Bone mineral density and fractures after risk-reducing salpingo-oophorectomy in women at increased risk for breast and ovarian cancer

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Abstract

Background: Risk-reducing salpingo-oophorectomy (RRSO) reduces ovarian cancer risk in *BRCA* mutation carriers. RRSO is assumed to decrease bone mineral density (BMD) and increase fracture risk more than natural menopause. We aimed to compare BMD and fracture incidence after premenopausal RRSO to general population data and identify risk factors for low BMD and fractures after RRSO.

Methods: In 212 women with RRSO at premenopausal age, BMD was measured by dual energy X-ray absorptiometry. Fractures and risk factors were assessed by self-administered questionnaire. Fracture incidence after RRSO was compared to general practitioner data by using standardized incidence ratios (SIRs). Risk factors for low standardized BMD-scores and fractures were identified by regression analyses.

Results: Median age at RRSO was 42 years (range 35 – 52) and duration of follow-up 5 years (2 - 8). Standardized lumbar spine (Z = 0.01, p = 0.870) and femoral neck BMD (Z = 0.15, p = 0.019) were not lower than population BMD. Higher age at time of RRSO and use of hormonal replacement therapy were associated with higher, and current smoking with lower standardized BMD-scores. Sixteen women reported 22 fractures. Fracture incidence was not higher than expected from the general population (all fractures: 25 – 44 years: SIR 2.12; 95% CI 0.85 - 4.37; 45 - 64 years: SIR 1.65; 95% CI 0.92 – 2.72).

Conclusion: Five years after RRSO, BMD and fracture incidence were not different than expected from the general population. Based on these data it appears safe not to intensively screen for osteoporosis within five years after RRSO, although prospective research on the long-term effects of RRSO on bone is warranted.

Introduction

Breast and ovarian cancer risk is elevated in women with a family history of breast cancer (hereditary breast ovarian cancer, HBOC), especially in women carrying a germ line mutation in the *BRCA1* and *BRCA2* genes (5,42). These women often opt for risk-reducing salpingo-oophorectomy (RRSO) to reduce ovarian cancer risk, as ovarian cancer screening is not effective (28). RRSO reduces ovarian cancer risk by up to 96% (31). RRSO is advised in *BRCA1* and *BRCA2* mutation carriers at the age of 35 – 40 years and 40 – 45 years respectively (37). The median age at RRSO, which leads to acute oestrogen deprivation, is about 10 years earlier than natural menopause (38).

When ovarian oestrogen production declines during natural menopause, bone mineral density (BMD) decrease accelerates and fracture incidence increases (309). Reports on the effect of early and surgical menopause on BMD and fracture incidence are inconclusive. Several studies suggested that BMD was lower and fracture incidence higher after early natural and surgical menopause, than after natural menopause at normal age (310,311). Others found a transient effect on fracture incidence or no effect at all (295,296). Several studies reported a high risk of osteoporosis after RRSO; however, these studies were all prone to bias, due to retrospective study designs, selected study populations and in some cases lack of a control group (64,234-236).

This study aimed to compare BMD and fracture incidence after RRSO before menopausal age in an unselected consecutive series of *BRCA1/2* mutation carriers and women with a family history of breast and ovarian cancer, to what can be expected from the general female population. The secondary aim was to identify risk factors for low BMD and fracture incidence after RRSO.

Patients and Methods

Study population and protocol

Since 1994, all women visiting the family cancer clinic at the University Medical Center Groningen are registered in a prospective database (312). Between February 2011 and May 2012, all *BRCA1/2* mutation carriers and women with a positive family history of breast and ovarian cancer with RRSO at the age of ≤ 52 were invited to attend osteoporosis and fracture screening if they were ≥ two years after RRSO. Women in whom ovarian cancer was detected at RRSO were excluded. Of the 254 invited women, 212 attended and gave written informed consent for the study (*Figure 5.1*).

Women were evaluated by a researcher, under supervision of a medical doctor, according to a standard protocol which included measurement of height, weight, a self-administered questionnaire, collection of blood samples, and BMD measurement. The institutional ethics review board considered this study extended standard care.

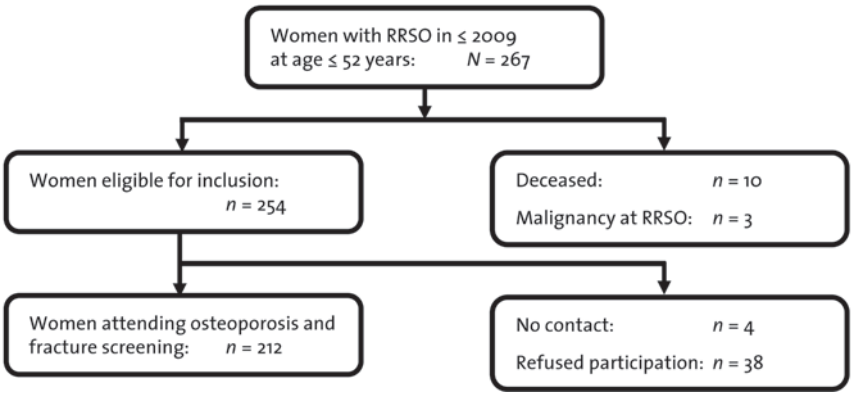

Figure 5.1: Flowchart on the recruitment and enrolment of participants eligible for osteoporosis and fracture screening after RRSO

Abbreviations: RRSO is risk-reducing salpingo-oophorectomy.

