

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

Investigation of the relationship between zinc supplementation and vaginal condition

by Péter Damjanovich, MD

Supervisor: Bence Kozma MD, Ph.D.



UNIVERSITY OF DEBRECEN

DOCTORAL SCHOOL OF NUTRITION AND FOOD SCIENCE

DEBRECEN, 2022

Investigation of the relationship between zinc supplementation and vaginal condition

Dissertation to obtain the degree of doctor of philosophy (PhD) in nutrition and food sciences

By Péter Damjanovich, MD

Written within the framework of the Doctoral School of Nutrition and Food Sciences (Doctoral Program in Nutritional Sciences) of the University of Debrecen

Supervisor: Bence Kozma, Ph.D.

Reviewers: Miklós Romics, Ph.D.

BalázsVarga, Ph.D.

Head of the **Defense Committee**: Miklós Vecsernyés, Ph.D.

Members of the Defense Committee: Miklós Romics, Ph.D.

BalázsVarga, Ph.D.

Zsuzsanna Molnár, Ph.D.

Szilárd Szatmári, Ph.D.

Live online access will be provided. If you wish to take part in the discussion, please send an e-mail to damjanovich.peter@med.unideb.hu not later than 12 pm on the day before the discussion (26 May 2022.). After the deadline, for technical reasons, it is no longer possible to join in to the defense.

1. Hypotheses and objectives of the doctoral dissertation:

In Hungary, as in other European countries, life expectancy has been steadily increasing over the last 30 years. While in the early 1990's the expected average age in the female population was 73 years, by 2019, according to KSH data, this has shifted to 79 years.

At the same time, the quality of life has gradually improved, which has led to a high demand for a high quality of life in the population. Patients are increasingly seeking medical attention for conditions such as menopausal genitourinal syndrome (GSM), which do not have a significant impact on life expectancy but quality of life. In light of this, there is still a small number of patients who actually seek medical attention for GSM with vaginal atrophy. postmenopausal women are affected by all 30-50%. Questionnaires, subjective patient complaints, and vaginal cytology are available to date for the investigation of unpleasant symptoms. The most commonly used treatments in its therapy are topical moisturizing and hydrating preparations, as well as hormone replacement, which is also administered topically. The latter is an obstacle in many cases, as general resistance to hormone-containing therapies is not uncommon in patients, and estrogen replacement is contraindicated in some hormone-dependent conditions. In addition, laser treatment has recently become an innovative treatment option for GSM. In 2014, the Food and Drug Administration (FDA) approved the use of fractionated microablative carbon dioxide laser therapy in urogenital atrophy.

We hypothesize that zinc levels measured in CVL correlate well with the presence or absence of GSM / VVA and that CVL zinc levels may represent a new potential test method for detecting vaginal atrophy. Thus, our primary objective was to explore a possible association between zinc levels measured in CVL and menopausal status and zinc levels measured in CVL and the presence of GSM / VVA. Zinc replacement is possible both orally and vaginally, however, we have very poor literature data on the appearance of orally administered zinc in the vagina. Our second hypothesis is that orally taken zinc may increase CVL zinc levels in both pre- and postmenopause. Accordingly, in the second phase of our studies, we examined CVL zinc levels before and after oral zinc supplementation.

2. Patient materials and methods:

2.1.1. Patient-CVL examination in vaginal atrophy

We conducted a prospective cohort study between March 2017 and September 2018 at the Department of Obstetrics and Gynecology, University of Debrecen. Selection criteria included the presence or absence of vaginal dryness and willingness to participate in the research. Exclusion criteria were pregnancy, hormone therapy (local or systemic) in the last 6 months, recent use of over-the-counter vaginal products, presence of a vaginal infection, urinary or faecal incontinence, grade 2 pelvic prolapse (according to POP-Q, see later) or any disease that may interfere with the test protocol. Our studies were performed with the approval of the Health Science and Research Ethics Committee (ETT TUKEB 18504-1 / 2017 / EKV). After enrollment, all enrolled patients signed a consent form prior to initiating treatment. There were no discontinuations due to withdrawn consent or other circumstances in patients.

