

University of Groningen

Beyond risk-reducing salpingo-oophorectomy

Fakkert, Ingrid Elizabeth

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2017

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Fakkert, I. E. (2017). *Beyond risk-reducing salpingo-oophorectomy: On breast cancer risk and bone health*. Rijksuniversiteit Groningen.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Elevated bone turnover markers after risk-reducing salpingo-oophorectomy in women at increased risk for breast and ovarian cancer

Ingrid Fakkert
Eveline van der Veer
Elske Marije Abma
Joop D. Lefrandt
Bruce H. Wolffenbuttel
Jan C. Oosterwijk
Riemer H.J.A. Slart
Iris G. Westrik
Geertruida H. de Bock
Marian J.E. Mourits

A version of this chapter was previously published in:

PLoS One 2017;12:e0169673

Abstract

Background: Risk-reducing salpingo-oophorectomy (RRSO) reduces ovarian cancer risk in *BRCA1/2* mutation carriers. Premenopausal RRSO is hypothesized to increase fracture risk more than natural menopause. Elevated bone turnover markers (BTMs) might predict fracture risk. We investigated BTM levels after RRSO and aimed to identify clinical characteristics associated with elevated BTMs.

Methods: Osteocalcin (OC), procollagen type I N-terminal peptide (PINP) and serum C-telopeptide of type I collagen (sCTX) were measured in 210 women ≥ 2 years after RRSO before age 53. BTM Z-scores were calculated using an existing reference cohort of age-matched women. Clinical characteristics were assessed by questionnaire.

Results: BTMs after RRSO were higher than age-matched reference values: median Z-scores OC 0.11, $p = 0.003$; PINP 0.84, $p < 0.001$; sCTX 0.53, $p < 0.001$ (compared to Z = 0). After excluding women with recent fractures or BTM interfering medication, Z-scores increased to 0.34, 1.14 and 0.88, respectively. Z-scores for OC and PINP were inversely correlated to age at RRSO. No correlation was found with fracture incidence or history of breast cancer.

Conclusions: Five years after RRSO, BTMs were higher than age-matched reference values. Since elevated BTMs might predict higher fracture risk, prospective studies are required to evaluate the clinical implications of this finding.

Introduction

Women from families with a high incidence of breast and ovarian cancer (hereditary breast and ovarian cancer; HBOC) have increased risks of both these cancers, especially women with a germline mutation in the *BRCA1* or *BRCA2* genes (4,5). Risk-reducing salpingo-oophorectomy (RRSO) is advised to all *BRCA1* and *BRCA2* mutation carriers between age 35 - 40 and 40 - 45 respectively (37,312). It is hypothesized that surgical menopause as induced by premenopausal RRSO increases fracture risk more than natural menopause, because of earlier age at menopause and acute and complete cessation of ovarian hormone production (234,297).

Current practice to identify women at risk of developing fractures is measurement of bone mineral density (BMD) by Dual-Energy X-ray absorptiometry (DXA) (227). Previous studies on BMD and fracture incidence after surgical menopause provided conflicting conclusions, as some suggested lower BMD and higher fracture incidence (234,235,327), while others did not find a difference compared to age-matched controls (296,328). Assessment of bone turnover by measuring bone turnover marker (BTM) levels after RRSO may be a useful addition to BMD measurement. BTMs may provide information on the influence of RRSO on both bone formation and resorption (232). Furthermore, BTMs in blood or urine might predict fracture risk independently of BMD (232,329).

It has been shown that BTMs increase rapidly within one month after surgical menopause and remain increased until at least one year after surgery (237-239,330). Bone resorption marker levels seem to increase faster than bone formation markers (237,238,240,330), but after several months to years, their ratios appear to normalise (237-240,330). Studies comparing BTMs after surgical and natural menopause report conflicting results; one study showed increased resorption marker levels after surgical menopause compared to natural menopause, while others found no differences in BTMs between the groups (241-243). Therefore, we compared BTMs in a group of women ≥ 2 years after RRSO at premenopausal age to age-matched reference values. Furthermore, we aimed to identify factors that characterize women with elevated BTMs after premenopausal RRSO.

Methods

Study population and protocol

At the University Medical Center Groningen family cancer clinic, all women with HBOC or *BRCA1/2* mutations have been registered since 1994 (312). Between

February 2011 and May 2012, all women with HBOC or *BRCA1/2* mutations who had undergone RRSO before the age of 53 at least two years before, were invited for osteoporosis screening. Women filled in a questionnaire and were screened according to a protocol including measurement of height, weight, collection of blood samples, and BMD measurement. Results on standard diagnostic procedures for osteoporosis in this study were described elsewhere (328). Two women were excluded from the current analyses because their BTMs were not measured. All women gave written informed consent for inclusion in the study.

The institutional ethics review board of the UMCG stated that the study did not fall under the scope of the Medical Research Involving Human Subjects Act as the study was considered as a part of standard care. A waiver for ethical approval was provided. IGW, IEF, MJEM, EMA, JL and EV provided individual patient care and were involved in data collection for this study. These authors had access to identifying patient data during data collection as they were involved in patient care. The other authors had no access to the identifying patient data.

Reference data for BTM levels were retrieved from an existing local reference cohort of 350 healthy Dutch women. Menopausal status of reference women aged ≤ 50 years was not known. Reference women aged > 50 were ≥ 5 years postmenopausal with serum $25(\text{OH})\text{D}_3$ (25OHD) levels > 50 nmol/L and LS and hip BMD T-score > -2.5 .

