Rheumatol Int (2017) 37:1891–1898
DOI 10.1007/s00296-017-3836-9

Rheumatology INTERNATIONAL

BIOMARKERS

Serum serotonin levels and bone in rheumatoid arthritis patients

Miguel Bernardes[1,2] · Tiago Vieira[3] · Raquel Lucas[4,5] · Jorge Pereira[3] · Lúcia Costa[1] · Francisco Simões-Ventura[6] · Maria João Martins[7,8]

Received: 1 May 2017 / Accepted: 23 September 2017 / Published online: 9 October 2017
© Springer-Verlag GmbH Germany 2017

Abstract In rheumatoid arthritis (RA), a disease characterized by bone loss, increased levels of serotonin have been reported. Recent studies have demonstrated a role for circulating serotonin as a regulator of osteoblastogenesis, inhibiting bone formation. Thus, we measured serum serotonin levels (SSL) in a Portuguese sample of 205 RA patients and related these to anthropometric variables, disease parameters, serum bone biomarkers, and bone mineral density (BMD) assessed by dual-energy X-ray absorptiometry at several sites (total proximal femur, lumbar spine, left hand, and left second proximal phalange). SSL were inversely associated with body mass index (BMI) in RA women ($r = -0.218$; $p = 0.005$), independent of exposure to biologics and/or bisphosphonates. Among biologic naïves, there was an inverse association between SSL and osteoprotegerin in RA women ($r = -0.260$; $p = 0.022$). Serum β-CTX and dickkopf-1 were strongly associated with SSL in RA men not treated with bisphosphonates ($r = 0.590$; $p < 0.001$/$r = 0.387$; $p = 0.031$, respectively). There was also an inverse association between SSL and sclerostin in RA men ($r = -0.374$; $p < 0.05$), stronger among biologic naïve or bisphosphonates-unexposed RA men. In crude models, SSL presented as a significant negative predictor of total proximal femur BMD in RA women as well as in postmenopausal RA women. After adjustment for BMI, disease duration, and years of menopause, SSL remained a significant negative predictor of total proximal femur BMD only in postmenopausal RA women. Our data reinforce a role, despite weak, for circulating serotonin in regulating bone mass in RA patients, with some differences in terms of gender and anatomical sites.

Electronic supplementary material The online version of this article (http://doi.org/10.1007/s00296-017-3836-9) contains supplementary material, which is available to authorized users.

✉ Miguel Bernardes
mbernardes09@gmail.com

Tiago Vieira
tiago.sampaio.vieira@gmail.com

Raquel Lucas
rlucas@med.up.pt

Jorge Pereira
jorgepgpereira@gmail.com

Lúcia Costa
dias.costa.ml@gmail.com

Francisco Simões-Ventura
fsventura@zonmail.pt

Maria João Martins
mmartins@med.up.pt

[1] Department of Rheumatology, São João Hospital Center, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal

[2] Medicine Department, Faculty of Medicine, University of Porto, Porto, Portugal

[3] Department of Nuclear Medicine, São João Hospital Center, Porto, Portugal

[4] EPIUnit-Institute of Public Health, University of Porto, Porto, Portugal

[5] Department of Clinical Epidemiology, Predictive Medicine and Public Health, Faculty of Medicine, University of Porto, Porto, Portugal

[6] Faculty of Medicine, University of Porto (FMUP), Porto, Portugal

[7] Unit of Biochemistry, Department of Biomedicine, Faculty of Medicine, University of Porto, Porto, Portugal

[8] Instituto de Investigação e Inovação em Saúde (i3s), University of Porto, Porto, Portugal

Keywords DXA · Biochemical markers of bone turnover · Wnt/β-catenin/LRPs · Other diseases and disorders of/related to bone · Osteoimmunology

Introduction

Low-density lipoprotein receptor-related protein 5 (LRP5), a co-receptor for Wnt proteins, is known to have an important role in skeletal metabolism. Osteoporosis pseudoglioma (OPPG) syndrome [1, 2] and inherited high bone mass (HBM) phenotypes are caused by LRP5 mutations [1, 3–6].

On the other hand, it was suggested that LRP5 regulates bone mass in part by modulating circulating levels of serotonin [1, 6–8]. OPPG patients present with several degrees of increase in circulating serotonin levels [7, 9–11] and Danish families with different HBM-causing LRP5 mutations exhibit significantly lower levels of platelet poor plasma (PPP) serotonin than controls [1, 12, 13]. Further clinical evidence was provided by a cross-sectional study in women from a population-based sample in whom serum serotonin levels are inversely correlated with bone mass [1, 14]. Phenylketonuria, a disease in which an increase in circulating serotonin levels occurs, is also associated with low bone mass [9, 15, 16].

In animal models, serotonin was shown to directly suppress osteoblast function [7, 17, 18] as well as to lead to cyclin genes repression [7, 19–21]. Serotonin can be reduced by inhibiting tryptophan hydroxylase 1 (Tph1) expression, the rate-limiting enzyme in serotonin synthesis [1, 7, 22].

Serotonin was also shown to be involved in the control of the anabolic response of appendicular skeleton to physical activity [23]. In the condition of reduced physical activity, tryptophan-free diet-treated rats exhibit a significant reduction of bone formation and dietary tryptophan supplementation improves bone mass by increasing osteoblast activity [23].