The POP-Q study, which precluded a greater degree of pelvic subsidence, was performed on the recommendation of the International Society for Continence. POP-Q status can be established based on physical examination of the genitals and vagina. Six points are required to describe the vagina, two points from the anterior vaginal wall, two points from the apical region, and two points from the posterior vaginal wall. During the measurements, the hymenal ring is considered as the reference plane. If the defined point is located proximally, i.e. above the hymenal ring, the resulting numerical value is denoted by a negative sign, while if it is located distally, i.e. a positive sign is used below the hyenal ring.

After reviewing historical data, women with a history of zinc or copper deficiency were also excluded. Patients who did not menstruate for 12 consecutive months without any other apparent cause and had elevated follicle-stimulating hormone levels (at least 30mIU / ml or higher) in their laboratories were considered to be of variable age.

Women enrolled in the study underwent a detailed pelvic examination, vaginal cytology was performed, and CVL was collected in a standardized manner (see later). The VMI and the VAS values were determined. Demographic and relevant clinical information was recorded prospectively and stored in a dedicated database. All patients signed a written consent before participating in our research. Laboratory measurements (CVL zinc levels and cytology) were performed in an independent laboratory without knowledge of clinical data.

2.1.2. CVL Collection

Vaginal assays were performed in each case with a plastic specimen that did not contain zinc or copper, thus minimizing the effect of external agents on the sample. Cervicovaginal lavage was collected by washing the cervix and vaginal walls with 10 ml of normal sterile saline, and the collected fluid was collected with a plastic syringe. The washings were injected onto the vaginal wall and cervix with a plastic syringe for 60 seconds, followed by three consecutive aspirations. The total amount of wash liquor was recovered from the posterior fornix by aspiration with a syringe. CVL samples were collected and stored at -80 ° C until analysis.

2.1.3. Visual Analog Scale (VAS)

Participants reported the intensity of their vaginal symptoms on a 10-point visual analog scale (VAS). They had to evaluate the symptoms of GSM: vaginal dryness, pain, burning-itching, dyspareunia, and dysuria. The left endpoint of the scale indicated complete absence of symptoms (0), while the right end indicated the strongest, worst possible symptoms. The VAS score was determined by summing the values.

2.1.4. Vaginal Health Index (VHI)

The Vaginal Health Index (VHI) is the sum of the scores given for the following properties: elasticity, fluid secretion, pH, epithelial mucosal integrity, and moisture. Each component is scored on a scale of 1 (worst) and 5 (best). The lower the score, the more severe atrophy it indicated.

2.1.5. Vaginal cytology

Vaginal smear samples were collected by a single scraping of the middle third of the lateral vaginal wall using a spatula. 200 cells per sample were analyzed and examined by an independent cytopathologist. We determined the parabasal (P) of the number of intermediate (I) and superficial (S) cells was multiplied by 0, 0.5, and 1.0, respectively. According to Meseils, the vaginal maturation value (VMV) was determined.

The increased percentage of P cells and I cells in the smear indicated a decrease in estrogen levels. VMV values between 0 and 49 indicated a low estrogenic effect, consistent with vaginal atrophy, i.e., if the VMV was less than 50, the sample was considered atrophic.

2.1.6. Sample preparation

The vaginal fluid samples contained large amounts of organic matter that would have affected the analysis, so pre-treatment of the samples was required. The 5 ml liquid samples were transferred without loss into glass beakers by washing the storage test tubes with 2 ml of concentrated nitric acid. The samples were heated to dryness on an electric hot plate and an additional 4 mL of 65% (w / w) nitric acid was added to wet digest the liquid samples. The samples were then heated to dryness and placed in plastic tubes without loss, diluted to 10 ml with 0.1 M nitric acid in ultra pure water.

The purity of the acids was checked by digesting blank samples that contained only the chemicals but no samples. All samples were kept in polypropylene tubes, diluted, and stored in a refrigerator at 48 ° C until measurement.

