Summary and general discussion
This thesis aims to increase knowledge on the effects of risk-reducing salpingo-oophorectomy (RRSO) on breast cancer risk and on bone health. Because RRSO is currently the only effective option to reduce ovarian cancer risk in BRCA1/2 mutation carriers, and because the procedure is most effective when performed at premenopausal age, knowledge on its long-term health effects is needed for adequate pre-operative counselling and post-operative follow-up and interventions.

This chapter contains a summary of the main findings of the studies included in this thesis, and a general discussion on these findings in their context of older and more recent literature. Strengths and limitations of the described studies in relation to their results and conclusions will be discussed. Subsequently, the implications for clinical care in women at increased risk of ovarian cancer who have to consider RRSO, and women who had RRSO in the past, are discussed. Lastly, knowledge gaps and areas for further research will be presented.

Summary

In premenopausal women, RRSO induces surgical menopause by acute elimination of ovarian steroid hormone production. Because surgical menopause decreases breast cancer risk in the general population (58,85,118,185-187), it is likely that also in BRCA1/2 mutation carriers, breast cancer risk decreases after premenopausal RRSO. Because after natural menopause bone mineral density (BMD) decreases and fracture risk increases (197,338), it is likely that after premenopausal RRSO, BMD will decrease faster at a younger age than after natural menopause, which will lead to higher fracture risk at older age.

Part I: Risk reducing salpingo-oophorectomy and breast cancer risk

In part I, the usefulness of breast cancer screening related to breast cancer incidence after RRSO in BRCA1/2 mutation carriers was explored.

BRCA1/2 mutation carriers are advised to opt for either intensive breast cancer screening with MRI, mammography and clinical breast examination, or for risk reducing mastectomy (RRM)(37). Breast cancer risk after RRSO has been reported to reduce with 50% (31). In addition, breast density decreases after surgical menopause (141). These factors may affect effectiveness and usefulness of breast cancer screening after RRSO. Therefore, after premenopausal RRSO, intensive breast cancer screening might need modification. In Chapter 2 we evaluated the effectiveness of intensive breast cancer screening in 88 BRCA1 and 51 BRCA2 mutation carriers after RRSO before 52 years of age. In 422 women years, 14 breast
Summary and general discussion

cancers were diagnosed: 2 prevalent, 10 screen detected and 2 interval cancers. No tumours were found with clinical breast examination. Sensitivity for MRI was 60% and for mammography 56%. Sensitivity for clinical breast examination and mammography were comparable to earlier findings, but MRI screening seemed less sensitive. We observed that breast cancer incidence after RRSO at premenopausal age remained high.

Breast cancer incidence in Chapter 2 seemed higher than expected and we hypothesized that this could be explained by a relatively high breast cancer incidence in BRCA1/2 mutation carriers in the northern Netherlands, or different effects of RRSO on breast cancer risk in BRCA1 compared to BRCA2 mutation carriers (4,188,189). Therefore, in Chapter 3 we compared observed breast cancer incidence after RRSO in 104 BRCA1 and 58 BRCA2 mutation carriers with RRSO before age 52 to expected incidence, based on regional reference data and assuming a 50% risk reduction. In 532 women-years, 13 incident breast cancers were observed, compared to 8 (range 6 - 10) expected (BRCA1: 12 observed, 5 [range 3 – 6] expected; BRCA2: 1 observed, 3 [range 2 – 5] expected).

The previously suggested breast cancer risk reduction of 50% after RRSO could not be confirmed in our cohort. Because also others have challenged the evidence on a RRSO-induced breast cancer risk reduction and it is postulated that previously reported risk reductions are the result of bias, this issue is inconclusive. Thus, we concluded that continued intensive breast cancer screening is warranted in BRCA1/2 mutation carriers after RRSO.