BMD measurement

BMD of the lumbar spine (LS; anterior-posterior projection at L1 – L4) and femoral neck (FN) were measured by dual energy x-ray absorptiometry (DXA) using a Hologic Discovery A densitometer (Hologic Inc., Bedford, MA). Vertebral fracture assessment (VFA) was performed with the same DXA machine as previously described (313).

Questionnaire

The questionnaire aimed at identifying history of bone fractures and risk factors for osteoporosis and low BMD. It was based on the clinical questionnaire used at the fracture and osteoporosis outpatient clinic at our centre (314). The questionnaire was sent to the patients before their osteoporosis and fracture screening visit. During the visit, missing or inconsistent answers were discussed by the researcher and the patient and corrected by the researcher if appropriate.

Laboratory assessments

A non-fasting blood sample was collected between 9:00 a.m. and 4:30 p.m. Calcium and albumin were measured by colorimetric assay (Roche Modular P, Mannheim, Germany; inter-assay coefficient of variation (IA–CV) < 2.0% and < 1.8%; lower detection limit 0.05 mmol/L and 10 g/L for calcium and albumin respectively). Calcium 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]). Serum 25(OH)D_3 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; IA-CV < 14.1%; recovery 93 – 98%; linearity r^2 = 0.9972. Accuracy was secured by the use of reference material from the National Institute of Standards & Technology (Gaithersburg, MD).

Study endpoints

Results of BMD measurement were expressed as BMD in grams/cm^2 and standardized by using Z- and T-scores. Z-scores present the number of standard deviations (SD) from the mean bone mineral density in woman of the same age, T-scores present the number of SDs from the mean peak BMD as reached in women between 20 – 30 years of age (201,316). According to the World Health Organisation definition, "osteoporosis" is defined as a T-score of ≤ -2.5; "osteopenia" as a T-score between -2.5 and -1.0; and "normal" as a T-score ≥ -1.0.

Fracture incidence after RRSO was evaluated by questionnaire. Fractures that were impossible based on clinical data were excluded. Aetiology of fractures was assessed to determine if they were fragility fractures, i.e. caused by low energy trauma. Low energy trauma was defined as a fall from standing position or a height of one meter or less (314).

To identify the prevalence of occult vertebral fractures, VFA data were used. Vertebral-shape deformities were classified using the Genant classification (grade 0: no deformities; grade 1: mild deformity, 20%–25% height decrease; grade 2: moderate deformity, 25% – 40% height decrease; and grade 3: severe deformity, > 40% height decrease) (317). In patients with a relative height reduction of any vertebra of ≥ 20% on VFA and no known previous vertebral fracture at that site, an X-ray of the thoracic and lumbar spine was made for further evaluation. According to the Dutch guidelines, a vertebral fracture was defined as a height reduction of > 25% on lateral X-ray of the spine or of > 40% on VFA scans (227).

Reference population

The standard Hologic reference databases for Caucasian women were used to calculate Z- and T-scores (201,316). For femoral neck references, data were retrieved from NHANES III (201). For lumbar spine, data were retrieved from a Hologic study on BMD in healthy American women (316).

Age-specific fracture incidence in Dutch women was obtained from a national survey on disease incidence in 4 general practices (318).

Statistical analysis

Analyses were conducted using IBM SPSS Statistics version 20 (IBM Corporation, Armonk, NY, USA). *P*-values < 0.05 were considered significant. Descriptive statistics were used to present patient characteristics using mean ± SD for parametric, median (range) for nonparametric, and number (%) for dichotomous data.

To compare BMD in the study population to the reference population, mean BMD Z-scores for the total group were compared to the mean Z-score in a healthy reference population (i.e. Z = 0, SD = 1) by using unpaired one-sample t-test.

Fracture incidence after RRSO was compared to age-specific fracture incidence in the Dutch female population by using Standardized Incidence Ratios (SIRs). SIRs were calculated for first and all incident fractures after RRSO.

Multiple imputation was applied to impute missing values for: BMD LS Z-score (n = 1), long term use of glucocorticosteroids (i.e. 7.5 mg prednisolone or equivalent ≥ 3 months; n = 1), corrected serum calcium (n = 2) and serum 25(OH)D_3 (n = 1), using all variables included in the regression models. Ten imputed datasets were used in the regression analyses and results were combined according to Rubin's rules (319).

