

Soluble RANKL and Risk of Nontraumatic Fracture

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BONE UNDERGOES A CONTINUOUS remodeling process, allowing optimal adaptation of microarchitecture to individual demands. Bone resorption triggered by osteoclasts is physiologically coupled and usually in balance with bone formation, which is mediated by osteoblasts.¹ Recently, essential physiological interactions between osteoclasts and osteoblasts have been unraveled and the receptor activator of nuclear factor κ B ligand (RANKL) was proposed as a key player in this scenario because (1) RANKL stimulates osteoclastogenesis and induces osteoclast activation²⁻⁵; (2) recent data suggest that RANKL directly activates osteoblasts and triggers bone formation at concentrations well below those necessary to induce osteoclastogenesis⁶; (3) RANKL is involved in coupling since its expression on osteoblasts stimulates osteoclastogenesis^{2,3}; and (4) juvenile Paget disease, a rare genetic condition of very high bone turnover, is associated with serum levels of RANKL more than

Context The receptor activator of nuclear factor κ B ligand (RANKL) is essential for osteoclast and, possibly, osteoblast activation and may represent a key link between bone formation and resorption.

Objective To determine the relationship between serum level of RANKL and the risk of nontraumatic fracture.

Design, Setting, and Participants As part of a prospective population-based study conducted in Bruneck, Italy, we recorded all fractures that occurred between 1990 and 2000 in 906 participants and classified them as traumatic (n=115) or nontraumatic (n=31). Serum levels of RANKL and osteoprotegerin and characteristics of bone metabolism and lifestyle were assessed in 1990 and at follow-up in 1995 and 2000.

Main Outcome Measure Incident nontraumatic fracture by levels of RANKL.

Results Levels of RANKL did not differ between sexes and were not related to age, menopausal status, lifestyle characteristics, or data from bone ultrasound at the heel. However, RANKL emerged as a significant predictor of nontraumatic fracture. In pooled logistic regression analysis, the relative risks of nontraumatic fracture in the lowest and middle vs highest tertile for RANKL were 10.0 (95% confidence interval [CI], 2.3-43.1) and 3.9 (95% CI, 0.8-19.0) ($P<.001$ for trend), respectively. Patients in the highest-tertile group had a low risk of fracture even in the presence of other predisposing factors, whereas women aged 60 years or older in the lowest tertile had a 5-year rate of nontraumatic fracture greater than 7%.

Conclusions A low level of RANKL is an independent predictor of nontraumatic fracture. This finding is consistent with the hypothesis of an important role of RANKL in human bone turnover and if confirmed in future investigations may gain relevance for assessment of fracture risk.

JAMA. 2004;291:1108-1113

www.jama.com

10 times those measured in healthy individuals.⁷

Functionally, RANKL, a protein with structural homology to tumor necrosis factor, acts through binding to the transmembrane receptor RANK.^{2-5,8,9} Competitive binding to the naturally occurring decoy receptor osteoprotegerin (OPG) blocks RANKL/RANK interaction at the ligand/receptor level.^{10,11}

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RANKL is expressed by osteoblasts, bone marrow stromal cells, and activated T cells and occurs in circulation as a soluble molecule,¹² making it suitable for laboratory assessment.

Despite the recent advances in understanding the significance of RANKL in experimental animal models, relevance in physiological bone metabolism of humans and potential conse-

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Financial Disclosure: A patent application has been filed in the name of Dr Schett by Auxeris Therapeutics Inc, St Louis, Mo, for the association of serum RANKL level and fracture risk.

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quences for bone quality and fracture risk remain to be clarified. In the current large, prospective study, we investigated whether RANKL qualifies as a laboratory marker of fracture risk in the general community.