Part II: Risk-reducing salpingo-oophorectomy and bone health

Part II explores the effect of surgical menopause due to premenopausal RRSO on bone health. Chapter 4 is a systematic review and meta-analysis on BMD and fractures after surgical menopause. Seventeen studies were included on BMD or fracture prevalence, in age-matched women with and without surgical menopause. BMD after surgical menopause was lower compared to premenopausal women (mean difference lumbar spine: -0.15 g/cm²; 95% CI -0.19 - -0.11, femoral neck: -0.17 g/cm²; 95% CI -0.23 - -0.11) but not to women with natural menopause (lumbar spine: -0.02 g/cm²; 95% CI -0.04 - 0.00; femoral neck: 0.04 g/cm²; 95% CI -0.09 - 0.16). Hip fracture rate was not increased after surgical compared to natural menopause (HR 0.85; 95% CI 0.70 - 1.04). However, included studies were heterogeneous in design and prone to bias, thus an effect of surgical menopause on BMD and fracture risk after menopausal age could not be excluded.
**Chapter 5** aimed to compare BMD and fracture incidence after premenopausal RRSO to general population data, and to identify risk factors for low BMD and fractures after RRSO. In 212 women with median age at RRSO of 42 (35 - 52) years and median time after RRSO of 5 (2 - 8) years, BMD measured by dual-energy X-ray absorptiometry (DXA) was compared to age-matched reference values by using DXA-provided BMD Z-scores. Fracture incidence after RRSO assessed with a questionnaire was compared to general practitioner (GP) data by using standardised incidence ratios (SIRs). Lumbar spine and femoral neck BMD Z-scores ($Z = 0.01, p = 0.870; Z = 0.15, p = 0.019$) after RRSO were not lower than population BMD ($Z = 0$). Higher age at RRSO and use of hormonal replacement therapy (HRT) were associated with higher, and current smoking with lower BMD Z-scores. Fracture incidence was not higher than in the general population (age 25 - 44: SIR 2.12; 95% CI 0.85 - 4.37, age 45 - 64: SIR 1.65; 95% CI 0.92 - 2.72).

To identify women at risk for fractures, alternative ways for BMD measurement have been suggested, such as measurement of serum bone turnover markers (BTMs). Elevated BTMs might predict fracture risk independently of BMD (232). **Chapter 6** describes BTM levels after RRSO and aimed to identify clinical characteristics associated with elevated BTMs. BTMs for bone formation (osteocalcin [OC] and procollagen type I N-terminal peptide [PINP]) and for bone resorption (serum C-telopeptide of type I collagen [sCTx]) were measured in 210 women ≥ 2 years after RRSO at age ≤ 52 and compared to an existing age-matched reference cohort by using Z-scores. 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, respective Z-scores increased to 0.34, 1.14 and 0.88. OC and PINP Z-scores were inversely correlated to age at RRSO. No correlation was observed with fracture incidence or history of breast cancer.

These results suggest that after premenopausal RRSO, BMD is lower compared to age-matched premenopausal women, but this BMD difference seems to attenuate with age and time after RRSO. These data don’t support intensive follow-up on BMD for all women with RRSO. However, BTMs five years after RRSO remain elevated and elevated BTMs might predict increased fracture risk. Prospective longitudinal research is required to evaluate the long-term clinical implications of elevated BTMs after RRSO.

**General discussion**

Because RRSO induces acute menopause at young age, a broad range of symptoms and health effects associated with natural menopause occur more severely and
at younger age. These symptoms and health effects are likely to affect quality of life and potentially life expectancy after RRSO and are thus possible indications for follow-up, prevention and therapy. Of the broad range of RRSO-induced short- and long-term symptoms and health effects, this research focuses on the effects of RRSO on breast cancer risk and bone health.

