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The decrease of soluble RAGE levels in rheumatoid arthritis patients following hormone replacement therapy is associated with increased bone mineral density and diminished bone/cartilage turnover: a randomized controlled trial

Rille Pullerits[1,*], Helena Forsblad d'Elia[1,*], Andrej Tarkowski[1,†] and Hans Carlsten[1]

Objective. The aim of the study was to prospectively investigate the effects of HRT on serum soluble receptor for advanced glycation end product (sRAGE) levels in RA patients and to determine whether sRAGE production is related to bone/cartilage metabolism.
Methods. Eighty-eight post-menopausal RA patients were randomized to receive vitamin D3 and calcium supplementation with or without HRT (oestradiol plus noretisterone acetate). The levels of total sRAGE in sera were measured before, 1 and 2 years after treatment initiation. Potential associations between sRAGE levels, bone/cartilage metabolic markers and BMD were investigated.
Results. Patients receiving HRT displayed significantly decreased levels of serum sRAGE at 1 and 2 years as compared with levels at study entry. The increase in serum oestradiol was associated with the decline in sRAGE levels. Importantly, sRAGE levels at baseline significantly correlated with bone/cartilage turnover markers including C-terminal propeptide of type I procollagen, carboxyterminal telopeptide of type I collagen and cartilage oligomeric matrix protein, and the decrease of sRAGE levels paralleled with diminished concentration of these molecules. BMD in hip and femoral neck and progression of Larsen score at 1 year were associated with baseline sRAGE levels. The decline in sRAGE levels significantly correlated with an increase in total BMD following 2 years of treatment in patients receiving HRT but not in the control group.
Conclusion. Our findings suggest that HRT decreases the levels of endogenous sRAGE in post-menopausal RA patients implicating its role in sRAGE regulation. In addition, serum sRAGE was associated with BMD and markers of bone/cartilage metabolism. These data suggest that sRAGE is involved directly or indirectly in bone metabolism.
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Key words: Soluble receptor for advanced glycation end products, Rheumatoid arthritis, Post-menopausal, Hormone replacement therapy.

Introduction

RA is a chronic systemic polyarticular inflammatory disease that involves not only joints but also affects several organs, and is associated with excessive disability, mortality and morbidity. It is characterized by chronic joint inflammation and variable degrees of bone and cartilage destruction leading to functional impairment. The pathogenesis of the disease is complex and extensive research in the past few decades has elucidated that a wide range of endogenous pro-inflammatory molecules play a role in the disease pathogenesis.

The receptor for advanced glycation end products (RAGE) is a multi-ligand member of the immunoglobulin superfamily and serves as a pattern recognition receptor for several endogenous ligands that are potent inducers of inflammation [1, 2]. The RAGE repertoire of ligands that could be found in the inflamed joints, as well as in the circulation of RA patients includes advanced glycoxidation end products (AGEs) [3], the β-amyloid protein [4], the S100/calgranulin family of pro-inflammatory molecules [5] and high mobility group box chromosomal protein 1 (HMGB1) [6]. Engagement of cell-bound RAGE by a ligand results in amplification of inflammatory response by many of the cells that participate in the development of RA, including macrophages, neutrophils and T cells [7, 8]. Furthermore, the endothelial cells and smooth muscle cells in RA patients' joints as well as inflamed synovial tissue show increased expression of RAGE [8, 9]. The receptor is also expressed by cells participating in bone turnover including osteoclasts and osteoblasts [10].

The soluble RAGE (sRAGE) is a truncated form of the receptor that lacks the cytosolic and transmembrane domains, i.e. signal transferring parts. It has been suggested to function as a 'decoy' protecting molecule because (i) it competes with cell-bound RAGE for ligand binding or (ii) it binds up excess of ligands destined for multiple receptors and therefore acts as an anti-inflammatory molecule [11].

