# Original Research

# Effects of total gastrectomy on plasma silicon and amino acid concentrations in men

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## Abstract

The aim of the study was to determine one-year effects of total gastrectomy on plasma silicon and free amino acid concentrations in patients and evaluate changes of volumetric bone mineral density (vBMD) in lumbar spine. Eight patients were enrolled to the control (CTR) group. Six patients subjected to total gastrectomy (GX group) were included to the experimental group. vBMD in trabecular and cortical bone was measured in lumbar vertebrae at baseline (before surgery) and one year later using quantitative computed tomography. Plasma concentrations of silicon and free amino acids were determined at baseline and one year later using photometric method and ion-exchange chromatography. Body weights within CTR and GX groups were not different after one-year follow-up when compared to the baseline values ($P > 0.05$). An average annual decrease of vBMD in the trabecular bone in the gastrectomized patients reached 15.0% in lumbar spine and was significantly different in comparison to the percentage changes observed in CTR group ($P = 0.02$). One-year percentage change of vBMD in the cortical bone in $L_1$ and $L_2$ has shown significantly decreased values by 10.5 and 9.1% in the GX group when compared to the percentage change observed in the controls ($P < 0.05$). Plasma concentration of adipic acid was significantly higher by 101.6% one year after total gastrectomy procedure in the patients when compared to the baseline value ($P = 0.01$). Plasma concentration of silicon was significantly lowered by 26.7% one year after the total gastrectomy when compared to the baseline value ($P = 0.009$). Total gastrectomy in patients has induced severe osteoporotic changes in lumbar spine within one-year period. The observed osteoporotic changes were associated with decreased plasma concentration of silicon indicating importance of exocrine and endocrine functions of stomach for silicon homeostasis maintenance. Gastrectomy-induced bone loss was not related to decreased amino acid concentration in plasma obtained from overnight fasted patients.

**Keywords:** Gastrectomy, amino acids, osteoporosis, silicon, quantitative computed tomography

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## Introduction

Total or partial gastrectomy impairs a variety of physiological functions of the stomach, disturbing internal systemic homeostasis of the body. The most common negative effects of gastrectomy such as impaired function of the gastric-hypothalamic-pituitary axis, abnormal food passage, disturbed nutrition intake, abnormal digestion and absorption, and micronutrient deficiencies result in decrease of body weight and skeletal system disorders such as severe osteopenia and osteoporosis.[1–6] Total gastrectomy and fundectomy (resection of the fundic part of the stomach) induce severe osteopenic effects in the trabecular and compact (cortical) bone compartments that are stronger than an osteopenia caused by ovariectomy, glucocorticoids, and long-term bone denervation.[2,7–9] Considering that approximately 50% of proteins is absorbed in the duodenum in humans, partial or total resection of the stomach must impair protein digestion dependent on gastric juice

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enzymes. Decreased amount or even lack of stomach mucosa-derived enzyme such as pepsinogen and its active form pepsin results in impaired protein digestion process and subsequent malabsorption. In case of partial resection of the stomach, time of the contact of pepsin with food ingredients is significantly reduced. Total resection of the stomach and following esophago-duodenal anastomosis negatively change pH value of intestinal juice in duodenum and jejunum influencing digestive enzyme activity.[10,11] In long-term studies on growing pigs, it was shown that performed fundectomy was associated with impaired protein digestion, limited amino acid absorption processes, and severe osteopenia development in trabecular and cortical bone compartments of lumbar spine.[5] Apart from consequences for protein metabolism, partial or total gastrectomy impairs carbohydrate, lipid, macro- and microelement (calcium, magnesium, iron, copper, zinc, potassium, and selenium), and vitamin (vitamins B1, B9, B12, A, D, E, and K) absorption within several parts of small intestine.[4,10]

The aim of the study was to determine one-year effects of total gastrectomy on silicon and free amino acid concentrations in plasma obtained from overnight fasted patients and evaluate their percentage changes of volumetric bone mineral density (vBMD) of trabecular and cortical bone compartments in lumbar spine.

