or rural (population density <150 inhabitants per km$^2$) areas. The study was approved by the Local Bioethical Committee of the Poznan University of Medical Sciences, Poznan, Poland, and every recruited subject undersigned a written consent.

### Metal Determination

Thawed tissues were dried at 40°C, flushed twice with MilliQ water (Millipore), dried to a constant weight, and then weighed in an electronic microscale (0.1 mg accuracy). Complete digestion was performed with the use of 10 mL of Suprapur 14 mol/L $HNO_3$ (Sigma-Aldrich) in sealed plastic tubes with the use of an oven (80°C). The concentration of metals (Al, Cd, Cr, Cu, Mn, Ni, Pb, and Zn) in the investigated samples was determined with the use of a microwave-induced nitrogen plasma atomic-emission spectrometer (MP-AES; Varian) equipped with a nitrogen generator. The limit of detection was 1.00 $\mu$g/kg for each analyzed metal. Detailed instrument settings were described previously (14). Before the analysis, the detection method was validated with reference materials BCR185R, NIST2709, and ERM-CE278K. The recovery rate exceeded 90% for all determined elements, at low relative standard deviation values (<10%). Moreover, a control without any tissue but containing $HNO_3$ was performed to exclude the interference of any procedure step on metal content determination: All analyzed elements were below limits of detection. The final concentrations were given as $\mu$g of metal per kg of dry tissue. Based on determined metal concentrations, Cu/Zn ratio, a previously proposed marker of tissue oxidative imbalance, was calculated (16).