In contrast to the data summarized above, Cui and colleagues provided evidence that serotonin does not interfere with the LRP5 effects on bone, but instead, LRP5 acts locally in the skeleton: (a) activation of a knock-in mutant LRP5 allele in the appendicular skeleton increases bone mass only in the limbs but not in the spine; (b) intestine-specific activation of HBM-causing LRP5 mutations has no effect on bone mass and measured serum serotonin levels are similar among HBM LRP5 knock-in, knock-out, or wild-type mice [1, 24]. Chang and colleagues also found similar serum serotonin levels in $LRP5^{-/-}$ mice and controls [1, 8].

In individuals from 2 kindreds with HBM-causing LRP5 mutations (G171V [13], N198S [2]) and using two different methodologies (ELISA and HPLC), no differences occur in serotonin levels in the PPP, serum, and platelet pellet (PP) between affected individuals and controls [1]. In carcinoid syndrome, a disease in which an increase in circulating serotonin levels is observed, there is neither association with lower bone density, poorer bone structure, nor lower bone formation markers [25].

In patients treated with selective serotonin reuptake inhibitors (SSRIs) [26–29], the previous studies show increased hip and wrist fractures [26, 27, 30, 31] and decreased bone mineral density (BMD) in femoral neck and total hip. In SSRI users, the association of compromised bone with low serum serotonin levels [26, 32–34] apparently contradicts the hypothesis of an inhibitory effect of circulating serotonin on bone formation as previously exposed [7, 26, 35]. One possible explanation is that SSRIs inhibit directly the serotonin transporter located on bone cell membranes, increasing local serotonin levels by reducing its removal from the bone cell microenvironment, despite the decrease in the circulating serotonin levels [19].

In rheumatoid arthritis (RA) patients, increased levels of serotonin versus healthy subjects have been reported, which may contribute to some extent to the excessive bone loss in this disease [36]. Recently, Klavdianou and colleagues confirmed these findings and showed that Ankylosing Spondylitis (AS) patients have lower serotonin levels than RA patients and healthy controls and that AS patients under anti-tumor necrosis factor alpha (TNFα) treatment exhibit even lower serotonin levels [37].

In view of these conflicting data, we revisited the relationship between bone mass and serum serotonin levels among a Portuguese cohort of RA patients to provide further evidence.

Patients and methods

Rheumatoid arthritis patients

During a 6-month period, RA patients, classified according to the American College of Rheumatology 1987 criteria, were consecutively included in the study protocol. Informed consent was obtained from each patient. The study protocol was approved by the local Ethical Committee in accordance with the principles of the 1964 Declaration of Helsinki [38].

Through a Rheumatology appointment, all subjects underwent clinical assessment using the Portuguese version of the Stanford Health Assessment Questionnaire (HAQ) and the disease activity score (DAS28) four variables (4v), to determine the RA disease state [39]. All past medication since admission to the outpatient clinic and ongoing medication were recorded. The smoking status (past and current) was registered and the body mass index (BMI) calculated.

Laboratory measurements

Between 08:00 and 10:00 h in the morning, a fasting blood sample was collected to evaluate C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies, creatinine, calcium, phosphorus, alkaline phosphatase, 25(OH) vitamin D3, intact parathormone (PTH), osteocalcin, β-isomerised carboxy-terminal cross-linking telopeptide of type I collagen (β-CTX), osteoprotegerin (OPG), receptor activator of nuclear factor–kappa B ligand (RANKL), dickkopf-1 (DKK-1), sclerostin, and serotonin levels. RA patients followed dietary restriction [of the following foods and drinks: tea, coffee, chocolate, avocados, bananas, tomatoes, plums, walnuts, eggplants, pineapples, citrus, and food with vanilla (namely candy from pastry shops)] and were asked not to take paracetamol or cough medicines for 48 h before and on the day of the blood sampling. Serum samples were stored at –70 °C for OPG, RANKL, DKK-1, sclerostin, and serotonin measurements by ELISA. All the specimens were measured in duplicate, according to the manufacturer's instructions and then averaged. OPG, DKK-1, RANKL, and sclerostin were determined as previously described [40]. Serotonin was measured using a kit from Labor Diagnostika Nord GmbH & Co, KG (Nordhorn, Germany); the intra- and inter-assay coefficients of variation were 3.9–5.4 and 6.0%, respectively.

Bone mineral density measurement

Dual-energy X-ray absorptiometry (DXA) (LUNAR Expert XL) was used to measure BMD at total left proximal femur, lumbar spine (L1–L4), left hand, and left second proximal phalange, according to a standardised procedure for each site. L1–L4 postero-anterior view was the spine region of interest for BMD measurements; all evaluable vertebrae were used and only were excluded those vertebrae affected by local structural change or artifact until a maximum of two. The entire hand (including the wrist bones but excluding the ends of the radius and ulna) was included in the analysis and all measurements were made using the "Hand" software package on GE Lunar scanners. One technician performed all DXA scans and the coefficients of variation of repeated measurements ranged from 0.9 to 1.5% for BMD at the different anatomical locations.

