



# The influence of habitual salt intake on bone remodelling in young healthy people

Vpliv dnevnega vnosa soli na preoblikovanje kosti pri mladih zdravih osebah

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## Abstract

**Introduction:** Sodium alters calcium metabolism by increasing calcium excretion, thus possibly influencing bone metabolism. The hypothesis of the present study is that amount of dietary sodium intake affects the bone remodelling. This study aimed to assess whether a habitual intake of sodium has an effect on peak bone mass and biochemical indicators of bone metabolism.

**Subjects and Methods:** In a cross-sectional study that involved 41 young men and women, six biochemical markers were assessed from blood samples using ELISA: osteocalcin, C-terminal procollagen type I peptide, receptor activator kappa B ligand, pyridinoline, parathyroid hormone, and osteoprotegerin, while bone mineral density (BMD) and bone mineral content (BMC) were measured by dual x-ray absorptiometry. Subjects were divided into two groups according to habitual sodium intake (low-Na and high-Na group) assessed by questionnaire.

**Results:** No difference was found between groups of low and high Na intake in BMD and BMC, or in biochemical markers of bone metabolism. Since the groups differed in Ca intake, energy and vitamin D, adjustments were made for those cofounders. Regression analysis showed that only the dietary intake of vitamin D was associated with dual femur BMD and BMC, and no correlation was found between bone remodelling indicators and Na intake after adjustment for vitamin D intake.

**Conclusion:** The present results could not confirm that habitual sodium intake above recommended levels affects bone remodelling processes or decreases bone mineral density in young healthy people if combined with adequate calcium intake.

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## Izvleček

**Uvod:** Natrij vpliva na presnovo kalcija tako, da poveča njegovo izločanje, s tem pa lahko vpliva na presnovo kosti. Naša hipoteza je bila, da količina zaužitega natrija v prehrani vpliva na presnovo kosti. V raziskavi smo želeli oceniti, ali običajni vnos natrija vpliva na kostno maso in biokemične kazalnike presnove kostnega tkiva.

**Preiskovanci in metode:** V presečni študiji, v kateri je sodelovalo 41 mladih moških in žensk, smo s pomočjo metode ELISA v vzorcih krvi določili šest biokemičnih označevalcev: osteokalcin, C-terminalni peptid prokolagena tipa I, ligand za aktivator receptorja jedrnega dejavnika kappa B, piridinolin, občutnični hormon in osteoprotegerin, z dvojno rentgensko absorpciometrijo pa smo izmerili mineralno gostoto kosti (BMD) in vsebnost mineralov v kosteh (BMC). S pomočjo vprašalnika smo ocenili količino običajnega vnosa natrija in glede na to preiskovance razdelili v dve skupini, tj. v skupino z nizkim ali v skupino z visokim vnosom natrija.

**Rezultati:** Med skupinama z nizkim in visokim vnosom natrija nismo ugotovili nobene razlike v BMD in BMC kot tudi ne v biokemičnih označevalcih presnove kostnega tkiva. Ker sta se skupini med seboj razlikovali po vnosu kalcija, energije in vitamina D, smo glede na te spremenljivke izvedli ustrezne prilagoditve. Regresijska analiza je pokazala pozitivno korelacijo samo med prehranskim vnosom vitamina D ter BMD in BMC stegenic, med tem ko po prilagoditvi za vnos vitamina D ni bila ugotovljena povezava med kazalniki presnove kosti in vnosom natrija.

**Zaključek:** Rezultati naše raziskave niso potrdili, da vnos natrija, ki je višji od priporočenega, vpliva na procese presnove kosti ali zmanjšanje mineralne gostote kosti pri mladih zdravih ljudeh, če je kombiniran z ustreznim vnosom kalcija.

## 1 Introduction

Although genetic factors appear to be much more important for bone health than the combination of environmental, nutritional, and lifestyle factors, the latter cannot be neglected (1). Numerous studies confirmed the importance of an adequate intake of calcium for maintaining bone quality and improving bone density, as could be seen in the meta-analysis made by Welten et al. (2). Since there are many mechanisms regulating the absorption of calcium from food, there is rising interest for cofounders that alter calcium metabolism and could influence calcium level and consequently bone health. One of the cofounders is contemporary Western diet that differs from dietary habits in the past because of much faster way of life; such diet is mostly industrially produced and thus loaded with salt and preservatives. Importantly, high sodium intake increases urinary calcium excretion (3), which could be detrimental for bones because of a possible increase in bone resorption. On the other hand, dietary salt may also trigger 1,25(OH)<sub>2</sub>D synthesis to increase alimentary calcium absorption (4).

