

University of Groningen

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

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

*Published in:*  
European Journal of Cancer

*DOI:*  
[10.1016/j.ejca.2014.11.022](https://doi.org/10.1016/j.ejca.2014.11.022)

**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:*  
2015

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

*Citation for published version (APA):*

Fakkert, I. E., Abma, E. M., Westrik, I. G., Lefrandt, J. D., Wolffenbuttel, B. H. R., Oosterwijk, J. C., Slart, R. H. J. A., van der Veer, E., de Bock, G. H., & Mourits, M. J. E. (2015). Bone mineral density and fractures after risk-reducing salpingo-oophorectomy in women at increased risk for breast and ovarian cancer. *European Journal of Cancer*, 51(3), 400-408. <https://doi.org/10.1016/j.ejca.2014.11.022>

**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).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**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.



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



Ingrid E. Fakkert<sup>a,1</sup>, Elske Marije Abma<sup>b,1</sup>, Iris G. Westrik<sup>c,1</sup>, Joop D. Lefrandt<sup>c,1</sup>, Bruce H.R. Wolffenbuttel<sup>d,1</sup>, Jan C. Oosterwijk<sup>e,1</sup>, Riemer H.J.A. Slart<sup>f,1</sup>, Eveline van der Veer<sup>g,1</sup>, Geertruida H. de Bock<sup>a,1</sup>, Marian J.E. Mourits<sup>h,\*</sup>

<sup>a</sup> University of Groningen, University Medical Center Groningen, Department of Epidemiology, Groningen, The Netherlands

<sup>b</sup> University of Groningen, University Medical Center Groningen, Department of Internal Medicine, Division of Geriatric Medicine, Groningen, The Netherlands

<sup>c</sup> University of Groningen, University Medical Center Groningen, Department of Internal Medicine, Division of Vascular Medicine, Groningen, The Netherlands

<sup>d</sup> University of Groningen, University Medical Center Groningen, Department of Endocrinology, Groningen, The Netherlands

<sup>e</sup> University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, The Netherlands

<sup>f</sup> University of Groningen, University Medical Center Groningen, Department of Nuclear Medicine and Molecular Imaging, Groningen, The Netherlands

<sup>g</sup> University of Groningen, University Medical Center Groningen, Department of Laboratory Medicine, Groningen, The Netherlands

<sup>h</sup> University of Groningen, University Medical Center Groningen, Department of Gynaecology, Groningen, The Netherlands

Received 29 August 2014; received in revised form 20 November 2014; accepted 27 November 2014

Available online 18 December 2014

### KEYWORDS

Genes  
*BRCA1*, *BRCA2*  
Ovariectomy  
Osteoporosis  
Fractures  
Bone

**Abstract** *Aim:* 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 standardised incidence ratios (SIRs). Risk factors for low standardised BMD-scores and fractures were identified by regression analyses.

\* Corresponding author at: University Medical Center Groningen, Department of Gynaecology, CB21, PO Box 30.001, 9700 RB Groningen, The Netherlands. Tel.: +31 50 361 3000; fax: +31 50 361 1806.

E-mail addresses: [i.e.fakkert@umcg.nl](mailto:i.e.fakkert@umcg.nl) (I.E. Fakkert), [e.m.abma@umcg.nl](mailto:e.m.abma@umcg.nl) (E.M. Abma), [iriswestrik@gmail.com](mailto:iriswestrik@gmail.com) (I.G. Westrik), [j.d.lefrandt@umcg.nl](mailto:j.d.lefrandt@umcg.nl) (J.D. Lefrandt), [b.h.r.wolffenbuttel@umcg.nl](mailto:b.h.r.wolffenbuttel@umcg.nl) (B.H.R. Wolffenbuttel), [j.c.oosterwijk@umcg.nl](mailto:j.c.oosterwijk@umcg.nl) (J.C. Oosterwijk), [r.h.j.a.slart@umcg.nl](mailto:r.h.j.a.slart@umcg.nl) (R.H.J.A. Slart), [e.van.der.veer@umcg.nl](mailto:e.van.der.veer@umcg.nl) (E. van der Veer), [g.h.de.bock@umcg.nl](mailto:g.h.de.bock@umcg.nl) (G.H. de Bock), [m.j.e.mourits@umcg.nl](mailto:m.j.e.mourits@umcg.nl) (M.J.E. Mourits).