Factors associated with BMD LS and FN Z-score were identified using linear univariate regression analyses. Factors individually characterizing the women who developed incident fractures after RRSO were identified using univariate logistic regression analysis. For the univariate analysis we selected factors from the questionnaire associated with breast cancer and clinical risk factors associated with fracture risk in the FRAX-tool (314,320). Multivariate regression analyses were performed with conditional stepwise backward inclusion of those variables with a p-value < 0.250 in univariate analysis. Multivariate analyses were corrected for ever use of anti-osteoporotic drugs (AOD) and calcium and vitamin D3 supplementation. Because fracture incidence was measured retrospectively, while questionnaire items and BMD were measured cross-

sectionally, we analysed risk factors that we assumed to be constant for a longer period of time, such as ever smoking, but not current smoking.

Results

Study population

In a total of 212 women, median age at RRSO was 42 years (range 30 - 53) and median current age was 49 years (36 - 65; *Table 5.1*). Follow-up time from RRSO to screening was 5 years (2 - 25). Of the 18 (9%) women who reported the use of anti-osteoporotic drugs after RRSO, 8 were using these drugs at the time of screening. Indications for AOD use are described in Table 5.1.

Bone mineral density

BMD LS and FN Z-score were not lower than Z = 0 (mean Z score 0.01 ± 1.09, p = 0.870 and mean Z-score 0.15 ± 0.93, p = 0.019, respectively; *Table 5.2*). Of all patients 6% (n = 13) had osteoporosis according to T-score, 12 of the lumbar spine and 1 of the femoral neck. Mean Z-scores for women with osteoporosis were -1.8 ± 0.44 and -0.08 ± 0.55 for lumbar spine and femoral neck respectively. Osteoporosis incidence was 9.2/1000 women-years.

Table 5.1: Demographics and main clinical features of the study population (*N* = 212)

Basic characteristics		M			M
Age in years	49 (36 – 65)	0	**Ever use calcium supplement**	63 (30)	0
Age at RRSO in years	42 (30 – 52)	0	**Current use**	46 (22)	
Follow-up in years	5 (2 – 25)	0	**Ever use vitamin D3 supplement**	61 (29)	0
BMI in kg/m²	26 (18 – 54)	0	**Current use**	49 (23)	
Menopausal status before RRSO		0	**Smoking – current**	41 (19)	0
Premenopausal	177 (84)		**Alcohol consumption in units/ week**	2 (0-35)	0
Regularly menstruating	94 (44)		**≤ 7 units/week**	174 (82)	
Irregularly menstruating	29 (14)		**> 7 units/week**	38 (18)	
OCP use	54 (26)		**Long-term use GCS[a]**	8 (4)	1
Postmenopausal	27 (13)		**Oncologic characteristics**		
Natural	11 (5)		**History of breast cancer**	80 (38)	0
Chemotherapy induced	16 (8)		**Chemotherapy**	60 (28)	0
Unknown: Hysterectomy	8 (4)		**Ever use AI**	11 (5)	0

Basic characteristics		M			M
Fracture before RRSO	64 (30)	0	**Ever use tamoxifen**	17 (8)	0
Parent with hip fracture	5 (2)		**Mutation status**		0
Ever use AOD	18 (9)	0	***BRCA*1**	121 (57)	
Current use	8 (4)		***BRCA*2**	60 (28)	
Indication AOD		0	**HBOC**	31 (15)	
AI use	3 (1)		**Ever use HRT**	100 (47)	0
GCS use	2 (1)		**Current use HRT**	51 (24)	0
Prevention	3 (1)		**Lab**		
Osteoporosis	3 (1)		**Corrected serum calcium in mmol/L**[b]	2.3 (2.0 – 2.5)	2
Osteopenia	5 (2)		**Serum 25(OH)D_3 in nmol/L**	64 (16 – 151)	1
Fracture after RRSO	2 (1)				

[a]Use of prednisone 7.5 mg or equivalent >3 months or >3 oral prednisolone courses per years;
[b]Calcium 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]).
Abbreviations: M is missing, RRSO is risk-reducing salpingo-oophorectomy, BMI is body mass index, AI is aromatase inhibitor, HBOC is hereditary breast ovarian cancer, HRT is hormonal replacement therapy, GCS is glucocorticosteroid, AOD is anti-osteoporotic drugs.
Values are median (range) and No. (%).

In the multivariate linear regression model, older age at RRSO, higher BMI and current hormonal replacement therapy (HRT) use were positively associated with BMD LS Z-score, while no covariates were significantly negatively associated (*Table 5.3*). For BMD FN Z-score, longer duration of follow-up, higher BMI and ever use of HRT were positively associated, while current smoking was negatively associated (*Table 5.3*).

Incident fractures

Seventeen (8%) women reported 23 fractures after RRSO. One fracture was excluded, as clinical data described a cartilage defect, but not a bone defect. Of the remaining 22 fractures in 16 women, 19 were considered fragility fractures (*Table 5.2*). Median follow-up to first fracture after RRSO was 5 years (1 - 15). Fracture incidence after RRSO was comparable to fracture incidence in the GP reference population (*Table 5.4*). In the multivariate analysis, alcohol consumption was positively associated with the occurrence of fractures after RRSO (*Table 5.5*).