Statistical analyses

Qualitative data are described as absolute counts and proportions and quantitative data as mean and standard deviation. Pearson correlation coefficients were estimated to assess pairwise correlations between serum serotonin levels and the anthropometric, disease, laboratory, and DXA parameters evaluated here. The magnitude of associations between serum serotonin levels and the distinct BMD outputs were estimated from linear regression coefficients and respective 95% confidence intervals. Associations are presented as crude measures and adjusted to BMI, disease duration, years since menopause and serotonin levels to reduce confounding by those. All analyses were two-sided and p values < 0.05 were considered statistically significant. Statistical analyses were performed using the STATA® software (V.11, Statacorp, College Station, Texas, USA).

Results

Patient's characteristics

Two hundred and five patients with RA were included [women represented 80.5% ($n = 165$) of the entire sample], with a mean (SD) age and BMI of 54 [11] years and 27.1 (4.8) kg/m^2, respectively. The duration of the disease, measured from the date of diagnosis, was 14 [10] years. In terms of medications that potentially could affect the circulating levels of serotonin, we can refer the use of beta-blockers in 18 (9%) patients and specific serotonin reuptake inhibitors in 13 (6%).

The majority of the patients were seropositive for RF ($n = 119$, 58%) and anti-CCP ($n = 163$, 80%) antibodies.

The mean (SD) DAS28 (4v) and HAQ were 4.282 (1.360) and 1.250 (0.710), respectively. DAS28 remission criteria were present in 19 (9%) patients.

One hundred and three patients (50%) were under biological therapy, mainly TNF-α blockers (41%), and 171 (83%) under non-biological disease-modifying antirheumatic drugs (DMARDs). Daily prednisone dosage was 5.126 (3.900) mg, with the mean duration of the corticotherapy being 11 [9] years. Twenty-eight (14%) patients were ever-smokers.

One hundred patients (49%) were osteopenic or osteoporotic, 68 (33%) under bisphosphonates and 43 (21%) under vitamin D supplements. None of the 98 postmenopausal women (59%, among female patients) were under selective estrogen receptor modulators or taking hormone therapy (oral or transdermal estrogen preparations with or without a progestin). We defined menopause as the absence of menses for greater than 1 year.

Distributions of clinical and laboratory variables are shown in Supplemental Table 1.

Relation of circulating serotonin with anthropometric, DXA, clinical, and laboratory parameters

Serum serotonin levels were inversely associated with BMI values in the whole sample ($r = -0.220$; $p = 0.002$) and in RA women ($r = -0.218$; $p = 0.005$) (Supplemental

Table 1 Linear regression coefficients for the crude and adjusted associations between serum serotonin level and bone mineral density outcomes in rheumatoid arthritis patients in different skeletal regions

Outcome	Group	Predictors	β β[a]	*p* value	95% CI for β	
					Lower	Upper
Proximal femur bmd (g/cm^2)	Women	Serotonin	− 0.308	0.008	− 0.5332	− 0.082
	Pre menopausal women	Serotonin	− 0.340	0.091	− 0.736	0.056
	Post menopausal women	Serotonin	− 0.396 − 0.303[a]	0.003 0.005[a]	− 0.649 − 0.514[a]	− 0.143 − 0.092[a]
	Men	Serotonin	− 0.104 − 0.213[a]	0.642 0.390[a]	− 0.554 − 0.713[a]	0.347 0.286[a]
Lumbar spine bmd (g/cm^2)	Women	Serotonin	− 0.144	0.305	− 0.419	0.132
	Post menopausal women	Serotonin	− 0.220[a]	0.080[a]	− 0.446[a]	0.027[a]
	Men	Serotonin	− 0.177 − 0.246[a]	0.436 0.341[a]	− 0.633 − 0.765[a]	0.279 0.273[a]
Left hand bmd (g/cm^2)	Women	Serotonin	− 0.075	0.212	− 0.184	0.027
	Post menopausal women	Serotonin	− 0.079[a]	0.143[a]	− 0.184[a]	0.027[a]
	Men	Serotonin	− 0.067 − 0.119[a]	0.528 0.320[a]	− 0.280 − 0.360[a]	0.146 0.122[a]
Second left proximal phalange bmd (g/cm^2)	Women	Serotonin	− 0.038	0.450	− 0.138	0.062
	Post menopausal women	Serotonin	− 0.052[a]	0.277[a]	− 0.147[a]	0.042[a]
	Men	Serotonin	0.022 − 0.022[a]	0.814 0.832[a]	− 0.167 − 0.233[a]	0.210 0.189[a]

[a]Adjusted for BMI, disease duration, serum serotonin levels, and years of menopause (the later only for the postmenopausal women subgroup) in a multivariable model. β and 95% CI for β are ×1000

Table 2). The same association was found in RA patients and RA women neither treated with biologics ($r = -0.278$; $p = 0.005/r = -0.278$; $p = 0.014$, respectively) nor with bisphosphonates ($r = -0.226$; $p = 0.008/r = -0.221$; $p = 0.023$, respectively), nor with both ($r = -0.332$; $p = 0.009/r = -0.335$; $p = 0.025$, respectively) (data not shown). In addition, serum serotonin levels were not associated with current age.