2.1.7. Sample analysis

Elemental analysis of CVL was performed by inductively coupled plasma optical emission spectrometry (ICP-OES 5100, Agilent Technologies, Santa Clara, CA, USA).

Atomic spectroscopic methods are instrumental analytical methods for the qualitative and high analytical sensitivity of 70-80 elements. In general, the element to be tested present in the sample is converted to free atoms. Free atoms can be generated by flames of different temperatures, electric arcs, sparks, direct current, inductive or capacitive coupled plasma, or high temperature graphite tubes. Depending on how we obtain data on the quality and quantity of free atoms, we are talking about atomic emission (AES), atomic absorption (AAS), and atomic fluorescence (AFS) methods.

In atomic emission spectrometry, the excited atoms (excited ions) of the element under study are generated using thermal or electrical energy. From the spectrum of the light emitted by these excited atoms, the quality of the given element is determined on the basis of the characteristic wavelength (qualitative analysis). From the relative intensity (I_{rel}) of the spectral line of a given wavelength, its concentration (c) is determined (quantitative analysis). The general relationship between the measured signal and the concentration is described by the Scheibe-Lomakin equation.

2.1.7.1. Inductively coupled atomic emission spectrometry

Inductively coupled plasma atomic emission spectrometry (ICP-AES) is one of the most advanced instrumental analytical methods. Due to the speed and low interference effect of ICP, it is ideal for the determination of analytical systems, chemical elements, from aqueous and non-aqueous solutions. The ICP-AES method is well automated, the automatic sample changer, computer-controlled analysis systems perform qualitative and quantitative analysis of 20-50 elements per minute from a sample of solution (approx. 2 cm³). The limit of detection is 10 to 10% ($\mu\text{g} / \text{cm}$ to $\mu\text{g} / \text{dm}$). The dynamic concentration range that can be covered by a single verification curve is 5-6 orders of magnitude.

The ICP-AES consists of a high-frequency (20-50 MHz) generator, a high-voltage (5-15 kV) rectifier unit, a water- or air-cooled triode and an oscillating circuit. The 2-3 turn induction coil is made of silver plated copper tube. The burner, which is made up of quartz tubes, is located in the axis of the coil, with an outer diameter of 2 to 3 cm. The input power of the generator is 2 kW, and the power output (output) in the light source is 0.7 kW on average.

The establishment of the high-frequency force field, after discharge, begins by directing a low-power spark discharge, called a Tesla spark, to the end of the quartz tube, which ionizes the argon. Oscillations and collisions of ions and electrons result in temperatures of 6-8000 K. The interior of the plasma is at a slightly lower temperature into which the aerosol produced by spraying the sample solution is introduced. The plasma jacket is used for emission detection at a temperature of 5-7000 K favorable for the excitation of metal atoms and atoms of some non-metals (S, P, As, Se, C) and single-charge metal ions.

The argon gas flowing upward through the inner tube “drills” a tunnel 1-2 mm in diameter through the plasma itself without disrupting its stability, thereby delivering the sample to the plasma in the form of an aerosol of the gas stream. The aerosol stays in the 5000 K tunnel for a relatively long time (approximately 1 ms), so the degree of atomization and ionization is very high.

In our study, plasma light was imaged directly into a vacuum polychromator with an argon gas purge light pathway to prevent short-wavelength (165-200 nm) spectral lines from being absorbed in air. Fiber optics around the plasma conduct light into three additional independent polychromators and one monochromator. The monochromator allows the measurement of the spectral lines of arbitrarily selected elements, the examination of wider (1-10 nm) spectral ranges. The four polychromators contain a channel of 25 elements that can be measured simultaneously.

The light signal is converted into an electrical signal by a photoelectron multiplier, and the data collection and processing is controlled by a computer.

With ICP approx. 70 elements can be determined from a single sample, principal components and trace contaminants can be tested simultaneously. The accuracy of the method is very good, self-absorption and other similar sources of error do not occur, so the analytical curve is straight within 4-6 order of magnitude.