Table 5.2: Bone mineral density and fracture incidence after RRSO

Bone mineral density					
				Lumbar spine (*n* = 211)[a]	**Femoral neck (*N* = 212)**
BMD in g/cm² [b]				0.97 ± 0.12	0.79 ± 0.11
Z-score – total study population				0.01 ± 1.09	0.15 ± 0.93*
Women with a history of breast cancer				-0.02 ± 1.00	0.011 ± 0.87
Women without a history of breast cancer				0.03 ± 2.24	0.18 ±0.039*
Women with age ≤ 52 years				-0.02 ± 1.07	0.11 ± 0.93
Women with age > 52 years				0.09 ± 1.13	0.24 ± 0.94*
T-score				-0.72 ± 1.11	-0.6 ± 0.96
Osteoporosis				13 (6)	
Osteopenia				89 (42)	
Normal BMD				110 (52)	
Fractures					
Questionnaire				**VFA (*n* = 206)**	
Women with fracture	16 (8)	**Other fractures**	17	**Height reduction > 20%**	12 (6)
No. of fractures	22	**Clavicle**	2	**Known abnormalities**	5
Fragility fractures[c]	19	**Elbow**	4	**VF before RRSO**	1
Vertebral	3	**Pelvis**	1	**VF after RRSO**	3
Hip	-	**Ankle/ Foot**	4	**Sheuermann's disease**	1
Wrist	2	**Toe/ finger**	6	**Height reduction > 25% on X-ray**	0

[a]For one woman no DXA of the lumbar spine was made because of the presence of ostheosynthesis material after scoliosis surgery; [b]For three women, DXA scans were performed outside the UMCG: one scan before the start of strontiumranelate therapy, one a year before study entry and one because of logistics close to her hometown; [c]Fragility fractures are fractures from a fall from standing position or a height < 1 metres
Abbreviations: RRSO is risk-reducing salpingo-oophorectomy; BMD is bone mineral density; VFA is vertebral fracture assessment; VF is vertebral fracture; RRSO is risk-reducing salpingo-oophorectomy
Values are mean ± SD or no. (%); *$p < 0.05$ with one-sample t-test.

Table 5.3: Uni- and multivariate linear regression analyses on Z-scores for bone mineral density of the lumbar spine and femoral neck

	Univariate analysis Z-score lumbar spine			Multivariate analysis Z-score lumbar spine[a]		
	β	*SE*	*p*-value	β	*SE*	*p*-value
Age at RRSO (per year)	0.02	0.01	0.101	0.06	0.02	< 0.001*
Follow-up (per year)	0.01	0.02	0.625			
BMI (per kg/m²)	0.04	0.01	0.004*	0.05	0.01	< 0.001*
Postmenopausal before RRSO	-0.24	0.23	0.287			
Fracture before RRSO	0.06	0.16	0.706			
Parent with hip fracture	0.97	0.49	0.047*			
Smoking – current	-0.23	0.19	0.234			
Alcohol per unit/wk	0.01	0.01	0.326			
Alcohol > 7 units/wk	-0.04	0.20	0.856			
Long-term use GCS[b]	0.24	0.38	0.527			
History of breast cancer	-0.06	0.16	0.699			
Chemotherapy	-0.08	0.17	0.644			
Ever use of AI	-0.53	0.34	0.117			
Ever use of tamoxifen	0.13	0.28	0.634			
Ever use of HRT	0.22	0.15	0.141			
Current use of HRT	0.33	0.17	0.056	0.76	0.19	< 0.001*
Corrected serum calcium	-0.39	0.99	0.692			
Serum 25(OH)D_3	0.00	0.00	0.182			

[a]Adjusted for AOD/calcium/vitamin D3 supplement use; [b]Use of prednisone 7.5 mg or equivalent > 3 months or > 3 oral prednisolone courses per years
Abbreviations: *SE* is standard error; RRSO is risk-reducing salpingo-oophorectomy; BMI is body mass index; AI is aromatase inhibitor; HRT is hormonal replacement therapy; GCS is glucocorticosteroids; AOD is anti-osteoporotic drugs.
*p < 0.05.

Univariate analysis Z-score femoral neck			Multivariate analysis Z-score femoral neck[a]		
β	*SE*	*p*-value	β	SE	*p*-value
0.00	0.01	0.969			
0.06	0.02	0.003*	0.04	0.02	0.043*
0.04	0.01	< 0.001*	0.04	0.01	< 0.001*
-0.11	0.19	0.582			
-0.07	0.14	0.601			
-0.11	0.42	0.788			
-0.54	0.16	0.001*	-0.52	0.15	0.001*
-0.01	0.01	0.655			
-0.11	0.17	0.522			
0.01	0.33	0.973			
-0.07	0.13	0.608			
-0.03	0.14	0.812			
-0.11	0.29	0.700			
0.14	0.24	0.563			
0.23	0.13	0.067	0.25	0.12	0.043*
0.12	0.15	0.417			
0.36	0.84	0.666			
0.00	0.00	0.806			

Table 5.4: Comparison of fracture incidence observed after RRSO to fracture incidence expected after RRSO from general practitioner data using standardized incidence ratios (SIRs)