The properties of sRAGE and the exact mechanisms by which this molecule regulates inflammatory responses remain unclear. Some experimental animal models demonstrated that treatment with sRAGE prevents cell-bound RAGE activation and thereby reduces inflammatory responses [12–14]. However, a body of evidence shows that sRAGE has properties other than only being a selective RAGE blocker or decoy receptor [15, 16]. Goova *et al*. [16] demonstrated that topical treatment with sRAGE significantly improved wound healing due to the accelerated inflammatory response in skin ulcers. We have recently shown in an animal model and *in vitro* that sRAGE on its own exerts strong pro-inflammatory activity that is mediated via Mac-1/NF-κB pathway [17].

A number of clinical studies have focused on the potential significance of circulating sRAGE levels in a variety of pathophysiological conditions [18, 19]. Several studies have demonstrated decreased sRAGE levels and its association with vascular complications in patients with cardiovascular and metabolic diseases such as diabetes [20, 21], coronary artery disease [22], essential hypertension [23] and atherosclerosis [24, 25]. We have recently demonstrated that patients with RA display lower serum levels of sRAGE as compared with patients with non-inflammatory joint disease and healthy controls [26]. On the other hand, elevated

[1]Department of Rheumatology and Inflammation Research, University of Gothenburg, Gothenburg, Sweden.

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Correspondence to: Rille Pullerits, Department of Rheumatology and Inflammation Research, Sahlgrenska Academy at Göteborg University, Guldhedsgatan 10A, 41346, Göteborg, Sweden.
E-mail: rille.pullerits@rheuma.gu.se
*Rille Pullerits and Helena Forsblad d'Elia equally contributed to this work.
†Deceased.

plasma sRAGE concentrations that were associated with prognostic outcome were detected in septic patients [27], and in patients with heart failure [28] and renal disease [29]. Furthermore, patients with SSc have been shown to display elevated sRAGE levels that are associated with disease severity and immunological abnormalities of the disease [30].

It has long been recognized that oestrogen has an immunomodulatory role in several rheumatic diseases including RA. During pregnancy, when oestrogen levels are increased, the disease activity is ameliorated in 75% of women with RA, whereas within 3 months after delivery, a relapse is observed in 90% of patients [31, 32]. HRT in RA patients has been shown to have beneficial effects in preventing bone loss, radiographic joint destruction and suppressing disease activity [33–36]. Recent observations implicate the involvement of oestrogen in RAGE/sRAGE signalling. It has been demonstrated that oestrogen inhibits the synthesis of AGEs, the substrate of RAGE in vaginal epithelial tissues of post-menopausal women [37, 38]. In addition, 17β-oestradiol (E_2) has been shown to induce the expression of RAGE in *in vitro*-cultured human endothelial cells [39].

It is unknown whether oestrogen affects sRAGE levels in RA patients. To address this issue, we examined post-menopausal women with RA in a prospective 2-year randomized, single blinded, controlled study where half of the patients received HRT (oestradiol plus noretisterone acatate) in addition to calcium and vitamin D substitution. Our aim was to investigate (i) how treatment with HRT influences serum sRAGE levels and (ii) whether baseline sRAGE levels and changes in its production are related to bone/cartilage metabolism and clinical disease characteristics.

Materials and methods

Study design and treatment regimen

Women with RA aged between 45 and 65 years were identified from Rheumatology Outpatient Clinic Registers in Gothenburg and Borås in Sweden. Included participants were all post-menopausal, fulfilled the American Rheumatism Association 1987 revised criteria for adult RA [40], and had active disease that met at least two of the following criteria: more than six painful joints; more than three swollen joints; ESR >20 mm/h; CRP >10 mg/l.

Treatment with DMARDs was stable for the past 3 months. A maximum daily dose of 7.5 mg of prednisolone was accepted and IA and intramuscular glucocorticosteroid injections were allowed during the study period. The patients had not been treated during the past 2 years with oestrogen or bisphosphonates and had no history of venous thrombosis, embolism or anamnesis for cancer in reproductive system or breasts.

The randomization process has been described in a previous publication [33]. Patients were allocated to one of the two treatment groups, the HRT group or the control group, using simple randomization, by an independent research nurse at the Department of Gynecology. A random number generator for up to 100 patients was used. Eighty-eight patients were included and at that time point 41 women had been allocated to the HRT group and 47 to the control group.