In the whole sample, no associations were found between the serum levels of bone metabolism biomarkers and serotonin, with the exception, although weak, of intact PTH (Supplemental Table 2). The levels of the bone resorption marker β-CTX were positively associated with serum serotonin levels in RA men ($r = 0.334$; $p = 0.035$) (Supplemental Table 2) and RA men not treated with bisphosphonates ($r = 0.590$; $p < 0.001$) (data not shown). Serum levels of DKK-1 and serotonin were also positively associated in RA men not treated with bisphosphonates ($r = 0.387$; $p = 0.031$) (data not shown). Among biologic naïves, there was also an inverse but weak association between serum levels of serotonin and OPG in RA patients and RA women ($r = -0.224$; $p = 0.024/r = -0.260$; $p = 0.022$, respectively) (data not shown). Serum levels of serotonin and sclerostin were negatively associated in RA men ($r = -0.374$; $p = 0.021$) (Supplemental Table 2) and, particularly, in RA men neither treated with biologics ($r = -0.457$; $p = 0.028$) nor with bisphosphonates ($r = -0.393$; $p = 0.035$) (data not shown).

In the whole sample, no associations were found between serotonin levels and disease-related parameters (RA disease duration, years under corticosteroids, prednisolone daily dose, months under biologicals, months under DMARDs, ESR, CRP, DAS28(4v), swollen joint count, tender joint count, global health, and HAQ) (Supplemental Table 2). Years of RA disease duration and years under corticosteroids were inversely associated with serum serotonin levels in RA men ($r = -0.340$; $p = 0.032/r = -0.326$; $p = 0.004$, respectively) (Supplemental Table 2). A negative association between years of RA disease duration and serum serotonin levels was also demonstrated for RA men not treated with bisphosphonates ($r = -0.432$; $p = 0.015$) (data not shown).

Inverse correlations were also found between serum serotonin levels and total proximal femur BMD values in the whole sample and in RA patients not treated with bisphosphonates [$r = -0.195$; $p = 0.010$ (Supplemental Table 2)/$r = -0.231$; $p = 0.013$ (data not shown), respectively], and similarly in RA women and RA women not treated with bisphosphonates [$r = -0.226$; $p = 0.008$ (Supplemental Table 2]/$r = -0.240$; $p = 0.023$ (data not shown), respectively]. No associations were found between lumbar spine (L1–L4), left hand, and left second proximal phalange BMD values and serum serotonin levels in any of the groups (Supplemental Table 2). Nevertheless, when we restricted the analysis to biologic-naïve RA patients not treated with bisphosphonates, there was an inverse and weak

association between serum serotonin levels and lumbar spine BMD ($r = -0.277$; $p = 0.046$) at the threshold of statistical significance (data not shown).

In crude analysis, serum serotonin levels presented as a significant negative predictor of total proximal femur BMD values in female RA patients as well as in postmenopausal RA women (Table 1 and Fig. 1). Serum serotonin level was not a significant predictor of lumbar spine, left hand, and left second proximal phalange BMD values in RA patients (Table 1).

Since BMI, disease duration, and menopause could represent additional confounders, we computed adjusted models with DXA parameters as the dependent variables and BMI, years of disease duration, years since menopause and serum serotonin levels as covariates. Thus, for total proximal femur BMD in postmenopausal RA women, serum serotonin level remained a significant negative predictor even after BMI, disease duration, and years since menopause had been accounted for (Table 1). Furthermore, when we also added steroid, vitamin D, and bisphosphonates usage to the model, serum serotonin level remained negatively associated with total proximal femur BMD in postmenopausal RA women (data not shown), although we must be cautious and question the validity of this last model with the inclusion of so many covariates.

In the whole sample, no significant associations were found between the serum bone biomarkers levels and BMD values, with the exceptions, although weak, of alkaline phosphatase and left hand BMD ($r = -0.196$; $p = 0.012$), Dkk-1 and lumbar spine BMD ($r = -0.140$; $p = 0.050$), OPG and total proximal femur BMD ($r = -0.155$; $p = 0.037$), OPG and left hand BMD ($r = -0.171$; $p = 0.030$) (data not shown).

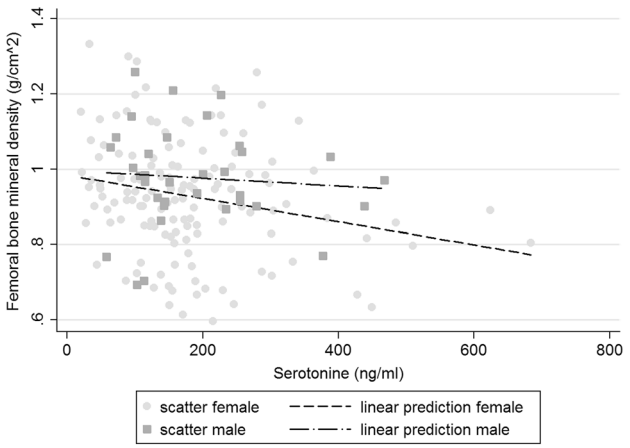

Fig. 1 Scatter plot and linear predictions of proximal femur bone mineral density according to serum serotonin levels in women and men with rheumatoid arthritis

Discussion

In our study, we demonstrate an inverse, despite weak, association between serum serotonin levels and total proximal femur BMD in postmenopausal RA women. This association was independent of BMI, disease duration, and years of menopause. Serum serotonin levels were also negatively correlated with proximal femur BMD in the subgroup of RA women not treated with bisphosphonates. This relation was not verified for premenopausal women or men, and serum serotonin level was not a significant predictor of lumbar spine, left hand, and left second proximal phalange BMD in RA patients. However, among biologic-naïve RA patients not treated with bisphosphonates, serum serotonin levels were also negatively correlated with lumbar spine BMD. Although anti-TNFα blockers may affect serotonin levels and BMD, simultaneously, and 40% of our RA patients were under these agents, serum serotonin levels did not correlate with the duration of biological treatment.