First incident fractures						
Age in years	No. of women[a]	No. of women-years	FI reference population[b]	Exp.	Obs.	SIR (95% CI)
25 - 44	139	557.38	5.7	3.2	5	1.56 (0.50 - 3.65)
45 - 64	158	807.56	10.7	8.6	11	1.28 (0.64 - 2.29)
65 - 74	1	0.33	25	0	0	-
All incident fractures						
25 - 44	139	570.14	5.7	3.3	7	2.12 (0.85 - 4.37)
45 - 64	160	845.34	10.7	9.1	15	1.65 (0.92 - 2.72)
65 - 74	2	1.23	25	0	0	-

[a]No. of women attributing to calculation of total no. of women-years per age group; [b]van de Lisdonk et al, 2008 (318).
Abbreviations: RRSO is risk-reducing salpingo-oophorectomy; No. is number; FI is fracture incidence/1000 women-years; Exp. is expected number of fractures; Obs. is observed number of fractures; SIR is standardized incidence ratio; CI is confidence interval.

Table 5.5: Uni- and multivariate logistic regression on women who reported fractures after RRSO

	Univariate analysis			Multivariate analysis[a]		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Age (per year)	1.0	0.9 – 1.1	0.511			
Age at RRSO (per year)	1.0	0.9 – 1.1	0.668			
Duration of follow-up (per year)	1.1	1.0 – 1.3	0.051			
BMI (per kg/m²)	1.0	0.9 – 1.1	0.847			
Postmenopausal before RRSO	0.4	0.1 – 3.3	0.405			
Fracture before RRSO	1.1	0.4 – 3.2	0.923			
Parent with hip fracture	-	-	-			
Ever smoking	0.5	0.2 – 1.3	0.130			
Alcohol (per unit/week)	1.1	1.0 – 1.1	0.179*			
Alcohol >7 units/week	3.1	1.0 – 9.1	0.042*	3.1	1.0 – 9.2	0.014*
Long-term use GCS[b]	1.7	0.2 – 14.5	0.638			
History of breast cancer	1.7	0.6 – 4.8	0.297			
Chemotherapy	1.6	0.5 – 4.6	0.399			
Ever use of AI	1.2	0.1 – 10.4	0.842			
Ever use of tamoxifen	1.7	0.4 – 8.3	0.497			
Ever use of HRT	0.7	0.2 – 1.9	0.423			

	Univariate analysis			Multivariate analysis[a]
Z-score BMD lumbar spine	1.0	0.6 – 1.6	0.993	
Z-score BMD femoral neck	1.0	0.6 – 1.7	0.974	
Corrected serum calcium	1.6	0.0 – 1167.0	0.896	
Serum 25(OH)D_3	1.0	1.0 – 1.0	0.892	

[a]Adjusted for AOD/calcium/vitamin D3 supplement use; [b]Use of prednisone 7.5 mg or equivalent > 3 months or > 3 oral prednisolone courses per years.
Abbreviations: CI is confidence interval; RRSO is risk-reducing salpingo-oophorectomy; BMI is body mass index; AI is aromatase inhibitor; HRT is hormonal replacement therapy; GCS is glucocorticosteroids; BMD is bone mineral density; AOD is anti-osteoporotic drugs.
*$p < 0.05$.

VFA outcomes

On VFA, 12 (6%) women had 13 relative vertebral height reductions of ≥ 20%. The affected vertebrae were: Th6, Th7 (n = 2), Th11 (n = 2), Th12 (n = 3), L1 (n = 3), L2 and L3. In five women, these vertebral deformities were diagnosed before study entry and were considered known vertebral fractures. Of the other seven women, four had a minimal deformity and three a moderate deformity according to the Genant classification on VFA. None of these seven women had height reductions of > 25% on X-ray, so none of the deformations detected with VFA fulfilled the Dutch definition of a vertebral fracture on X-ray (227).

Secondary causes of osteoporosis

Several women reported co-morbid diseases that may have contributed to a higher fracture risk according to the QFracture algorithm, either in medical history or at the time of study participation (321). Of all women, 37% (n = 79) reported one or more co-morbid diseases, of the women with fractures this was 41% (n = 7, p = 0.728; *Table S5.1*).

Discussion

In an unselected consecutive series of 212 women with RRSO at age ≤ 52, after a median follow-up of five years, BMD was not lower (BMD LS Z-score: 0.01, p = 0.870 and BMD FN Z- score: 0.15, p =0.019) and fracture incidence was not higher (all fractures: 25 – 44 years: SIR 2.12; 95% CI 0.85 - 4.37; 45 - 64 years: SIR 1.65; 95% CI 0.92 – 2.72) than what can be expected from an age-matched reference population.

These findings are in contrast to the common hypothesis that after RRSO BMD decreases and fracture incidence rises faster than after natural menopause. However, these are in line with the results of previous studies that failed to find an effect of early and surgical menopause on bone mineral density and fractures (296,297), and studies that found an effect of early or surgical menopause only in the first years after the procedure, which suggests that the effect of RRSO might eventually be overruled by chronological age (293,295).