All patients received a daily dose of 500 mg calcium and 400 international units vitamin D_3. The HRT group was treated additionally with either: (i) continuously with 2 mg E_2 plus 1 mg norethisterone acetate (NA) daily (patients who were >2 years post-menopausal, $n=19$); or (ii) only 2 mg E_2 daily (those with a previous history of hysterectomy, $n=4$); or (iii) sequentially with 2 mg micronized oestradiol for 12 days followed by 10 days of E_2 plus 1 mg NA followed by 6 days of 1 mg E_2 (remaining patients, $n=12$).

The investigators at the rheumatology departments were blinded to the HRT. Regular medication for RA could be altered by the clinician but not by the investigator.

The evaluation of the effect of the HRT on circulating sRAGE levels in these patients was added as a secondary endpoint of the study. Serum samples to detect sRAGE levels were available for 81 patients that were included in analysis: 35 patients receiving HRT and 46 patients control treatment. Three patients (HRT: $n=3$) did not leave the blood samples at 1 year but completed the study at 2 years. Serum samples were not available for four patients at 2 years (HRT: $n=1$; controls: $n=3$). Data regarding other diseases that could potentially influence sRAGE levels were analysed. Twelve patients had been diagnosed with essential hypertension; the distribution of patients with hypertension was similar between groups [HRT: $n=5$ (14%) and control group: $n=7$ (15%)]. Only three patients included in the study had type II diabetes and two patients had previously known cardiovascular disease that was considered not to affect the statistical analysis. None of the patients had renal insufficiency. For further information see previous report [33].

All the patients gave informed consent and the study was approved of by the Ethics Committee at Sahlgrenska Academy at Gothenburg University.

Patient population

The baseline characteristics of patients are summarized in Table 1.

At baseline, there were no significant differences between the study groups regarding treatment with DMARDs, NSAIDs, glucocorticoids and disease activity measurements.

The majority of patients, 66/81 (81%) received treatment with different DMARDs. Among DMARDs, MTX dominated and was used by 30 patients (37%), 10 women (12%) were treated with SSZ and 10 patients (12%) received treatment with oral or parenteral gold salt compounds. Other different DMARDs were used by few patients. The mean dosage of prednisolone was 4.6 mg daily.

None of the patients received anti-TNF agents or anti-B-cell therapy during the study period. The proportions of the patients treated with DMARDs, MTX and corticosteroids did not differ between the groups at any time point.

Assessment of clinical and laboratory parameters

Venous blood samples. These were obtained at study entry, after 12 and 24 months. The serum samples were stored at −70°C until the time of analysis. Serum samples from all time points were analysed simultaneously regarding sRAGE levels. The routine laboratory parameters (ESR, CRP, creatinine, blood cell count) were measured consecutively.

TABLE 1. The baseline characteristics of patients in the HRT group and control group

	HRT group ($n=35$)	Control group ($n=46$)	*P*-value
Serum sRAGE, pg/ml	1504 (1225–1828)	1395 (962–1966)	0.45
Age, mean ± S.D., years	56.7 ± 5.5	58.2 ± 4.7	0.36
Disease duration, mean ± S.D., years	16.2 ± 11.9	15.6 ± 11.8	0.82
Duration of menopause, mean ± S.D., years	7.9 ± 5.3	8.4 ± 5.4	0.79
DMARDs	30 (86)	36 (78)	0.57
NSAIDs	29 (36)	35 (43)	0.59
Glucocorticoids at present	8 (23)	9 (20)	0.79
Positive RF	24/30 (80)	29/36 (81)	0.99
Erosive disease	31/35 (89)	40/44 (90)	0.73
Larsen score	1.15 (0.31–2.14)	1.31 (0.70–2.07)	0.64
DAS28	5.3 (4.6–6.0)	5.2 (4.7–6.0)	0.67
ESR, mm/h	26 (17–47)	24 (16–38)	0.27
Creatinine, μmol/l	79 (72–87)	77 (71–84)	0.41

Values are median (25th–75th percentiles) if not otherwise indicated. Regarding treatment and disease characteristics, the number of patients (%) in each group is shown.