## Materials and methods

This study was conducted according to the guidelines laid down in the Declaration of Helsinki. The experimental procedures used throughout this study were approved by The Local Ethics Committee of Medical University in Lublin, Poland—agreement number KE-0254/4/2008 on 31 January 2008.

### Patients qualified to the study

Fourteen patients treated at the II Department of General and Gastroenterological Surgery and Surgical Oncology of the Alimentary Tract of Medical University in Lublin were included in the one-year study. The control group consisted of eight men (CTR Group; $N = 8$) at mean age of $49.5 \pm 4.9$ years undergoing abdominal surgery due to cardiospasmus. Six men at mean age of $56.7 \pm 4.5$ years subjected to total gastrectomy procedure due to gastric cancer (GX Group; $N = 6$) belonged to the experimental group. Patients have undergone surgery between February 2008 and January 2011. Just before the abdominal and gastric surgeries in patients from both groups, baseline vBMD measurement of lumbar spine was performed and fasted overnight blood samples were collected (between 8:00 and 9:00 AM) for plasma analysis. One year from the baseline (one year after the surgery), both control and experimental patients were subjected to vBMD of lumbar spine measurement and fasted overnight blood sample collection (between 8:00 and 9:00 AM) for plasma analysis. Blood samples were collected into heparinized tubes using standard venipuncture of the *cephalic vein* to avoid hemolysis. Immediately after centrifugation, plasma samples were frozen and stored at $-80^{\circ}C$ for further biochemical analyses. Patients suffering from coexisting additional diseases to cardiospasmus or gastric cancer and impaired metabolic/hormonal homeostasis of the body were eliminated from the investigated participants at the initial stage of the study. Poor health status, death of patient, neoplastic disease recurrence, or metastases were additional factors limiting participation in the study.

### Densitometry of lumbar spine

vBMD of trabecular and cortical bone compartments of four lumbar vertebrae ($L_1$–$L_4$) was determined using quantitative computed tomography (QCT) method and Somatom Emotion—Siemens apparatus (Siemens, Erlangen, Germany) supplied with Somaris/5 VB10B software (Version B10/2004A). vBMD was measured separately for the trabecular and the cortical bone of the vertebral body using 10-mm thick, cross-sectional scans, placed at 50% of each vertebral body length. Both the trabecular (Td) and cortical vBMD (Cd) were measured using Osteo CT application package (Software Version B10/2004A) and expressed quantitatively as calcium hydroxyapatite (Ca-HA) density in the trabecular ($Tb_{Ca\text{-}HA}$) and cortical bone ($Cb_{Ca\text{-}HA}$) compartments. One-year percentage changes of $Tb_{Ca\text{-}HA}$ and $Cb_{Ca\text{-}HA}$ in each patient were used for statistical comparisons. All patients were scanned together with the water- and bone-equivalent calibration phantom provided by the scanner producer and serving as a standard for such measurements.

### Biochemical analyses of plasma

Determination of free amino acid concentration in plasma from patients was performed with the use of ion-exchange chromatography and an apparatus for the automatic analysis of amino acids (INGOS AAA-400, Ingos Corp., Prague, Czech Republic). Amino acids were separated using analytic column OSTION LG FA $3\,mm \times 200\,mm$. Five lithium citrate buffers (pH 2.9, 3.1, 3.35, 4.05, and 4.9, respectively) were used for amino acid separation. Amino acids were derivatized with ninhydrin and their determination was performed on the basis of retention time in comparison to the standards, using photocell combined with a computer. The software MIKRO (version 1.8.0) was used for amino acids evaluation (Ingos Corp., Prague, Czech Republic). Silicon (Si) concentration in plasma samples was determined using spectrophotometric method. In this method, reaction of silicate ions with molybdenic acid is followed by the reduction of the obtained silicomolybdenic acid into silicomolybdenic blue. The absorbance of such compound was measured at 800 nm. The concentration of Si in plasma was read from the standard curve prepared using silicon standard Titrisol® (1000 mg of Si as $SiCl_4$ in 14% NaOH solution; Merck KGaA, Darmstadt, Germany) and was expressed in μmol/L.

