The relationship between histamine and bone metabolism

Doctoral thesis

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1. Introduction

Several data confirm that the skeletal and immune system are relatd to each other. The pathogenesis of autoimmune disorders, systemic and local infections, metastases and local inflammation after bone fractures involve both bone and immune system. The cells of the adaptive immmune system and the mineralised skeleton have similar development and the regulatory factors are mostly the same. The anatomical location of the beginning of skeletogenesis and hematopoiesis is the same, which assumes similar regulatory factors. The hormonal and local factors of bone metabolism together with the immune regulators influence the local skeletal function.

The skeleton is dynamically rebuilding during the adulthood. If the balance ceases between bone formation and resorption – the synthesis of regulatory factors change (inflammation, oestrogen deficiency, etc.) – the intgrity of skeleton injures. According to several researches the bone homeostasis can be influenced by factors of immune system. The systemic and local immun reactions influence bone metabolism. The pathogensis of bone disese cauesed by immun disorders is not fully understood. Recent data suppose the role of histamine in bone metabolism. I examine this possible metabolic way in animal and human models.

2. Aims

In my researches I examined the relationship between histamine and bone metabolism.

The skeletal effect of histamine deficiency was examined in histidine-decarboxylase knock-out mice. In this way indirect information can be obtained about histamine effect in bone metabolism.

Allergic patients were examined to detect the possible bone effect of histamine overproduction. Allergic chilren were selected to show the effect of abundant histamine secretion on bone maturation and postmenopausal women and men were recruited to examine the rebuilding of the skleton in histamin overproduction.

Partial histamine deficiency was modelled by antihistmine treated allergic patients (children, postmenopausal women and men). The effect of H1 histamine receptor antagonists were tested on bone metabolism in this manner.

3. Material, patients and methods

3.1. Histamine deficiency was made by the knock-out of the histidine-decarboxylase gene (HDC-KO) the only histamine-synthesizing enzyme and by the inhibition of histmine consumption, which is less responsible for histamine presence. Oestrogen deficiency was produced by ovariectomy. The bone mineral content of the isolated femora was measured using single photon absorptiometric method (Gamma NK-364, Gamma, Hungary). Anteroposterior soft X-ray radiograms of the femora were made by Siemens Mammomat 3000 (Siemens, Germany) for morphologic examinations. Serum calcium and phosphorus was measured by routin biochemical methods (Hitachi 912 analyzer, Hitachi, Japan). Vitamin $1.25(OH)_2D_3$ was determined by RIA (Gamma-B 1.25-Dihydroxy Vitamin D RIA Kit, Germany). HDC-KO (n=15) and wild type (n=15) animals were compared. Mice were sham-operated (n=8), ovariectomized (n=7) or no operation was performed (n=15). Mice were placed on a histamine-free diet (n=8 in the HDC-KO and n=7 in the WT group; 0.6 nmol histamine per g food, 500 units/kg vitamin D; Altormin, Lage, Germany). One group of mice (n=4 in the HDC-KO and n=4 in the WT group) was kept on a diet without vitamin D and reduced in calcium (0.4% calcium vs. 2% in normal diet, D-deficient diet; Harlan Teklad, Madison, WI, USA). Normal diet was also used as a control (Charles River, Hungary). Eight groups of mice were made in this way. Mann-Whitney test was used for comparisons (SPSS 10.0, Chicago, IL, USA).

3.2. Fifty two multiplex allergic children were involved in the study from an outpatient department. The diagnosis of allergy was based on characteristic clinical and laboratory findings (skin prick tests, specific IgE assays, and food challenges). They had not taken drugs affecting bone metabolism. Some children were treated with inhalated corticosteroids during the acute allergic symptoms but none of them received systemic corticosteroids. Potential participants with diseases affecting bone metabolism were excluded. Thirty eight children suffered from cow's milk protein allergy, 32 children had multiplex food allergy, 42 children had allergic rhinitis and 10 children had atopic dermatitis. Fifteen allergic children (mean age: 7.93 ys, range: 5.9-9.7, 10 boys and 5 girls) were not treated either with H1R antagonists or inhalated corticosteroids, because allergic symptoms were prevented by avoiding allergic agents. Thirty seven recruited children (mean age: 9.02 ys, range: 6.5-11.7, 21 boys and 16 girls) received systemic H1R antagonists (either of cetirizine, maximal 5-10 mg per day, loratidine, maximal 5-10 mg per day or ketotifen, maximal 2 mg per day) for the allergic