Oestradiol. E_2 levels in serum were measured (~12 h after tablet intake) at baseline and yearly thereafter using RIA (Clinical Assays™, DiaSorin, Vercelli, Italy). The detection limit of the test is 18 pmol/l. Some data were missing due to technical problems and were available in 68 patients at study entry (HRT: $n=28$, control: $n=40$), 65 patients at 1 year (HRT: $n=25$, control: $n=40$) and 67 patients at 2 years (HRT: $n=30$, control: $n=37$).

Detection of sRAGE. The levels of sRAGE were determined using a specific sandwich ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. ELISA plates coated with mouse monoclonal antibody against RAGE were used for quantitative detection of sRAGE. After incubation with serum samples, polyclonal capture antibody against the extracellular portion of RAGE was used. The minimum detectable concentration of sRAGE was 4 pg/ml. According to the manufacturer, no significant cross-reactivity to EN-RAGE, HMGB1, S100A10 or S100Baa is observed.

Markers of bone and cartilage turnover. RIA was used for the quantitative determination in serum of the bone resorption marker, carboxyterminal telopeptide of type I collagen (ICTP) and for the collagen type I turnover marker, C-terminal propeptide of type I procollagen (PICP) (Orion Diagnostica, Espoo, Finland). The detection limit of the tests were ICTP 0.5 μg/l and PICP 1.2 μg/l. Cartilage oligomeric matrix protein (COMP), a cartilage-turnover marker, was measured in serum by ELISA (AnaMar Medical, Lund, Sweden). The detection limit of the test was 0.1 U/l.

Radiographs. Radiographs of the hands, wrists and forefeet were evaluated at baseline, and after 12 and 24 months according to Larsen [41]. Briefly, 40 joints were scored in each patient from 0 (normal) to 5 (maximal destruction). The scores for each patient were summarized and then divided by the number of examined joints to give the mean Larsen score for each patient ranging from 0 to 5.

BMD. BMD at left forearm, left total hip, left femoral neck, lumbar spine and total BMD were measured at study entry, at 12 and 24 months by dual energy X-ray absorptiometry (DXA; Hologic®, Bedford, MA, USA). Regarding total BMD, patients with prostheses and previous joint replacement surgery with osteosynthetic materials were excluded from statistical analysis in order to avoid false values.

Statistical analysis

Descriptive statistics is presented as medians (25th–75th percentiles).

The majority of data were not normally distributed and thus non-parametrical tests were employed. Comparisons between the groups were made using Mann–Whitney U-test and Fisher's exact test, as appropriate. The changes within the treatment groups were calculated using Wilcoxon sign rank test for paired samples. Associations between variables were assessed by Spearman's rank correlation test and coefficient expressed as Spearman's ρ. Patients who dropped out were included in calculations until withdrawal. Analyses were performed using StatView version 5.0.1. for Microsoft Windows. P-value <0.05 was considered statistically significant.

Results

The effect of HRT on serum levels of sRAGE

Serum levels of sRAGE decreased significantly in patients receiving HRT following 1 year [median 1402 (1053–1731) pg/ml, $P=0.0008$, $n=32$] and 2 years [median 1312 (1081–1810) pg/ml, $P=0.0075$, $n=34$] of treatment as compared with levels at study entry [median at baseline 1504 (1225–1828) pg/ml, $n=35$] (Fig. 1). By contrast, such a decrease was not observed in the control group [median at baseline 1395 (962–1966) pg/ml, $n=46$; at 1 year 1403 (882–1966) pg/ml, $n=46$; and at 2 years 1287 (964–1712) pg/ml, $n=43$].