2.1.8. Statistical analysis of data

In each case, the normality of the continuous variables was examined using graphical diagrams. For continuous variables, mean and standard deviation (SD) were determined. Student's t-test (for parametric data) and paired t-test were performed before and after zinc supplementation comparison. Correlations between serum trace element concentrations and CVL were evaluated using the Pearson correlation coefficient. Differences were considered significant if the P value was below 0.05. All statistical analyzes were performed using SAS 9.4 statistical software (SAS Institute Inc., NC, USA).

2.2.1. Patient group - Oral zinc supplementation

We are conducting a prospective intervention cohort study between March 2017 and September 2018 at the Department of Urogynology, Department of Obstetrics and Gynecology, University of Debrecen. Our studies were performed with the approval of the Scientific and Research Ethics Committee of the Health Science Council (ETT TUKEB 18504-1 / 2017 / EKU). After enrollment, all enrolled patients signed a consent form prior to initiating treatment. There was no discontinuation of treatment due to withdrawal or adverse effects in patients. Women older than 18 years were included in the study. Exclusion criteria were: pregnancy, hormone therapy (local or systemic), recent use of over-the-counter vaginal products, vaginal infection, sexual activity in the last 48 hours, cytological atypia, dysmenorrhea, pelvic organ prolapse (POP-Q> 2 std .), severe urinary or faecal incontinence, or any disease that interferes with the test protocol. Patients with a history of zinc or copper deficiency were also excluded. The menopausal group included those who had not menstruated for at least 12 consecutive months without any other apparent cause, had elevated follicular stimulating hormone (FSH) levels above 30 mIU / ml, or had previously had both ovaries removed surgically. Vaginal cytology and CVL were collected in a standardized manner after a detailed gynecological examination.

Serum samples were taken to measure zinc and copper levels. Participants were asked to take 30 mg zinc tablets daily in the form of zinc acetate and zinc gluconate for two weeks (20 mg zinc acetate (BioCo Hungary Kft, Budapest, Hungary) and 10 mg zinc gluconate (Jamieson Laboratories)). Ontario, Canada)]. At the end of the two-week treatment period, patients were asked to reapply for gynecological examination, vaginal cytology, and CVL. Demographic and relevant clinical information was recorded prospectively and stored in a dedicated, separate database.

2.2.2. Sample Preparation-Analysis

As in the measurement of CVL zinc levels, it was necessary to prepare the samples, which was performed as previously described, and then the samples were analyzed by inductively coupled plasma optical emission spectrometry, after which the data were statistically analyzed.

3. Results

3.1. Examination of CVL in vaginal atrophy

120 women were selected, with a mean age of 54 ± 13 years. Of the patients, 68% (82) were menopausal and the median of previous pregnancies and births was 2. The VAS total score was 14 ± 14 before CVL collection. Of the participating women, 62% (74 subjects) reported vaginal dryness, with a mean vaginal dryness of 4 ± 4 . Vaginal atrophy was detected in 37 (31%) women, and these patients had a VMI of less than 50. The mean VHI was 15 ± 6 and the mean CVL zinc level was $0.10 \text{ ml / L} \pm 0.10$. In the statistical analysis, we found significant differences in CVL zinc levels between age groups, menopausal status, the presence of vaginal dryness, and vaginal atrophy (VMV less than 50). In the correlation analysis, a moderately positive correlation was also found between the zinc levels of VMV and CVL ($r = 0.495$, $P\text{-value} < 0.01$). However, in the multivariate regression model, taking into account all significant factors, only vaginal atrophy was significant ($P \text{ value} = 0.02$). Based on all these, our multivariate regression model revealed a significant association between vaginal atrophy (VMV < 50) and CVL zinc levels.