<sup>1</sup> Address: PO Box 30.001, 9700 RB Groningen, The Netherlands.

<http://dx.doi.org/10.1016/j.ejca.2014.11.022>

0959-8049/© 2014 Elsevier Ltd. All rights reserved.

**Results:** Median age at RRSO was 42 years (range 35–65) and duration of follow-up 5 years (2–8). Standardised 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 standardised 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% confidence interval (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.

© 2014 Elsevier Ltd. All rights reserved.

## 1. 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 [1,2]. These women often opt for risk-reducing salpingo-oophorectomy (RRSO) to reduce ovarian cancer risk, as ovarian cancer screening is not effective [3]. RRSO reduces ovarian cancer risk by up to 96% [4]. RRSO is advised in *BRCA1* and *BRCA2* mutation carriers at the age of 35–40 years and 40–45 years, respectively [5]. The median age at RRSO, which leads to acute oestrogen deprivation, is about 10 years earlier than natural menopause [6].

When ovarian oestrogen production declines during natural menopause, bone mineral density (BMD) decrease accelerates and fracture incidence increases [7]. 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 [8,9]. Others found a transient effect on fracture incidence or no effect at all [10,11]. 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 [12–15].

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.

## 2. Patients and methods

### 2.1. 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 [16]. 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  $\leq 52$  were invited to attend osteoporosis and fracture screening if they were  $\geq$  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 (Fig. 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.

### 2.2. 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 [17].

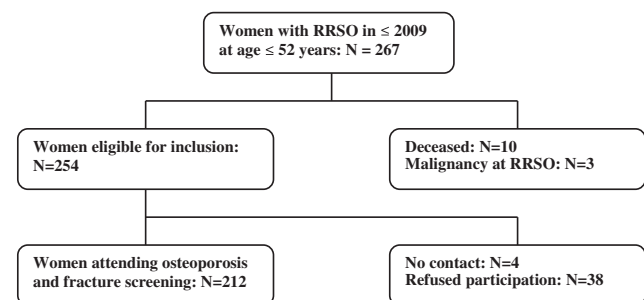


Fig. 1. Flowchart on the recruitment and enrolment of participants eligible for osteoporosis and fracture screening after RRSO. Abbreviations: RRSO is risk-reducing salpingo-oophorectomy.

### 2.3. 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 [18]. 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.

### 2.4. 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<sub>3</sub> was measured by isotope dilution–online solid phase extraction liquid chromatography–tandem mass spectrometry [19]. 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).

### 2.5. Study end-points

Results of BMD measurement were expressed as BMD in g/cm<sup>2</sup> and standardised 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 [20,21]. T-scores present the number of SDs from the mean peak BMD as reached in women between 20 and 30 years of age [20,21]. According to the World Health Organisation definition, ‘osteoporosis’ is defined as a T-score of  $\leq -2.5$ ; ‘osteopenia’ as a T-score between  $-2.5$  and  $-1.0$ ; and ‘normal’ as a T-score  $\geq -1.0$ .

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 metre or less [18].

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) [22]. In patients with a relative height reduction of any vertebra of  $\geq 20\%$  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 [23].

### 2.6. Reference population

The standard Hologic reference databases for Caucasian women were used to calculate Z- and T-scores [20,21]. For femoral neck references, data were retrieved from NHANES III [20]. For lumbar spine, data were retrieved from a Hologic study on BMD in healthy American women [21].

Age-specific fracture incidence in Dutch women was obtained from a national survey on disease incidence in four general practices [24].