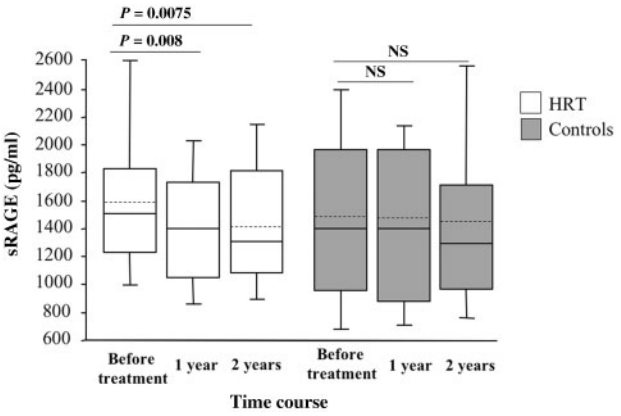

FIG. 1. The sRAGE levels decrease significantly following 1 and 2 years of oestrogen treatment, whereas no significant changes were observed in the control group. Box plots show the 25th and 75th percentiles. Horizontal solid lines within boxes indicate medians, hatched lines indicate means. Vertical bars indicate the 5th and 95th percentiles.

The change from baseline sRAGE levels (Δchange) was significantly different in the HRT group compared to the control group after 1 year following treatment initiation (median decrease in sRAGE levels 184 *vs* 19 pg/ml, respectively, $P=0.024$). The differences between study groups did not reach the level of statistical significance following 2 years of treatment (median decrease in sRAGE levels in HRT group 172 *vs* 56 pg/ml in controls, respectively, $P=0.079$).

Oestradiol levels in serum were measured before entering the study and at 12 and 24 months thereafter. As expected, serum oestradiol increased significantly in the HRT group at 1 year [median 126 (83–245) pmol/l] as compared with the levels at study entry [median 34 (26–40) pmol/l, $P<0.001$] and remained high at 24 months of treatment [median 149 (81–284) pmol/l], whereas in control patients serum oestradiol levels remained low during the study period [median 33 (26–39) pmol/l at 1 year and median 32 (25–36) pmol/l at 2 years, respectively, *vs* median 30 (25–43) pmol/l at baseline] as previously reported [33].

There was a significant inverse correlation between alterations (Δchange) in sRAGE and serum oestradiol levels (Fig. 2) following 2 years of treatment ($\rho=-0.409$, $P=0.0014$) in the studied RA population. No correlation was observed between changes in sRAGE and oestradiol levels at 1 year [$\rho=-0.2$, statistically not significant (NS)].

The association of sRAGE with markers of bone/cartilage turnover and BMD at baseline

Next, we wanted to study a potential association between serum sRAGE levels and markers of bone/cartilage turnover. Interestingly, we found that at baseline, serum sRAGE levels were significantly positively associated with marker of bone formation, PICP ($\rho=0.294$, $P=0.01$) and bone resorption ICTP ($\rho=0.318$, $P=0.006$). Serum levels of COMP, an indicator of cartilage destruction in RA patients, significantly correlated with sRAGE levels ($\rho=0.345$, $P=0.002$) at baseline. Furthermore, sRAGE levels at baseline were negatively associated with BMD in total hip ($\rho=0.241$, $P=0.03$) and femoral neck ($\rho=0.245$, $P=0.03$).

The association between changes in sRAGE levels and changes in markers of bone/cartilage turnover and BMD

We wanted to further explore whether a decrease in sRAGE levels was associated with changes in biochemical markers of bone/cartilage turnover and BMD. Our results showed that the decrease of sRAGE levels (ΔsRAGE) was significantly associated with decline of COMP ($\rho = 0.475$, $P < 0.0001$) and PICP ($\rho = 0.297$, $P = 0.014$) levels following 1 year of treatment, and with ICTP ($\rho = 0.297$, $P = 0.014$) and COMP levels ($\rho = 0.403$, $P = 0.0005$) following 2 years of treatment in the whole-studied RA patient population. A groupwise analysis at 2 years showed a significant positive correlation between changes in serum levels of sRAGE and ICTP ($\rho = 0.410$, $P = 0.03$), and of sRAGE and COMP ($\rho = 0.636$, $P = 0.0003$), in patients receiving HRT, whereas no such an association was found in control patients (Fig. 3A).

In addition, the reduction in sRAGE levels was associated with elevation of BMD in lumbar spine ($\rho = -0.283$, $P = 0.014$) at 1 year after treatment initiation. Furthermore, there was a significant correlation between a decrease in sRAGE levels and an increase in total BMD in RA patients receiving HRT ($\rho = -0.411$, $P < 0.05$) following 2 years of treatment, which was not observed in the control group (Fig. 3B).