Laboratory assessments

Non-fasting blood samples were obtained between 9:00 a.m. and 4:30 p.m. Serum samples were stored within 1 hour after collection at -20°C until analysis. Calcium and albumin were measured by colorimetric assay (Roche Modular P, Mannheim, Germany; inter-assay coefficient of variation (IE-CV) $< 2.0\%$ and $< 1.8\%$; lower detection limit 0.05 mmol/L and 10 g/L for calcium and albumin respectively). Phosphate was measured by photometric UV assay (Roche Modular P, Mannheim Germany; IE-CV $< 2.6\%$; lower detection limit 0.1 mmol/L). Serum 25OHD was measured by isotope dilution-online solid phase extraction liquid chromatography-tandem mass spectrometry (315). Method specifications were: level of quantification 4.0 nmol/L; IE-CV $< 14.1\%$; recovery $93 - 98\%$; linearity $r^2 = 0.997$. Accuracy was secured by the use of reference material from the National Institute of Standards & Technology (Gaithersburg, MD). Serum 25OHD was considered low when < 50 nmol/L between October and April and < 75 nmol/L between April and October. Thyroid stimulating hormone (TSH) was measured by electrochemiluminescence immunoassay (Roche Modular E; Mannheim, Germany; IE-CV $< 2.3\%$; lower detection limit 0.005 mU/L). PTH was measured by

immunoluminometric assay (Cobas e 601, Roche; Mannheim, Germany; IE-CV < 3.2%, lower detection limit 0.040 pg/L).

Bone turnover was assessed by measurement of the levels of bone formation markers osteocalcin (OC), intact procollagen type I N-terminal propeptide (PINP) and bone resorption marker serum C-telopeptide of type I collagen (sCTX).

OC was measured by immunoradiometric assay (BioSource Europe S.A, Nivelles, Belgium; IE-CV 9.4%). OC levels were expressed in $\mu\text{g/L}$ and Z-scores to correct for the influence of age. PINP levels were measured by using a radioimmunoassay (Orion Diagnostica, Espoo, Finland; IE-CV 9.0%) PINP levels were expressed in $\mu\text{g/L}$ and Z-scores. sCTX was measured by Electrochemiluminescence immunoassay (Elecsys 2010; Roche, Mannheim, Germany; IE-CV 10.8%). sCTX levels were expressed in pg/ml and Z-scores.

Clinical measurements

Fractures and risk factors for osteoporosis were assessed by questionnaire, based on the questionnaire used at our fracture and osteoporosis outpatient clinic (314). The questionnaire was sent to the women before the visit and missing or inconsistent answers were discussed and corrected if indicated during the visit.

BMD of the lumbar spine (LS; anterior-posterior projection at L1 – L4) and femoral neck (FN) were measured by DXA using a Hologic Discovery A densitometer (Hologic Inc., Bedford, MA). BMD was expressed in grams per cm^2 , BMD Z-scores and T-scores. BMD Z-scores present the number of standard deviations (SD) that an individual's BMD differs from the mean BMD in a cohort of white age-matched women. T-scores present the numbers of SD from the mean peak BMD in women aged 20 - 30 years. Z- and T-scores were retrieved from the Hologic DXA machine and calculated using the standard reference databases for Hologic in the Netherlands were used (201,316). A T-score ≤ -2.5 was considered as osteoporosis, based on the lowest T-score of either LS or FN.

Statistical analysis

Results were expressed as median (inter quartile range [IQR: 25th and 75th percentile]) for continuous data and as number (%) for dichotomous data. BTM Z-scores were used to correct for the influence of age on bone turnover marker levels. A BTM Z-score represents the number of SDs an individual's BTM level differs from the mean BTM level in a cohort of age-matched women. Z-scores were calculated by the following formula: (BTM value of individual patient – mean BTM value / SD of matched 10-year reference cohort. The difference between

PINP and sCTX Z-scores was calculated to determine the relative difference in bone formation and resorption. To compare BTMs after RRSO with BTMs in age matched controls, median Z-scores for BTMs were compared with a hypothetical median Z-score of 0 in the general population using Wilcoxon's signed-rank test. Medians were used as BTM Z-scores did not follow a normal distribution in our study cohort.

To identify factors associated with elevated BTM Z-scores, linear univariate and multivariate regression analyses were performed. Women with recent fractures (i.e. within 12 months before BTM level measurement) or BTM affecting medication (current use of HRT or aromatase inhibitors (AI) or ever use of anti-osteoporotic drugs (AOD)) were excluded from the regression analyses, because these factors are supposed to strongly affect BTMs and therefore could mask relations between BTM levels and other independent variables. Factors included for univariate regression analyses were study population specific characteristics (e.g. factors related to RRSO and history of breast cancer), bone related characteristics and factors associated with BTMs in literature. Dichotomous variables with $N \geq 5$ for each option were included. Multivariate regression analyses were performed with manual conditional stepwise backward inclusion of variables that had a p -value < 0.25 in univariate analysis. Comparison of BTM Z-scores between women that were included versus those that were excluded from regression analyses was done by using the Mann-Whitney U test, Chi Square Test or Fisher's Exact Test when applicable.

Statistical analyses were performed with IBM SPSS Statistics 20 software (SPSS, Chicago, III). p -values < 0.05 were considered significant.

Results

Study population

Of the 254 women eligible for inclusion, 212 women agreed to participate (*Figure 6.1*). Two women were excluded because blood sampling and thus measurement of BTMs was not possible. The median age at time of study participation was 44 years (IQR 41 - 49 years; *Table 6.1*). Median age at RRSO was 42 years (38 - 46) and median time since RRSO was 5 years (4 - 8). Of all women, 75 (36%) had a history of fractures, 21 (10%) had fractures at adult age and 16 (8%) had one or more fractures after RRSO. Details on fracture type in women before and after RRSO are provided in *Table S6.1*.