symptoms, during our study. Twenty nine children were treated with inhalated corticosteroids of no more than 400μg per day [either of budenosid (maximum 400 μg/nap), fluticason (maximum 100 μg/nap), mometasone (maximum 18 mg/nap)] together with H1R antagonists during the acute allergic symptoms. Twenty one age and gender matched healthy children (mean age: 9.3 ys, range: 5.8-10.5, 12 boys and 9 girls), who were admitted to the hospital because of banal reason served the control group. We measured the total osteocalcin (OCN) serum level, the β-Crosslaps (β-CTx) serum concentration and the intact parathyroid hormone (PTH) level (electrochemiluminescence immunoassay [ECLIA]; Roche Elecsys System, Mannheim, Germany) by Elecsys 2010 immunoassay system (Hitachi, Japan). The 25-OH-vitamin D_3 serum level was investigated by fluorescence detection after high-pressure liquid chromatography (Bio-Rad Laboratories GmbH, München, Germany). Blood was also drawn from all patients and controls for analysis of serum calcium and ionised calcium (routine laboratory methods, Hitachi 912 analyser, Roche). Peripheral blood samples were analysed by FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) using CellQuest software version 3.1 (Becton Dickinson). The immunophenotype of the circulating white blood cells were determined by fluorochrome labelled monoclonal antibodies CD3, CD4, CD8, CD16, CD56, CD19 (Becton Dickinson Immunology Systems, San Jose, CA, USA), using double-colour or three-colour stainings. Cell surface expression of H1R and H2R on white blood cells were assessed by indirect immunofluorescence technique, by monoclonal H1R and H2R antibodies (developed by Molecular Immunology Research Group, Hungarian Academy of Sciences, Budapest, Hungary). The measurements were carried out without cell separation from whole blood. The leukocyte populations, (granulocytes, monocytes, lymphocytes) were defined after size and structure, using FS/SS dot blot histograms. Intracellular concentration of histamine and HDC of granulocytes, monocytes, lymphocytes were measured from permeabilized cells by indirect immunofluorescence staining method. Monoclonal anti-histamine antibody (Sigma Chemical Co., St. Louis, MO) and polyclonal anti-HDC antibody (Promega Corp., Madison, WI) were used as primary antibodies and FITC-labelled anti-mouse or anti-chiken IgGs were used as secondary antibodies in indirect immunostaining method. To control for the specificity of the antibodies, the autofluorescence of the cells and aspecificity of the secondary antibodies were also noted. Data are expressed as the relative fluorescence intensities against class-matched controls. Mann-Whitney test and Pearson correlation test were used (SPSS version 10.0, Chicago, IL, USA).