METHODS

Study Participants

The Bruneck Study is a prospective population-based survey of the epidemiology and pathogenesis of atherosclerosis and disorders of the brain and bone.¹³ The study protocol was reviewed and approved by the appropriate ethics committees, and all study participants gave written informed consent. At baseline in 1990, the study population was recruited as a random sample, stratified according to sex and age, of all inhabitants of Bruneck, Italy (125 women and 125 men in each of the fifth to eighth decades of age). A total of 93.6% participated, with data assessment completed in 919 participants. Reevaluations were performed after 5 years (1995) and 10 years (2000).¹³ Blood samples for measurement of RANKL and other parameters were available from 919 (1990), 826 (1995; 96.5% of those alive), and 700 (2000; 97.7% of those alive) participants, respectively. For the current analysis, 1 participant with a pathologic fracture due to bone metastasis and 12 who had experienced nontraumatic fractures before baseline were excluded. In the remaining 906 participants, determination of clinically apparent nontraumatic fractures was nearly complete (>99%) between 1990 and 2000.

Clinical History and Examination

Lifetime peripheral and clinically apparent vertebral fractures were carefully recorded for all study participants using the participant's self-report and a standardized reevaluation of all radiographs ever taken in study participants. The situation in Bruneck is unique in that (1) the only radiography facility in the district is located at the hospital and all radiographs were available for review; (2) it is convenient to perform radiography in virtually all cases of injury and moderate to severe or long-lasting back pain;

and (3) population mobility in the survey area was extremely low during the study. None of the reported fractures occurred outside of the Bruneck area. For all radiologically confirmed fractures, localization, date of occurrence, and associated circumstances were recorded. Fractures were classified as nontraumatic if resulting from a fall from standing height or less or manifesting without any trauma.¹⁴ Other fractures, especially those of fingers, toes, skull, face, cervical vertebrae, and chest/sternum, were considered traumatic.¹⁵ Vertebral fractures were radiologically defined by a decrease of at least 20% and at least 4 mm of anterior, medial, or posterior vertebral height (compared with the posterior margin of the same vertebra or, if reduced, above adjacent vertebra) in lateral thoracic and lumbar spine radiographs (segments T4 to L5).¹⁶

All lifestyle variables were assessed in 1990, 1995, and 2000. Body mass index was calculated as weight in kilograms divided by the square of height in meters. Smoking status and alcohol consumption were recorded as described previously.¹³ A physical activity score was calculated from the scores for work (3 categories) and sports/leisure activities (0, ≤ 2 , or > 2 h/wk).¹³ Socioeconomic status was defined by a 3-category scale (low, medium, or high) based on information about occupational status and educational level of the person with the highest income in the household.¹³ Diabetes was diagnosed according to World Health Organization criteria. Bone ultrasound data (broadband ultrasonic attenuation and speed of sound) were assessed in 2000 at the heel using quantitative ultrasound equipment (SAHARA, Hologic Inc, Bedford, Mass).