For the regression analyses 75 women were excluded because of recent fractures ($n = 6$) and/or BTM affecting medication ($n = 71$). Compared to the women

included for regression analyses, these excluded women were significantly younger at the time of the study and at time of RRSO, had lower BMIs, had lower absolute BMD in grams/cm² but not BMD Z-score for both LS and FN and had higher calcium and phosphate levels (Table S6.2).

Bone formation markers OC and PINP

Median OC level for all women was 13.0 ng/ml (IQR 9.8 - 15.3). Median OC Z-score was 0.11 (-0.65 - 1.34; Figure 6.2), which was significantly higher than the theoretical median Z-score of 0 in the reference cohort ($p = 0.003$). Median OC Z-score in women included for regression analysis was significantly higher than in the women excluded for regression analysis (Figure 6.2). Results for univariate analyses are shown in Table 6.2. In multivariate analysis, OC Z-scores were higher in women who were younger at time of RRSO ($\beta -0.098$ per year), had lower femoral neck BMD Z-score ($\beta -0.425$ per SD) and had higher corrected serum calcium ($\beta 5.573$ per mmol/L) and phosphate levels ($\beta 3.193$ per mmol/L; Table 6.3).

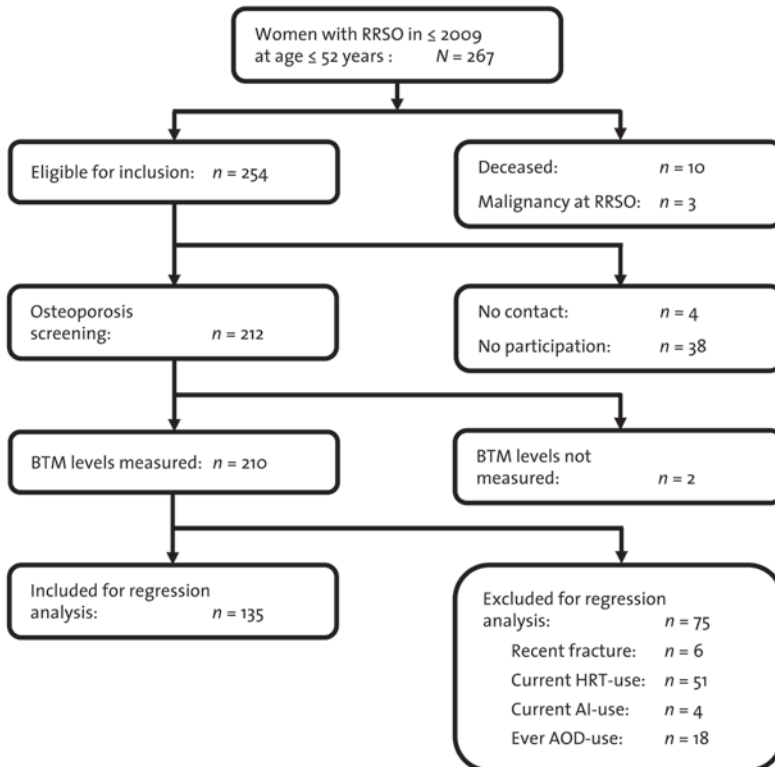


Figure 6.1: Flowchart of the inclusion process

Abbreviations: RRSO is risk-reducing salpingo-oophorectomy; BTM is bone turnover marker; HRT is hormone replacement therapy; AI is aromatase inhibitor; AOD is anti-osteoporotic drugs.

Table 6.1. Study population characteristics (N = 210)

Basic characteristics		Lifestyle characteristics	
Age in years	44 (41-49)	Exercise	169 (81)
Age at RRSO in years	42 (38-46)	Sports	133 (63)
Time since RRSO in years	5 (4-8)	Current smoking	40 (19)
BMI in kg/m ²	25.9 (22.9-29.5)	Alcohol consumption in units/week	2.5 (0-6)
Parity	2 (1.8-3.0)	> 7 units/week	38 (18)
Menopausal status before RRSO		Drug use	
Premenopausal	176 (84)	Ever use HRT	100 (48)
Postmenopausal	26 (12)	Current use	51 (24)
Hysterectomy	8 (4)	Ever use AI	11 (5)
Oncologic characteristics		Current use	4 (2)
Mutation status		Ever use tamoxifen	15 (7)
<i>BRCA1</i>	121 (58)	Current use	-
<i>BRCA2</i>	58 (28)	Ever use AOD	18 (9)
HBOC ^a	31 (15)	Current use	8 (4)
History of breast cancer	78 (37)	Current use of GCS	19 (9)
Chemotherapy	59 (28)	Longterm use of GCS ^{c,d}	8 (4)
Radiotherapy	49 (23)	Current use of calcium	34 (16)
Bone related characteristics		Current use of vitamin D ₃	34 (16)
History of fractures	75 (36)	Current use of multivitamin	26 (12)
Fracture at adult age ^b	21 (10)	Laboratory measurements	
Fracture after RRSO	16 (8)	Corrected calcium in mmol/L ^{c,e}	2.26 (2.22-2.30)
Recent fracture	6 (3)	Serum 25OHD in nmol/L	65 (50-82)
BMD LS in g/cm ^{2c}	0.97 (0.88-1.06)	Low 25OHD ^f	91 (43)
LS Z-score	0.00 (-0.85-0.85)	Phosphate in mmol/L	1.10 (0.98-1.20)
BMD FN in g/cm ²	0.78 (0.71-0.84)	PTH in pmol/L ^c	4.8 (4.0-6.1)
FN Z-score	0.10 (-0.60-0.80)	TSH in mE/L ^c	1.55 (1.11-2.21)
Osteoporosis (T-score ≤ -2.5)	13 (6)		