### 2.7. Statistical analysis

Analyses were conducted using IBM SPSS Statistics version 20 (IBM Corporation, Armonk, NY, United States of America (USA)). *p*-Values <0.05 were considered significant. Descriptive statistics were used to present patient characteristics using mean  $\pm$  SD for parametric, median (range) for non-parametric 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 standardised 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  $\geq 3$  months;  $N = 1$ ), corrected serum calcium ( $N = 2$ ) and serum 25(OH)D<sub>3</sub> ( $N = 1$ ), 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 [25].

Factors associated with BMD LS and FN Z-score were identified using linear univariate regression analyses. Factors individually characterising 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

Table 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)
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)
BMI in kg/m <sup>2</sup>	26 (18–54)	0	Current use	49 (23)
Menopausal status before RRSO		0	Smoking – current	41 (19)
Premenopausal	177 (84)		Alcohol consumption in units/week	2 (0–35)
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 <sup>a</sup>	8 (4)
Postmenopausal	27 (13)		Oncologic characteristics	
Natural	11 (5)		History of breast cancer	80 (38)
Chemotherapy induced	16 (8)		Chemotherapy	60 (28)
Unknown: Hysterectomy	8 (4)		Ever use AI	11 (5)
Fracture before RRSO	64 (30)	0	Ever use tamoxifen	17 (8)
Parent with hip fracture	5 (2)		Mutation status	0
Ever use AOD	18 (9)	0	<i>BRCA1</i>	121 (57)
Current use	8 (4)		<i>BRCA2</i>	60 (28)
Indication AOD		0	HBOC	31 (15)
AI use	3 (1)		Ever use HT	100 (47)
GCS use	2 (1)		Current use HT	51 (24)
Prevention	3 (1)		Lab	
Osteoporosis	3 (1)		Corrected serum calcium in mmol/L <sup>b</sup>	2.3 (2.0–2.5)
Osteopaenia	5 (2)		Serum 25(OH)D <sub>3</sub> in nmol/L	64 (16–151)
Fracture after RRSO	2 (1)			

Values are median (range) and No. (%); M is missing, RRSO is risk-reducing salpingo-oophorectomy, BMI is body mass index, AI is aromatase inhibitor, HBOC is hereditary breast ovarian cancer, HT is hormonal replacement therapy, GCS is glucocorticosteroid, AOD is anti-osteoporotic drugs.

<sup>a</sup> Use of prednisone 7.5 mg or equivalent >3 months or >3 oral prednisolone courses per years.

<sup>b</sup> 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]).

factors associated with fracture risk in the FRAX-tool [18,26]. 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.

### 3. Results

#### 3.1. Study population

In a total of 212 women, median age at RRSO was 42 years (range 30–52) and median current age was 49 years (36–65; Table 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, eight were using these drugs at the time of screening. Indications for AOD use are described in Table 1.

#### 3.2. Bone mineral density

BMD LS and FN  $Z$ -scores were not lower than  $Z = 0$  (mean  $Z$  score  $0.01 \pm 1.09$ ,  $p = 0.870$  and mean  $Z$ -score  $0.15 \pm 0.93$ ,  $p = 0.019$ , respectively; Table 2). Of all patients 6% ( $n = 13$ ) had osteoporosis according to  $T$ -score, 12 of the lumbar spine and one of the femoral neck. Mean  $Z$ -scores for women with osteoporosis were  $-1.8 \pm 0.44$  and  $-0.08 \pm 0.55$  for lumbar spine and femoral neck respectively. Osteoporosis incidence was 9.2/1000 women-years.

In the multivariate linear regression model, older age at RRSO, higher BMI and current hormonal replacement therapy (HT) use were positively associated with BMD LS  $Z$ -score, while no covariates were significantly negatively associated (Table 3). For BMD FN  $Z$ -score, longer duration of follow-up, higher BMI and ever use of HT were positively associated, while current smoking was negatively associated (Table 3).