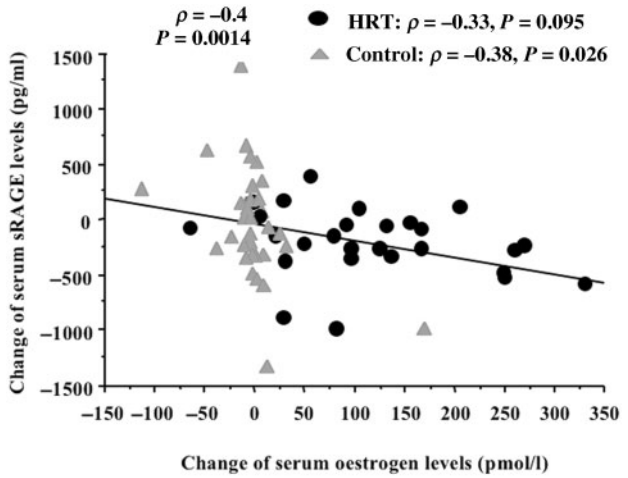

FIG. 2. The change of sRAGE levels is significantly associated with changes in serum oestrogen levels in RA patients following 2 years of treatment with oestrogen. The regression line and correlation coefficient for all patients and for each group are presented separately on the plot.

The association of sRAGE with radiological joint destruction

Next, we wanted to examine whether sRAGE levels at baseline correlated with radiological joint destruction (Larsen score) and thus could be a predictive marker. Interestingly, we found that progression of Larsen score (Δ-Larsen) at 1 year following initiation of the treatment was correlated with sRAGE levels at baseline ($\rho = -0.248$, $P = 0.033$) and at 1 year of treatment ($\rho = -0.350$, $P = 0.003$) in the whole-studied RA population. Moreover, group analysis showed that radiological progression (Δ-Larsen) during the first year was associated with sRAGE levels at baseline in those patients receiving HRT ($\rho = -0.430$, $P = 0.014$) but not in controls ($\rho = -0.089$, NS). We did not find any significant association between sRAGE levels and Larsen score at baseline. No statistically significant differences were detected regarding mean sRAGE levels in patients with erosive disease [median 1452 (1031–1964) pg/ml, $n = 71$] as compared with those without radiological erosions [median 1450 (1015–1724) pg/ml, $n = 8$] at baseline.

Disease activity indices, treatment options and sRAGE

We also assessed sRAGE levels in RA patients at study entry with respect to treatment options. We observed that patients receiving treatment with peroral corticosteroids ($n = 17$) had significantly lower ($P < 0.05$) levels of sRAGE in serum [median 1243 (952–1441) pg/ml] as compared with RA patients ($n = 64$) without corticosteroid treatment [median 1589 (1119–2084) pg/ml]. Patients who received treatment with DMARDs before entering the study had comparable sRAGE levels [median 1453 (1013–1966) pg/ml, $n = 66$] with those not receiving DMARD treatment [median 1504 (1180–1922) pg/ml, $n = 15$]. The patients receiving monotherapy with MTX ($n = 30$) had a tendency towards higher sRAGE levels [median 1642 (1276–2098) pg/ml] as compared with those without DMARD treatment, as reported previously [26].

We did not find any association between sRAGE levels and disease activity indices such as ESR, CRP and disease activity score (DAS28) at any studied time point.