In our sample, serum serotonin levels (mean ± SD ng/mL, 179.3 ± 108.5) were similar compared with healthy subjects (mean ± SEM ng/mL, 177.4 ± 24.58) and lower compared with other RA patients (mean ± SEM ng/mL, 244.8 ± 37.5) from the Klavdianou study [37]. However, in this last study, there was a lack of control for dietary intake of tryptophan as in ours, and for serotonin measurement, they used a radioimmunoassay, while we used ELISA. The ideal situation would have been the inclusion of a healthy control group to corroborate Klavdianou findings of higher serum serotonin levels in RA patients versus healthy subjects, and this fact is a weakness of our study.

Our results are in accordance with those of Mödder and colleagues, who [14, 19] found negative associations between serum serotonin levels and femur neck total, trabecular volumetric BMD, and trabecular thickness in 275 women. They also found an inverse association with femur neck trabecular volumetric BMD in the premenopausal subgroup ($n = 185$) [14, 19]. Although these associations were statistically weaker than ours, unlike in our study, they did not control for dietary intake of tryptophan in the subjects submitted to serotonin measurements. Frost and colleagues have also reported an inverse association between serum serotonin and cortical thickness among 19 controls [13].

On the other hand, our study appears to contradict that of Wang and colleagues, who also investigated associations between serum serotonin and DXA and quantitative computed tomography bone traits in a Finnish sample (235 young women, 121 premenopausal women, 124 postmenopausal women, and 168 men) [26]. In postmenopausal women, they observed that serotonin was positively correlated with whole body and femur areal BMD, as well as with distal radius bone mineral content and volumetric BMD, and that these findings remained significant after adjustment for

weight [26]. In another study, Kim and colleagues reported no significant association between serotonin and lumbar or femoral BMD in 80 postmenopausal women, not on hormone therapy [41]. The discrepancy between the obtained results in the several population studies can be explained by differences in terms of sample size and dietary habits, associated with lack of diet control prior to the blood draw. In fact, serum serotonin level is deeply affected by tryptophan intake [26, 41, 42].

In our study, serum serotonin levels were inversely associated with BMI among RA women, independent of the cotreatment with bisphosphonates and/or biologics. However, this trend has already been described [14, 43–47]. Mödder and colleagues revealed that fat mass was the major driver of this association [14]. In addition, Wang and colleagues observed that serum serotonin was negatively correlated with weight, BMI, lean, and fat mass in women but positively with height and lean mass in men [26]. In line with this evidence, it has been suggested that serotonin plays an important role in the regulation of appetite, causing a reduction in the caloric intake. Fluoxetine, an SSRI, has been shown to result in significantly greater weight loss than placebo treatment [14, 48–51]. In counterpoint, blocking serotonin synthesis resulted not only in the prevention of serotonin-induced hypophagia, but also in an increased food intake [14, 52].

Regarding the proposed mechanisms of action of serotonin at bone metabolism, Yadav and colleagues [7, 14] demonstrated that gut-derived serotonin principally regulates bone formation in vivo and osteoblast proliferation in vitro, with no clear effect on bone resorption in vivo. However, these findings were in contrast with earlier studies, revealing that serotonin can enhance osteoclast differentiation in vitro [14, 53]. Positive associations between serum serotonin and aminoterminal propeptide of procollagen I (PINP) levels as well as between serum serotonin, CTX levels, and osteocalcine have also been reported in different subgroups [13, 14, 25, 26]. By contrast, Kim and colleagues observed that plasma serotonin level was correlated with serum total alkaline phosphatase but not with serum osteocalcin or CTX levels [41].

Interestingly, in our study and in RA men, we found a positive association of serum serotonin levels with β-CTX and a negative one with sclerostin, independent of the cotreatment with bisphosphonates and biologics/bisphosphonates, respectively. DKK-1 and serum serotonin levels were also positively associated in RA men not treated with bisphosphonates. Among biologic-naïves RA women, there was also an inverse association between serum serotonin levels and OPG. Our findings reflect a higher level of complexity in what concerns to serotonin effects on bone metabolism in humans, which seems to be not exclusively restricted to bone formation.