3.2. - Oral zinc supplementation

Twelve premenopausal and ten postmenopausal women without significant gynecological pathology were included in our prospective intervention cohort study. In the variable age group, the mean age was 35 ± 7 years, whereas in the variable age group was 60 ± 3 years. Women in the postmenopausal group were significantly older than participants in the premenopausal group. (mean \pm SD, years, 60 ± 3 vs. 35 ± 7 , $P < 0.01$). Both vaginal health index (VHI) and vaginal maturation (VMV) were significantly higher in premenopausal women than in postmenopausal women before and after zinc supplementation. In the premenopausal group, the VHI was 23 ± 2 before treatment and 23 ± 2 after treatment. The VMV in the same group was 58 ± 6 before treatment and 61 ± 12 after treatment. The VHI was 11 ± 5 in the postmenopausal group and 13 ± 4 after treatment. The VMV was 26 ± 24 in patients of variable age before treatment and 27 ± 20 after treatment. It is clear from the data that there is no significant difference in VHI or VMV values in either group before and after zinc supplementation.

Serum zinc levels were significantly elevated in both the premenopausal and postmenopausal groups (mean \pm SD, mg / L, 0.88 ± 0.17 vs. 1.06 ± 0.23 , $P < 0.01$ and 0.83 ± 0.24 vs. 0.96 ± 0.33 , $P < 0.01$) after two weeks of daily zinc supplementation. CVL zinc levels were also significantly higher in the premenopausal group than in the postmenopausal group before and after the study (mean \pm SD, mg / L, 0.13 ± 0.05 vs. 0.06 ± 0.04 , $P < 0.01$ and 0.10 ± 0.03 vs. 0.05 ± 0.01 , $P < 0.01$), but zinc supplementation had no significant effect on CVL zinc levels in either group. Overall, therefore, oral supplementation of 30 mg zinc daily for two weeks did not significantly increase CVL zinc levels, despite a significant increase in serum zinc levels. Zinc levels in CVL were significantly higher in premenopausal women than in postmenopausal women, but copper levels were similar. Zinc levels in CVL were 15–20-fold higher than copper levels in CVL.

4. Discussion

Menopausal Genitourinary Syndrome (GSM), or formerly known as vulvovaginal atrophy (VVA), can affect about half of middle-aged women. GSM / VVA was first defined by Portman and Gass as a syndrome due to declining levels of estrogen and other steroid hormones. In 2014, the North American Menopause Society (NAMS) changed the nomenclature of a syndrome previously known primarily as vulvovaginal atrophy and proposed VVA is part of GSM in which vaginal wall atrophy leads to symptoms that can have a significant impact on quality of life and sexual health. Untreated symptoms such as vaginal pain, dryness and burning, itching, and loss of elasticity worsen over time in the affected patient group.

The development of GSM is due to estrogen deficiency after changing ages, which initiates morphological and secretory changes in the vulva and vagina, resulting in decreased blood supply, lubrication and elasticity, and glycogen production. The period during which no further bleeding occurs within 12 months after the last monthly bleeding and no other pathological abnormality is present is called a menopause. The variable age occurs on average around 47.5 years, but it is not uncommon for it to be 52-53 years old.

Symptoms of GSM / VVA develop as a consequence of morphological and secretory changes during menopause due to decreased estrogen levels. Decreased blood supply leads to a decrease in elasticity, while a decrease in glycogen content adversely affects the vaginal wall epithelium, thereby adversely affecting the vaginal flora and consequently shifting the vaginal pH in an alkaline direction.

The incidence of GSM after a variable age is 40-57%, but the incidence of symptoms varies widely. The presence of vaginal dryness varies between 27-55%; dyspareunia is reported in 40-77% of those affected, and other urological symptoms and other vaginal complaints (burning-itching, vaginal discharge) occur in 6-37%.

The diagnosis of VVA / GSM is based on medical history and clinical trials. There are several scoring systems available to determine female urogenital health (e.g., VHI). Vaginal wall biopsy is the most direct method to examine changes in the vaginal wall; however, it is invasive and therefore ethically of concern for research purposes. Due to the invasiveness of biopsy, cytological examination of the vaginal wall is used to objectively examine atrophy in both research and clinical practice. Meisels introduced vaginal maturation value (VMV) for objective measurement of vaginal atrophy. A VMV below 50 indicates a low estrogenic effect, which corresponds to vaginal atrophy. A value between 65 and 100 indicates a satisfactory estrogenic effect, while a value between 50 and 64 indicates a moderate estrogenic effect. Apart

from this method, there is no other reliable and easy-to-use marker for laboratory diagnosis of VVA.