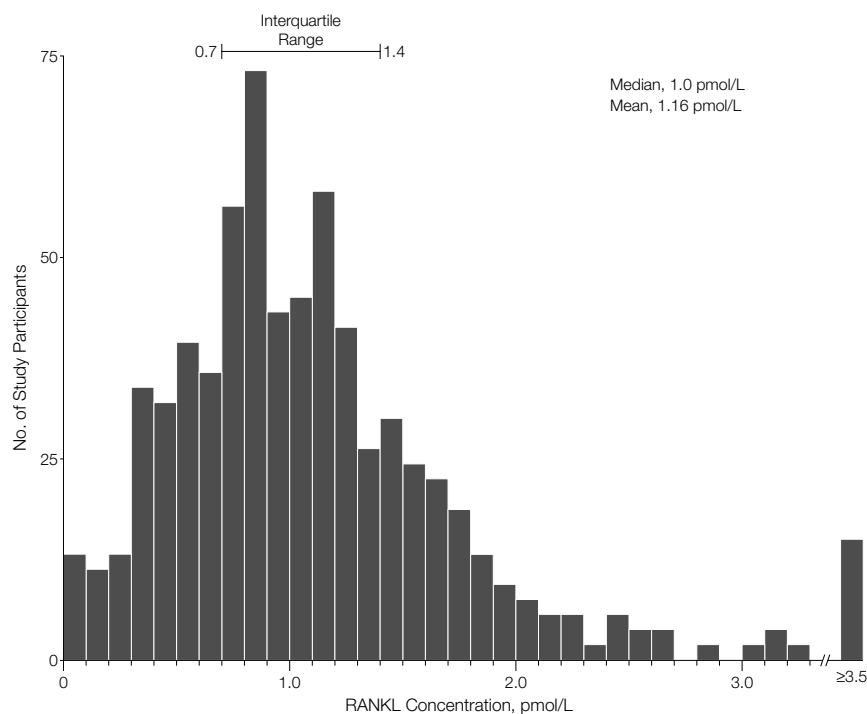
Laboratory Methods

Blood samples were drawn in 1990, 1995, and 2000 after an overnight fast and 12 hours of abstinence from smoking.¹³ Serum was immediately frozen and stored at -70°C without any cycle of thawing-freezing until analysis. Serum levels of osteocalcin, parathyroid hormone, and β -crosslaps were measured by electrochemiluminescence immunoas-

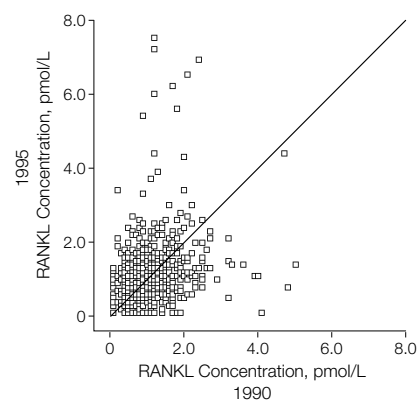
say (ECLIA, Roche Diagnostics, Mannheim, Germany). 25-Hydroxyvitamin D was analyzed by automated chemiluminescence assay (Nichols Advantage, Nichols Institute Diagnostics, San Juan Capistrano, Calif). Intra-assay coefficients of variation for osteocalcin, parathyroid hormone, β -crosslaps, and 25-hydroxyvitamin D testing were low at 0.6%, 1.4%, 3.1%, and 3.7%, respectively. Serum levels of soluble uncomplexed RANKL were measured by a commercial sandwich enzyme-linked immunosorbent assay (Biomedica, Vienna, Austria), as described previously.¹² All measurements were performed by a single experienced technician who was unaware of any characteristics of study participants. Chimeric OPG-Fc protein (R & D Systems, Minneapolis, Minn) was coated on microtiter plates and used to bind free RANKL in the samples. In a second step, RANKL captured by OPG was detected by a specific affinity-purified and biotinylated rabbit antibody (Leinco Technologies, St Louis, Mo) followed by incubation with streptavidin peroxidase and visualization with tetramethylbenzidine. Biosynthetic RANKL (Peprotech, Rocky Hill, NJ) diluted in human serum was used as a standard. Intra-assay and interassay coefficients of variation were 6% and 8%, respectively. The lower detection limit of the test was 0.1 pmol/L. Osteoprotegerin was measured using a sandwich enzyme immunoassay (R & D Systems). Recombinant OPG from Research Diagnostics Inc (Flanders, NJ) served as a standard. Intra-assay and interassay coefficients of variation were less than 10%.

Statistical Analyses

Person-years of follow-up for each participant were accrued from the 1990 baseline until diagnosis of nontraumatic fracture, death, or August 1, 2000, whichever came first. Participants who had nontraumatic fractures were censored with respect to subsequent follow-up. Participants were divided into 3 approximately equally sized groups according to tertiles of RANKL. Relative risks (RRs) were estimated with rate ratios compar-

Figure 1. Baseline Distribution of Serum Concentration of RANKL

RANKL indicates receptor activator for nuclear factor κ B ligand.