The results of studies investigating the influence of sodium intake on bone metabolism are inconsistent and often conflicting. Some researchers concluded that sodium intake significantly influences bone density (5,6), while others did not notice any changes in bone density with a different sodium load (7,8). The available data are mostly from studies of older population with

salt intake usually much higher (5) or lower (6) than in a usual diet. Among the studies of effects of sodium chloride on bone metabolism markers, the reported results are also inconsistent. The studies investigating postmenopausal women report that an increased load in sodium increases bone resorption (9,10), while the studies of younger adults report no effect of sodium on bone metabolism (9,11). The Dietary Reference Intakes for sodium are 1500 mg/day, and the recommendation is not to exceed the limit of 2300 mg/day (12), while the recommended daily intakes of calcium and vitamin D according to The World Health Organization (13) are 1000 mg for Ca and 5 µg for vitamin D (13).

At present, there are a limited number of studies investigating connections of nutritional intakes and lifestyle to bone health and bone quality in young people, and most of the published studies are focusing on young people of Asian origin. These studies reported low bone density and high prevalence of osteoporosis in young people, which is in correlation with the contemporary way of life (14-16). Thus, it seemed important to conduct a similar research among a population of young people in Europe, especially because of great prevalence of Western nutritional habits, which are characterized by increased consumption of processed and prepared foods with a high sodium content. The aim of this study was to establish a possible influence of habitual sodium intake on the peak bone mass and

bone metabolism in healthy young population by assessing possible relationships between salt intake and bone density and bone mineral content, and salt intake and biochemical markers of bone metabolism. The hypothesis of the present study is that the amount of dietary sodium intake affects bone remodelling. Different amounts of sodium intake will affect the biochemical markers of bone turnover as well as bone density and bone mineral content. The lower values of biochemical markers of bone metabolism and the higher values of bone density and bone mineral content may be expected in the group consuming under 2300 mg sodium/day (low sodium group) comparing to the values of the group consuming 2300 mg sodium/day or more (high sodium group).

## 2 Methods and materials

### 2.1 General study design

This cross-sectional study was performed during spring months (March-May) in 2013. The sample size was calculated based on published differences in concentration of one of the most important outcomes - biochemical marker of bone metabolism: osteoprotegerin (OPG) (17). For effect size (Cohen D) 0.698 with  $\alpha=0.05$  and strength of 80% sample size was 8 per group. The effect size quantifies the size of the difference between two groups. It calibrates the difference between two groups in terms of standard deviation. Sample size was increased because more parameters were used for analysis. The exclusion criteria in sample forming process were any diagnosed bone diseases or chronic illnesses that could influence bone metabolism, as well as the use of hormone replacement therapy or contraceptives, diuretics or antacids, and any mineral/vitamin supplements during the study. For women, the additional exclusion criteria were any hormonal problems within one-year period before the research. Forty-one healthy students at the College of Applied Sciences Lavoslav Ruzicka in Vukovar (21 men and 20 women) aged between 20 and 30 years participated in the study. Every participant gave his or her written informed consent for participation in the research study. The study protocol and procedures conformed to the standards set by the latest revision of the *Declaration of Helsinki* and national legislation, and the approval of the Ethical Committee of the Faculty of Medicine University of Osijek was obtained in November 2012 (number of approval 2158-61-07-12-42).

### 2.2 Bone density assessment

All participants were measured for body mass and height. Bone mass density (BMD) and bone mineral content (BMC) were measured by dual x-ray absorptiometry at three sites: at lumbar spine L1-L4, and at hips: dual femur (total mean) and femoral neck (mean). Measurements were taken by a trained technician using Lunar Prodigy 64575 G.E.S. S.A. (GE Healthcare, Boston, Massachusetts, USA). The technician was blinded to other parameters obtained in the study.

### 2.3 Physical fitness assessment

Physical activity level (PA) was assessed by a questionnaire and confirmed by performing Cooper test of assessing  $VO_2$ max. Physical activity was assessed by short version of IPAQ questionnaire (International Physical Activity Level Questionnaire) (18) by assessing the time spent in physical activity during 7 days in several aspects of life: at work, transportation, housework, house maintenance and caring for family, recreation, sport and leisure time. MET- score (metabolic equivalent) was calculated for each activity and the sum of all activities during one-week period, following the instructions for IPAQ processing results (19). According to the MET-score, the participants were grouped by the level of their physical activity into three categories: low (below 600 MET-min/week), moderate (minimum 600 MET-min/week), and high (minimum 3000 MET-min/week).