**Part I: Risk-reducing salpingo-oophorectomy and breast cancer risk**

**Breast cancer risk after RRSO**

In 2009, in a meta-analysis it was concluded that RRSO reduced breast cancer risk in BRCA1/2 mutation carriers by approximately 50% (31). However, in our study in Chapter 3, more new breast cancers were detected after RRSO than expected given this 50% risk reduction, especially in BRCA1 mutation carriers. For BRCA2 mutation carriers, fewer breast cancers were observed than expected, however the number of women and the number of incident breast cancers were low. A different effect of RRSO-induced depletion of ovarian steroid hormones on breast cancer risk in BRCA1 and BRCA2 mutation carriers was hypothesized before, because most breast cancers in BRCA1 mutation carriers are hormone receptor negative, while BRCA2 mutation carriers most often have hormone receptor positive tumours (188). The findings in Chapter 3 support the hypothesis that RRSO affects breast cancer risk differently in BRCA1 and BRCA2 mutation carriers. Other contemporary studies provided inconsistent results. A large prospective study in BRCA1/2 mutation carriers in the United Kingdom and Ireland reported no overall breast cancer reduction after RRSO, but did report a risk reduction for contralateral breast cancer in BRCA2 mutation carriers in women with RRSO before age 45 (7). Several other studies after the meta-analysis of Rebbeck et al. confirmed a 50% breast cancer risk reduction after RRSO for both first, ipsi- and contralateral breast cancer, in both BRCA1 and BRCA2 mutation carriers (34,192,339-341). In 2015, Heemskerk et al. evaluated existing evidence on breast cancer risk after RRSO and concluded that previously reported risk reductions were likely the result of bias (193). After conducting a more advanced analysis aiming at maximal bias correction, they could not confirm a reduction of breast cancer risk after RRSO (193). Also no effect of RRSO on contralateral breast cancer risk was found by this unbiased analysis (342). A recent large prospective study with adequate bias correction did not report an overall risk reduction after RRSO, but observed a reduced risk of breast cancer before age 50, only in BRCA2 mutation carriers (343).

Differences in the effect of oestrogens on the aetiology of hormone receptor positive and negative breast cancers are also supported by the finding that other
breast cancer risk factors associated with higher levels of oestrogens, such as BMI, HRT use and reproductive factors, were mainly associated with an increased risk of ERα and/or PR positive breast cancer, while for triple negative breast cancer, risk associations are often less clear (122,128,344-346). However, current theories on the development of breast cancer support the contradicting hypothesis that oestrogens are important in the tumorigenesis of ERα negative breast cancers. ERα negative breast cancer might develop under the influence of paracrine ERα dependent mechanisms or toxic effects of metabolites of oestrogens on ERα negative progenitor or stem cells in breast tissue (116,117,157,347). Recent studies suggested that BRCA1 associated basal-like breast cancer originates from luminal progenitor cells, which can be either ERα negative or positive (157,348). Earlier it has been suggested that ERα expression is high in BRCA1 associated breast cancer during development but decreases during tumour progression (349). The latter hypothesis is supported by data indicating in breast cancer development, ERα and PR expression decrease with progression from in situ to invasive carcinoma (350,351).

Methodological considerations
Breast cancer risk after RRSO would ideally be studied after random allocation of RRSO to healthy BRCA1/2 mutation carriers, irrespective of family history or age. This would result in similar baseline mean breast cancer risks for women with and without RRSO. However, a randomised design is unethical and unfeasible, because RRSO is currently the only safe strategy to prevent ovarian cancer and related mortality in BRCA1/2 mutation carriers. Thus, breast cancer risk after RRSO can only be studied in observational studies, with a high risk of selection bias, as described by Heemskerk et al (193). In Chapter 3 we aimed to study breast cancer risk after RRSO at premenopausal age in BRCA1 and BRCA2 mutation carriers in the Northern Netherlands in a prospective cohort, and our study design was liable to similar types of bias as described by Heemskerk et al (193).