Although we know the importance of hormone replacement therapy in bone, in our study, it was not possible to evaluate the impact of such therapy, because none of our postmenopausal RA women were under hormone therapy. Meanwhile, some studies tried to elucidate whether serotonin may modulate the skeletal effects of estrogen on bone. Kim and colleagues revealed that the changes of circulating serotonin levels after 3 months of initiating hormone therapy did not associate with lumbar or femoral BMD variations determined after 1 year under hormone therapy, suggesting that the estrogen bone-preserving effect is independent of the peripheral serotonin actions [41]. In addition, the cross-sectional study by Mödder and colleagues did not detect a significant difference in serum serotonin level among postmenopausal women treated or not treated with estrogen replacement therapy [14, 41]. In another report of the same group and after a 4-month period of study, plasma levels of serotonin did not differ between controls and women treated with transdermal estradiol, either in peripheral or bone-marrow (BM) plasma samples [41, 54]. Moreover, the plasma concentration of peripheral serotonin was significantly higher than that of BM serotonin. We should note that serotonin concentrations may actually be very different, depending on the type of sample used, and this is, undoubtedly, an important issue to take into account when we compare several population studies.

Circulating serotonin derived mainly from the gastrointestinal enterochromaffin cells is rapidly taken up by platelets [14, 27, 55]. During serum collection, the platelets-stored serotonin is released; consequently, serum serotonin levels are approximately 100-fold higher than in PPP [14, 27, 36]. Nowadays, it is still unclear which is the best ‘‘index’’ of gut serotonin production: serum or PPP serotonin levels. Thus, it is possible that the weak associations we noted may have been different if we had used PPP instead of serum for our measurements. In addition, serotonin may be released from platelets in blood samples when the concentration of anticoagulant is inadequate [41, 56, 57]. In a previous small study, the recommended optimum concentration of EDTA2K was 7.4–9.9 mmol/L [41, 56], but we did not evaluate the concentration of EDTA2K in our collection samples.

Conclusion

Our study does provide support for a possible, despite weak, physiologic role for circulating serotonin levels in regulating femoral bone mineral density in postmenopausal women with RA, but not in premenopausal women or men with RA. The fact that we demonstrated that circulating serotonin is an independent predictor of femoral BMD in a disease that, by itself or by its treatments, largely influences the bone mass, is of some importance. These findings also suggest

that gender and, eventually, hormone status in women may contribute to the mechanism behind this link. In our sample, there is also a weak connection between circulating serotonin levels and bone metabolism markers. The clinical significance of these weak associations remains unclear. However, these data reinforce recent findings indicating that increased serotonin signalling has negative effects on bone. Further studies in RA cohorts are needed to validate our findings, elucidating the potential role of serotonin in bone metabolism regulation in humans.

Acknowledgements Authors' roles: MB conceived the idea of the study, designed the study, coordinated the project, participated in patient recruitment and in data acquisition, drafted the manuscript, and takes responsibility for the integrity of the data analysis. RL performed the statistical analysis, revised the manuscript, and takes responsibility for the integrity of the data analysis. TV and JP performed DXA evaluations and approved the final version of the manuscript. LC participated in patient recruitment and approved the final version of the manuscript. FSV coordinated the project, participated in patient recruitment and revised the manuscript. MJM conceived the idea of the study, coordinated the experimental work (ELISA), assisted in manuscript drafting, and takes responsibility for the integrity of the data analysis. The authors wish to acknowledge Associação Nacional de Reumatologia for the doctoral grant, Conceição Gonçalves (MSc) from the Laboratório Nobre in the Faculty of Medicine of the University of Porto, the nursing Service of Rheumatology Day Hospital of São João Hospital Center, and the clinicians from the Rheumatology Department of São João Hospital Center, especially Alexandra Bernardo (M.D.) and Sofia Pimenta (M.D.).

Funding Associação Nacional de Reumatologia, the study sponsor, had no role in the study design; in the collection, analysis, and interpretation of the data; in the writing of the report; and in the decision to submit the paper for publication.

Compliance with ethical standards

Conflict of interest The authors declare there is no conflict of interests regarding the publication of this paper.

Ethical approval The study protocol was approved by the local Ethical Committee, *Comissão de Ética para a Saúde do Centro Hospitalar de São João do Porto*, in accordance with the principles of the 1964 Declaration of Helsinki [38].