Figure 2. Scatterplot of RANKL Measurements in 1990 and 1995

RANKL indicates receptor activator for nuclear factor κ B ligand.

ing the incidence of nontraumatic fractures in each tertile with that in the highest (referent) tertile using pooled logistic regression.^{17,18} This technique treated each observation period (1990-1995 and 1995-2000) as a follow-up substudy in which updated risk factor measure-

ments were used to predict fracture risk. Observations in both periods were pooled into a single sample. This approach has been shown to be asymptotically equivalent to the Cox regression model with time-dependent covariates given short intervals between reevaluations and low rates of events.^{17,18} Multivariate models were adjusted for age (years), sex (men, premenopausal women, or postmenopausal women), follow-up period (1990-1995 or 1995-2000), socioeconomic status (low, medium, or high), smoking (cigarettes per day), alcohol consumption (grams per day), physical activity score, diabetes (no vs yes), body mass index, creatinine levels (milligrams per deciliter), hormone therapy (no vs yes), and, facultatively, other types of medication and parameters of bone metabolism. We performed tests for linear trend by treating the medians in each category of RANKL as a continuous variable. Regression-adjusted rates of nontraumatic fractures according to tertiles of RANKL, age, sex, and menopausal status were calculated with the marginal

method of the regression adjustment technique.¹⁹ All reported *P* values are 2-sided; *P* < .05 was considered statistically significant. SPSS software version 11.5 (SPSS Inc, Chicago, Ill) was used for all analyses.

RESULTS

During 8087 person-years of follow-up, 31 cases of nontraumatic and 115 cases of traumatic fractures were documented. Baseline distribution of RANKL and main descriptive characteristics are depicted in FIGURE 1. Individual levels of RANKL emerged as comparatively stable over time, as indicated by a high correlation between 1990 and 1995 measurements ($r=0.63$) and a mean absolute difference of 0.077 pmol/L (95% confidence interval [CI], -0.001 to 0.157 pmol/L) (SD, 1.14 pmol/L). A scatterplot is shown in FIGURE 2. Age, sex and menopausal state were equally distributed among tertiles of baseline RANKL level (TABLE 1). Participants with lower levels of RANKL tended to be of lower socioeconomic status, less active, and more likely to be current smokers and have diabetes. However, none of these trends achieved significance considering the performance of multiple comparisons. Furthermore, serum levels of RANKL were unrelated to bone ultrasound data assessed at the heel and various parameters of bone metabolism except for OPG (modest inverse relation; *P* < .001).

The incidence of nontraumatic fractures varied from 0.7 per 1000 person-years in the highest tertile to 8.1 per 1000 person-year in the lowest tertile of RANKL (TABLE 2). In pooled logistic regression analyses adjusted for age, sex, menopausal status, and period of follow-up ($n=1712$), the 5-year risk of nontraumatic fracture increased with decreasing tertiles of RANKL (RR, 10.0; 95% CI, 2.3-43.1 in the lowest tertile and RR, 3.9; 95% CI, 0.8-19.0 in the middle tertile compared with the highest tertile; *P* for trend < .001) (Table 2). In multivariate analyses, after simultaneous control for a variety of demographic and lifestyle variables, creatinine concentration, and hormone therapy, results did

not change appreciably (RR, 9.7; 95% CI, 2.2-42.1 in the lowest tertile and RR, 4.0; 95% CI, 0.8-19.7 in the middle tertile compared with the highest tertile; *P* for trend <.001). In this model, the RR of nontraumatic fractures was 1.4 (95% CI, 0.9-2.3; *P* = .15) for a 10-year increase in age and 4.2 (95% CI, 0.4-47.3; *P* = .25) and 9.7 (95% CI, 2.8-33.3; *P* <.001), respectively, for premenopausal and postmenopausal women vs men. Additional adjustment for levels of OPG and other parameters of bone metabolism (Table 2) and concomitant medication had no further effect. As expected, corresponding Cox regression models with time-dependent covariates yielded results very similar to those of the pooled logistic regression models (Table 2).