3.3. One hundred and twenty-five postmenopausal women with pollen allergy (mean age ± standard error: 61.26±0.57 ys) were recruited from an allergologist's practice for this cross-sectional study. The diagnosis of allergy was based on characteristic clinical and laboratory findings (skin prick tests, specific IgE assays). The 125 women were placed into four cohort groups on the basis of type of allergy treatment, or the lack of treatment. One group consisted of 43 allergic women who received neither inhalated corticosteroids nor H1R antagonist; the average duration of the pollen allergy among these women was 22.3 years (range: 7–45 years). A second group consisted of 53 allergic women who were being treated only with an H1R antagonist [either cetirizine (10 mg/day), loratidine (10 mg/day), or ketotifen (2 and 4 mg/day)]; the duration of pollen allergy among these women was 16.5 years (range: 7–15 ys). The third group consisted of 17 allergic patients who received an H1R antagonist (at the same doses mentioned above) and inhalated corticosteroid [either budesonide (maximal 512 μg/day), fluticasone (maximal 400 μg/day), or mometasone (maximal 36 μg/day)]; the allergic disease had existed for 8.5 years (range: 5–16 years) in this group of patients. The fourth group consisted of 12 allergic women who were treated only with an inhalated corticosteroid (at the doses mentioned above); the average duration of the pollen allergy was 7.4 years (range: 5–14 years) among these women. The minimal duration of allergic symptoms in each of the patients participating in the study was 5 years and, where relevant, the treatment had been seasonally used for 5 years or more. Most allergic symptoms occurred in every group, however they were of a shorter duration. One hundred non-allergic subjects from general practice served as controls; these were matched for age, body mass index (BMI), and age at menopause to the allergic subjects. Potential participants in both the allergic and non-allergic control groups with secondary causes of osteoporosis or those on medication likely to affect skeletal metabolism (with the exception of inhalated corticosteroids and H1R antagonists) were excluded. Bone mineral density (BMD, g/cm2) was measured for all of the allergic patients and controls at lumbar 2–4 vertebrae, left femoral neck [XR-46, Norland, Fort Atkinson, Wis.; coefficient of variation (CV %): 0.9 on vertebrae; 1.3 on femoral neck] and at the radius of the nondominant side (P-DEXA, Norland; CV%: 0.464) by the dual-energy X-ray absorptiometry method (DXA). Broadband ultrasound attenuation of the heel (BUA, dB/MHz, CV%: 1.92), speed of sound (SOS, m/s, CV%: 0.14), quantitative ultrasound index (QUI, CV%: 1.85) and estimated calcaneal BMD (CV%: 2.19) and it's T-score were assessed by the quantitative ultrasound (QUS) method (Sahara bone sonometer, Hologic, Waltham, Mass.). According to the World Health Organization (WHO) guidelines, BMD measurements were categorized as normal (T-score 1.0 or above), osteopenia (Tscore between -1.0 and -2.5), or osteoporosis (T-score 2.5 or

below). Diagnosis of osteopenia or osteoporosis was set up if T-score values were lower than -1.0 or ≤2.5 at one or more measured sites. Distal forearm, hip and validated (by morphometry) vertebral fractures with clinical symptoms that resulted from mild to moderate trauma (typically a fall to the floor from standing height or less) and had occurred within the past 10 years, and were confirmed by medical record were considered to be low-energy fractures. Fractures following severe trauma (a fall from greater than standing height, traffic accidents) and other fracture sites that are not listed above were excluded. Morphological vertebral fractures without relevant clinical symptoms were not considered. The patients were asked about skeletal pain, especially about back pain, by means of a questionnaire. T-test, Mann-Whitney test, Chi-squared test, Pearson's correlation test and multivariate logistic regression analysis was applied for statistical analysis (SPSS 10.0, Chicago, Ill., USA).

3.4. Nineteen pollen allergic men (previously diagnosed) were enrolled in this study (mean age ± SE: 56.63 ± 2.71 ys) from an allergology outpatient department. The diagnosis of allergy was based on characteristic clinical and laboratory findings. Four men did not receive H1R antagonist treatment. Fifteen men were on antihistamine therapy (the previously mentioned types and doses). The minimal duration of allergic symptoms in each of the patients participating in the study was 5 years and the treatment had been seasonally used for 5 years or more. Two patients in both allergic groups used inhalated corticosteroid therapy (types and doses mentioned above), however neither of them received systemic corticosteroid treatment. Most allergic symptoms occurred in every group, however they were of a shorter duration among the untreated allergic patients (2-3 weeks) than among antihistamine or inhalated corticosteroid treated patients (1-2 months). Neither antihistamine, nor corticosteroid treatment was recommended for patients with mild, or short period of allergic symptomes. Nineteen age and BMI matched non allergic control patients were selected from a general practice. Potential participants with diseases affecting bone metabolism or receiving medication likely to affect skeletal metabolism were excluded. Bone mass was measured by osteodensitometric methods (at femoral neck, lumbar spine and forearm) and non mass bone parameters were assessed by quantitative bone ultrasound (at heel) by the above mentioned devices. Bone fractures were determined according to patients' history and by vertebral morphometry. Antropometric data were also recorded. T-test, Mann Whitney test, Kruskal-Wallis test, Chi-squared test and Pearson's correlation test was used for analysis (SPSS 10.0, Chicago, Ill., USA).