The first type of bias that is likely to influence the results of studies on breast cancer risk after RRSO is cancer-induced testing bias. Women with a proven BRCA1/2 mutation and ovaries in situ, may have opted for genetic testing for two reasons: 1) because they had breast cancer and genetic testing was performed either for the complete BRCA1 and BRCA2 genes, or for a known familial BRCA1/2 mutation or 2) because they considered risk reducing surgery and surveillance for a known familial BRCA1/2 mutation, when they were unaffected with cancer themselves. This is likely to result in a different selection of mutation carriers with RRSO, who have their DNA-result before RRSO when they are asymptomatic, and mutation
carriers without RRSO with DNA-testing after breast cancer diagnosis. This might lead to an overestimation of breast cancer risk in women without RRSO and thus an overestimation of the RRSO-induced breast cancer risk reduction. This cancer-induced testing bias can be avoided by starting follow-up at time of genetic testing and exclude women with breast cancer before genetic testing (193). In Chapter 3, the expected breast cancer incidence was based on penetrance curves for the northern Netherlands high risk families, including index cases who are likely to have had breast cancer before genetic testing (4), which might have caused overestimation of the number of breast cancers expected and thus overestimation of the RRSO-induced breast cancer risk reduction. However, we did not observe a RRSO-induced breast cancer risk reduction and cancer-induced testing bias does not explain that breast cancer risk after RRSO in Chapter 3 was higher than expected.

The second type of bias that might be of influence is immortal person-time bias. Immortal person-time refers to the period that women must have survived event-free to become eligible for the exposure, in this case RRSO. Women with breast cancer before RRSO, but after genetic testing, will be allocated to the non-RRSO group. Immortal person-time bias may occur when the person-time before RRSO is not allocated to the non-RRSO group, which will lead to less observation time in this group, increased breast cancer rates, and overestimation of breast cancer risk reduction after RRSO. Immortal person-time bias can be avoided by allocating the person-time before RRSO in the RRSO group to the non-RRSO group (193). This bias is unlikely to be applicable in Chapter 3, because follow-up for women in the study of van der Kolk et al. ended at time of RRSO, which results in allocation of follow-up until RRSO to the non-RRSO group (4).

Women with RRM are censored at time of RRM because at that moment the increased breast cancer risk ends. This censoring might be informative, because RRM might be chosen by women at an extra high risk of breast cancer, for example due to a positive family history for breast cancer at young age. This informative censoring might lead to biased estimations when women with higher breast cancer risks choose RRM before RRSO and women with a relatively low breast cancer risk opt for RRSO without RRM (193). Breast cancer risk reduction after RRSO may then incorrectly be attributed to RRSO, while it actually is a consequence of informative censoring for RRM. This type of bias may be hard to avoid, and it is unknown how much it affected our results. In our family cancer clinic, RRM was chosen more often and earlier after genetic testing in 1) women under 50, 2) those having a mother with breast cancer or 3) those having a personal history of breast cancer (352). This might indicate that
informative censoring was of influence in our study, but it does not explain why we did not find a breast cancer risk reduction after RRSO.

Another bias is introduced in Chapter 3 by including women with breast cancer before RRSO. This may induce confounding, because adjuvant systemic therapy after breast cancer reduces contralateral breast cancer risk. If more women with a history of breast cancer opt for RRSO, breast cancer risk after RRSO may be reduced due to adjuvant systemic therapy as well as a possible RRSO effect. If a reported RRSO-induced breast cancer risk reduction is not corrected for the possible influence of adjuvant systemic therapy, this will cause overestimation of the actual risk reduction due to RRSO. This type of confounding can be avoided by excluding women with breast cancer before genetic testing or perform separate analyses for women with and without breast cancer before RRSO (193). Of all women with RRSO in Chapter 3, 20% had a previous breast cancer. As adjuvant systemic therapy would enlarge an observed risk reduction after RRSO, it is unlikely that this type of bias explains our results.