The treatment of GSM / VVA varies depending on the severity of the symptoms. For milder symptoms, lifestyle changes and over-the-counter preparations (lubricants, hydrates).

can be used effectively. Regular use of non-hormonal, long-acting vaginal moisturizers can significantly improve the symptoms of vaginal dryness, lowering the pH of the vagina to premenopausal levels, but not as effectively as hormonal treatments.

Although many lubricants and moisturizers are commercially available, very few clinical trials have been conducted to demonstrate their safety and efficacy.

In a study examining the safety of moisturizers and lubricants, the authors found that many water-based gels are hyperosmolar and can lead to epithelial cell damage and toxicity. Estrogen therapy (ET) is the most successful treatment option for moderate to severe symptoms. Both systemic and vaginal forms are effective in ameliorating the symptoms of GSM / VVA. However, hormone therapy should only be used if all risk factors have been ruled out due to the increased risk of developing endometrial carcinoma due to high estrogen levels.

Zinc is one of the most common trace elements in the human body. It is a major component of many metalloenzymes and plays a critical role in wound healing, biosynthesis, and homeostasis of various connective tissues. Zinc plays a prominent physiological role in collagen metabolism, and zinc deficiency results in a decrease in the healing tendency of many organs. Zinc is absorbed from the small intestine through carrier-mediated carriers. Once absorbed, it enters the enterocytes in the duodenum and jejunum and then through the portal system into the systemic circulation, where 70% is bound to albumin.

Animal experiments have shown that zinc has a beneficial effect on the extracellular matrix (ECM) of the vaginal wall. Plasma zinc concentrations in buffaloes are significantly lower in animals with antepartum vaginal prolapse compared to normal pregnant buffaloes. Histological and histochemical images of the sheaths of mice fed a zinc-deficient diet are similar to those of ovariectomized animals. Under ex vivo conditions, Takács et al. Were able to significantly stimulate elastin production in vaginal smooth muscle cells with zinc sulfate. In a study published in 2019, Takács et al. Examined the effect of zinc-containing vaginal moisturizing gel on postmenopausal vulvovaginal symptoms. Their studies have shown that zinc-containing vaginal moisturizing gel improves post-menopausal vulvovaginal symptoms without significant side effects.

Vaginal changes can be most accurately monitored by sampling the entire thickness of the vaginal wall or by vaginal wall biopsy, however, these methods are invasive and ethically

questionable in a study because the studies are not only inconvenient but also painful for the patient. Information obtained from cervicovaginal lavage (CVL) is widely used to examine changes in the vagina. It has been used successfully to detect a number of viral pathogens such as HIV, HSV, CMV. CVL provides a significant advantage, as not only does it provide information about a specific area of the vagina, such as a biopsy, but lavage can be used to obtain information about the mucosa of the entire vaginal wall. Collecting lavage is a safe, simple, and non-invasive procedure that is easy to perform during a vaginal examination. The assay provides information on all components of the mucosal surface, including small molecules, extracellular materials, peptides, cells, and trace elements.

We hypothesize that zinc levels measured in CVL correlate well with the presence or absence of GSM / VVA and that CVL zinc levels may represent a new potential test method for detecting vaginal atrophy. Thus, our primary objective was to explore a possible association between zinc levels measured in CVL and menopausal status and zinc levels measured in CVL and the presence of GSM / VVA. 120 patients were included in our prospective cohort study. 68% of participants were menopausal, and 62% reported vaginal dryness in our studies. For each participant, we determined the VHI (vaginal health index), an objective examination method that measures the condition of the vagina based on five characteristics. These include elasticity, fluid secretion, vaginal pH, condition of the epithelial mucosa, and vaginal moisture. Based on these, we found vaginal atrophy in 31% of participants. We determined VAS (visual analog scale), in which patients reported the intensity of their vaginal symptoms on a 10-point scale. The left endpoint of the scale indicated complete absence of symptoms (0), while the right end indicated the strongest, worst possible symptoms. The women involved underwent a detailed pelvic examination, underwent vaginal cytology, and CVL zinc concentrations were quantified from the samples by 5-point calibration with inductively coupled plasma optical emission spectrometry. Sterile saline was used as a lavage fluid for our study, as other fluids, such as hypotonic water, can lead to cell lysis, which can interfere with accurate measurement of vaginal fluid components. In the analysis of our data, we found significant differences in CVL zinc levels between age groups, menopausal status, and vaginal dryness. between and the presence of vaginal atrophy (VMV less than 50). In the further analysis, a correlation analysis was performed, where a moderately positive correlation was found between the zinc levels of VMV and CVL, however, in the multivariate regression model, taking into account all significant factors, only vaginal atrophy (VMV <50) was significant for CVL zinc. level. Based on our results, measuring the amount of zinc in the CVL may help in the diagnosis of GSM /