^aHBOC women had RRSO because of family history of breast or ovarian cancer, 15 had breast cancer, 23 had negative *BRCA* testing in the family, two were negative and one was not tested for their familial *BRCA* mutation, four had no *BRCA* testing and *BRCA* status of the family was unknown, ^bAdult age is ≥ 20; ^cMissing values for: BMD LS *n* = 1, long-term use of GCS *n* = 1, Corrected calcium *n* = 1, PTH *n* = 4, TSH *n* = 26; ^dUse of prednisone 7.5 mg or equivalent > 3 months or > 3 oral prednisolone courses per years; ^eCalcium was corrected for albumin levels with the following formula: Corrected calcium (mmol/L) = measured total Calcium (mmol/L) + 0.02 (41 – serum albumin [g/L]); ^fLow for season: < 50 nmol/L October - April; < 75 nmol/L April – October.

Abbreviations: IQR is interquartile range (i.e. 25th percentile – 75th percentile); RRSO is risk-reducing salpingo-oophorectomy; BMI is body mass index; HBOC is hereditary breast ovarian cancer; BMD is bone mineral density; LS is lumbar spine; FN is femoral neck; HRT is hormonal replacement therapy; AI is aromatase inhibitor; AOD is anti-osteoporotic drugs; GCS is glucocorticosteroids; PTH is parathyroid hormone; TSH is thyroid stimulating hormone.
 Values in median (IQR) or No. (%)

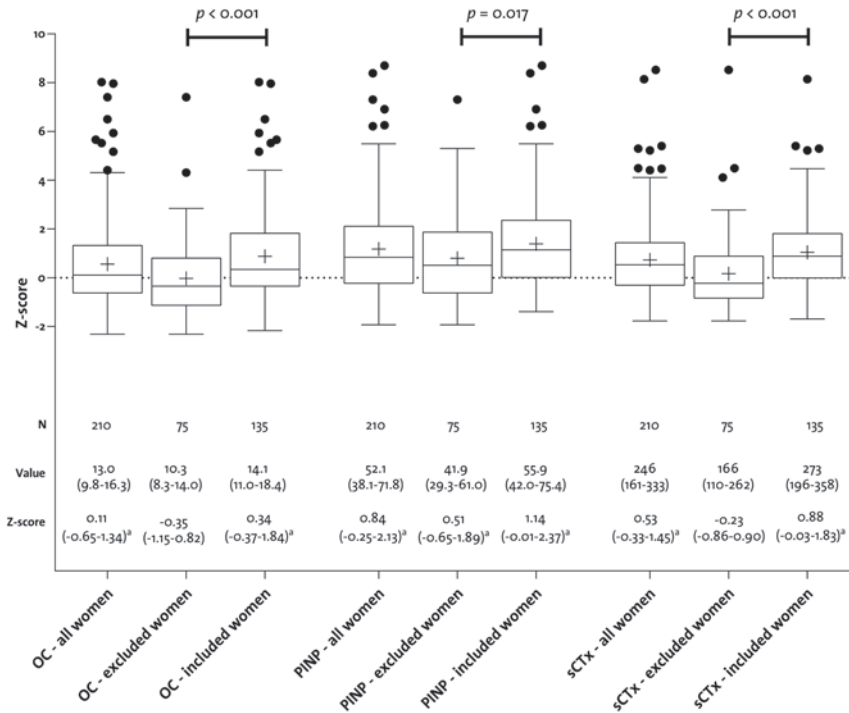


Figure 6.2: Levels of Osteocalcin, PINP and sCTX after RRSO in all women (N = 210), excluded women with recent fractures or currently using HRT or AI or ever using AOD (n = 75) and women included for regression analyses (n = 135).

^ap < 0.05; median Z-scores are compared to a median Z-score of 0.
 Box-and whisker plots (Tukey): boxes indicate medians with interquartile ranges; + indicate means; whiskers indicate 1.5 times the interquartile distances; • indicate outliers.

Table 6.2: Determinants related to BTM Z-scores as assessed by univariate linear regression analyses after exclusion of women with recent fractures, ever AOD use and current HRT or AI use (n = 135)