4. Results

4.1. Ovariectomy: Lower BMC was measured in the ovariectomized WT mice than in the sham operated group (26.54±0.52 vs 32.9±1.19; p=0.001). The architecture of femora was more transparent and the cortical thickness was lower in the ovariectomized mice.

Ovariectomy and histidine-decarboxylase gene knock-out: Increased BMC was found among ovariectomized HDC-KO mice on histamine free diet in comparison with ovariectomized WT mice receiving histamine free diet (30.54±1.48 vs 26.54±0.52; p=0.02). No significant differences in BMC were found between sham operaed WT and OVX HDC-KO mice (32.9±1.19 vs 30.54±1.48). X-ray sheet film showed lesser transparency of femora and greater cortical thickness in HDC-KO, and no differences were detected between OVX HDC-KO and sham operated HDC-KO mice. Moreover, WT femora were thinner than HDC-KO femora.

Low calcium diet and histidine-decarboxylase gene knock-out: Lower BMC was detected in HDC-KO mice on low calcium diet than in HDC-KO on normal diet (29.08±0.58 vs 35.92±2.46; p=0.007). No BMC difference was measured between HDC-KO and WT animals receiving low calcium diet (29.08±0.58 vs 28.58±2.3).

Normal diet and histidine-decarboxylase gene knock-out: Statistical differences were not found in BMC between the two genotypic mice consuming normal diet, however, HDC-KO mice have slightly increased values than WT animals (35.92±2.46 vs 33.0±1.66).

Laboratory parameters: Serum calcium and phosphorus levels were similar in HDC-KO and WT mice, however, vitamin $1.25(OH)_2D_3$ serum level in HDC-KO significantly exceeded (even twice) the values of WT mice, independently from treatment.

4.2. Bone turnover markers: OCN, PTH and calcidiol did not differ between the antihistamine treated allergic patients and the controls. However, the H1R antagonist treated allergic children had lower serum β-CTx level than the untreated allergic (1090.82±80.25 vs 1275.76±51.72, p=0.047) and the control group (1456.58±95.81, p=0.001). β-CTx correlated with OCN in healthy subjects (r=0.845, p<0.001), and in H1R antagonist treated allergic children (r=0.519, p=0.005), however no correlation was found in untreated allergic patients. In all healthy and allergic subjects examined, calcidiol negatively correlated with PTH (r=-0.31, p=0.012) and β-CTx positively correlated with PTH concentration (r=0.226, p=0.046). In the separated groups no correlations were detected. All the patients and the controls had normal serum calcium (2.14-2.65 mmol/l) and normal serum ionised calcium level (1.1-1.3mmol/l).

Flow cytometry: We detected increased leukocyte (lymphocytes, monocytes, granulocytes) HDC expression in allergic patients, independently from antihistamine administration, in comparison with the control group. There was no statistical significant difference in the intracytoplasmatic histamine concentration between the groups. The lymphocyte surface H1R expression was lower in the allergic groups than in the controls. Lymphocyte H2R surface expression was found to be lower in H1R antagonist treated allergic children in comparison with controls. T cells with CD3+/CD16-56+ immunophenotype were in lower percentage in allergic children than in controls. We did not find statistically confirmed difference between the groups neither in the percentage distribution of the other T, NK and B cells (CD3+/CD4+, CD3+/CD8+, CD3-/CD16+56+, CD3-/CD8+, CD19+, Table 2.) nor in the CD4+/CD8+ ratio. Serum calcidiol negatively correlated with CD4+/CD8+ ratio (r=-0.803, p=0.016) in control children.