References

1. Lee GS, Simpson C, Sun BH, Yao C, Foer D, Sullivan B et al (2014) Measurement of plasma, serum, and platelet serotonin in individuals with high bone mass and mutations in LRP5. J Bone Miner Res 29:976–981
2. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM et al (2001) LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 107:513–523
3. Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA et al (2002) High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med 346:1513–1521
4. Van Wesenbeeck L, Cleiren E, Gram J, Beals RK, Benichou O, Scopelliti D et al (2003) Six novel missense mutations in the LDL receptor-related protein 5 (LRP5) gene in different conditions with an increased bone density. Am J Hum Genet 72:763–771
5. Whyte MP, Reinus WH, Mumm S (2004) High-bone-mass disease and LRP5. N Engl J Med 350:2096–2099 (**author reply -9**)
6. Goltzman D (2011) LRP5, serotonin, and bone: complexity, contradictions, and conundrums. J Bone Miner Res 26:1997–2001
7. Yadav VK, Ryu JH, Suda N, Tanaka KF, Gingrich JA, Schutz G et al (2008) Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. Cell 135:825–837
8. Chang MK, Kramer I, Keller H, Gooi JH, Collett C, Jenkins D et al (2014) Reversing LRP5-dependent osteoporosis and SOST deficiency-induced sclerosing bone disorders by altering WNT signaling activity. J Bone Miner Res 29:29–42
9. Karsenty G, Yadav VK (2011) Regulation of bone mass by serotonin: molecular biology and therapeutic implications. Annu Rev Med 62:323–331
10. Yadav VK, Arantes HP, Barros ER, Lazaretti-Castro M, Ducy P (2010) Genetic analysis of Lrp5 function in osteoblast progenitors. Calcif Tissue Int 86:382–388
11. Saarinen A, Saukkonen T, Kivela T, Lahtinen U, Laine C, Somer M et al (2010) Low density lipoprotein receptor-related protein 5 (LRP5) mutations and osteoporosis, impaired glucose metabolism and hypercholesterolaemia. Clin Endocrinol 72:481–488 **(Oxf)**
12. Frost M, Andersen TE, Yadav V, Brixen K, Karsenty G, Kassem M (2010) Patients with high-bone-mass phenotype owing to Lrp5-T253I mutation have low plasma levels of serotonin. J Bone Miner Res 25:673–675
13. Frost M, Andersen T, Gossiel F, Hansen S, Bollerslev J, van Hul W et al (2011) Levels of serotonin, sclerostin, bone turnover markers as well as bone density and microarchitecture in patients with high-bone-mass phenotype due to a mutation in Lrp5. J Bone Miner Res 26:1721–1728
14. Modder UI, Achenbach SJ, Amin S, Riggs BL, Melton LJ 3rd, Khosla S (2010) Relation of serum serotonin levels to bone density and structural parameters in women. J Bone Miner Res 25:415–422
15. Giovannini M, Valsasina R, Longhi R, Cesura AM, Galva MD, Riva E et al (1988) Serotonin and noradrenaline concentrations and serotonin uptake in platelets from hyperphenylalaninaemic patients. J Inherit Metab Dis 11:285–290
16. Modan-Moses D, Vered I, Schwartz G, Anikster Y, Abraham S, Segev R et al (2007) Peak bone mass in patients with phenylketonuria. J Inherit Metab Dis 30:202–208
17. Gustafsson BI, Thommesen L, Stunes AK, Tommeras K, Westbroek I, Waldum HL et al (2006) Serotonin and fluoxetine modulate bone cell function in vitro. J Cell Biochem 98:139–151
18. Galli C, Macaluso G, Passeri G (2013) Serotonin: a novel bone mass controller may have implications for alveolar bone. J Negat Results Biomed 12:12
19. Bliziotes M (2010) Update in serotonin and bone. J Clin Endocrinol Metab 95:4124–4132
20. Yadav VK, Ducy P (2010) Lrp5 and bone formation: a serotonin-dependent pathway. Ann N Y Acad Sci 1192:103–109
21. Kode A, Mosialou I, Silva BC, Rached MT, Zhou B, Wang J et al (2012) FOXO1 orchestrates the bone-suppressing function of gut-derived serotonin. J Clin Invest 122:3490–3503
22. Tamai K, Zeng X, Liu C, Zhang X, Harada Y, Chang Z et al (2004) A mechanism for Wnt coreceptor activation. Mol Cell 13:149–156
23. Sibilia V, Pagani F, Dieci E, Mrak E, Marchese M, Zarattini G et al (2013) Dietary tryptophan manipulation reveals a central role for serotonin in the anabolic response of appendicular skeleton to physical activity in rats. Endocrine 44:790–802
24. Cui Y, Niziolek PJ, MacDonald BT, Zylstra CR, Alenina N, Robinson DR et al (2011) Lrp5 functions in bone to regulate bone mass. Nat Med 17:684–691