	Z-score OC			Z-score PINP		
	β	SE	p-value	β	SE	p-value
Basic characteristics						
Age > 50	-0.732	0.317	0.023*	-1.430	0.306	< 0.001*
Age at RRSO (per year)	-0.092	0.032	0.004*	-0.157	0.031	< 0.001*
Time since RRSO (per year)	-0.132	0.051	0.011*	-0.122	0.052	0.021*
BMI (per kg/m ²)	-0.057	0.027	0.038*	-0.019	0.028	0.502
Parity	-0.047	0.135	0.728	-0.265	0.136	0.054
Postmenopausal before RRSO	-0.943	0.454	0.040*	-0.926	0.461	0.047*
Oncologic characteristics						
History of breast cancer	-0.132	0.331	0.689	-0.161	0.338	0.634
Chemotherapy	-0.213	0.349	0.541	-0.244	0.356	0.494
Radiotherapy	-0.204	0.372	0.585	-0.288	0.379	0.449
Bone related characteristics						
Fracture at adult age ^a	0.079	0.548	0.885	-0.757	0.555	0.175
Fracture after RRSO	0.669	0.727	0.359	1.116	0.738	0.133
LS Z-score	-0.456	0.141	0.002*	-0.377	0.144	0.010*
FN Z-score	-0.531	0.171	0.002*	-0.315	0.179	0.080
Osteoporosis (T-score \leq -2.5)	-0.263	0.648	0.685	-0.006	0.661	0.993
Lifestyle characteristics						
Exercise ^b	-0.044	0.423	0.917	-0.345	0.430	0.424
Sports	0.123	0.328	0.708	-0.239	0.334	0.475
Current smoking	-0.012	0.423	0.978	0.228	0.431	0.597
Alcohol consumption in units/week	-0.023	0.029	0.428	-0.019	0.030	0.527
> 7 units/week	-0.218	0.422	0.607	-0.091	0.431	0.833
Drug use						
Past use of HRT	0.598	0.337	0.078	1.010	0.337	0.003*
Ever use of tamoxifen	-0.270	0.684	0.694	-0.428	0.698	0.541
Current use of GCS	-0.114	0.530	0.829	-0.305	0.540	0.573
Current use of calcium	0.440	0.463	0.344	0.138	0.474	0.771
Current use of vitamin D3	0.504	0.473	0.289	0.163	0.485	0.738
Current use of multivitamin	-0.057	0.455	0.900	-0.769	0.460	0.097

Z-score sCTx			Z-score PINP-sCTx		
β	SE	p-value	β	SE	p-value
-0.573	0.247	0.022*	-0.857	0.280	0.003*
-0.058	0.025	0.022*	-0.099	0.028	0.001*
-0.044	0.041	0.276	-0.078	0.046	0.097
-0.038	0.021	0.080	0.019	0.025	0.454
-0.078	0.105	0.459	-0.187	0.120	0.123
-0.473	0.357	0.188	-0.453	0.412	0.274
-0.186	0.257	0.471	0.025	0.297	0.933
-0.295	0.271	0.278	0.051	0.313	0.870
-0.011	0.290	0.970	-0.277	0.333	0.407
0.111	0.427	0.795	-0.868	0.485	0.076
0.386	0.567	0.497	0.730	0.650	0.263
-0.362	0.109	0.001*	-0.015	0.132	0.909
-0.280	0.136	0.041*	-0.035	0.159	0.825
0.216	0.505	0.670	-0.222	0.580	0.703
0.335	0.328	0.310	-0.680	0.374	0.071
0.224	0.255	0.380	-0.464	0.291	0.114
-0.451	0.327	0.171	0.679	0.374	0.072
0.004	0.023	0.859	-0.023	0.026	0.381
0.044	0.329	0.893	-0.135	0.379	0.721
0.493	0.262	0.062	0.516	0.302	0.090
-0.733	0.530	0.169	0.305	0.613	0.619
0.339	0.412	0.412	-0.645	0.472	0.174
-0.075	0.362	0.836	0.213	0.416	0.609
0.066	0.370	0.860	0.097	0.426	0.820
-0.387	0.353	0.274	-0.381	0.406	0.350

	Z-score OC			Z-score PINP		
	β	SE	p-value	β	SE	p-value
Laboratory measurements						
Corrected calcium^c	5.436	2.237	0.016*	3.241	2.314	0.164
Serum 25OHD	0.011	0.007	0.091	0.005	0.007	0.453
Low 25OHD^d	-0.553	0.320	0.087	-0.250	0.330	0.450
Phosphate	3.166	1.084	0.004*	1.890	1.130	0.097
PTH	0.076	0.076	0.319	-0.010	0.079	0.897
TSH	-0.011	0.139	0.940	-0.052	0.142	0.715

^aAdult age is ≥ 20 ; ^b ≥ 30 minutes of exercise a day; ^cCalcium was corrected for albumin levels with the following formula: Corrected calcium (mmol/L) = measured total calcium (mmol/L) + 0.02 (41 – serum albumin [g/L]); ^dLow for season: < 50 nmol/L October - April; < 75 nmol/L April – October.

Abbreviations: BTM is bone turnover marker; OC is osteocalcin, PINP is procollagen type I N-terminal propeptide; sCTX is serum C-telopeptide of type I collagen; SE is standard error; RRSO is risk-reducing salpingo-oophorectomy, BMI is body mass index; LS is lumbar spine; FN is femoral neck; HRT is hormonal replacement therapy; AI is aromatase inhibitor; GCS is glucocorticosteroids; PTH is parathyroid hormone; TSH is thyroid stimulating hormone.

p-values were calculated using univariate linear regression analysis. * $p < 0.05$.

Table 6.3: Determinants related to BTM Z-scores as assessed by multivariate linear regression analyses

	Multivariate analysis on Z-score OC (<i>n</i> = 134)			Multivariate analysis on Z-score PINP (<i>N</i> = 135)		
	β	SE	p-value	β	SE	p-value
Age at RRSO (per year)	-0.098	0.029	0.001*	-0.157	0.030	$< 0.001^*$
Time since RRSO (per year)				-0.121	0.048	0.012*
LS BMD Z-score						
FN BMD Z-score	-0.531	0.158	0.001*			
Past use of HRT						
Corrected calcium^a	5.573	2.024	0.007*			
Phosphate	3.193	1.002	0.002*			

^aCalcium was corrected for albumin levels: Corrected calcium (mmol/L) = measured total calcium (mmol/L) + 0.02 (41 – serum albumin [g/L])

Abbreviations: BTM: is bone turnover marker; OC is osteocalcin, PINP is procollagen type I N-terminal propeptide; sCTX is serum C-telopeptide of type I collagen; SE is standard error; RRSO is risk-reducing salpingo-oophorectomy, LS is lumbar spine; FN is femoral neck; HRT is hormonal replacement therapy p-values were calculated using multivariate linear regression analysis with manual conditional stepwise backward inclusion of variables that had a p-value < 0.25 in univariate analysis.