However, an effect of RRSO on BMD or fracture risk in this study population cannot be completely excluded because of several reasons. Firstly, a large proportion of women had used bone protective medication, such as HRT (47%), tamoxifen (8%) and AOD (9%). This might have attenuated the effect of RRSO on both BMD and fracture incidence. HRT use was associated with higher BMD LS and FN Z-scores in multivariate analysis, which confirms the known protective effect of HRT on BMD (322). Secondly, a positive relation between age at RRSO and BMD Z-score was shown, which might indicate that BMD is lower in women who have RRSO at younger age. Thirdly, it is known that fracture incidence is relatively low at young age and increases significantly with older age (295). A long-term effect of RRSO on fracture risk at older age needs to be further investigated in a prospective longitudinal study. Lastly, changes in bone characteristics might increase fracture risk independently of actual BMD, such as structural changes in bone architecture and changes in bone loss rate. BMD measurement by DXA might not be sensitive enough to evaluate bone architecture and detect structural changes in bone after RRSO. It is possible that these changes are visible on more advanced techniques like quantitative computed tomography, which measures cortical and trabecular BMD separately or on DXA using trabecular bone score software (323,324). Also, a higher rate of bone loss has been shown to be associated with fracture risk independent of actual BMD and a higher rate of bone loss in surgical menopause compared to natural menopause has been reported by others (325,326). As BMD was measured cross-sectionally in this study, BMD before and after RRSO could not be compared in the same woman to calculate bone loss rates. Bone turnover marker measurement might be useful to estimate bone loss rate after RRSO (232).

In addition, this study has several limitations. Questionnaires were used to assess fracture incidence and risk factors, which might have induced a risk of selective reporting. Fracture incidence was not systematically confirmed with hospital data. We considered a questionnaire a reliable tool to measure fracture incidence. Clinical confirmation was available for 16/22 fractures, which might be considered a limitation.

Fracture incidence in the control population was assessed trough general practitioner (GP) reports, which might have resulted in under reporting of the actual fracture risk, as some fractures are directly seen at the emergency department. However, as almost all Dutch citizens are registered with a GP who records all their diagnoses, and because GPs function as the gatekeepers for specialized medical care, one can assume that GPs have the most complete file on the incidence of health problems in the general population. Moreover, within this study, GPs are trained for adequately registering health problems and quality of the registration was monitored (318). This was further supported by the finding that fracture incidence before RRSO was comparable to fracture incidence in the reference population (*Table S5.2*).

To our knowledge, this study is the largest study on the effects of RRSO at premenopausal age on bone mineral density and fracture incidence. Participation rate was as high as 83%. Also, we are the first to measure BMD after RRSO in an unselected consecutive series of women with RRSO, which makes this study population representative for actual practice without selection bias.

In this study, women with RRSO at premenopausal age did not have lower BMD and higher fracture incidences compared to an age-matched control population. Based on these results, it cannot be advised to offer BMD measurements to all women after RRSO. Prospective research remains warranted to evaluate long-term fracture incidence after RRSO.

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Conflict of interest statement

The following authors disclosed possible conflicts of interest. Bruce H.R. Wolffenbuttel has received grant support for clinical studies 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

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Supplements

Supplementary tables

Table S5.1: Quality control of fracture reference database by using expected and observed fracture incidence before RRSO

All incident fractures						
Age in years	No. of women[a]	No. of women-years	FI reference population[b]	Exp.	Obs.	SIR (95% CI)
0 - 4	212	1060	5	5.3	7	1.32 (0.53 - 2.72)
5 - 14	212	2120	25.7	54.5	52	0.95 (0.71 - 1.25)
15 - 24	212	2120	11.4	24	20	0.83 (0.51 - 1.29)
25 - 44	212	3497.22	5.7	19.9	22	1.11 (0.69 - 1.67)
45 - 64	73	277.75	10.7	3.0	5	1.67 (0.54 - 3.89)

[a]No. of women attributing to calculation of total no. of women-years per age group; [b]van de Lisdonk et al, 2008 (318).
Abbreviations: RRSO is risk-reducing salpingo-oophorectomy; No. is number; FI is fracture incidence/1000 women-years; Exp. is expected number of fractures; Obs. is observed number of fractures; SIR is standardized incidence ratio; CI is confidence interval.

Table S5.2: Comorbidities associated with elevated fracture risk in the QFracture algorithm for women with and without fractures after RRSO

	Women with fractures after RRSO (*n* = 16)	Women without fracture after RRSO (*n* = 169)	*p*-value
One or more comorbidities	7 (44)	72 (37)	0.577
Asthma/COPD	2 (13)	12 (6)	0.285
Any cancer[a]	0 (0)	8 (4)	1.000
Cardiovascular disease	1 (6)	7 (4)	0.472
Dementia	-	-	-
Epilepsy	1 (6)	5 (3)	0.379
Chronic liver disease	0 (0)	1 (1)	1.000
Parkinsons' disease	-	-	-
Reumatoid arthritis/SLE	0 (0)	2 (1)	1.000
Chronic renal disease	-	-	-
Diabetes	0 (0)	5 (3)	1.000
Endocrine disorders	0 (0)	12 (6)	0.606
Gastro-intestinal malabsorption	0 (0)	7 (4)	1.000
Antidepressants use	3 (19)	36 (18)	1.000