#### 3.3. 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

Table 2  
Bone mineral density and fracture incidence after RRSO.

	Bone mineral density				
	Lumbar spine ( <i>N</i> = 211) <sup>a</sup>	Femoral neck ( <i>N</i> = 212)			
BMD in g/cm <sup>2b</sup>	0.97 ± 0.12	0.79 ± 0.11			
Z-score – total study population	0.01 ± 1.09	0.15 ± 0.93*			
Women with history of breast cancer	−0.02 ± 1.00	0.011 ± 0.87			
Women without 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)				
Osteopaenia	89 (42)				
Normal BMD	110 (52)				
Fractures					
Questionnaire		VFA ( <i>N</i> = 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 <sup>c</sup>	19	Elbow	4	VF before RRSO	1
Vertebral	3	Pelvis	1	VF after RRSO	3
Hip	–	Ankle/Foot	4	Scheuermann's disease	1
Wrist	2	Toe/finger	6	Height reduction >25% on X-ray	0

Values are mean ± SD or No. (%); 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.

<sup>a</sup> For one woman no DXA of the lumbar spine was made because of the presence of osteosynthesis material after scoliosis surgery.

<sup>b</sup> For three women, DXA scans were performed outside the UMCG: one scan before the start of strontium ranelate therapy, one a year before study entry and one because of logistics close to her hometown.

<sup>c</sup> Fragility fractures are fractures from a fall from standing position or a height <1 m.

\*  $p < 0.05$  with one-sample *t*-test.

the remaining 22 fractures in 16 women, 19 were considered fragility fractures (Table 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 4). In the multivariate analysis, alcohol consumption was positively associated with the occurrence of fractures after RRSO (Table 5).

### 3.4. 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 [23].

### 3.5. 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 [27]. 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 on co-morbid diseases [Supplementary Online](#)).

## 4. Discussion

In an unselected consecutive series of 212 women with RRSO at age ≤52, after a median follow-up of 5 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% confidence interval (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 hypotheses 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 [11,28], 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 [10,29].

However, an effect of RRSO on BMD or fracture risk in this study population cannot be completely

Table 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 <sup>a</sup>			Univariate analysis Z-score femoral neck			Multivariate analysis Z-score femoral neck <sup>a</sup>		
	$\beta$	SE	p-Value	$\beta$	SE	p-Value	$\beta$	SE	p-Value	$\beta$	SE	p-Value
Age at RRSO (per year)	0.02	0.01	0.101	0.06	0.02	0.000*	0.00	0.01	0.969			
Follow-up (per year)	0.01	0.02	0.625				0.06	0.02	0.003*	0.04	0.02	0.043*
BMI (per kg/m <sup>2</sup> )	0.04	0.01	0.004*	0.05	0.01	0.000*	0.04	0.01	0.000*	0.04	0.01	0.000*
Postmenopausal before RRSO	-0.24	0.23	0.287				-0.11	0.19	0.582			
Fracture before RRSO	0.06	0.16	0.706				-0.07	0.14	0.601			
Parent with hip fracture	0.97	0.49	0.047*				-0.11	0.42	0.788			
Smoking – current	-0.23	0.19	0.234				-0.54	0.16	0.001*	-0.52	0.15	0.001*
Alcohol per unit/wk	0.01	0.01	0.326				-0.01	0.01	0.655			
Alcohol >7 units/wk	-0.04	0.20	0.856				-0.11	0.17	0.522			
Long-term use GCS <sup>b</sup>	0.24	0.38	0.527				0.01	0.33	0.973			
History of breast cancer	-0.06	0.16	0.699				-0.07	0.13	0.608			
Chemotherapy	-0.08	0.17	0.644				-0.03	0.14	0.812			
Ever use of AI	-0.53	0.34	0.117				-0.11	0.29	0.700			
Ever use of tamoxifen	0.13	0.28	0.634				0.14	0.24	0.563			
Ever use of HT	0.22	0.15	0.141				0.23	0.13	0.067	0.25	0.12	0.043*
Current use of HT	0.33	0.17	0.056	0.76	0.19	0.000*	0.12	0.15	0.417			
Corrected serum calcium	-0.39	0.99	0.692				0.36	0.84	0.666			
Serum 25(OH)D <sub>3</sub>	0.00	0.00	0.182				0.00	0.00	0.806			