* $p < 0.05$.

Z-score sCTx			Z-score PINP-sCTx		
β	SE	p-value	β	SE	p-value
1.173	1.781	0.511	2.068	2.037	0.312
0.007	0.005	0.175	-0.002	0.006	0.748
-0.412	0.250	0.101	0.162	0.290	0.577
1.017	0.867	0.243	0.873	0.999	0.384
0.064	0.060	0.287	-0.074	0.069	0.282
0.007	0.105	0.945	-0.059	0.126	0.638

Multivariate analysis on Z-score sCTx (n = 134)			Z-score PINP - sCTx (N = 135)		
β	SE	p-value	β	SE	p-value
			-0.099	0.028	0.001*
-0.373	0.107	0.001*			
0.565	0.250	0.025*			

Median PINP level for all women was 52.1 ng/ml (IQR 38.1 - 71.8). Median Z-score was 0.84 (-0.35 - 2.13; *Figure 6.2*), which was significantly higher than the theoretical median Z-score of 0 in the reference cohort ($p < 0.001$). Median PINP Z-score in women included for regression analysis was significantly higher than in the women excluded for regression analysis (*Figure 6.2*). In multivariate analysis, PINP Z-scores were higher in women who were younger at the time of RRSO (β -0.157 per year) and had a shorter time since RRSO (β -0.121 per year; *Table 6.3*).

Bone resorption marker sCTx

Median sCTx level for all women was 246 pg/ml (IQR 160 - 133). Median Z-score of 0.53 (-0.33 - 1.45; *Figure 6.2*), which was significantly higher than the hypothetical median Z-score of 0 in the reference cohort ($p < 0.001$). Median sCTx Z-score in women included for regression analysis was significantly higher than in the women excluded for regression analysis (*Figure 6.2*). In multivariate analysis, sCTx Z-scores were higher in women who had lower lumbar spine Z-scores (β -0.373 per SD) and used HRT in the past (β 0.565 for users compared to non-users; *Table 6.3*).

Difference between Z-scores for PINP and sCTx

The median difference between Z-scores for PINP and sCTx for all women was 0.27 (-0.48 - 1.14) (*Figure 6.3*). This difference was similar in the women that were in- and excluded for regression analyses. Younger age at RRSO was correlated with an increasing positive difference between PINP and sCTx Z-score.

Discussion

Within this consecutive series of 210 women that underwent RRSO before age 53, with a median time after RRSO of 5 years, OC, PINP and sCTx levels were significantly higher than age-matched reference values. After excluding women with recent fractures and those using BTM affecting medication, Z-scores for bone formation markers were higher in women with younger age at time of RRSO. No significant correlation was found between BTM Z-scores and history of fractures or breast cancer.

Our finding that BTMs after RRSO were higher than age-matched reference values is partially in line with the results of Morgante *et al.* (242), who reported higher plasma OC and urinary deoxypyridoline levels in women with surgical

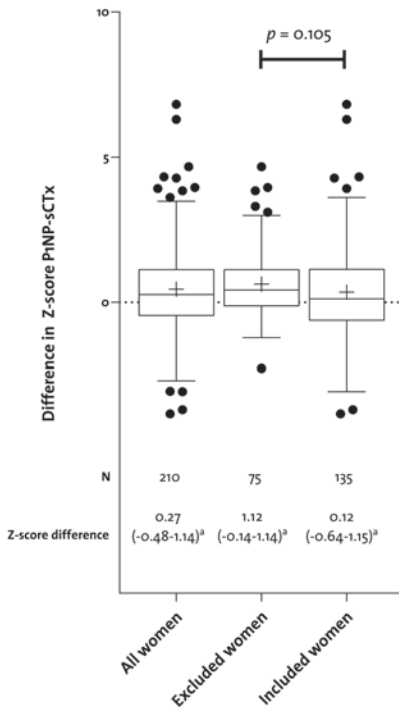


Figure 6.3: Difference in Z-scores for PINP and sCTx after RRSO in all women ($N = 210$), excluded women with recent fractures or currently using HRT or AI or ever using AOD ($n = 75$) and women included for regression analyses ($n = 135$)

^a $p < 0.05$; difference in Z-scores are compared to a median difference of 0.

Box-and whisker plots (Tukey): boxes indicate medians with interquartile ranges; + indicate means; whiskers indicate 1.5 times the interquartile distances; • indicate outliers.

menopause compared to age-matched premenopausal women, but not to age-matched women with natural menopause. In univariate regression analyses, Z-scores for all BTMs were significantly higher in women aged ≤ 50 years compared to women aged > 50 , which is in line with the finding that BTM levels after surgical menopause differ more from premenopausal than from natural menopausal women. Also partially in line with our results, are the observations of Ohta *et al.* (241) who reported higher serum levels of type I carboxy-terminal pyridinoline cross-linked telopeptide within three years after surgical menopause compared to natural menopause, but not after three years and not for other bone resorption markers. In this study, women more than three years after surgical menopause appeared to be older and had a longer time interval after menopause than our study population. Garcia-Perez *et al.* (243) showed similar levels of serum OC and urinary N-telopeptide of type I collagen in women

with surgical and natural menopause, but in that study women with natural menopause were significantly older than women with surgical menopause, thus it cannot be ruled out that women with surgical menopause had a higher bone turnover than women after natural menopause matched for age.