### Statistical analysis

Statistical analysis of data was performed using Statistica software (version 6.0). Data are presented as means ± SEM or percentage changes of vBMD from the baseline values.

The percentage changes of $Tb_{Ca\text{-}HA}$ and $Cb_{Ca\text{-}HA}$ one year from the baseline within both groups of patients were compared using paired Student's *t*-test for dependent variables. Comparisons of one-year changes of body weight, amino acid, and silicon concentrations within each group were performed using paired Student's *t*-test for dependent variables. Non-paired Student's *t*-test for non-dependent variables was used to compare plasma body weight, amino acid, and Si concentrations between the control and experimental groups of patients at the baseline and one year later. For all comparisons a $P$ value $\leq 0.05$ was considered as statistically significant.

## Results

### Body weight of patients

Body weight of control group of patients was not significantly different at the one-year follow-up ($82.4 \pm 1.6$ kg) when compared to the baseline value ($81.8 \pm 2.0$ kg; $P = 0.81$). Body weight of gastrectomized group was not significantly different one year after the surgical procedure ($76.7 \pm 3.2$ kg) when compared to the baseline value ($73.3 \pm 2.9$ kg; $P = 0.49$). At the baseline, no significant difference between body weight in the GX and CTR group was observed ($P = 0.18$), while one year after the surgical procedure the difference was statistically significant ($P = 0.05$).

### vBMD of lumbar vertebrae

Results presenting one-year percentage changes of vBMD within the trabecular and cortical bone compartments of lumbar vertebrae from the baseline values in the control and gastrectomized groups of patients are presented in Table 1. Comparison of one-year percentage changes of $Tb_{Ca\text{-}HA}$ in $L_1$, $L_2$, $L_3$, and $L_4$ has shown its lower values in the gastrectomized group of patients when compared to the controls and the differences were statistically significant ($P \leq 0.05$). Comparison of one-year percentage changes of $Cb_{Ca\text{-}HA}$ in $L_1$ and $L_2$ has shown significantly lower values in the gastrectomized group of patients when compared to the controls ($P < 0.05$). Similar tendency of the one-year percentage changes of $Cb_{Ca\text{-}HA}$ between the investigated groups was observed in $L_3$ ($P = 0.11$). Comparison of one-year percentage changes of $Tb_{Ca\text{-}HA}$ (average values for all lumbar vertebrae $L_1$–$L_4$) has shown significantly lower values in the gastrectomized group of patients when compared to the CTR group ($P = 0.02$). Similar tendency of the comparison of one-year percentage changes of $Cb_{Ca\text{-}HA}$ in all the examined lumbar vertebrae ($L_1$–$L_4$) in both the groups of patients was observed ($P = 0.08$).

### Free amino acid concentration in plasma

Results of amino acid analyses in plasma of patients from the CTR group are shown in Table 2. Plasma concentrations of all the analyzed amino acids from overnight fasted control group of patients were not significantly different after one-year follow-up when compared to the baseline values (all $P > 0.05$). Results of amino acid analyses in plasma of patients from the GX group are presented in Table 3. Plasma concentration of adipic acid was significantly higher by 101.6% one year after the total gastrectomy procedure in the GX group of patients when compared to the baseline value ($P = 0.01$). Plasma concentrations of all the other analyzed amino acids from overnight fasted GX group of patients were not significantly different after one year when compared to the corresponding baseline values (all $P > 0.05$).