There was no evidence of a differential association between RANKL and nontraumatic fracture in men vs women (interaction term, *P* = .66) or in different age groups (interaction term, *P* = .42), and the findings were consistent in analyses that separately focused on hip and vertebral fractures. The findings were essentially the same after exclusion of participants with diabetes (Table 2). Four persons with diabetes had a nontraumatic fracture. Three of these had a RANKL level in the lowest tertile and 1 in the middle tertile.

Next, we calculated regression-adjusted rates of nontraumatic fractures in subgroups according to age, sex, and menopausal status (FIGURE 3). In brief, participants in the highest tertile of RANKL faced a low risk of nontraumatic fracture irrespective of age and sex. However, fracture risk steeply increased from the highest to the lowest tertile of RANKL, especially in postmenopausal women.

In contrast with nontraumatic fractures, traumatic fractures were not related to RANKL level in multivariate pooled logistic regression analysis (RR in the lowest and middle vs highest tertiles of RANKL, 1.1 [95% CI, 0.7-1.8] and 1.0 [95% CI, 0.6-1.7], respectively; *P* for trend = .72) (see Table 2 for adjustment). Results were virtually identical after exclusion of participants with diabetes. The intervals between blood sampling and oc-

currence of nontraumatic and traumatic fractures were a mean of 2.5 and 2.8 years, respectively. After a fracture, levels of RANKL did not change significantly.

As anticipated, the relationship between RANKL and nontraumatic fracture was independent of serum OPG. Osteoprotegerin per se was associated with risk of nontraumatic fracture (RR in the lowest and middle vs highest tertiles of OPG, 0.2 [95% CI, 0.1-0.6] and 0.4 [95% CI, 0.2-0.9]; *P* for trend = .001)

but this relationship disappeared after adjustment for age (RR, 0.5 [95% CI, 0.2-1.8] and 0.6 [95% CI, 0.2-1.6]; *P* for trend = .23) and other variables (RR, 0.5 [95% CI, 0.2-1.8] and 0.6 [95% CI, 0.2-1.5]; *P* for trend = .20) due to a high correlation between OPG and age (Spearman *r* = 0.52; *P* <.001).

COMMENT

Our prospective study demonstrates that a low serum level of RANKL is a highly

Table 1. Baseline Demographic and Lifestyle Characteristics, Indicators of Bone Metabolism, Bone Ultrasound Data, and Medications According to Tertile of RANKL*