4.3. Descriptive statistics: Forty-six allergic patients (36.8% of all allergic) were obese (BMI>30 kg/m^2), and 49 allergic women (39.2% of all allergic) were overweight (25≤BMI≤30 kg/m^2). Of the non-allergic postmenopausal women that had been matched with the allergic ones for age, BMI, and age at menopause, 35% were obese and 41% were overweight.

Fracture prevalence and back pain: Allergic women who did not receive any treatment for pollen allergy had almost triple the rate of prevalent fractures [34.9% (15/43): 12 distal forearm, 1 clinical vertebral, 2 hip fractures] compared to non-allergic women [13% (13/100), p=0.003] and a 1.6-fold higher rate of back pain [72.1% (31/43) vs 44% (44/100), respectively; p=0.002]. Distal forearm fracture occurred more often among H1R-antagonist-only treated allergic women than among non-allergic controls [30.19% (16/53) vs 9% (9/100), respectively; p=0.001], and back pain was more frequent among H1R-antagonist-only treated allergic than among controls [73.6% (39/53) vs 44% (44/100), respectively; p<0.001]. Neither allergic patients treated only with H1R antagonist nor allergic patients treated with both an antagonist as well as an inhalated steroid had clinical vertebral or hip fractures. The bone fractures [50% (6/12) vs 29.4% (5/17), respectively] and the clinical vertebral and hip fractures in particular [8.3% (1/12) versus 0% (0/17), respectively] were more frequent in those receiving only inhalated steroid treatment than in those receiving both an inhalated steroid and antihistamine. Back pain appeared more often in allergic patients receiving only an inhalated steroid than in those on an inhalated steroid and H1R antagonist treatment [91.6% (11/12) vs 64.7% (11/17), respectively], however the differences were not statistically significant. Compared to the

controls, both the H1R-only treated group and the inhalated-corticosteroid-only group had significantly more low-trauma bone fractures. No documented hip or clinical vertebral fracture was found in those pollen allergic women who received only H1R antagonist or both H1R antagonist and inhalated corticosteroid.

Densitometric and quantitative ultrasound findings: Allergic patients who received neither inhalated corticosteroids nor H1R had significantly lower T- and Z-scores at the femoral neck than the non-allergic controls (T-score: -2.33±0.13 vs -1.93±0.12; Z-score: -0.67±0.13 vs -0.27±0.1), and in most of the allergic patients the other DXA and QUS parameters were lower (not statistically significant) than those of the nonallergic controls. Osteopenia was diagnosed more often among untreated pollen allergic women than among the controls, and normal bone density was not found at all in the former group. No differences were found with respect to the DXA nor in QUS results between allergic patients receiving H1R antagonist treatment and the nonallergic controls. Furthermore, some results of the allergic patients were slightly higher. A modest correlation was found between QUS and DXA results at all measurement sites (range: r=0.276–0.584) in the non-allergic controls, the untreated allergic women, and the H1R-only treated allergic women. However, no correlation was revealed between the QUS and DXA results in corticosteroid-treated allergic subjects, irrespective of the H1R antagonist treatment.

Prediction of fractures: In the control group, BMI-adjusted values for a 1 SD reduction in the lumbar 2–4 vertebral T-score predicted prevalent low-energy fractures with a significant (p=0.034) odds ratios of 2.01 (95% CI: 1.06–3.817). In allergic patients receiving no treatment, only the body mass index was able to predict prevalent fractures at 1.278 (95% CI:1.047–1.559, p=0.016) for a 1 kg/m2 increase in BMI, and this effect remained significant after an adjustment for age (OR=1.193; 95% CI: 1.017–1.4; p=0.031). The femoral neck BMD positively correlated with BMI in both the control group and in untreated allergic subjects, with a higher number of fractured patients found with higher BMD in the untreated allergic group. Neither DXA nor QUS values were able to predict bone fractures in any of the allergic groups. No significant differences were observed in age, BMI, and age at menopause between fractured and non-fractured subjects in the control group. Fractured patients had lower lumbar and forearm BMD and QUS values. In contrast, the fractured untreated pollen allergic patients did not differ from non-fractured untreated allergic women with respect to BMD at any of the skeletal sites tested and QUS values. The fractured allergic women were, however, older and more obese, and they had earlier menopause than their non-fractured counterparts. The fractured untreated allergic women had a higher lumbar BMD and a higher lumbar T-score

than the nonallergic non-fractured women. The non-fractured allergic patients were younger, and they had a lower femoral neck Z-score than the non-fractured control patients.