Comparison of free plasma amino acid concentration between the GX and CTR groups at the baseline has shown significantly lower concentration of glutamine and cystine in the patients undergoing gastrectomy procedure

**Table 1** Comparison of one-year percentage changes of the volumetric bone mineral density (vBMD) of trabecular and cortical bone of lumbar vertebrae ($L_1$–$L_4$) from the baseline between the control patients (CTR group) and the patients subjected to total gastrectomy (GX group)*

| | One-year percentage change | | |
|---|---|---|---|
| **Investigated parameter** | **CTR group** | **GX group** | **P value** |
| $L_1$ vertebra | | | |
| Calcium hydroxyapatite density in the trabecular bone (mg/mL) | –1.9% | –16.6%† | 0.016 |
| Calcium hydroxyapatite density in the cortical bone (mg/mL) | –0.3% | –10.5%† | 0.048 |
| $L_2$ vertebra | | | |
| Calcium hydroxyapatite density in the trabecular bone (mg/mL) | –2.7% | –15.7%† | 0.006 |
| Calcium hydroxyapatite density in the cortical bone (mg/mL) | –0.7% | –9.1%† | 0.048 |
| $L_3$ vertebra | | | |
| Calcium hydroxyapatite density in the trabecular bone (mg/mL) | –4.0% | –15.5%† | 0.042 |
| Calcium hydroxyapatite density in the cortical bone (mg/mL) | +2.3% | –5.7% | 0.112 |
| $L_4$ vertebra | | | |
| Calcium hydroxyapatite density in the trabecular bone (mg/mL) | –3.7% | –11.9%† | 0.055 |
| Calcium hydroxyapatite density in the cortical bone (mg/mL) | +1.4% | –3.0% | 0.523 |
| Average $L_1$–$L_4$ vertebrae | | | |
| Calcium hydroxyapatite density in the trabecular bone (mg/mL) | –3.0% | –15.0%† | 0.017 |
| Calcium hydroxyapatite density in the cortical bone (mg/mL) | +0.5% | –7.2% | 0.080 |

*Values are means.
†Statistically significant differences versus percentage changes in CTR group for $P \leq 0.05$ by non-paired Student's *t*-test for non-dependent variables.

**Table 2** Free amino acid concentrations (nmol/mL) in plasma of control patients (CTR group) at the baseline and one year after the surgical procedure*

| Amino acid | CTR group (baseline) | CTR group (one year) | P |
|---|---|---|---|
| Taurine | 58.0 ± 4.4 | 66.9 ± 9.5 | 0.41 |
| Aspartate | 44.0 ± 5.1 | 58.5 ± 5.3 | 0.07 |
| Threonine | 146.8 ± 7.7 | 141.8 ± 12.2 | 0.73 |
| Serine | 141.6 ± 6.2 | 135.3 ± 9.7 | 0.58 |
| Glutamate | 169.6 ± 31.5 | 193.3 ± 27.4 | 0.59 |
| Glutamine | 1096.0 ± 86.3 | 1066 ± 80.4 | 0.80 |
| Adipic acid | 4.9 ± 0.6 | 5.1 ± 1.1 | 0.84 |
| Glycine | 266.0 ± 28.9 | 274.1 ± 27.8 | 0.84 |
| Alanine | 328.4 ± 29.9 | 346.3 ± 39.5 | 0.72 |
| Ethanoloamine | 79.8 ± 6.4 | 70.0 ± 9.5 | 0.41 |
| Alpha-amino-butyrate | 30.9 ± 6.3 | 33.1 ± 3.6 | 0.76 |
| Valine | 270.6 ± 30.1 | 246.8 ± 32.2 | 0.59 |
| Cystine | 22.3 ± 5.4 | 14.3 ± 2.7 | 0.20 |
| Methionine | 27.5 ± 1.9 | 30.1 ± 4.7 | 0.60 |
| Isoleucine | 94.0 ± 12.5 | 89.8 ± 11.7 | 0.80 |
| Leucine | 209.5 ± 23.3 | 217.3 ± 21.3 | 0.80 |
| Tyrosine | 94.6 ± 5.1 | 97.3 ± 8.7 | 0.79 |
| Phenylalanine | 89.3 ± 5.5 | 97.0 ± 8.4 | 0.45 |
| Tryptophan | 59.8 ± 5.6 | 60.4 ± 7.8 | 0.94 |
| Ornithine | 63.8 ± 5.9 | 74.5 ± 8.7 | 0.32 |
| Lysine | 232.9 ± 11.8 | 245.9 ± 25.8 | 0.65 |
| Histidine | 69.3 ± 7.4 | 82.5 ± 10.5 | 0.32 |
| Arginine | 127.4 ± 7.7 | 116.8 ± 9.6 | 0.40 |