The types of bias mentioned described above might have been present in our studies, but are likely to cause an overestimation of RRSO-induced breast cancer risk reduction. Thus they provide no explanation for our findings in Chapter 3, were we could not confirm a RRSO-induced breast cancer risk reduction. Because ovarian steroid hormones might influence breast cancer risk in BRCA1 mutation carriers in a paracrine way or through direct toxic effects of metabolites of oestrogens (65,116), sources of bias that might have caused underestimation of a risk reduction in BRCA1 mutation carriers were explored in Chapter 3. Median follow-up time in our study was 28 months, which might be too short to observe a risk reduction in BRCA1 mutation carriers. If the growth rate of prevalent tumours decreases after RRSO, this might cause overestimation of newly diagnosed breast cancer after RRSO by delaying the growth velocity of prevalent tumours. However, although a less aggressive breast cancer phenotype after RRSO was suggested, breast cancer growth rate seemed similar in women with and without RRSO (353). Breast cancer incidence in Chapter 3 might also be increased compared to breast cancer incidence in the reference population due to the introduction of MRI screening for BRCA1/2 mutation carriers in 2008, while follow-up in our reference study ended in March 2008 (4,159). Breast cancer incidence was higher after the introduction of MRI screening in BRCA1/2 mutation carriers and after introduction of population-based breast cancer screening with mammography (1,263,264). Furthermore, as with RRM, RRSO may also be chosen by women at particularly high risk of breast cancer, because RRSO is tended to be chosen more often by women who also opt RRM, by women with a personal
history of breast cancer and by women with a family history of breast cancer (354-356). Lastly, 47% of included women used HRT, which may have reversed the achieved breast cancer risk reduction. Several studies reported no adverse effect of short-term HRT on breast cancer risk after RRSO, however, no studies on long-term HRT use or with long-term follow-up are available (357). Thus, although our results are in line with other studies not observing a RRSO-induced breast cancer risk reduction, it cannot be ruled out that this risk reduction is underestimated in Chapter 3 due to sources of bias and confounding that cause overestimation of breast cancer risk after RRSO compared to before RRSO.

In Chapter 2, breast cancer screening with MRI, mammography and clinical breast examination was evaluated in women after RRSO. The overall screening protocol was less sensitive in detecting breast cancer than in previously published studies. This difference might be caused by a difference in screening sensitivity between a protocled study setting, were MRI and mammography are always performed alternating every six months, and a clinical practice setting, were physician, hospital and patient related factors influence timing and uptake of screening visits. However, it can also be a consequence of altered breast cancer risk and breast density after RRSO. To evaluate the isolated effect of RRSO on screening sensitivity, MRI and mammography screening should be performed every six months adherent to current guidelines, which can be achieved by performing breast cancer surveillance in an intervention study setting or by excluding women in whom screening was not performed according to protocol from observational studies.

We observed that none of the breast cancers that occurred during breast cancer surveillance after RRSO were detected primarily by CBE. This is in line with other studies on the effectiveness of CBE in screening of women with hereditary increased breast cancer risk and it has been hypothesized in studies that do observe an additional cancer yield by CBE, this might rather be a reflection of relative poor performance of mammography and MRI than a reflection of the discriminative power of CBE (358).

Clinical implications

Literature on breast cancer risk reduction after RRSO provides inconsistent conclusions. Recent studies indicate that the previously reported breast cancer risk reduction of 50% probably is an overestimation and this is in line with our findings in the first part of this thesis. However, individual studies indicated that there might be a risk reducing effect when RRSO is performed at younger age, especially in BRCA2 mutation carriers (7,343). In line with these observations,
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it was proposed to update the Dutch guideline on breast cancer with the advice to counsel BRCA1/2 mutation carriers on an uncertain effect of RRSO on breast cancer risk and to perform RRSO primarily for ovarian cancer risk reduction (36). Furthermore, as breast cancer risk remains significantly elevated after RRSO, and effectiveness of the intensive screening is similar before and after RRSO, there is currently no reason to modify breast cancer surveillance schemes after RRSO. However, as none of the incident breast cancers in Chapter 2 were detected with clinical breast examination and its sensitivity is generally low, MRI or mammography should always be performed during a breast cancer screening visit for BRCA1/2 mutation carriers (359,360).