SE is standard error; RRSO is risk-reducing salpingo-oophorectomy; BMI is body mass index; AI is aromatase inhibitor; HT is hormonal replacement therapy; GCS is glucocorticosteroids; AOD is anti-osteoporotic drugs.

<sup>a</sup> Adjusted for AOD/calcium/vitamin D<sub>3</sub> supplement use.

<sup>b</sup> Use of prednisone 7.5 mg or equivalent >3 months or >3 oral prednisolone courses per years.

\*  $p < 0.05$ .

excluded because of several reasons. Firstly, a large proportion of women had used bone protective medication, such as HT (47%), tamoxifen (8%) and AOD (9%). This might have attenuated the effect of RRSO on both BMD and fracture incidence. HT use was associated with higher BMD LS and FN Z-scores in multivariate analysis, which confirms the known protective effect of HT on BMD [30]. 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 [10]. 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 [31,32]. 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 [33,34]. 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 [35].

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 question-

Table 4

Comparison of fracture incidence observed after RRSO to fracture incidence expected after RRSO from general practitioner data using standardised incidence ratios (SIRs).

Age in years	No. of women <sup>a</sup>	No. of women-years	FI reference population <sup>b</sup>	Exp.	Obs.	SIR (95% CI)
<i>First incident fractures</i>						
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	–
<i>All incident fractures</i>						
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	–

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 standardised incidence ratio; CI is confidence interval.

<sup>a</sup> No. of women attributing to calculation of total no. of women-years per age group.

<sup>b</sup> van de Lisdonk et al. [24].

Table 5

Uni- and multivariate logistic regression analyses on women who reported fractures after RRSO.

	Univariate analysis			Multivariate analysis <sup>a</sup>		
	OR	95% CI	<i>p</i> -Value	OR	95% CI	<i>p</i> -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/m <sup>2</sup> )	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 <sup>b</sup>	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 HT	0.7	0.2–1.9	0.423			
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 <sub>3</sub>	1.0	1.0–1.0	0.892			

CI is confidence interval; RRSO is risk-reducing salpingo-oophorectomy; BMI is body mass index; AI is aromatase inhibitor; HT is hormonal replacement therapy; GCS is glucocorticosteroids; BMD is bone mineral density; AOD is anti-osteoporotic drugs.

<sup>a</sup> Adjusted for AOD/calcium/vitamin D3 supplement use.

<sup>b</sup> Use of prednisone 7.5 mg or equivalent >3 months or >3 oral prednisolone courses per years.

\* *p* < 0.05.

naire 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 through 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 specialised 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 [24]. This was further supported by the finding that fracture incidence before RRSO was comparable to fracture incidence in the reference population (Supplementary table online).

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.

### Funding sources

This study was supported by unrestricted grants by Amgen, Menarini, Nycomed and WillPharma and by the Junior Scientific Masterclass MD/PhD funds of the University Medical Center Groningen. None of the sponsors was involved in study design; in collection, analysis and interpretation of data; in the writing of the report and in the decision to submit the article for publication.

### 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 from Boehringer Ingelheim. All remaining authors have declared no conflict of interest.

### Acknowledgements

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

### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ejca.2014.11.022>.