*Values are means ± SEM.

**Table 3** Free amino acid concentrations (nmol/mL) in plasma of patients subjected to total gastrectomy (GX group) at the baseline and one year after the surgical procedure*

| Amino acid | GX group (baseline) | GX group (one year) | P |
|---|---|---|---|
| Taurine | 57.0 ± 5.6 | 73.5 ± 7.1 | 0.12 |
| Aspartate | 43.3 ± 4.2 | 45.8 ± 5.2 | 0.74 |
| Threonine | 130.7 ± 15.8 | 140.0 ± 26.3 | 0.78 |
| Serine | 140.7 ± 25.5 | 135.2 ± 15.4 | 0.87 |
| Glutamate | 176.0 ± 48.9 | 162.8 ± 27.0 | 0.83 |
| Glutamine | 790.7 ± 75.5 | 817.8 ± 151.4 | 0.88 |
| Adipic acid | 6.2 ± 0.8 | 12.5 ± 1.8† | 0.01 |
| Glycine | 259.0 ± 22.5 | 355.8 ± 63.4 | 0.21 |
| Alanine | 394.0 ± 27.8 | 445.3 ± 71.4 | 0.55 |
| Ethanoloamine | 83.8 ± 26.0 | 106.7 ± 40.0 | 0.67 |
| Alpha-amino-butyrate | 26.7 ± 3.9 | 17.0 ± 4.7 | 0.17 |
| Valine | 243.7 ± 50.4 | 250.0 ± 47.6 | 0.93 |
| Cystine | 9.0 ± 1.6 | 11.2 ± 4.6 | 0.72 |
| Methionine | 47.0 ± 20.7 | 28.5 ± 5.0 | 0.44 |
| Isoleucine | 92.7 ± 26.4 | 94.2 ± 21.0 | 0.96 |
| Leucine | 163.6 ± 36.3 | 185.3 ± 42.8 | 0.72 |
| Tyrosine | 79.0 ± 9.4 | 88.5 ± 8.1 | 0.50 |
| Phenylalanine | 121.2 ± 30.6 | 97.2 ± 15.6 | 0.53 |
| Tryptophan | 54.0 ± 3.9 | 62.2 ± 13.5 | 0.64 |
| Ornithine | 60.8 ± 6.9 | 98.2 ± 22.4 | 0.17 |
| Lysine | 216.0 ± 33.0 | 240.7 ± 39.1 | 0.66 |
| Histidine | 66.2 ± 7.6 | 85.2 ± 13.4 | 0.28 |
| Arginine | 139.7 ± 24.3 | 152.5 ± 23.6 | 0.73 |

*Values are means ± SEM.
†Mean values in a row differ significantly for $P < 0.05$ tested by paired Student's *t*-test for dependent variables.

by 28% ($P = 0.01$) and 60% ($P = 0.04$), respectively. One year after the surgery, plasma concentration of adipic acid was significantly higher by 144% in the gastrectomized group of patients when compared to the controls ($P = 0.01$). Plasma concentration of alpha-amino-butyrate was significantly lower by 49% one year after the gastrectomy in patients when compared to the control group after one year from the abdominal surgery ($P = 0.02$).