VVA. Based on our studies, we suggested that zinc levels in CVL may be useful as a new marker of vaginal wall atrophy.

Zinc replacement is possible both orally and vaginally, however, there is very little literature data on the appearance of orally administered zinc in the vagina. Our second hypothesis is that orally taken zinc may increase CVL zinc levels in both pre- and postmenopause. Accordingly, in the second phase of our studies, we examined CVL zinc levels before and after oral zinc supplementation. It was not previously known what the relationship was between serum and vaginal zinc levels, or whether an increase in serum zinc levels would consequently increase the zinc content of the vaginal tissue. We conducted a prospective intervention cohort study that included 12 premenopausal and 10 postmenopausal women. Vaginal cytology was performed and CVL was collected in a standardized manner after pelvic examination. Zinc and copper levels were determined from serum samples. Women in the study were asked to take 30 mg of zinc tablets a day in the form of zinc acetate and zinc gluconate for two weeks. After completing the two weeks, participants underwent a repeated gynecological examination, underwent vaginal cytology, underwent CVL, and had a blood sample. CVL zinc concentration was quantified from the samples by 5-point calibration by inductively coupled plasma optical emission spectrometry. This method is reproducible and is based on standard laboratory methods. Based on our results, zinc supplementation had no significant effect on CVL zinc levels in either the pre- and postmenopausal groups. In addition, neither serum nor CVL copper levels were affected by zinc supplementation.

No significant association was found between serum and CVL zinc or copper levels. To the best of our knowledge, our group first investigated the effect of oral zinc supplementation on CVL zinc levels and the role of CVL zinc levels in vaginal atrophy.

In our results, we found a significant association between vaginal atrophy (VMV <50) and CVL zinc levels. We found that zinc levels in CVL can be used as a new marker of vaginal atrophy. Based on our results, it appears that oral 30 mg zinc supplementation had no effect on the symptoms of GSM / VVA, which may also be an effect of short-term zinc supplementation, so further studies are needed to demonstrate this.

Vaginal atrophy with variable age complicates the lives of peri- and postmenopausal women with a number of unpleasant symptoms and significantly affects the quality of life of these patients. To date, physicians have access to questionnaires, subjective patient complaints, and vaginal cytology.

5. New scientific results of the dissertation

1. In our study, we found a significant correlation between CVL zinc levels and the menopausal status of the subjects studied: CVL zinc content was significantly higher in premenopause than in postmenopause.
2. In contrast to zinc, menopausal status does not affect CVL copper content: pre- and postmenopausal CVL copper content does not differ significantly.
3. The zinc content of CVL is about 15-20 times that of copper.
4. In the results of our studies we found a significant correlation between vaginal atrophy (Vaginal maturation value <50) and zinc levels in CVL.
5. We were the first to use CVL zinc levels as a new marker of vaginal wall atrophy.
6. To the best of our knowledge, our research group first investigated the effect of oral zinc supplementation on CVL zinc levels in both the pre- and postmenopausal groups.
7. Oral administration of 30 mg zinc daily orally for two weeks did not significantly alter the zinc content of CVL, despite a significant increase in serum zinc content.