Characteristics	RANKL Level Tertile		
	1 (Low) (n = 310)	2 (Medium) (n = 292)	3 (High) (n = 304)
RANKL level, median (range), pmol/L	0.60 (0.10-0.80)	1.00 (0.85-1.25)	1.60 (1.30-16.95)
Demographic variables			
Age, y	59.6 (10.8)	59.3 (11.8)	58.3 (11.6)
Sex, No. (%)			
Men	165 (53.2)	149 (51.0)	143 (47.1)
Premenopausal women	33 (10.7)	43 (14.7)	56 (18.4)
Postmenopausal women	112 (36.1)	100 (34.3)	105 (34.5)
Socioeconomic status, No. (%)			
Low	207 (66.8)	180 (61.6)	176 (57.9)
Medium	56 (18.0)	62 (21.2)	70 (23.0)
High	47 (15.2)	50 (17.2)	58 (19.1)
Lifestyle variables			
Smoking, cigarettes/d	5.3 (9.0)	3.3 (7.1)	3.6 (7.6)
Alcohol consumption, g/d	31.3 (41.9)	28.4 (36.7)	25.0 (35.7)
Physical activity score†	4.2 (1.6)	4.3 (1.5)	4.5 (1.5)
Diabetes mellitus, No. (%)	30 (9.7)	20 (6.8)	15 (4.9)
Body mass index‡	24.8 (3.8)	25.2 (3.7)	24.8 (3.8)
Bone metabolism/renal function			
Osteocalcin, ng/mL	27.3 (15.5)	28.1 (18.6)	27.4 (14.4)
Osteoprotegerin, pmol/L	3.9 (1.7)	3.7 (1.0)	3.5 (0.8)
β-Crosslaps, ng/mL	0.47 (0.36)	0.46 (0.28)	0.46 (0.26)
Parathyroid hormone, pg/mL	55.3 (30.5)	49.5 (27.9)	47.8 (25.5)
25-Hydroxyvitamin D, ng/mL	31.1 (13.7)	30.5 (13.5)	32.3 (11.4)
Bone ultrasound data§			
Broadband ultrasound attenuation, dB/MHz	72.4 (19.4)	71.5 (17.2)	71.9 (18.3)
Speed of sound, m/s	151.1 (3.2)	154.2 (3.0)	154.3 (3.0)
Creatinine, mg/dL	0.94 (0.15)	0.91 (0.23)	0.90 (0.14)
Medications, No. (%)			
Corticosteroids	3 (1.0)	0	2 (0.7)
Warfarin	1 (0.3)	1 (0.3)	0
Bisphosphonates	0	0	0
Statins	0	0	0
Hormone therapy (women)	17 (11.7)	13 (9.1)	18 (11.2)

Abbreviation: RANKL, receptor activator for nuclear factor κB ligand.

SI conversion: To convert creatinine to μmol/L, multiply by 88.4.

*Data are expressed as mean (SD) unless otherwise noted.

†Physical activity score was calculated from the scores for work (3 categories) and sports/leisure activities (0, ≤2, or >2 h/wk).¹³ The possible range of scores is 2 to 6.

‡Body mass index was calculated as weight in kilograms divided by the square of height in meters.

§Broadband ultrasound attenuation and speed of sound were measured in 2000 at the left and right heels (n = 683).

significant risk predictor for nontraumatic fractures in the general population independent of age, sex, menopausal status, level of OPG, and lifestyle characteristics. Study participants in the highest tertile of RANKL faced a low risk of nontraumatic fractures irrespective of the presence or absence of other predisposing factors (incidence of <1 per 1000 person-years). In contrast, women aged 60 years or older with a serum RANKL level in the lowest tertile showed a re-

gression-adjusted 5-year rate of fracture greater than 7% (Figure 3). Although RANKL was significantly associated with fracture risk in all subgroups, absolute risk differences and, accordingly, predictive relevance were highest in postmenopausal women (Figure 3). Importantly, the serum level of RANKL was comparatively stable over time and emerged as unrelated to bone ultrasound data, which may be regarded as a surrogate of bone mass. How-

ever, the latter observation should be regarded as preliminary because bone ultrasound data were assessed at the heel and standard radiological measurements of bone density at predilection sites of osteoporosis were not available.

Many of the previously established risk predictors of nontraumatic fractures such as advanced age, postmenopausal status, impaired neuromuscular function, and gene polymorphisms in the estrogen receptor α and collagen type 1 genes are considered to be responsible for loss of bone mass.²⁰⁻²⁶ It is commonly assumed, however, that resistance against fracture also depends on the quality of bone.²⁷ Although nature and causality of the relationship between RANKL and fracture risk in our study are speculative at this time, we believe that RANKL plays a key role in human bone remodeling and, thus, affects bone quality. Hypothetically, low levels of RANKL may be associated with a low degree of bone remodeling, unfavorable bone microarchitecture, and, therefore, enhanced fracture risk. Lack of an association of RANKL with osteocalcin and β -crosslaps in our population is not in contradiction to this concept because continuous adaptation of bone microarchitecture putatively occurs within a physiological range of bone turnover.

