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# OPG and sRANKL serum levels and incident hip fracture in postmenopausal Caucasian women in the Women's Health Initiative Observational Study

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ABSTRACT

*Purpose:* The osteoprotogerin/receptor activator of NF-kappa β/receptor activator of NF-kappa β ligand (OPG/RANK/RANKL) pathway plays a critical role in bone remodeling. This study investigated associations between serum levels of OPG, soluble RANKL (sRANKL), and the ratio of OPG/sRANKL to risk of incident hip fracture.
*Methods:* A nested case–control study was conducted among postmenopausal, Caucasian women aged 50–79 at baseline (1993–1998), followed for hip fracture through March 2005 in the Women's Health Initiative Observational Study. 400 incident hip fracture cases were selected and individually matched to 400 controls with no prior fracture or incident hip fracture. Matching factors were baseline age, enrollment date and hormone therapy (HT) exposure. Baseline serum OPG and sRANKL levels were measured using high sensitivity ELISA. Odds ratios were computed for quartiles of each biomarker adjusting for matching factors and hip fracture risk factors.
*Results:* Serum OPG was significantly associated with older age, low physical activity and poorer physical function in control women. sRANKL was inversely associated with total calcium intake in control women, but not associated with age or other fracture risk factors. The odds ratio for hip fracture comparing the highest to lowest quartiles of OPG was 2.28 (95% confidence interval (CI), 1.45–3.61) after adjusting for the matching variables (p-value for linear trend <0.001), and 1.87 (95% CI, 1.15–3.04; p for linear trend = 0.02) after adjusting for self-rated health status, physical activity and physical functioning. No significant associations between sRANKL or the ratio of OPG/sRANKL and hip fracture risk were observed.
*Conclusion:* Serum OPG levels were independently associated with a nearly twofold increased risk of hip fracture in postmenopausal women.



## Introduction

The discovery of osteoprotegerin (OPG) in 1997 led quickly to the understanding that the OPG/RANKL/RANK System is central to the coupling of osteoclasts and osteoblasts in bone biology [1]. At the level of the bone remodeling unit, receptor activator of NF-kappa β ligand (RANKL) is expressed on the cell surface of both osteoblasts and stromal cells. RANKL binds to RANK and leads to differentiation and maturation of osteoclasts, leading to bone resorption. OPG is a protein made by osteoblasts that binds to RANKL as a decoy receptor and blocks its interaction with RANK, thus blocking osteoclast formation and bone resorption. Other tissues besides osteoblasts produce RANKL and OPG and thus, measurements of these biomarkers in the serum may reflect both skeletal and non-skeletal sources, as well as age-related differences in clearance [2]. The balance between RANKL and OPG may be regulated by other cytokines or hormones that promote bone resorption.

Associations between OPG, bone turnover markers, and BMD have been inconsistent [2–4]. There are limited data examining the

*Abbreviations:* OPG, osteoprotogerin; RANK, receptor activator of NF-kappa β; RANKL, receptor activator of NF-kappa β ligand; sRANKL, soluble receptor activator of NF-kappa β ligand; WHI, Women's Health Initiative.

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relationship between OPG and/or RANKL and fracture risk. OPG levels were not associated with fracture in one study [5], but high levels were associated with increased risk in a small case–control study of hip fracture [6] and height loss in postmenopausal women [7]. sRANKL has been associated with fracture in one study with 31 fractures [8], but no association was seen between sRANKL and height loss in a larger cohort of postmenopausal women [7]. The early studies are limited by small sample sizes, low numbers of fracture events, and lack of sensitivity of early generation assays.

The sole epidemiologic study to examine a high sensitivity marker of OPG in relation to incident hip fracture found that the highest levels of OPG were associated with hip fracture risk [9]. We conducted a nested case–control study within the Women's Health Initiative Observational Study (WHI-OS) to examine serum OPG and soluble RANKL (sRANKL) levels and the ratio of OPG to sRANKL to risk of incident hip fracture risk. A second objective was to determine if associations between these biomarker levels and incident hip fracture differed for women who were users vs. nonusers of HT, those with high and low body mass index, those with high vs. low total calcium intake (diet plus supplement intake) and those with high and low FRAX scores (a measure of 10-year fracture risk).

## Materials and methods

### *Study group*

The WHI-OS is a prospective cohort study that enrolled 93,676 women ages 50–79 years from 1994–1998 at 40 clinical centers throughout the United States [10]. The women were eligible if they were postmenopausal, unlikely to move or die within three years, not enrolled in the WHI Clinical Trials and not currently participating in any other clinical trial. At baseline, the women completed screening and enrollment questionnaires by interview and self-report, a physical examination and blood specimen collection. The study was reviewed and approved by the Human Subjects Review Committees at each participating institution.