Cooper test was performed by running on a treadmill for 12 minutes, and  $VO_2$ max was calculated from the distance passed by running in this period of time (20). According to the calculated  $VO_2$ max expressed as ml/kg/min the subjects were assigned to one of the following five categories of physical fitness (20): very poor (male<33, female<23.6), poor (male 33-36.5; female 23.6-28.9), fair (male 36.5-42.4; female 29.0-32.9), good (male 42.5-46.4; female 33.0-36.9), excellent (male 46.5-52.4; female 37.0-41.0) and superior (male >52.4; female>41.0).

### 2.4 Nutritional intake assessment

The subjects also completed a food frequencies questionnaire from EPIC-Norfolk study for assessment of nutrients intake (21). The questionnaire consists of a list of foods, with amount shown for each food, either a "medium serving" or a common household unit such

as a slice or teaspoon. Subjects are asked to indicate, by putting a tick in the box, how often, on average, they have eaten the specified amount of each food during the past year. The average use is indicated from never or less than once/month to 6+ servings per day. Foods are organized in categories: meat and fish, bread and savory biscuits, cereals, potatoes, rice and pasta, dairy products and fats, bread and vegetables, sweets and snacks, soups, sauces and spreads, drinks, fruits, and vegetables. After processing the data with FETA software version 2.49 (22), the results are given as average quantities of consumed food types or group of food in units of mass/day (as g or mg).

## 2.5 Bone remodelling marker measurements

Blood samples were taken after overnight fast for analysis of bone remodelling biomarkers: osteocalcin (OC), C-terminal procollagen type I peptide (PICP), pyridinoline (PYD), parathyroid hormone (PTH), osteoprotegerin (OPG) and receptor activator of nuclear kappa B ligand (RANKL). The serum was removed after centrifugation at 1500g for 10 min and stored at  $-80^{\circ}\text{C}$  before analysis. OC concentration was assessed by using ELISA (Elabscience Biotechnology Co, Beijing, NR China), CV<10%. Concentration of PICP was measured by ELISA (USCN Life Science Inc, Hubei, NR China), intra-assay CV<10%, inter-assay CV<12%. PYD concentration was assessed by ELISA (Qayee-Bio, Shanghai, NR China), CV<15%. PTH concentration was measured by ELISA (Calbiotech, Spring Valley, CA) with intra-assay CV<10%, inter-assay CV<12%. OPG concentration was determined by ELISA (Kamiya Biomedical Company, Seattle, WA, USA) with intra-assay CV<12%, inter-assay CV<14%. Concentration of RANKL was measured by ELISA (Cusabio Biotech, Hubei, NR China), intra-assay CV<8%, inter-assay CV<10%.

## 2.6 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 20. Kolmogorov-Smirnov test was used to assess normal distribution, variables that met normal distribution are presented as arithmetic mean  $\pm$  SD, and other are presented as median and range. The subjects were divided into two groups differentiated by consumption of Na in their usual diet: a group of low Na intake (less than 2300 mg of Na/day), and a group of high Na intake (2300 mg Na/day or more). Differences between low Na and high Na groups in anthropometric

variables, physical activity and fitness level and nutritional intakes of Calcium, Vitamin D and energy were tested by independent t-test. ANCOVA was used to assess the differences between the groups in the concentration of biomarkers of bone turnover and the results of densitometry. Since groups also differed in energy, calcium and vitamin D intake, those are considered confounding variables. The correlation of Na intake with BMD, BMC and biochemical markers of bone metabolism was assessed by partial correlation. As the results of partial correlation analyses, we presented zero-order correlations for all tested variables and partial correlations for Na with bone characterizing variables, i.e. correlations adjusted for all other dietary intakes. Linear regression analyses were performed for those bone characterizing variables that were correlated with Na intake. The level of statistical significance was set at two-sided  $p=0.05$  for all performed tests.

## 3 Results

### 3.1 General groups' characteristics and nutrients intake

Forty-one students participated in this study, both men (N=21) and women (N=20). The median age was 24 years. The median BMI (body mass index) in the sample was  $23.3\text{ kg/m}^2$ , which was within the range from  $17.7\text{ kg/m}^2$  to  $33.2\text{ kg/m}^2$ . Most of the subjects were in the category of normal body mass, with 4 underweight (BMI<20) and 7 overweight (BMI>25) subjects.