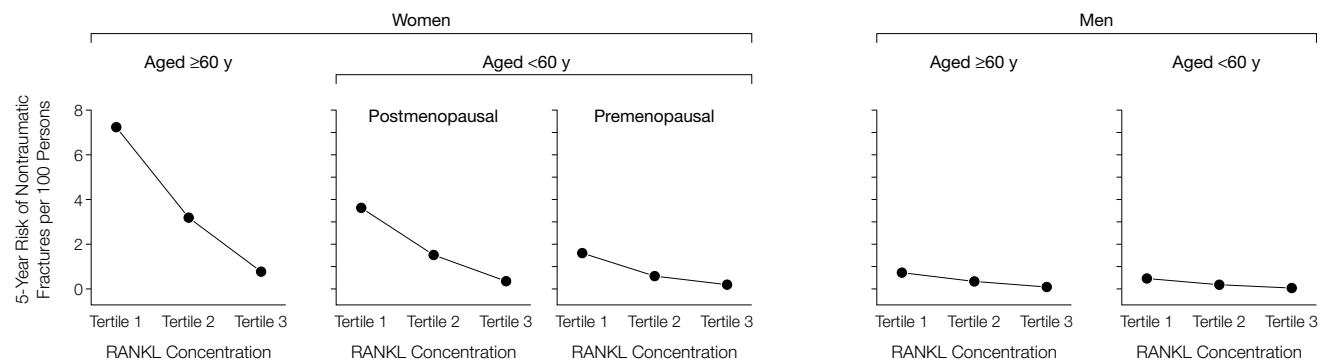
In recent years, several laboratory parameters have been identified which predict or modify the risk of osteoporotic fractures, including β -crosslaps and the

Table 2. Relative Risk of Nontraumatic Fractures According to Tertile of RANKL

	Tertile of RANKL Level		
	1 (Low)	2 (Medium)	3 (High)
RANKL level, median (range), pmol/L	0.60 (0.10-0.80)	1.00 (0.85-1.25)	1.60 (1.30-16.95)
No. of nontraumatic fractures	22	7	2
Person-years of follow-up*	2726	2545	2816
Incidence, events per 1000 person-years	8.1	2.8	0.7
Relative risk (95% confidence interval)†			
Age- and sex-adjusted‡	10.0 (2.3-43.1)	3.9 (0.8-19.0)	1.0
Multivariate§	9.7 (2.2-42.1)	4.0 (0.8-19.7)	1.0
Multivariate, including bone metabolism variables	9.4 (2.2-40.8)	3.8 (0.8-18.6)	1.0
Multivariate, Cox model§¶	9.4 (2.2-40.1)	3.8 (0.8-18.6)	1.0
Multivariate, nondiabetic participants§	9.1 (2.1-39.9)	3.7 (0.7-18.8)	1.0

Abbreviation: RANKL, receptor activator for nuclear factor κ B ligand.
 *Cutoff values for tertile groups were defined based on the baseline distribution of RANKL. Allocation of person-time to each tertile group was based on the 1990 level of RANKL for the first 5-year period (1990-1995) and 1995 level of RANKL for the second 5-year period (1995-2000).
 † $P < .001$ for trend across tertiles in all analyses.
 ‡This model included variables for age (years), period during the study (two 5-year periods), and sex/menopausal status (men, premenopausal women, or postmenopausal women).
 §The multivariate relative risk was adjusted for age (years), follow-up period (1990-1995 or 1995-2000), sex/menopausal status (men, premenopausal women, or postmenopausal women), socioeconomic status (low, medium, or high), smoking (cigarettes per day), alcohol consumption (grams per day), physical activity score, diabetes (no vs yes), body mass index (weight in kilograms divided by the square of height in meters), creatinine level (milligrams per deciliter), and hormone therapy (no vs yes).
 ||This model was additionally adjusted for levels of osteoprotegerin, osteocalcin, β -crosslaps, parathyroid hormone, and 25-hydroxyvitamin D.
 ¶Cox regression analysis with time-dependent covariates.