### Reference

- [1] Claus EB, Risch N, Thompson WD. Autosomal dominant inheritance of early-onset breast cancer. Implications for risk prediction. *Cancer* 1994;73:643–51.
- [2] Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet* 2003;72:1117–30.
- [3] van der Velde NM, Mourits MJ, Arts HJ, de Vries J, Leegte BK, Dijkhuis G, et al. Time to stop ovarian cancer screening in BRCA1/2 mutation carriers?. *Int J Cancer* 2009;124:919–23.
- [4] Rebbeck TR, Kauff ND, Domchek SM. Meta-analysis of risk reduction estimates associated with risk-reducing salpingo-oophorectomy in BRCA1 or BRCA2 mutation carriers. *J Natl Cancer Inst* 2009;101:80–7.
- [5] NABON (Nationaal borstkanker overleg Nederland). Breast cancer Dutch guideline Version 2.0 [internet]. IKNL (Comprehensive Cancer Centre the Netherlands); 2012 [cited 2014 august 22]. Available from: <<http://www.oncoline.nl/uploaded/docs/mammacarcinoom/Dutch%20Breast%20Cancer%20Guideline%202012.pdf>>.
- [6] Groeneveld FP, Bareman FP, Barentsen R, Dokter HJ, Drogen-dijk AC, Hoes AW. The climacteric and well-being. *J Psychosom Obstet Gynaecol* 1993;14:127–43.
- [7] Luisetto G, Zangari M, Tizian L, Nardi A, Ramazzina E, Adami S, et al. Influence of aging and menopause in determining vertebral and distal forearm bone loss in adult healthy women. *Bone Miner* 1993;22:9–25.
- [8] Kritz-Silverstein D, Barrett-Connor E. Early menopause, number of reproductive years, and bone mineral density in postmenopausal women. *Am J Public Health* 1993;83:983–8.
- [9] Hreshchshyn MM, Hopkins A, Zylstra S, Anbar M. Effects of natural menopause, hysterectomy, and oophorectomy on lumbar spine and femoral neck bone densities. *Obstet Gynecol* 1988;72:631–8.
- [10] Banks E, Reeves GK, Beral V, Balkwill A, Liu B, Roddam A, et al. Hip fracture incidence in relation to age, menopausal status, and age at menopause: prospective analysis. *PLoS Med* 2009;6:e1000181.
- [11] Vesco KK, Marshall LM, Nelson HD, Humphrey L, Rizzo J, Pedula KL, et al. Study of Osteoporotic Fractures. Surgical menopause and nonvertebral fracture risk among older US women. *Menopause* 2012;19:510–6.
- [12] Michelsen TM, Dorum A, Dahl AA. A controlled study of mental distress and somatic complaints after risk-reducing salpingo-oophorectomy in women at risk for hereditary breast ovarian cancer. *Gynecol Oncol* 2009;113:128–33.
- [13] Challberg J, Ashcroft L, Lalloo F, Eckersley B, Clayton R, Hopwood P, et al. Menopausal symptoms and bone health in women undertaking risk reducing bilateral salpingo-oophorectomy: significant bone health issues in those not taking HRT. *Br J Cancer* 2011;105:22–7.
- [14] Cohen JV, Chiel L, Boghossian L, Jones M, Stopfer JE, Powers J, et al. Non-cancer endpoints in BRCA1/2 carriers after risk-reducing salpingo-oophorectomy. *Fam Cancer* 2012;11:69–75.
- [15] Pezaro C, James P, McKinley J, Shanahan M, Young MA, Mitchel G. The consequences of risk reducing salpingo-oophorectomy: the case for a coordinated approach to long-term follow up post surgical menopause. *Fam Cancer* 2012;11:403–10.
- [16] De Bock GH, Hesselink JW, Roorda C, De Vries J, Hollema H, Jaspers JP, et al. Model of care for women at increased risk of breast and ovarian cancer. *Maturitas* 2012;71:3–5.
- [17] Hoppers IC, van der Laan JG, Zeebregts CJ, Nieboer P, Wolffenbuttel BH, Dierckx RA, et al. Vertebral fracture assessment in supine position: comparison by using conventional semiquantitative radiography and visual radiography. *Radiology* 2009;251:822–8.
- [18] Hegeman JH, Willemsen G, van Nieuwpoort J, Kreeftenberg HG, van der Veer E, Slaets JP, et al. Effective tracing of osteoporosis at a fracture and osteoporosis clinic in Groningen; an analysis of the first 100 patients. *Ned Tijdschr Geneesk* 2004;148:2180–5.
- [19] Doorenbos CR, de Cuba MM, Vogt L, Kema IP, van den Born J, Gans RO, et al. Antiproteinuric treatment reduces urinary loss of