The anthropometric data and dietary intake of calcium, sodium, vitamin D, and caloric intake as well, according to the intake of sodium are presented in Table 1. The intakes of calcium and vitamin D are mostly within the values recommended by WHO for Ca, and a little below recommended for vitamin D (13). Overall daily intake of sodium in the investigated sample ranged from 1087 mg to 7233 mg. The arithmetic mean was  $3607.77\text{ mg} \pm 1619\text{ mg}$ , which is not unusual for contemporary Western diets with great deal of processed food rich in salt. After dividing the participants into two groups based on the average daily consumption of sodium (low sodium group with consumption <2300 mg Na/day, and high sodium group with consumption  $\geq 2300\text{ mg Na/day}$ ) we found that those groups differ also in caloric intake ( $F=7.01$ ,  $p<0.001$ ), the intake of calcium ( $F=10.47$ ,  $p=0.002$ ) as well as vitamin D ( $F=30.42$ ,  $p<0.001$ ). Namely, low sodium intake groups had lower intake of calcium and vitamin D compared to high sodium intake group.

### 3.2 Fitness and physical activity levels

The mean value of VO<sub>2</sub>max in the sample is 32.30 ml/kg/min and it ranges from 14.4 ml/kg/min to 55.310 ml/kg/min, with both extreme values; the subject with the lowest value and the subject with the highest value of VO<sub>2</sub>max both being in the high sodium group. Differences in physical activity and fitness level between low-salt and high-salt group are presented in Table 1.

### 3.3 Bone density and bone remodelling markers values

As expected, the subjects of this study had healthy bones, with the mean densities of 1.143 g/cm<sup>3</sup> for both femurs, 1.31 g/cm<sup>3</sup> for L1-L4 lumbar spine, and 1.16 g/cm<sup>3</sup> for femoral necks, meaning that T-scores were for femurs 0.65, for L1-L4 lumbar spine 0.88, and for femoral necks 0.78. Table 2 presents the results of densitometry measurements and biomarkers of bone remodelling according to sodium intake. BMD and BMC at the hips were higher with higher intakes of sodium. After adjustments for caloric intake as well as intakes of calcium and vitamin D, we found no statistically significant differences in BMD or BMC at the hips (dual femur and neck mean) or the lumbar spine in the groups of different sodium intake, or in concentration of biochemical markers of bone remodelling between the groups as well, as showed in Table 2.

### 3.4 Correlations of salt intake with bone density and bone remodelling markers values

Correlation and partial correlation coefficients are presented in Table 3. Sodium intake showed positive correlation to intake of Ca, vitamin D and energy intake. Statistically significant positive correlation was obtained for sodium intake with BMD and BMC dual femur, which after controlling for calcium, vitamin D and energy intake, changed in correlation coefficient value from positive to negative correlation and was no longer statistically significant (Table 3).

Regression analysis for dual femur BMD (dependent variable) and dietary intake of Na, Ca, vitamin D and energy (independent variables) showed statistically significant (F=4.97, p=0.003) moderate (R=0.597, R<sup>2</sup>=0.356) association with vitamin D as the only statistically significant predictor (B=1.853, p=0.008).

Regression analysis for dual femur BMC (dependent variable) and dietary intake of Na, Ca, vitamin D

**Table 1:** Mean values for PA level (METmin/week), fitness level (VO<sub>2</sub>max), anthropometric measurements and intake of energy, calcium and vitamin D according to sodium intake.

	Low Na (<2300 mg) (N=12)			High Na (>2300 mg) (N=29)			mean difference (Low Na-High Na)		95%CI of difference		
	Mean	Std. Deviation	min	max	Mean	Std. Deviation	min	max	lower	upper	P
MET-min/week	5602.67	4744.03	817.5	14622	5218.05	5185.94	99	23904	-3132.03	3901.26	0.826
VO <sub>2</sub> max (ml/kg/min)	30.65	8.91	19.33	47.49	35.78	9.51	14.41	55.31	-11.6	1.36	0.118
Height (cm)	170.33*	10.21	149	186	178.14*	9.13	160	200	-14.36	-1.24	0.021*
Body mass (kg)	67.79	11.31	44.1	83.2	76.31	17.5	47.7	131.2	-19.6	2.58	0.129
BMI (kg/m <sup>2</sup> )	23.2	1.91	19.9	25.9	23.87	4.13	17.7	34.6	-3.2	1.86	0.594
Vitamin D – ergocalciferol intake (mcg)	1.76*	0.42	1.02	2.24	4.38*	2.45	1.54	12.27	-3.6	-1.66	0.001*
Calcium intake (mg)	819.40*	244.55	470.8	1141.57	1215.04*	391.46	651.51	2063.87	-642.95	-148.32	0.002*
Energy intake (kcal)	1368.21*	315.69	920.54	1861.85	2746.78*	938.02	1642.43	5392.61	-1776.48	-980.65	<0.001*