Table S5.3: Linear regression analyses on bone mineral density Z-score in women with and without a history of breast cancer

Linear regression analyses on bone mineral density Z-score in women with a history of breast cancer (*n* = 80)						
	Univariate analysis Z-score lumbar spine			Multivariate analysis Z-score lumbar spine[a]		
	β	*SE*	*p*-value	β	*SE*	*p*-value
Age at RRSO (per year)	0.027	0.023	0.224			
Follow-up (per year)	0.018	0.030	0.554			
BMI (per kg/m²)	0.036	0.021	0.094			
Postmenopausal before RRSO	-0.014	0.267	0.958			
Fracture before RRSO	-0.293	0.252	0.245			
Parent with hip fracture	0.953	0.718	0.184			
Smoking – current	-0.211	0.289	0.466			
Alcohol per unit/wk	0.033	0.017	0.050			
Alcohol > 7 units/wk	0.202	0.270	0.455			
Long-term use GCS[b]	0.222	0.559	0.691			
History of breast cancer						
Chemotherapy	-0.065	0.257	0.801			
Ever use of AI	-0.536	0.323	0.097			
Ever use of tamoxifen	0.201	0.276	0.467			
Ever use of HRT	0.191	0.317	0.547			
Current use of HRT	0.393	0.466	0.399			
Corrected serum calcium	1.574	1.377	0.261			
Serum 25(OH)D_3	0.003	0.005	0.466			
Linear regression analyses on bone mineral density Z-score in women without a history of breast cancer (*n* = 131)						
Age at RRSO (per year)	0.024	0.019	0.200	0.095	0.022	< 0.001*
Follow-up (per year)	0.008	0.034	0.826			
BMI (per kg/m²)	0.041	0.018	0.021*	0.057	0.018	0.001*
Postmenopausal before RRSO	-0.746	0.444	0.093	-1.143	0.452	0.011*
Fracture before RRSO	0.255	0.213	0.232			
Parent with hip fracture	0.991	0.663	0.135			
Smoking – current	-0.235	0.250	0.346			
Alcohol per unit/wk	-0.008	0.021	0.696			
Alcohol > 7 units/wk	-0.219	0.280	0.435			
Long-term use GCS[b]	0.259	0.521	0.620			
History of breast cancer						
Chemotherapy	-0.132	1.149	0.908			

Univariate analysis Z-score femoral neck			Multivariate analysis Z-score femoral neck[a]		
β	*SE*	*p*-value	β	*SE*	*p*-value
0.026	0.019	0.172			
0.061	0.025	0.015*	0.050	0.024	0.041*
0.054	0.018	0.002*	0.050	0.017	0.041*
0.165	0.230	0.473			
-0.112	0.218	0.606			
-0.265	0.623	0.670			
-0.495	0.243	0.042*			
0.006	0.015	0.687			
0.060	0.233	0.796			
-0.226	0.513	0.660			
0.076	0.221	0.730			
-0.073	0.283	0.795			
0.213	0.273	0.369			
0.058	0.273	0.831			
-0.244	0.401	0.543			
1.899	1.165	0.103			
-0.001	0.004	0.778			
-0.013	0.016	0.416			
0.059	0.029	0.039			
0.036	0.015	0.017	0.039	0.015	0.008*
-0.630	0.375	0.093			
-0.056	0.182	0.757			
-0.010	0.569	0.986			
-0.569	0.207	0.006	-0.625	0.202	0.002*
-0.017	0.018	0.340			
-0.232	0.236	0.326			
0.170	0.444	0.702			
-0.178	0.978	0.856			

Linear regression analyses on bone mineral density Z-score in women without a history of breast cancer (*n* = 131)						
	Univariate analysis Z-score lumbar spine			Multivariate analysis Z-score lumbar spine[a]		
	β	*SE*	*p*-value	β	*SE*	*p*-value
Ever use of AI						
Ever use of tamoxifen						
Ever use of HRT	0.278	0.211	0.186			
Current use of HRT	0.339	0.207	0.101	0.890	0.231	< 0.001*
Corrected serum calcium	-1.859	1.368	0.174			
Serum 25(OH)D_3	0.004	0.004	0.286			

[a]Adjusted for AOD/calcium/vitamin D3 supplement use; [b]Use of prednisone 7.5 mg or equivalent > 3 months or > 3 oral prednisolone courses per years.
Abbreviations: *SE* is standard error; RRSO is risk-reducing salpingo-oophorectomy; BMI is body mass index; AI is aromatase inhibitor; HRT is hormonal replacement therapy; GCS is glucocorticosteroids; AOD is anti-osteoporotic drugs.
*$p < 0.05$.