25. Walsh JS, Newell-Price JD, DeBono M, Adaway J, Keevil B, Eastell R (2013) Circulating serotonin and bone density, structure, and turnover in carcinoid syndrome. J Clin Endocrinol Metab 98:2902–2907
26. Wang Q, Chen D, Nicholson P, Cheng S, Alen M, Mao L et al (2014) The associations of serum serotonin with bone traits are age- and gender-specific. PLoS ONE 9:e109028
27. Richards JB, Papaioannou A, Adachi JD, Joseph L, Whitson HE, Prior JC et al (2007) Effect of selective serotonin reuptake inhibitors on the risk of fracture. Arch Intern Med 167:188–194
28. Diem SJ, Blackwell TL, Stone KL, Yaffe K, Haney EM, Bliziotes MM et al (2007) Use of antidepressants and rates of hip bone loss in older women: the study of osteoporotic fractures. Arch Intern Med 167:1240–1245
29. Williams LJ, Henry MJ, Berk M, Dodd S, Jacka FN, Kotowicz MA et al (2008) Selective serotonin reuptake inhibitor use and bone mineral density in women with a history of depression. Int Clin Psychopharmacol 23:84–87
30. Liu B, Anderson G, Mittmann N, To T, Axcell T, Shear N (1998) Use of selective serotonin-reuptake inhibitors or tricyclic antidepressants and risk of hip fractures in elderly people. Lancet 351:1303–1307
31. Vestergaard P, Rejnmark L, Mosekilde L (2006) Anxiolytics, sedatives, antidepressants, neuroleptics and the risk of fracture. Osteoporos Int 17:807–816
32. Karege F, Widmer J, Bovier P, Gaillard JM (1994) Platelet serotonin and plasma tryptophan in depressed patients: effect of drug treatment and clinical outcome. Neuropsychopharmacology 10:207–214
33. Rothman RB, Zolkowska D, Baumann MH (2008) Serotonin (5-HT) transporter ligands affect plasma 5-HT in rats. Ann N Y Acad Sci 1139:268–284
34. Zolkowska D, Baumann MH, Rothman RB (2008) Chronic fenfluramine administration increases plasma serotonin (5-hydroxytryptamine) to nontoxic levels. J Pharmacol Exp Ther 324:791–797
35. Yadav VK, Balaji S, Suresh PS, Liu XS, Lu X, Li Z et al (2010) Pharmacological inhibition of gut-derived serotonin synthesis is a potential bone anabolic treatment for osteoporosis. Nat Med 16:308–312
36. Kopp S, Alstergren P (2002) Blood serotonin and joint pain in seropositive versus seronegative rheumatoid arthritis. Mediators Inflamm 11:211–217
37. Klavdianou K, Liossis SN, Papachristou DJ, Theocharis G, Sirinian C, Kottorou A et al (2016) Decreased serotonin levels and serotonin-mediated osteoblastic inhibitory signaling in patients with ankylosing spondylitis. J Bone Miner Res 31:630–639
38. Reynolds T (2000) Declaration of Helsinki revised. J Natl Cancer Inst 92:1801–1803
39. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL (1995) Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 38:44–48
40. Bernardes M, Vieira TS, Martins MJ, Lucas R, Costa L, Pereira JG et al (2017) Myocardial Perfusion in Rheumatoid Arthritis Patients: Associations with Traditional Risk Factors and Novel Biomarkers. Biomed Res Int 2017:6509754
41. Kim HY, Park MH, Yoon HK, Han KO (2012) The changes in plasma serotonin levels after hormone therapy and their relationship with estrogen responsiveness on bone in postmenopausal women. J Clin Endocrinol Metab 97:1986–1994
42. Gershon MD, Tack J (2007) The serotonin signaling system: from basic understanding to drug development for functional GI disorders. Gastroenterology 132:397–414
43. Blundell JE (1984) Serotonin and appetite. Neuropharmacology 23:1537–1551
44. Blundell JE (1986) Serotonin manipulations and the structure of feeding behaviour. Appetite 7(Suppl):39–56
45. Curzon G (1990) Serotonin and appetite. Ann N Y Acad Sci 600:521–530 (**discussion 30-1**)
46. Leibowitz SF, Weiss GF, Suh JS (1990) Medial hypothalamic nuclei mediate serotonin's inhibitory effect on feeding behavior. Pharmacol Biochem Behav 37:735–742
47. Simansky KJ (1996) Serotonergic control of the organization of feeding and satiety. Behav Brain Res 73:37–42
48. McGuirk J, Silverstone T (1990) The effect of the 5-HT re-uptake inhibitor fluoxetine on food intake and body weight in healthy male subjects. Int J Obes 14:361–372
49. Pijl H, Koppeschaar HP, Willekens FL, Op de Kamp I, Veldhuis HD, Meinders AE (1991) Effect of serotonin re-uptake inhibition by fluoxetine on body weight and spontaneous food choice in obesity. Int J Obes 15:237–242
50. Lawton CL, Wales JK, Hill AJ, Blundell JE (1995) Serotoninergic manipulation, meal-induced satiety and eating pattern: effect of fluoxetine in obese female subjects. Obes Res 3:345–356
51. Ward AS, Comer SD, Haney M, Fischman MW, Foltin RW (1999) Fluoxetine-maintained obese humans: effect on food intake and body weight. Physiol Behav 66:815–821
52. MacKenzie RG, Hoebel BG, Ducret RP, Trulson ME (1979) Hyperphagia following intraventricular p-chlorophenylalanine-, leucine- or tryptophan-methyl esters: lack of correlation with whole brain serotonin levels. Pharmacol Biochem Behav 10:951–955
53. Battaglino R, Fu J, Spate U, Ersoy U, Joe M, Sedaghat L et al (2004) Serotonin regulates osteoclast differentiation through its transporter. J Bone Miner Res 19:1420–1431
54. Modder UI, Roforth MM, Hoey K, McCready LK, Peterson JM, Monroe DG et al (2011) Effects of estrogen on osteoprogenitor cells and cytokines/bone-regulatory factors in postmenopausal women. Bone 49:202–207
55. Jonnakuty C, Gragnoli C (2008) What do we know about serotonin? J Cell Physiol 217:301–306
56. Hirowatari Y, Hara K, Kamihata H, Iwasaka T, Takahashi H (2004) High-performance liquid chromatographic method with column-switching and post-column reaction for determination of serotonin levels in platelet-poor plasma. Clin Biochem 37:191–197
57. Pussard E, Guigueno N, Adam O, Giudicelli JF (1996) Validation of HPLC-amperometric detection to measure serotonin in plasma, platelets, whole blood, and urine. Clin Chem 42:1086–1091