Legend: p – statistical significance; \* – significant difference.

**Table 2:** Results of densitometry measurements and biomarkers of bone remodelling in groups of different sodium intake (p = level of statistical significance for difference between groups of low and high Na intake obtained by ANCOVA test after adjustments for energy, Ca and Vitamin D intake).

	Low Na (<2300 mg) N = 12	High Na (>2300 mg) N=29	95%CI of difference (Low Na-High Na) <sup>a</sup>		
	Arithmetic mean ± SD	Arithmetic mean ± SD	lower	upper	p <sup>a</sup>
DF TOTAL BMC (g)	33.9± 4.8	42.28±9.3	-9.869	3.872	0.171
DF TOTAL BMD (g/cm <sup>3</sup> )	1.1±0.1	1.2±0.1	-0.161	0.093	0.423
L1-L4 BMC (g)	70.8±13.3	81.5±16.9	-17.607	9.405	0.363
L1-L4 BMD (g/cm <sup>3</sup> )	1.3±0.2	1.3±0.1	-0.138	0.119	0.507
NECK MEAN BMC (g)	5.3±0.7	6.5±1.3	-1.565	0.46	0.152
NECK MEAN BMD (g/cm <sup>3</sup> )	1.1±0.1	1.2±0.2	-0.196	0.073	0.145
OC (ng/ml)	13.7±8.9	10.4±5.6	-3.282	9.936	0.606
OPG (ng/ml)	0.9±0.3	1.1±0.3	-0.572	0.043	0.150
PICP (ng/ml)	108.6±50.6	89.9±44.7	-37.371	56.257	0.792
PTH (pg/L)	39.6±10.9	41.3±15.5	-12.93	14.16	0.687
PYD (nmol/L)	57.2±19.3	49.6±25.6	-1.714	42.04	0.183
RANKL (pg/ml)	105.4±17.3	114.8±43.8	-37.492	21.432	0.822

<sup>a</sup>Based on estimated marginal means with covariates evaluated at the following values: Vitamin D=3.168, Ca=1057.87, Energy=2204.7

Abbreviations: BMC – bone mineral content; BMD – bone mineral density; DF TOTAL – dual femur total; OC – osteocalcin; OPG – osteoprotegerin; PICP C – terminal procollagen type I peptide; PTH – parathyroid hormone; PYD – pyridinoline; RANKL – receptor activator of nuclear kappa B ligand.

and energy (independent variables) also showed statistically significant (F=3 p=0.031) moderate (R=0.500, R<sup>2</sup>=0.250) association with vitamin D as the only statistically significant predictor (B=0.17, p=0.015).

## 4 Discussion

The most important finding in the present study is that despite the differences in diet, there were no statistically significant differences in BMD, BMC or concentration of bone remodelling biomarkers between the groups of low and high sodium intake, and no correlation for sodium intake with BMD and BMC and markers of bone metabolism after adjustments for confounding dietary intake.

According to the acid-ash hypothesis, bones erode to provide alkali when it is needed for the maintenance of physiological pH in the blood. Sodium is one of the nutrients in a usual high acid-producing diet that negatively influences metabolism of calcium by increasing

calcium excretion (23). It has been estimated that in healthy population the loss of calcium is 1 mmol for 100 mmol increment of a sodium intake (3). Some groups of investigators showed that intestinal absorption of calcium rises with increasing excretion of calcium, as could happen with sodium overload (24). This could be accomplished through different mechanisms and pathways (4). It seems that human organism can accommodate to its needs in different ways. However, most of the data available in published studies are the data pertinent to postmenopausal women and older men. In the present study subjects were young, healthy people, and intakes of sodium are calculated on behalf of habitual diet for one year, with no interference or entanglement to intake of any nutrient or food type. Diet that subjects of the study are practicing is rich in milk and dairy products, but also containing a great deal of processed food that is loaded with salt and preservatives. The Dietary Reference Intakes recommendation for sodium consumption has been set at 2300 g/day, and that criterion was

**Table 3:** Zero-order correlation coefficients (r) and partial correlation coefficients (r<sub>xy-z</sub>) between dietary intakes and BMD, BMC and biochemical markers of bone metabolism (z-controlling variables: Ca (mg) & Energy (kcal) & Vitamin D - ergocalciferol (mcg)).