### Silicon concentration in plasma

Concentrations of silicon in plasma of control patients and patients subjected to total gastrectomy at the baseline and one year after the surgical procedure are shown in Table 4. Plasma concentration of silicon in overnight fasted control group of patients was not significantly different after one year when compared to the baseline value ($P = 0.46$). Plasma concentration of Si was significantly lowered by 26.7% one year after the total gastrectomy procedure in the GX group of patients when compared to the baseline value ($P = 0.009$). Plasma concentration of Si measured one year after the surgical procedures was significantly lower in the Gx group of patients when compared to the CTR group ($P < 0.0001$). No significant difference of plasma Si concentration was found between the CTR and GX groups at the baseline ($P = 0.18$).

**Table 4** Concentration of silicon (μmol/L) in plasma of control patients (CTR group) and patients subjected to total gastrectomy (GX group) at the baseline and one year after the surgical procedure*

| Silicon concentration | Baseline | One year after surgical procedure |
|---|---|---|
| CTR group (N = 6) | 54.70 ± 9.0 | 47.70 ± 1.50 |
| GX group (N = 8) | 40.87 ± 2.73 | 29.96 ± 1.52†,‡ |

*Values are means ± SEM.
†Statistically significant difference versus baseline value for $P < 0.01$ tested by paired Student's *t*-test for dependent variables.
‡Statistically significant differences versus CTR group for $P < 0.0001$ tested by non-paired Student's *t*-test for non-dependent variables.