Table S5.4: Linear regression analyses on bone mineral density Z-score in women aged ≤ 52 years and > 52 years

Linear regression analyses on bone mineral density Z-score in women aged ≤ 52 years (*n* = 148)						
	Univariate analysis Z-score lumbar spine			Multivariate analysis Z-score lumbar spine[a]		
	β	*SE*	*p*-value	β	*SE*	*p*-value
Age at RRSO (per year)	0.029	0.022	0.187	0.073	0.023	0.001*
Follow-up (per year)	0.022	0.036	0.529			
BMI (per kg/m²)	0.039	0.015	0.009	0.053	0.014	< 0.001*
Postmenopausal before RRSO	-0.224	0.494	0.651			
Fracture before RRSO	0.055	0.194	0.774			
Parent with hip fracture	1.005	0.539	0.062			
Smoking – current	-0.113	0.213	0.596			
Alcohol per unit/wk	-0.001	0.017	0.942			
Alcohol > 7 units/wk	-0.344	0.241	0.153			
Long-term use GCS[b]	-0.031	0.417	0.941			
History of breast cancer	-0.168	0.192	0.382			
Chemotherapy	-0.241	0.212	0.254			
Ever use of AI	-0.841	0.485	0.083			
Ever use of tamoxifen	-0.053	0.370	0.885			
Ever use of HRT	0.374	0.177	0.035*			
Current use of HRT	0.420	0.185	0.023*	0.766	0.195	< 0.001*
Corrected serum calcium	-1.318	1.171	0.260			
Serum 25(OH)D_3	0.002	0.004	0.575			

Univariate analysis Z-score femoral neck			Multivariate analysis Z-score femoral neck[a]		
β	*SE*	*p*-value	β	*SE*	*p*-value
0.340	0.177	0.055	0.342	0.170	0.044*
0.163	0.177	0.359			
-0.777	1.170	0.507			
0.001	0.003	0.669			

Univariate analysis Z-score femoral neck			Multivariate analysis Z-score femoral neck[a]		
β	*SE*	*p*-value	β	*SE*	*p*-value
-0.004	0.019	0.837			
0.073	0.030	0.016*			
0.052	0.013	< 0.001*	0.053	0.012	< 0.001*
0.017	0.424	0.968			
0.015	0.168	0.929			
0.142	0.472	0.764			
-0.566	0.178	0.001*	-0.552	0.167	0.001*
-0.038	0.015	0.009*			
-0.591	0.202	0.003*			
-0.253	0.360	0.483			
-0.132	0.166	0.426			
-0.059	0.184	0.750			
-0.406	0.423	0.337			
-0.025	0.321	0.938			
0.285	0.154	0.064	0.368	0.142	0.010*
0.147	0.162	0.367			
0.847	1.016	0.405			
0.001	0.003	0.817			

Linear regression analyses on bone mineral density Z-score in women aged >52 years (*n* = 64)						
	Univariate analysis Z-score lumbar spine			Multivariate analysis Z-score lumbar spine[a]		
	β	*SE*	*p*-value	β	*SE*	*p*-value
Age at RRSO (per year)	0.034	0.041	0.406			
Follow-up (per year)	-0.007	0.034	0.829			
BMI (per kg/m²)	0.043	0.032	0.171			
Postmenopausal before RRSO	-0.460	0.304	0.130			
Fracture before RRSO	0.070	0.308	0.821			
Parent with hip fracture	0.922	1.144	0.420			
Smoking – current	-0.577	0.425	0.175			
Alcohol per unit/wk	0.035	0.023	0.123			
Alcohol > 7 units/wk	0.504	0.339	0.137			
Long-term use GCS[b]	1.745	1.117	0.120			
History of breast cancer	0.061	0.286	0.830			
Chemotherapy	0.116	0.288	0.688			
Ever use of AI	-0.322	0.488	0.508			
Ever use of tamoxifen	0.309	0.429	0.472			
Ever use of HRT	0.010	0.345	0.977			
Current use of HRT	0.369	0.818	0.652			
Corrected serum calcium	1.405	1.819	0.440			
Serum 25(OH)D_3	0.008	0.006	0.152			

[a]Adjusted for AOD/calcium/vitamin D3 supplement use; [b]Use of prednisone 7.5 mg or equivalent > 3 months or > 3 oral prednisolone courses per years.
Abbreviations: *SE* is standard error; RRSO is risk-reducing salpingo-oophorectomy; BMI is body mass index; AI is aromatase inhibitor; HRT is hormonal replacement therapy; GCS is glucocorticosteroids; AOD is anti-osteoporotic drugs.
*$p < 0.05$.

Univariate analysis Z-score femoral neck			Multivariate analysis Z-score femoral neck[a]		
β	*SE*	*p*-value	β	*SE*	*p*-value
-0.052	0.034	0.126			
0.046	0.028	0.096			
0.009	0.027	0.722			
-0.333	0.255	0.192			
-0.277	0.252	0.271			
-1.057	0.942	0.262			
-0.404	0.353	0.253			
0.045	0.018	0.013*	0.045	0.018	0.013*
0.753	0.269	0.005*			
1.390	0.835	0.096			
-0.039	0.237	0.869			
-0.070	0.239	0.768			
0.084	0.405	0.836			
0.282	0.355	0.427			
0.378	0.281	0.179			
0.835	0.670	0.212			
-0.809	1.497	0.589			
< 0.001*	0.005	0.997			