	Zero-order correlation				Partial correlation
	Na(mg)	Ca (mg)	Energy (kcal)	Vitamin D – ergocalciferol (mcg)	Na (mg)
	r (p)	r (p)	r (p)	r (p)	r <sup>xy-z</sup> (p)
Na (mg)		<b>0.688 (0.000*)</b>	<b>0.852 (0.000*)</b>	<b>0.527 (0.000*)</b>	
L1-L4 BMD	0.051 (.753)	0.006 (0.971)	0.072 (0.653)	0.277 (0.079)	-0.015 (0.931)
L1-L4 BMC	0.226 (0.156)	0.222 (0.164)	0.273 (0.084)	<b>0.508 (0.001*)</b>	0.003 (0.987)
D.F.TOTAL BMD	<b>0.322 (0.04*)</b>	0.304 (0.054)	<b>0.434 (0.005*)</b>	<b>0.444 (0.004*)</b>	-0.100 (0.549)
D.F.TOTAL BMC	<b>0.351 (0.024*)</b>	<b>0.365 (0.019*)</b>	<b>0.455 (0.003*)</b>	<b>0.583 (0.000*)</b>	-0.071 (0.672)
NECK MEAN BMD	0.263 (0.097)	<b>0.204 (0.020*)</b>	<b>0.390 (0.012*)</b>	<b>0.357 (0.022*)</b>	-0.153 (0.358)
NECK MEAN BMC	0.305 (0.052)	0.283 (0.073)	<b>0.398 (0.010*)</b>	<b>0.499 (0.001*)</b>	-0.066 (0.694)
RANKL (pg/ml)	0.106 (0.551)	0.080 (0.652)	0.013 (0.941)	0.082 (0.644)	0.249 (0.178)
PYD (nmol/L)	0.263 (0.133)	0.187 (0.290)	0.209 (0.235)	0.265 (0.130)	0.215 (0.246)
PTH (pg/L)	-0.107 (0.547)	0.082 (0.645)	-0.072 (0.687)	-0.083 (0.639)	-0.042 (0.825)
PICP (ng/ml)	0.006 (0.974)	-0.013 (0.940)	-0.065 (0.714)	-0.227 (0.197)	0.109 (0.558)
OPG (ng/ml)	0.217 (0.217)	<b>0.342 (0.048*)</b>	0.265 (0.129)	0.075 (0.672)	0.005 (0.977)
OC (ng/ml)	-0.196 (0.267)	-0.101 (0.570)	-0.198 (0.261)	-0.173 (0.329)	-0.034 (0.858)
Ca (mg)	<b>0.688 (0.000*)</b>		<b>0.821 (0.000*)</b>	<b>0.493 (0.001*)</b>	
Energy (kcal)	<b>0.852 (&lt;0.001*)</b>	<b>0.821 (&lt;0.001*)</b>		<b>0.634 (&lt;0.001*)</b>	
Vitamin D – ergocalciferol (mcg)	<b>0.527 (&lt;0.001*)</b>	<b>0.493 (0.001*)</b>	<b>0.634 (&lt;0.001*)</b>		

Legend: p – statistical significance; \* – significant difference.

met by only 12 subjects, while the remaining 29 subjects were consuming sodium in quantities that were above the recommended values. Since there are numerous mechanisms of increasing calcium absorption in order to maintain the level needed for normal function in cases of increased calcium loss, the excretion of calcium was not measured in this study. The association between sodium intake in habitual diet and bones was assessed by measuring their density and concentration of biomarkers of remodelling. The results of this study do not show that sodium intake may be detrimental for the bones of young healthy people, because no difference is established between the results of densitometry measurements of subjects with high and subjects with low sodium intake. It is possible that the difference is imperceptible, or that in a sample of renal patients would be more noticeable, but we did not find indices to

conclude detrimental effects of Na intake on bones. Also, we found no correlation of sodium intake with bone density and mineral content and biochemical markers of bone metabolism. It is possible that the results show no correlation because of the dietary habits of the sample, because we found a strong correlation between sodium and calcium intake, so it is possible that high calcium intake can annul the additional calcium excretion which happens with high sodium intake, like some have stated (23).