## Discussion

Total gastrectomy performed in patients in this study has induced severe osteoporotic changes of lumbar spine within one-year period. The most negative effects of the gastrectomy were observed within trabecular bone compartment where one-year bone loss expressed by decreased Ca-HA density in the trabecular bone was between 11.9 and 16.6%. An average one-year decrease of $Tb_{Ca\text{-}HA}$ in $L_1$–$L_4$ has reached 15.0%. In the cortical bone compartment of lumbar spine, the gastrectomy-induced osteopenia was less dramatic than in the trabecular bone; however, the decrease of $Cb_{Ca\text{-}HA}$ in $L_1$ and $L_2$ reached 10.5 and 9.1%, respectively. Similar tendency was observed in the other lumbar vertebrae of the gastrectomized patients. All these observations indicate very intensive bone resorption process within both the trabecular and cortical bone compartments. The difference between bone loss rate within trabecular and cortical bone is not surprising. In experimental studies it was shown that metabolic turnover rate of the trabecular bone compartment is more intensive when compared to cortical bone. Metabolic response of the trabecular bone to physiological, pharmacological, and nutritional factors is faster than in the cortical bone.[12,13] Moreover, annual metabolic turnover rate of the trabecular and cortical bone compartments was assessed at 20–25 and 1–3%, respectively.[14] All these differences result from various structural characteristics, modeling and remodeling capacity, and physiological functions in the body. Cortical bone has higher mineral density than trabecular and forms skull bones and shafts of long bones mainly counteracting to bending forces and providing protective functions. Trabecular bone is present as the internal structural network of metaphyses and epiphyses of long bones, pelvis, and vertebral bodies neutralizing compressive forces.[15] Considering that structure of lumbar vertebrae in humans is formed of the cortical shell and trabecular core, it is methodologically advantageous to investigate mineral density in both these tissue compartments separately, especially for precise evidence of the expected changes in response to experimental factors.[13,16] In contrast to the gastrectomized group of patients, abdominal surgery performed due to cardiospasmus in the control group has not induced significant changes of vBMD of lumbar spine. It was observed that one-year changes of $Tb_{Ca\text{-}HA}$ of lumbar vertebrae in the control patients were between −4.0 and −1.9%, reaching an average value for all lumbar segment −3.0%. In the cortical bone compartment of lumbar vertebrae, one-year changes of $Cb_{Ca\text{-}HA}$ were within −0.7 and +2.3%, reaching an average value for all lumbar vertebrae +0.5%. However, all these changes of vBMD within the trabecular and cortical bone compartments were not statistically significant. The results of vBMD measurements obtained in this study are in accordance with the data presented in previous studies on gastrectomized patients in Korea. Mean bone loss in lumbar spine one year after gastrectomy reached −5.7% and was expressed as the percentage change of bone mineral density (BMD) from the baseline values measured before the surgical procedure. However, it should be pointed out that the previous study was performed using dual-energy X-ray absorptiometry (DEXA) method and instead of separately determined changes of vBMD for the trabecular and cortical bone compartments, areal BMD measurements for all vertebrae were presented.[17] Other studies in USA population on gastrectomized patients with the use of DEXA method have revealed 14.1% bone loss in lumbar spine, expressed as a decrease of BMD value. Male patients in those investigations were at mean age of 71.5 years exhibiting nearly 15 years more advanced age in comparison to the current study.[18] Lack of significant differences of vBMD in the control group of patients one year from abdominal surgery is in accordance with previously reported data in humans showing age-related annual bone loss rate between 0.5 and 0.75%. In case of rapid phase of postmenopausal bone loss, annual decrease in trabecular bone density is assessed on 2–4%. Annual cortical bone density loss rate in postmenopausal women was determined between 1 and 2%.[17] The observed gastrectomy-induced significant bone loss in the current study was not related to decreased amino acid concentration in plasma obtained from overnight fasted patients. It is interesting to perform determination of amino acid concentration in plasma collected from gastrectomized patients within shorter periods from food intake than the period of overnight fasting. Thus, further studies with non-fasted gastrectomized patients are necessary to better explain the metabolic consequences of gastrectomy for amino acid and protein turnover. Significant differences of amino acid concentration were observed in case of comparison between two groups performed at the baseline and one year later. It was observed that plasma concentrations of glutamine and cystine at the baseline were significantly lower in patients from the GX group when compared to the controls. Results of glutamine evaluation in the current study are different from data reported by Pernow *et al.* (2010) which revealed 12.5% higher concentration of glutamine in plasma from fasted male patients suffering from idiopathic osteoporosis when compared to healthy weight- and sex-matched controls. In those studies, plasma concentrations of threonine, alanine, asparagine, glycine, and whole sum of non-essential amino acids were significantly higher in men with idiopathic osteoporosis (MIO) than in controls. However, the analysis of fasting erythrocyte amino acid concentrations in MIO patients revealed significantly lower concentration of histidine, lysine, phenylalanine, tryptophan, citruline, ornithine, sum of essential amino acids, and sum of aromatic amino acids than in the healthy control group.[19] Regardless of the differences and similarities existing between previous report and the current study, further investigations are necessary to explain amino acid metabolism changes both in relation to osteopenia/osteoporosis pathophysiology and treatment. This statement is supported by experimental studies on animals in which changes of plasma amino acid concentrations were also observed. In studies on growing pigs, it was shown that surgical removal of the fundic part of stomach induced severe osteopenia in lumbar spine associated with significantly decreased plasma concentrations of taurine, threonine, glycine, valine, methionine, leucine, tyrosine, tryptophan, and arginine when compared to the sham-operated group.[5] Assuming that total or partial gastrectomy may impair protein digestion processes leading to osteopenia/osteoporosis development, the endocrine and exocrine functions of the stomach seem to be crucial for proper development and homeostasis maintenance of the skeletal system.[4,5] In other studies on animals, it was shown that long-term amino acid metabolites administration improves BMD, morphological parameters of bones, and their mechanical endurance and these effects are combined with improved plasma amino acid status. Such effects were observed in studies on physiologically growing animals and in animals with developing osteopenia.[5,20–22] In the current study, plasma concentration of alpha-amino-butyrate was shown to be significantly lower in the GX group of patients one year after the gastric surgery when compared to the control group. At this stage of the follow-up, plasma concentration of adipic acid was significantly higher in the gastrectomized group of patients when compared to both the baseline value and to the control group. Even known functions of both these amino acids in relation to cellular, organ, and systemic metabolism, their particular role in bone metabolism regulation processes was not explained.[23–25] As shown in the current study, rapid development of lumbar spine osteoporosis was associated with decreased silicon concentration in plasma of the gastrectomized patients. It was observed that one year after the performed total gastrectomy, plasma silicon concentration in the patients was significantly decreased by nearly 27% when compared to the baseline concentration. Thus, impaired silicon metabolism in the gastrectomized patients may be one of important causative factors responsible for osteoporosis development. Considering that gastrointestinal tract is the main entry of silicon to the body, stomach resection seems to impair its intestinal absorption. The absorption of silicon is related to production rate of soluble and absorbable forms of silica in the gastrointestinal tract for which proper exocrine and endocrine functions of the stomach are necessary.[26,27] Poor plasma silicon status one year after the gastrectomy was also confirmed by direct comparison of the concentration of this element in GX and CTR groups. It was shown that plasma silicon concentration was significantly lowered by over 37% in the GX group when compared to the controls. The results obtained in the current study are in accordance with other reports showing beneficial effects of silicon administration to humans and animals with normal or impaired bone metabolism. Retrospective study on osteoporotic women with silicon administration (100 mg/week) for four months has induced significant increase of hip BMD by 4.7% when compared to controls.[28] In studies on osteoporotic subjects, oral (5.5 mg/day by 20 days a month for three months) and intramuscular (16.5 mg/week for four months) administrations of silicon significantly increased trabecular bone volume and this effect was combined with an increase in circulating lymphocytes and immunoglobulins.[29] As shown in studies on women with low bone mass, oral administration of silicon (6 mg/day) significantly increased BMD in femur.[30] Moreover, cross-sectional studies of the Framingham Offspring cohort (1251 men and 1596 women) have shown positive association between dietary silicon intake and hip BMD both in men and premenopausal women.[31] Positive association of dietary intake of silicon with BMD in spine and femur was confirmed also in cross-sectional study of the Aberdeen Prospective Osteoporosis Screening Study including nearly 3200 premenopausal and early postmenopausal women undergoing hormone replacement theraphy.[32,33] In studies on animals, dietary silicon depletion in growing rats resulted in an increased longitudinal bone growth, thinner bone growth plate, and increased chondrocyte density.[34]