Discussion

This is the first prospective randomized controlled study evaluating the effect of HRT on circulating sRAGE levels in post-menopausal patients with RA. Our finding indicated that HRT decreased circulating sRAGE levels in these patients. In addition, higher sRAGE levels were associated with elevated

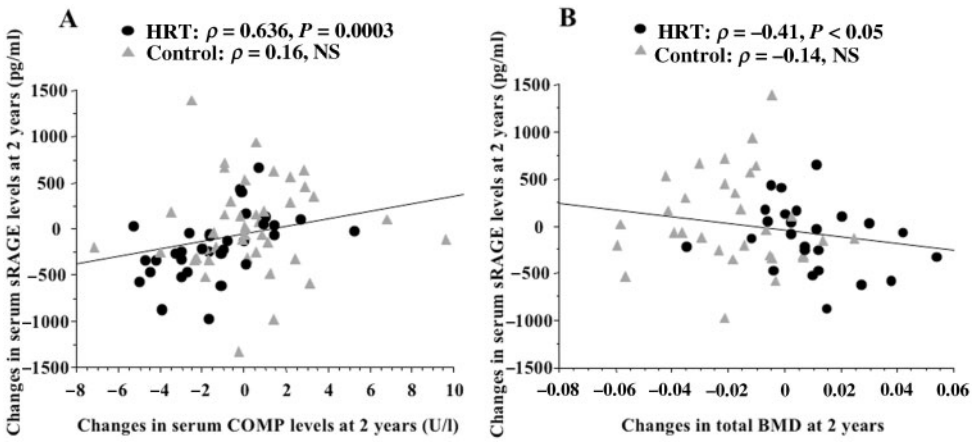

FIG. 3. The decrease of sRAGE levels is significantly associated with a decline in COMP levels (**A**) and an increase in BMD (**B**) in RA patients following 2 years of treatment with HRT but not in controls. The regression line for all patients and correlation coefficients for each group are presented separately on the plot.

levels of bone resorption and bone turnover markers at baseline, whereas the decrease of sRAGE levels paralleled with diminished concentration of these molecules indicating a possible role of sRAGE in bone/cartilage metabolism.

Recent studies implicate the involvement of oestrogens in RAGE/sRAGE signalling. Indeed, it has been demonstrated that 17β-E_2, an oestrogen predominantly found in the circulation of pre-menopausal women, induces the expression of membrane-bound RAGE in *in vitro*-cultured human endothelial cells at doses that are found *in vivo* during pregnancy [39]. These data are confirmed and extended by other authors using physiological concentrations of oestrogen [37, 42]. To date, it is not known how oestrogens regulate sRAGE levels. Buhimschi *et al.* [43] reported that while oestrogen levels are high during pregnancy, sRAGE concentrations increase exponentially about 100-fold up to 30 weeks of gestation in amnionic fluids, and after that the rate of change virtually plateaus. In contrast to this, we demonstrate a decrease of sRAGE levels in serum of RA patients receiving HRT in comparison with control group. In addition, we observed a significant correlation between decline of sRAGE and increase of serum oestradiol levels at the end of study. These associations, together with work by Buhimishi, suggest that oestrogens might be directly involved in the regulation of sRAGE levels. The expression of RAGE isoforms varies largely between tissues [44, 45] and these contradictory results are probably obtained because different compartments and distinct physiological conditions were studied.

Cell-bound RAGE serves as a trophic factor regulating osteoclast formation in mice. Mice devoid of the RAGE gene have increased BMD and decreased bone resorptive activity *in vivo* [46, 47]. In addition, blockade of cellular RAGE using sRAGE diminished alveolar bone loss in diabetic periodontal disease model in mice [48]. However, the impact of sRAGE on bone physiology in man is unknown.

In our study, we found at baseline a significant negative correlation between sRAGE levels and BMD in femoral neck and total hip in our RA population. In addition, higher sRAGE levels correlated with increased concentrations of bone turnover markers at baseline, whereas the decrease of sRAGE levels paralleled with attenuated levels of these molecules. These associations provide evidence that the sRAGE/RAGE pathway might be involved also in the regulation of osteoclast function and bone remodelling in man.