It is widely accepted that an increase in calcium absorption in the intestine is mediated through an increase in parathyroid hormone (PTH), but Mayer et al. (25) found increase in calcium absorption with loading with sodium, despite the impaired function of the parathyroid gland in a patient with hypoparathyroidism, suggesting other mechanisms of calcium absorption increase. We

found no difference between PTH concentrations in groups of different sodium intake, which is consistent with report of Mayer et al. (25). The confidence interval for difference between low Na and high Na group indicates that the difference (with 95% confidence) is between -12.93 and 14.16 pg/L. Considering this interval span, we cannot conclude anything else but that there is not enough data to estimate whether the difference could be clinically significant with sufficient precision. Difference may exist, but we were not able to prove it with our data. Lin et al. (26) reported that fasting PTH was related to sodium intake and was reduced significantly when sodium was reduced in the control group that consumed usual diet, but there was no difference in the group consuming DASH (Dietary Approaches to Stop Hypertension) diet. They concluded that hypertensive subjects may be particularly susceptible to the effects of a high sodium intake with greater calcium losses and elevated PTH levels. In the present study, no correlation between sodium intake and any of the 6 tested biochemical markers of bone metabolism was found. In addition, there was no difference in the concentration of any of biomarkers of bone remodelling, or in BMD and BMC between the groups of different sodium intake after adjustment for caloric, calcium and vitamin D intake. Studies performed in older populations showed that loading with sodium is accompanied with changes in bone metabolism, especially in salt-sensitive population (10). Our findings suggest that in healthy people these changes could be lower, or minimal or none.

Markers of bone formation osteocalcin (OC) and C-terminal procollagen type I peptide (PICP) are involved in bone formation. An increase in markers is usually connected to an increase in bone turnover process. Differences between the groups of low Na and high Na intake in the concentration of OC are within 95% confidence interval, from -3.28 to 9.936 ng/ml. The mean value of OC concentration for high Na group was lower than the mean value for low Na group, and 95% confidence interval showed that there could be 3.28 ng/ml lower value of OC in low Na group compared to high Na group, which we found not clinically significant, especially considering that the same confidence interval included the possibility of 9.936 ng/ml higher OC in low Na group than in high Na group. This result lead us to a conclusion that high Na intake is not connected to higher OC level. These results are in agreement with the results reported by Ginty et al. (11). Lin et al. (26) also found no effect of sodium intake on serum OC in the control group, contrary to the group with DASH diet, in which low level of sodium significantly decreased serum

OC level. Similar situation is with PICP. With 95% confidence, we estimated the difference between groups in PICP concentration to be between -37.37 and 56.257, so the difference may exist, but we do not have sufficient evidence to prove it. One of the markers of bone degradation is pyridinoline (PYD). In cases related to increased bone degradation its level should rise (27). Therefore, if there was some detrimental effect of consumed salt in subjects that consume salt in quantities higher than recommended levels, concentrations of biochemical markers of bone turnover should be higher compared to the levels in persons that consume less salt in their diet, because excretion of calcium decreases its level and activates bone degradation to release calcium stored in the bones. Our results showed higher mean value of PYD concentration in low Na group than in high Na group. We are 95% confident that mean PYD value for low Na group could be 1.714 nmol/L less to 42.04 nmol/L more than in high Na group. This confidence interval could not confirm our expectation of higher PYD concentration in high Na group than in low Na group. Considering its asymmetry towards the positive difference (lower value of PYD concentration in high Na group than in low Na group), there is very small possibility of increased bone degradation in high Na group. Ginty et al. (11) reported similar results. They concluded that the lack of effect of Na on urinary pyridinium crosslinks suggests that sodium-induced Ca loss is compensated for by increased Ca absorption rather than bone resorption.