Figure 3. Regression-Adjusted Rates of Nontraumatic Fracture According to Sex, Menopausal Status, and Age



RANKL indicates receptor activator for nuclear factor κ B ligand. Calculations are based on multivariate analysis as described in Table 2. Tertile 1 = 0.10 to 0.80 pmol/L; tertile 2 = 0.85 to 1.25 pmol/L; tertile 3 = 1.30 to 16.95 pmol/L.

forementioned gene polymorphisms in the estrogen receptor α and collagen type I genes.^{25,26,28} However, no blood test has so far found broad access to the routine estimation of fracture risk in the general community. RANKL is a promising candidate for closing this gap, provided that our findings will hold true in future investigations.

The strengths of this study include its representative nature for the general community (near-complete participation and follow-up), the broad age range studied (40-89 years), and the high degree of accuracy in assessing and classifying fractures in the setting of a population study. Several potential limitations deserve consideration as well. First, clinically inapparent vertebral fractures were not assessed. However, such events are of little relevance from a clinical viewpoint, and nonassessment of minor disease phenotypes may be expected to weaken evident relations rather than to create spurious ones. Second, the study cohort consisted of whites only and was population-based. Thus, results should not be extrapolated to other races or chronically ill or immobilized patients. Third, the number of participants with nontraumatic fractures in our study was comparatively low ($n=31$), and, as a consequence, 95% CIs for risk estimates are broad. It must be acknowledged, however, that P values for the main findings are at a comfortable range at $<.001$, data are highly consistent in subgroup and confirmation analyses, and the lower limit of the 95% CI of fracture risk for the lowest vs highest tertile group still exceeds 2 (Table 2). Finally, in this study beginning in 1990, only a few participants were treated with drugs affecting bone metabolism. Although this may be considered to be an advantage for data analysis and accuracy, it should be noted that prescription of these drugs is much more common currently.

In conclusion, our study identifies a low serum level of RANKL as a novel and significant risk predictor of nontraumatic fractures in the general community. This finding fits well into the hypothesis of a crucial role of RANKL in human bone turnover and quality

and may gain relevance in the routine assessment of fracture risk. Clinical application of our findings awaits prior confirmation in independent population samples. Moreover, standardized thresholds and the sensitivity and specificity of the test remain to be defined for a reliable interpretation of RANKL measurements in individual patients.

Author Contributions: Dr Schett had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Schett, Kiechl, Redlich, Oberholzer, Weger, Egger, Mayr, Jocher, Xu, Pietschmann, Teitelbaum, Smolen, Willeit.

Acquisition of data: Schett, Kiechl, Redlich, Oberholzer, Weger, Egger, Mayr, Jocher, Xu, Pietschmann, Teitelbaum, Smolen, Willeit.

Analysis and interpretation of data: Schett, Kiechl, Redlich, Egger, Mayr, Teitelbaum, Willeit.

Drafting of the manuscript: Schett.

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Obtained funding: Schett, Mayr, Willeit.

Administrative, technical, or material support: Schett, Kiechl, Mayr, Jocher, Xu, Smolen.

Supervision: Schett, Willeit.

Funding/Support: This study was supported by a grant from the Austrian National Bank (project 8715), the Pustertaler Verein zur Prävention von Herz- und Hirngefäßerkrankungen, the Sanitätseinheit Ost, the Assessorat für Gesundheit, and the START prize of the Austrian Ministry of Sciences. There was no industry support for this study.

Role of the Sponsor: The study's sponsors had no role in the design and conduct of the study, in the collection, analysis, and interpretation of the data, and in the preparation, review, or approval of the manuscript.

Acknowledgment: We are indebted to Margareta Tryniecki, Annamaria Raffetseder, and Wolfgang Woloszczuk (Ludwig Boltzmann Institute for Experimental Endocrinology, Vienna, Austria) for technical support.

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