The generalized bone loss in RA is largely caused by increased osteoclast activation as a result of systemic inflammatory state. Cellular RAGE is expressed by many of the cells that directly participate in the development of osteoporosis and cartilage destruction in RA including osteoclasts, osteoblasts and synovial fibroblasts [10]. In patients with RA, a wide diversity of pro-inflammatory RAGE ligands is found in the circulation as well as locally in the joints [5–7] that contribute to osteoclast activation and further increase receptor expression. For example, accumulation of AGEs in bone tissue has been proposed to contribute to disturbed bone remodelling [10]. HMGB1, another RAGE ligand, enhances RANKL-induced osteoclastogenesis by its interaction with RAGE [49], and is chemotactic to osteoclasts [50]. Since sRAGE has the same ligand-binding specificity, it can bind these RAGE ligands and (i) prevent them from reaching cell surface receptor and (ii) prevent ligands from interacting with other yet unidentified receptors of importance in bone metabolism than RAGE. In our study, higher sRAGE levels at baseline correlated with less progression in the Larsen score during the first year of treatment, indicating the protective role for sRAGE, especially in patients receiving HRT.

It is known that oestrogen has an immuno-modulatory effect [51]. HRT in RA patients has been shown to suppress disease activity [33, 51]. Several reports have shown that oestrogens affect the function of endothelial cells [51, 52] previously shown to induce production and release of sRAGE [45]. With this background and considering that (i) oestrogen displays anti-inflammatory and anti-arthritic properties [51], and (ii) serum sRAGE might also reflect tissue RAGE expression in inflammatory conditions like RA as it is suggested in diabetes [53], it is plausible to assume that less sRAGE is produced by endothelial/inflammatory cells and/or shed via proteolytic cleavage leading ultimately to decreased sRAGE levels. Consistent with this concept, RA patients receiving peroral treatment with low-dose prednisolone, one of the most efficient and fast-acting anti-inflammatory drugs, displayed significantly lower sRAGE levels as compared with patients without glucocorticoid treatment.

In addition, oestrogen has been found to display pro-apoptotic effects on osteoclasts and anti-apoptotic effects on osteoblasts as well as to decrease the level of several important cytokines for osteoclastogenesis such as IL-6, IL-1, TNF-α M-CSF [51] thereby counteracting inflammation and bone loss. Hypothetically, as the osteoclast activation, bone turnover and inflammation declines with oestrogen treatment, less sRAGE is generated and the balance is restored. In accordance with this, we found a relationship between decreased sRAGE levels and increased BMD in patients receiving HRT.

Some limitations of the study should be considered. In our study, the majority of patients received combined HRT with oestradiol and NA as a synthetic form of progesterone. Only four patients were treated with oestrogen alone and subgroup analysis was not applicable. Therefore, it was not possible to determine to what extent the different sex hormones contributed to the decrease in sRAGE levels.

The change from baseline sRAGE levels (Δchange) was significantly different in the HRT group compared with the control group after 1 year and did not reach the level of significance ($P = 0.079$) following 2 years of treatment. The probable explanation for the absence of significance, despite noticeable differences, could be missing data for patients in both groups and in the control group in particular at 2 years ($n = 3$), which influences the statistical power of analysis. There was also no correlation between changes in sRAGE and oestradiol levels after 1 year of treatment as compared with 2 years. Some of the data regarding serum oestradiol levels were missing in both groups due to technical problems and in particular in the HRT group only data on 25 women were available at 1 year, which certainly might affect the statistical analysis. Studies with a larger sample size than our RA population could possibly clarify this issue. On the other hand, HRT has been shown to increase the rate of neoplasm and increase the risk of cardiovascular side effects and is no longer recommended for long-term therapy [54]. Therefore, it also remains to be elucidated in future studies whether alternative therapies (selective oestrogen receptor modulator, bisphosphonates) reproduce the same beneficial effect of oestrogen, but without its side effects.

Although 'cause or effect' relationships are not established in this report, we suggest that HRT medication in post-menopausal RA patients leads to decreased sRAGE levels indicating its involvement in sRAGE regulation. Moreover, our data suggest that sRAGE is involved directly or indirectly in bone and cartilage metabolism. Further, studies are needed to provide insights into the clinical significance of HRT in sRAGE regulation.

Rheumatology key messages

- We evaluated the effect of HRT on circulating sRAGE levels in post-menopausal RA patients.
- HRT decreased sRAGE levels in these patients, suggesting involvement of sex hormones in sRAGE regulation.
- The sRAGE was associated with BMD and may be involved in bone metabolism.

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