Another lack of confirmation of influence of sodium intake on bone remodelling was difference in the levels of RANKL and OPG, two very important molecules for initiation of bone remodelling (27). Even though mean value of RANKL concentration was higher in high Na group than in low Na group, with 95% confidence we estimated difference between groups to be from -37.492 to 21.432 pg/ml. That means that there is a possibility that high Na group has RANKL concentration higher for 37.492 pg/ml compared to low Na group, which would indicate increased remodelling in high Na group, but also that difference between low Na and high Na group may indicate value of RANKL in low Na group higher for 21.432 pg/ml, which could mean that remodelling is more increased with low sodium intake. Considering this result, we can say that the difference in RANKL concentration may exist, but we were not able to prove it, or to determine its direction with our data. We are 95% confident that the difference in OPG concentration between low Na and high Na group is between -0.572 and 0.043 ng/ml. After analyzing our data, we obtained an effect size (calculated based on eta squared and transformed



to Cohen D) 0.667 for OPG, which was used for sample size calculation. Confidence interval for difference in OPG concentration between low Na and high Na group is indicating a possibility of lower concentration of OPG in low Na group for 0.572 ng/ml compared to high Na group, which could be considered a clinically relevant result, but since the OPG is considered to be a protective factor against bone loss, this result could not support the hypothesis of increased bone loss with higher Na consumption. We cannot disregard that difference may exist, but it could not indicate increased remodelling with higher Na intake. And there is also a possibility of 0.043 ng/ml lower concentration of OPG in high Na group, but we regard this difference magnitude not clinically significant. Since we found no statistically significant differences in any of biochemical parameters, and 95% CI for differences showed no increase in bone degradation in high Na group compared to low Na group, there is no evidence to conclude that the processes of remodelling were influenced by sodium intake. This is in agreement with the finding of Ilich et al. (7), who reported no detrimental effects of high sodium intake in older adults if calcium intake is adequate.

The only statistically significant correlation between diet and biochemical markers we found was positive correlation between calcium intake and OPG level. Since it became statistically insignificant when adjusted for other dietary intakes, it is probably affected by some other nutrient in the diet. The subjects with higher dietary intake of sodium had the higher consumption of calcium and vitamin D. A similar difference could be seen in dietary consumption of vitamin D as well. Although sodium intake was higher than recommended in most subjects in this trial, this did not influence their bones negatively. It is unquestionable and well established that sodium has hypercalciuric effect, but the main question is whether the increase in gastrointestinal absorption is quantitatively adequate to compensate for the loss of calcium initiated by an increase in sodium level. This may be related to age and menopausal status. In young men and premenopausal women hypercalciuria induces an increase in 1,25-dihydroxy vitamin D level and increase in intestinal calcium absorption, but in postmenopausal women that is not the case, so they may be unable to compensate for calcium loss in that way (10). The findings of our study support the findings and conclusions of others (25) that young people can handle the challenge of increase in sodium intakes without any or with minor skeletal changes as opposed to the older population, in whom a detrimental impact of high dietary salt intake on the bones could be a factor that contributes

to postmenopausal osteoporosis. The present results could not support the hypothesis that dietary salt intake is a factor that influences peak bone mass by altering remodelling process in cases of adequate calcium consumption. If Ca intake is in the range of recommended levels, increased calcium loss from high sodium intake evokes responses that result in improved dietary calcium extraction that is able to compensate for loss and to assure adequate amounts of calcium necessary for metabolic needs without needs for bone degradation (25).

There are several investigations that confirm the importance of regular physical activity for bone health, as explained by Toshihiro (29). It was shown that physical fitness is in positive correlation with bone density (30). Most of the subjects in this study are moderately active, and their  $VO_{2max}$  values are in the category of fair fitness. It is very important for the results of this study that physical fitness was not different for groups that differ in sodium consumption, so physical activity and its influence on bone metabolism in this sample is unsequential.

Limitation of the study: the present study is cross-sectional, which allowed to establish mutual dependence of the examined variables (salt intake and bone metabolism), but not causal relationships and impacts. Also, we cannot discuss possible effects of long-term variations of the amount of sodium intake on the measured parameters. Another limitation is the use of questionnaires for determining nutritional intakes and physical activity level. To avoid bias or wrong answers of subjects as a result of memory impairments, or misstatements, the standardized questionnaire with good metric characteristics was used. Finally, although hormonal status of female subjects was not checked by measuring hormonal levels and was assessed only on the basis of questionnaire answers on health status, these results showed random phases of menstrual cycling of participants in both groups, thus hormonal status has not biased the results.

## 5 Conclusion

The present results could not confirm that habitual sodium intake above recommended levels affects bone remodelling processes or decreases bone mineral density of young healthy people if combined with adequate calcium intake. This is based on the possibility that the influence of sodium intake prevailing in usual diet of the investigated population is offset by adequate intake of calcium.

## Conflict of interest

None declared.

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