4.4. Overweight and obesity (25kg/m^2≤BMI) was very common among allergic men (57.9%) and among BMI matched controls. In the group of all allergic patients a slightly higher bone mass at lumbar spine and femoral neck was measured, however significantly higher bone mineral density was found at forearm region in comparison with non allergic controls (0.32±0.04 vs 0.38±0.018, $p<0.05$). The quantitative bone ultrasound parametrs were also higher (not significantly) in the allergic, considering all patients than in the controls. Only one patient suffered from bone fracture among the allergic, however 8 subjects had fracture in the control group (Chi-squared test, $p=0.007$). Slightly lower bone mass and significantly lower QUS parameters were measured in the untreaed allergic than in the H1R antagonist treated allergic men (SOS 1507±4.4 m/s vs 1539±7.6, $p=0.018$). The positive correlation between bone mass and BMI was confirmed both in the control ($r=0.548$, $p=0.015$), and in the entire allergic group ($r=0.484$, $p=0.036$). Weak positive correlation was calculated between DXA and QUS parameters in both the above mentioned groups (range: $r=0.283$ – $r=0.544$ for different parameters).

5. Summary, new findings

1. Our results showed a moderately increased bone mass in histidine-decarboxylase gene knock-out mice in comparison with control animals. Our data proved that oestogen depletion after ovariectomy did not cause decreased bone mineral content in histamine deficiency. Therefore, the role of histamine is presumable in the pathogenesis of bone loss in oestogen deficiency. The histamine depletion in histidine-decarboxylase gene knock-out did not protect against low calcium diet, moreover, elevated calcitriol serum level was measured in histamine deficiency. In conclusion the skeletal effect of histamine deficiency can be explained by an increased vitamin D activation.

2. No correlation was found between bone formation and resorption in multiplex allergic children without antihistamine administration, therefore coupling could be injured among this patients. On the contrary, the healthy controls had correlation between bone formation and resorption and H1R antagonists could restore this relationship in allergy, moreover antihistamines decrease bone resorption. The allergic disease and H1R antagonist treatment could have importance in skeletal maturation.

3. T cells with CD3+/CD16-56+ phenotype occurred in lower rate in allergic groups than in control blood samples. The mechanism by which CD3+/CD56+ cells may influence bone metabolism is, however, unknown. An increased tendency was found in CD4+/CD8+ (T helper/T cytotoxic-suppressor) ratio among allergic children. In our study vitamin D supply showed strong inverse association between CD4+/CD8+ ratio in healthy subjects. It is possible that increased CD4+/CD8+ ratio reflects a compromised vitamin D status.

4. Pollen allergic postmenopausal patients who received neither inhalated corticosteroids nor H1R antagonists had significantly lower bone density at the femoral neck than the non-allergic controls matched for age, gender and antropometric parameteres. In our cross-sectional analysis, we have found a higher bone fracture rate in pollen allergic postmenopausal women who had not been receiving treatment for pollen allergy in comparison to the controls. The bone fractures were more frequent in those receiving only inhalated steroid treatment than in those receiving antihistamine only or both an inhalated steroid and antihistamine.

The higher fracture rate in untreated pollen allergic patients, however, can not be explained by the possible unfavorable effects of inhalated corticosteroids. Our results show that an allergy, independent of corticosteroid administration, has an adverse effect on fracture risk.

5. We hypothesize that postmenopausal women who are at a higher risk for bone fractures due to allergic rhinitis and inhalated steroid therapy could be protected by an H1R antagonist since neither clinical vertebral nor hip fracture was found in the antihistamine-treated patient group independently from corticosteroid administration.