Several factors were significantly correlated with BTMs in multivariate analyses. Most importantly, there was an inverse correlation between age at RRSO and BTM Z-scores for OC and PINP. This might indicate that every year a woman is younger at time of RRSO, is associated with significantly higher bone formation marker levels after correction for chronological age. To our knowledge, this association was not investigated in previous studies. The finding might indicate a more profound effect of RRSO on bone turnover when surgery is performed at a younger age. Also, there was a positive correlation between OC Z-scores and corrected serum calcium levels, which is in contrast with earlier findings (331,332) and a positive correlation between OC levels and phosphate levels, which is in line with some (331), but not all earlier findings (332). PINP Z-scores were negatively correlated with time since RRSO, which might indicate that with a longer time interval after RRSO BTMs will normalize to age-appropriate values. Higher OC and sCTX Z-scores were correlated with lower BMD, which indicates that high bone turnover is associated with bone loss after RRSO. This was seen before for OC (242) and sCTX (237).

Last, we found a correlation between past HRT use and higher sCTX Z-score, which is in line with earlier findings that after cessation of HRT use, sCTX levels increase significantly (333). In contrast, current HRT use is associated with a significant increase in BMD and decrease in BTMs (334,335). In our study population, the 24% of the included women using HRT had significantly lower BTM Z-scores and were excluded for regression analysis. Although, HRT is effective in improving BMD and preventing fractures, prescribing HRT after the age of natural menopause is not advised in *BRCA* mutation carriers with RRSO after the age of natural menopause (336).

Treatment with AI increases bone turnover and decreases BMD (337), and women using AI often receive AOD for prevention, thus we excluded these women from the regression analyses. Of the women included for regression analyses, 53 (39%) had a history of breast cancer, but this was not correlated with BTMs. There were no women with clinical symptoms of bone metastases. However, because of the exclusion of a significant number of breast cancer patients from the regression analyses, we cannot exclude an effect of history of breast cancer on BTMs.

The median difference between Z-scores for PINP and sCTx reflects the difference in formation and resorption of collagen relative to age-matched controls. This difference can be considered as a marker for absolute bone turnover. We found a median difference of 0.12 (-0.64 - 1.15), which was significantly higher than 0 in the reference population. There was an inverse relation between age at RRSO and the difference between Z-score for PINP and sCTx. The clinical implications of this finding are unknown; however it does not suggest an imbalance favouring bone resorption after RRSO. As there are no earlier studies that have reported on this difference between PINP and sCTx Z-scores, there is a need for more studies on the clinical interpretation of findings on the relative difference between these markers.

Strengths of this study are the unselected sample that is representative of a patient population with surgical menopause due to RRSO, and the large study population compared to earlier studies. Furthermore, BTMs were presented as Z-scores to increase comparability with other studies, as was advised by Vasikaran *et al.* (232). Limitations of the study are that we were not able to fully correct for several factors that influence BTMs, such as diurnal variation and the timing of food intake. As it is known that BTMs decrease during the afternoon and after ingestion of a meal (232), not correcting for these factors could have attenuated elevated BTMs. The percentage of women with surgical menopause in the reference cohort was unknown, but the prevalence of surgical menopause in the general population is low. In addition, women with RRSO before age 53 were assumed to be premenopausal before RRSO, but some reported to be postmenopausal at that time. Women who were postmenopausal before RRSO had lower OC and PINP Z-scores in univariate analyses. Both factors might have caused underestimation of the effect of RRSO on BTMs.

The clinical implications of BTM elevation after RRSO are unknown, but elevated BTMs have been shown to predict elevated fracture risk in longitudinal population studies, independent of BMD (232,329). This might tone down the reassuring findings of our previous study in this study population, which showed that BMD and fracture incidence after RRSO were comparable to population data (328). Although we did not find a correlation between BTMs and fracture incidence in this cross-sectional study, longitudinal studies are needed to evaluate the long-term clinical implications of elevated BTMs after RRSO.

In conclusion, this study shows that after a median time of 5 years after premenopausal RRSO, BTMs are elevated compared to age-matched reference values, especially in women with RRSO at younger age. In this cross-sectional

study, no relation between elevated BTMs and fracture incidence was shown. However, as elevated BTMs predict elevated fracture risk in the general population, prospective studies are required to evaluate the long-term clinical implications of elevated BTMs after RRSO.

Acknowledgements

We thank the staff from the department of Gynaecology, the University Center for Geriatric Medicine, the department of Nuclear Medicine and Molecular Imaging and the department of Laboratory Medicine of the University Medical Center Groningen for their help with the data collection.

Funding

Unrestricted grants by Amgen, Menarini, Nycomed and Willpharma. Junior Scientific Masterclass MD/PhD funds of the University Medical Center Groningen. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Competing interests

Bruce H.R. Wolffenbuttel has received grant support for clinical studies in the diabetes field and also consulting fees for serving on advisory boards and as a speaker for Eli Lilly and Company, GlaxoSmithKline, Novo Nordisk, and Pfizer. He has also received consulting fees from Eli Lilly and Company as a member of the 4B study and the DURABLE Trial Data Monitoring Committee. Joop D. Lefrandt has received grant support from Boehringer Ingelheim..