In conclusion, this study has shown that total gastrectomy in patients has induced severe osteoporotic changes in lumbar spine within one-year period. The most negative effects were observed in the trabecular bone where the decrease of Ca-HA density in the trabecular bone was up to 16.6%. In the cortical bone of lumbar vertebrae, the gastrectomy-induced bone mineral loss was up to 10.5%. The observed negative changes of vBMD were associated with decreased concentration of silicon by almost 27% in comparison to baseline values indicating importance of exocrine and endocrine functions of stomach for silicon homeostasis maintenance. Gastrectomy-induced bone loss in lumbar spine was not related to changes of amino acid concentration in plasma obtained from overnight fasted patients.

**Authors' contributions:** MRT was responsible for the concept and experimental design of the study, data collection, statistical evaluation of data and their interpretation, supervised all stages of the experiment and was responsible for manuscript preparation. WK was responsible for the concept and experimental design of the study, data collection, supervised all stages of the experiment. WK was also responsible for radiological evaluation of patients with the use of QCT technique and supervised sample collection. MS was responsible for biochemical evaluation of plasma amino acid concentration. AD was responsible for the concept and experimental design of the study, as well as gastric and abdominal surgical procedures and postoperative care of patients. PB was responsible for plasma samples collection, gastric and abdominal surgical procedures, and postoperative care of patients. AS was responsible for statistical evaluation of data and their interpretation and manuscript preparation. AC was responsible for data interpretation, literature collection, and manuscript preparation. AB-C was responsible for determination of silicon concentration in plasma samples. RM was responsible for the concept and experimental design of the study and participated in data interpretation. GW was responsible for gastric and abdominal surgical procedures and postoperative care of patients. All authors participated in the preparation of, and have approved the final version of the manuscript.

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