- vitamin D-binding protein but does not affect vitamin D status in patients with chronic kidney disease. *J Steroid Biochem Mol Biol* 2012;128:56–61.
- [20] Looker AC, Wahner HW, Dunn WL, Calvo MS, Harris TB, Heyse SP, et al. Updated data on proximal femur bone mineral levels of US adults. *Osteoporos Int* 1998;8:468–89.
- [21] Kelly TL. Bone Mineral Density reference databases for American men and women. *J Bone Min Res* 1990;5:S249.
- [22] Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. *J Bone Miner Res* 1993;8:1137–48.
- [23] CBO Kwaliteitsinstituut voor de gezondheidszorg, Nederlandse vereniging voor de reumatologie: Richtlijn osteoporose en fractuurpreventie, derde herziening [internet]. Utrecht: CBO/NVR; 2011 [cited 2014 august 22]. Available from: <<http://www.diliguide.nl/document/1015/osteoporose-en-fractuurpreventie.html>>.
- [24] van de Lisdonk EH, van den Bosch WJHM, Lagro-Janssen ALM, Schers HJ. *Ziekten in de huisartspraktijk*. 5th ed. Maarsse: Elsevier gezondheidszorg; 2008.
- [25] Rubin DB. *Multiple imputation for non-response in surveys*. New York: Wiley; 1987.
- [26] Kanis JA, Oden A, Johansson H, Borgström F, Ström O, McCloskey E. *FRAX and its applications to clinical practice*. *Bone* 2009;44:734–43.
- [27] Hippisley-Cox J, Coupland C. Derivation and validation of updated QFracture algorithm to predict risk of osteoporotic fracture in primary care in the United Kingdom: prospective open cohort study. *BMJ* 2012;344:e3427.
- [28] Parker WH, Broder MS, Chang E, Feskanich D, Farguhar C, Liu Z, et al. Ovarian conservation at the time of hysterectomy and long-term health outcomes in the nurses' health study. *Obstet Gynecol* 2009;113:1027–37.
- [29] Kritz-Silverstein D, von Mühlen DG, Barrett-Connor E. Hysterectomy and oophorectomy are unrelated to bone loss in older women. *Maturitas* 2004;47:61–9.
- [30] Castelo-Branco C, Pons F, González-Merlo J. Bone mineral density in surgically postmenopausal women receiving hormonal replacement therapy as assessed by dual photon absorptiometry. *Maturitas* 1993;16:133–7.
- [31] Adams JE. Quantitative computed tomography. *Eur J Radiol* 2009;71:415–24.
- [32] Briot K, Paternotte S, Kolta S, Eastell R, Reid DM, Felsenberg D, et al. Added value of trabecular bone score to bone mineral density for prediction of osteoporotic fractures in postmenopausal women: the OPUS study. *Bone* 2013;57:232–6.
- [33] Sornay-Rendu E, Munoz F, Duboeuf F, Delmas PD. Rate of forearm bone loss is associated with an increased risk of fracture independently of bone mass in postmenopausal women: the OFELY study. *J Bone Miner Res* 2005;20:1929–35.
- [34] Yildiz A, Sahin I, Gol K, Taner Z, Ulutürk A, Biberoglu K. Bone loss rate in the lumbar spine: a comparison between natural and surgically induced menopause. *Int J Gynaecol Obstet* 1996;55:153–9.
- [35] Vasikaran S, Eastell R, Bruyere O, Foldes AJ, Garnero P, Griesmacher A, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int* 2011;22:391–420.