Supplements

Supplementary tables

Table S6.1: Details on fractures reported in the study population ($N = 210$)

	Before RRSO	After RRSO
Women reporting fractures - No. (%)	64 (30)	16 (8)
Fractures – Total (No).	105	23
Fracture types - No. (%)		
Hip	0	0
Wrist	16 (15)	2 (9)
Vertebra	4 (4)	3 (13)
Nose	6 (6)	0
Clavicle	2 (2)	2 (9)
Rib	5 (5)	0
Sternum	1 (1)	0
Arm	10 (10)	1 (4)
Elbow	6 (6)	3 (13)
Hand	4 (4)	1 (4)
Pelvis	1 (1)	1 (4)
Leg	9 (9)	0
Knee	1 (1)	0
Ankle/foot	17 (16)	4 (17)
Toe/finger	23 (22)	6 (26)

Table S6.2: Characteristics of the women who were in- and excluded for regression analysis on BTMs after RRSO

	Included women (n = 135)	Excluded women (n = 75)	p-value
Basic characteristics			
Age in years	50 (47 - 54)	46 (42 - 50)	< 0.001*
Age at RRSO in years	44 (40 - 47)	39 (36 - 42)	< 0.001*
Time since RRSO in years	5 (4 - 8)	6 (4 - 8)	0.728
BMI in kg/m²	26.2 (23.3 - 30.0)	24.8 (22.3 - 29.1)	0.030*
Parity	2 (1 - 3)	2 (2 - 3)	0.862
Menopausal status before RRSO			0.137
Premenopausal	109 (81)	67 (89)	
Postmenopausal	20 (15)	6 (8)	
Hysterectomy	6 (4)	2 (3)	
Oncologic characteristics			
Mutation status			0.153
BRCA1	72 (53)	49 (65)	
BRCA2	39 (29)	19 (25)	
HBOC	24 (18)	7 (9)	
History of breast cancer	53 (39)	25 (33)	0.394
Chemotherapy	42 (31)	17 (23)	0.192
Radiotherapy	34 (25)	15 (20)	0.395
Bone related characteristics			
History of fractures	43 (32)	32 (43)	0.117
Fracture at adult age^a	13 (10)	8 (11)	0.810
Fracture after RRSO	7 (5)	9 (12)	0.073
Recent fracture	N/A	6 (8)	N/A
BMD LS in g/cm^{2b}	0.95 (0.86 - 1.04)	0.99 (0.90 - 1.08)	0.029*
LS Z-score^c	-0.10 (-0.90 - 0.83)	0.10 (-0.70 - 0.90)	0.371
BMD FN in g/cm²	0.76 (0.69 - 0.83)	0.80 (0.73 - 0.88)	0.017*
FN Z-score	0.00 (-0.60 - 0.70)	0.20 (-0.40 - 0.80)	0.329
Osteoporosis (T-score ≤ -2.5)	9 (7)	4 (5.3)	0.775

^aAdult age is ≥ 20; ^bMissing values for: BMD LS n = 1, longterm use of GCS n = 1, Corrected calcium n = 1, PTH n = 4, TSH n = 26; ^cUse of prednisone 7.5 mg or equivalent > 3 months or > 3 oral prednisolone courses per years; ^dCalcium was corrected for albumin levels with the following formula: Corrected calcium (mmol/L) = measured total calcium (mmol/L) + 0.02 (41 - serum albumin [g/L]); ^eLow for season: <50 nmol/L October - April; <75 nmol/L April - October.

	Included women (n = 135)	Excluded women (n = 75)	p-value
Lifestyle characteristics			
Exercise	111 (82)	58 (77)	0.392
Sports	79 (59)	54 (72)	0.052
Current smoking	24 (18)	16 (21)	0.530
Alcohol consumption in units/week	3 (0-6)	2 (0-6)	0.926
> 7 units/week	24 (18)	14 (19)	0.873
Drug use			
Ever use HRT	46 (34)	54 (72)	< 0.001*
Current use	N/A	51 (68)	N/A
Ever use AI	4 (3)	7 (9)	0.058
Current use	N/A	4 (5)	
Ever use tamoxifen	8 (6)	7 (9)	0.358
Current use	-	-	
Ever use AOD	N/A	18 (24)	N/A
Current use	N/A	8 (11)	N/A
Current use of GCS	14 (10)	5 (7)	0.370
Longterm use of GCS^{b,c}	4 (3)	4 (5)	0.396
Current use of calcium	19 (14)	15 (20)	0.264
Current use of vitamin D₃	18 (13)	16 (21)	0.132
Current use of multivitamin	20 (15)	6 (8)	0.151
Laboratory measurements			
Corrected calcium in mmol/L^{b,d}	2.27 (2.23 - 2.32)	2.23 (2.19 - 2.29)	0.003*
Serum 25OHD in nmol/L	64 (49 - 83)	66 (54 - 79)	0.431
Low 25OHD^e	64 (47)	27 (36)	0.110
Phosphate in mmol/L	1.13 (1.04 - 1.21)	1.00 (0.88 - 1.12)	< 0.001*
PTH in pmol/L^b	5.2 (4.1 - 6.4)	4.55 (3.87 - 5.60)	0.050
TSH in mE/L^b	1.54 (1.09 - 2.21)	1.59 (1.19 - 2.21)	0.371

Abbreviations: IQR is interquartile range (i.e. 25th percentile – 75th percentile); RRSO is risk-reducing salpingo-oophorectomy; BMI is body mass index; HBOC is hereditary breast ovarian cancer; BMD is bone mineral density; LS is lumbar spine, FN is femoral neck; HRT is hormonal replacement therapy; AI is aromatase inhibitor; AOD is anti-osteoporotic drugs; GCS is glucocorticosteroids; PTH is parathyroid hormone; TSH is thyroid stimulating hormone.

Values in median (IQR) or No. (%). Comparisons by Mann-Whitney U test for non-parametric data and Chi Square or Fisher's Exact Test for dichotomous or categorical data.