### *Follow-up and outcome ascertainment*

The women were sent questionnaires annually to report the occurrence of any hospitalization and a wide variety of outcomes including clinical fractures of any type. The median (interquartile range) of follow-up time was 8.0 (7.0–9.0) years per participant as of September, 2005. At that time, 4.8% of WHI-OS participants had withdrawn or were lost to follow-up and 6.7% had died. Hip fractures were verified by review of radiological, magnetic resonance imaging, or operative reports by trained physicians at each clinical center and then confirmed by blinded central adjudicators [11]. Hip fractures with a possible or confirmed pathological cause (from malignancy, infection or focal bone lesion) were excluded. There was no attempt to distinguish fractures caused by excessive trauma from other hip fractures.

### *Nested case–control study design*

The study population was restricted to Caucasian women who had been part of a previous whole genome association study. From this group, 400 randomly selected incident cases of hip fracture and their matched controls were identified. Controls were selected using random sampling in a 1:1 ratio with cases from the subpopulation of women who reported no postmenopausal fractures (self-reported fracture at age ≥55 years) at baseline and no incident hip fracture through the planned study closeout (March 31, 2005) with individual matching by age (+/−1 year), enrollment date (+/1 year) and current hormone therapy use at baseline (exact). Matching by enrollment date serves the dual purpose of matching on length of follow-up and length of frozen storage for the serum specimens. Biomarker levels were obtained in 396 cases and 397 controls. This study design provided 80% power to detect a difference of 0.20 standard deviations in mean biomarker levels between cases and controls at the 0.05 level of statistical significance. This corresponds to 80% power to detect a statistically significant odds ratio of 1.22 for hip fracture per 1 standard deviation difference in biomarker levels.

### *Baseline clinical variables*

Baseline questionnaires ascertained information on race/ethnicity, treated diabetes, history of myocardial infarction, coronary revascularization or stroke, current and past smoking, parental history of hip fracture, and self-rated health status. Detailed information on current and past HT use was ascertained by questionnaire. Alcohol consumption was estimated using questionnaire items as servings per week. Physical activity was classified on the basis of frequency and duration of four speeds of walking and mild, moderate and strenuous activities in the prior week. Kilocalories of energy expended in a week on leisure time activity was calculated (MET score = kcal h/week/kg) [12]. Physical function was measured using the 10-item Rand-36 physical function scale which includes items measuring whether health now limits physical function in moderate/vigorous activities, strength to lift, carry, stoop, or bend, stair climb, ability to walk various distances without difficulty, and self-care [13]. Weight was measured to the nearest 0.1 kg on a balance beam scale with the participant dressed in indoor clothing without shoes. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body mass index was calculated as weight (kg)/height ($m^2$).

Current use of prescription medications including corticosteroids was recorded by clinic interviewers at the first screening visit by direct inspection of medicine containers. Prescription names were entered into the WHI database which assigned drug codes using Medispan software. Dietary supplements, including calcium preparations, taken at least twice weekly for the prior two weeks were entered directly from medicine containers as described above. Dietary intake of calcium was measured using a semi-quantitative food frequency questionnaire [14]. Total calcium intake was defined as the sum of calcium from diet, supplements, and medications. FRAX scores were provided by the WHO Collaborative Group based on women's individual clinical risk factors without bone mineral density [15].

Cases and controls did not have previous measurements of bone mineral density, serum 25-hydroxyvitamin D (Vitamin D) levels or bone metabolism markers, except as detailed below.

### *Laboratory procedures*

Laboratory personnel were blinded to case–control status for all measurements. OPG and sRANKL were measured by ELISA (Osteoprotegerin EIA, and ampli-sRANKL EIA, Biomedica kits distributed by American Laboratory Products Company, LTD; Salem, NH) at Ohio State University in baseline serum that had been stored at −70° Celsius within two hours of collection. The OPG assay measures both free and complexed OPG-RANKL and detects both the monomeric and dimeric forms of OPG. The minimum detection limit is 0.14 pmol/L (2.8 pg/ml). The range of OPG levels in serum sample from normal healthy individuals is 0.65–4.2 pmol/L (13.0–84 pg/ml). The intra-assay and inter-assay coefficients of variation are 6% and 8%, respectively. The sRANKL ELISA measures free RANK-L and uses an enzyme catalyzed amplification cycle to enhance the detection limit. The median value for normal healthy women is 0.37 pmol/L. The detection limit of the assay is 0.001 pmol/L. The intra-assay and inter-assay coefficients of variation are 8% and 6%, respectively. Serum sRANKL levels were undetectable in one-third of cases and controls overall (35% of cases, 28% of controls).

Plasma “whole” parathyroid hormone (PTH) levels were measured by immunoradiometric assay utilizing a polyclonal 1–84 PTH antibody (Whole PTH™) at Ohio State University. This third generation assay specifically measures the entire biologically active 1–84 PTH molecule.