Registry number: DEENK/91/2022.PL
Subject: PhD Publication List

Candidate: Péter Gábor Damjanovich
Doctoral School: Doctoral School of Nutrition and Food Sciences
MTMT ID: 10068575

List of publications related to the dissertation

1. **Damjanovich, P. G.**, Sipos, A. G., Larson, K., Cunningham, T. D., Takács, P., Kozma, B.:
Cervicovaginal lavage fluid zinc level as a marker of vaginal atrophy.
Menopause. 27 (7), 776-779, 2020.
DOI: <http://dx.doi.org/10.1097/GME.0000000000001536>
IF: 2.953
2. Takács, P., **Damjanovich, P. G.**, Sipos, A. G., Kozma, B.: The effect of oral zinc supplementation
on cervicovaginal lavage fluid zinc level.
Eur. J. Obstet. Gynecol. Reprod. Biol. 248, 106-109, 2020.
DOI: <http://dx.doi.org/10.1016/j.ejogrb.2020.03.026>
IF: 2.435

List of other publications

3. Molnár, S., Maka, E., **Damjanovich, P. G.**, Török, P., Lampé, R., Krasznai, Z. T.: A hámeredetű
petefészek-daganatok sebészeti ellátása és annak hatása a betegségmentes túlélésre a
Debreceni Egyetem adatai alapján.
Nőgyógy. Onkol. 25, 7-12, 2020.
4. Póka, R., Barna, L., **Damjanovich, P. G.**, Farkas, Z., Orosz, G. B., Orosz, M., Ördög, L., Sipos, A.
G., Török, O.: Emelkedő anyai életkor részesedése a császármetszés-frekvencia
növekedésében.
Magyar Nőorv. L. 83 (1), 275-281, 2020.
5. Póka, R., Barna, L., **Damjanovich, P. G.**, Farkas, Z., Molnár, S., Orosz, M., Ördög, L., Sipos, A.
G., Juhász, A. G., Török, O.: Large fetal weight alone in Robson-1 parturients doesn't
translate into a risk of Caesarean delivery higher than that of a vaginal birth.
J. Obst. Gynecol. Rep. Biol. 239, 7-10, 2019.
IF: 1.868





6. Póka, R., Barna, L., Csehely, S., **Damjanovich, P. G.**, Farkas, Z., Molnár, S., Nagyházi, O., Orosz, G. B., Orosz, M., Ördög, L., Sipos, A. G., Juhász, A. G., Török, O., Tóth, Z.: Szülés módja és neonatális eredmények: terminuson túl szinguláris fejküvel magzattal spontán vajúdó, először szülő nők körében.
Magyar Nőrv. Lap. 82, 228-234, 2019.
7. Póka, R., Barna, L., Csehely, S., **Damjanovich, P. G.**, Farkas, Z., Molnár, S., Nagyházi, O., Orosz, G. B., Orosz, M., Ördög, L., Sipos, A. G., Juhász, A. G., Török, O., Tóth, Z.: Születési súly és császármetszés kapcsolata: 37. hetet betöltött, szinguláris fejküvel magzattal spontán vajúdó először szülő nőkben.
Magyar Nőrv. Lap. 82 (2), 56-61, 2019.
8. Póka, R., Barna, L., Csehely, S., **Damjanovich, P. G.**, Farkas, Z., Molnár, S., Nagyházi, O., Orosz, G. B., Orosz, M., Ördög, L., Sipos, A., Újvári, B., Tóth, Z.: A császármetszés frekvenciájának Robson-féle klasszifikáció szerinti elemzése a Debreceni Egyetem Szülészeti és Nőgyógyászati Klinika tízéves anyagában.
Magy. Nőrv. Lapok. 80, 76-85, 2017.
9. Póka, R., **Damjanovich, P. G.**, Károlyi, P. K., Miszti-Blasius, K., Kerényi, A., Kappelmayer, J.: A firinogénszint referenciaértékei várandósságban.
Magy. Nőrv. Lapok. 79, 10-19, 2016.

Total IF of journals (all publications): 7,256

Total IF of journals (publications related to the dissertation): 5,388

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

22 February, 2022