6. The low-energy fractures were not related to bone mass and quantitative ultrasound results among pollen allergic postmenopausal women. Our study confirms previously reported data concerning the association of fracture risk with BMD in postmenopausal non-allergic control patients; however, in allergic subjects, fracture risk is not related to bone density. In comparison with non-allergic subjects, untreated allergic patients had almost a threefold increase in low-energy fracture rate despite only a mild difference in bone mass. Since this result suggests an involvement of bone in pollen allergic postmenopausal patients that is independent of mineral density. QUS at the heel, with respect to SOS values, showed an ability to discriminate between women with or without fractures in the postmenopausal control group; this result is in concordance with that of other studies. However, this finding was not confirmed among allergic postmenopausal women. We found only moderately lower QUS values among non-treated fractured allergic patients in comparison with non-treated, non-fractured allergic women. A novel finding of this study is the moderately decreased QUS results in every allergic group. To date, no QUS data have been reported for patients being treated with inhalated corticosteroids. Fracture risk could be estimated by QUS in postmenopausal osteoporosis, however, the clinical usefulness of the method in different patient groups, such as in those with an allergy, has not established.

7. Low BMI is one of the main determinants of low bone mass according to previous results – and confirmed by our study – and it was found to be associated with low-energy fractures as an independent risk factor in primary osteoporosis. Despite the fact that overweight and obesity were very common among allergic postmenopausal women, we found a high prevalence of fractures in this group. The greater BMI by 1 kg/m2 was accompanied by almost a 1.3-fold higher risk for bone fractures among non-treated, pollen allergic, postmenopausal women.

8. Most of the recruited allergic men were on antihistamine therapy in our study. A slightly higher bone mass at lumbar spine and femoral neck was measured, and significantly higher bone density was found at forearm region in the allergic group in comparison with non allergic controls. The quantitative bone ultrasound parametrs were also slightly higher in the allergic group. Lower DXA and QUS parameters were measured in the untreaed allergic than in the H1R antagonist treated allergic men. Therefore, it is possible that antihistamine treatment not only protects against the unfavourable skeletal effect of histamin overproduction is allergy, but also further increases bone mass. We confirmed the positive correlation between DXA and QUS parameters in the non allergic and allergic men, similar to the previous findings in postmenopausal women.

9. We have found significantly lower bone fracture rate in the allergic group of patients (most of them were on antihistamine therapy) than in the controls. The bone fractures were not principally related to lower bone mass as it was established among postmenopausal pollen allergic women. The greates QUS parameters, BUA showing ultrasound attenuation, and SOS characterising speed of ultrasound, were measured in the antihistamine treated group of allergic men. In the antihistamine treated group SOS exceeded the value which was measured among allergic men who did not received antihistamine therapy. We conclude that antihistamines have advantageous effect on bone strength, which is mostly approached by SOS value. Our results suggest that H1R antagonists have favourable skeletal effect increasing bone elasticity, therefore protect against bone fractures among pollen allergic patients.

6. List of Papers

Publications related to the PhD thesis

1. Fitzpatrick L, Búzás E, Gagne TJ, Nagy A, Horváth C, **Ferencz V**, Mester Á, Kári B, Ming R, Falus A, Bársony J. Targeted deletion of histidine decarboxylase gene in mice increases bone formation and protect against ovariectomy-induced bone loss. Proc Natl Acad Sci U S A. 2003 13;100:6027-32.

2. **Ferencz V**, Meszaros S, Csupor E, Toth E, Bors K, Falus A, Horvath C.: Increased bone fracture prevalence in postmenopausal women suffering from pollen-allergy. Osteoporos Int 2006;24:1-8.

3. **Ferencz V**, Mészáros S, Csupor E, Tóth E, Bors K, Falus A, Horváth C. Nagyobb csonttörési prevalencia postmenopausas pollenallergiás nőkben. Ca és Csont 2007;10:22-30.

4. **Ferencz V,** Bojszkó Á, Pallinger É, Lakatos P, Falus A, Horváth Cs. Csökkent csontbontás H1 hisztamin receptor antagonistákkal kezelt allergiás gyermekekben Ca és Csont 2007; közlésre elfogadva

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