Future perspectives
Due to recent publications, serious doubt has arisen on the presence and size of an RRSO-induced breast cancer risk reduction. More knowledge on this subject can be achieved by observing breast cancer incidence during a longer follow-up after RRSO and in larger study populations, for example by performing individual patient data meta-analysis on existing cohorts and collecting prospective data in international collaborations. In addition, breast cancer risk reduction for separate histological breast cancer subtypes and for individual BRCA1 and BRCA2 mutations need to be studied in more detail. Other subjects for investigation are the influence of age at RRSO on breast cancer risk reduction and the long-term effects of HRT on breast cancer risk in BRCA1/2 mutation carriers. Furthermore, a pilot study suggested that breast cancer in BRCA1/2 mutation carriers detected more than one year after RRSO has a less aggressive phenotype than breast cancer in age-matched BRCA1/2 mutation carriers without RRSO (353). If this finding can be replicated in larger studies, this might indicate a reason for adaptation of breast cancer screening after RRSO after longer follow-up. Another interesting new development is that currently, a national prospective study is investigating the effect of risk-reducing salpingectomy with delayed oophorectomy on quality of life, with ovarian cancer incidence as secondary outcome (361). If this strategy proves effective in improving quality of life, the next, and most important step will be to prove that salpingectomy with delayed oophorectomy is as safe as RRSO at the recommended age. Only then, oophorectomy can be delayed until age of natural menopause. In that case its effect on breast cancer risk reduction is expected to become minimal.
Part II: Risk-reducing salpingo-oophorectomy and bone health

Bone health after RRSO

*Chapter 4*, summarises literature on BMD and fracture prevalence after surgical menopause in the general population, while *Chapters 5* and *6* study BMD, fractures and BTMs in women with RRSO because of hereditary increased risk of ovarian cancer. In *Chapter 4*, BMD was lower in women with surgical menopause compared to age-matched premenopausal women. This is in line with the findings in *Chapter 5* that women with longer follow-up after RRSO have higher BMD Z-scores and in *Chapter 6* that women aged 50 years or younger have higher BTM Z-scores than women older than 50 years. It seems plausible that BMD is lower and BTMs are higher shortly after RRSO, because age-matched controls are still pre- or perimenopausal. However, the clinical relevance of relatively low BMD and high BTMs at this young age is unknown. A difference of one SD in BMD, as shown in the meta-analysis for pre- and perimenopausal reference groups, in *Chapter 4* was associated with two to three fold increase of absolute fracture risk, which is in line with the SIR for fractures of 2.12 in women aged 25 - 44 in *Chapter 4* (228-230). Although this SIR increase was not significant, this might be explained by the low number of fractures in our study and an associated lack of power. However, fracture incidence in women younger than 50 is generally low and it is unclear if a two to three fold increase in fracture risk is of clinical relevance in this age-category (208).

Although the individual studies on BMD after surgical compared to natural menopause that were included in *Chapter 4* present inconclusive results, overall meta-analyses did not show a difference in BMD and fracture rate after surgical compared to natural menopause. Combined with the finding that BMD is significantly lower after surgical menopause compared to pre- and perimenopausal age-matched controls, we concluded that the effect of surgical menopause on BMD might attenuate after age of natural menopause. This is in line with our results in *Chapter 5*, where we observed higher femoral neck BMD Z-scores after longer follow-up after RRSO and in *Chapter 6* where we observed lower PINP Z-scores after longer time since RRSO. Furthermore, SIRs for fractures after RRSO decreased from 2.12 in women aged 25 - 44 to 1.65 in women aged 45 - 64. In contrast, in *Chapter 1*, we hypothesized that after surgical menopause through premenopausal RRSO, BMD is likely to decrease and fracture risk to increase stronger than after natural menopause, due to the acute and complete cessation of ovarian steroid hormone production at young age. We were not able to confirm this hypothesis, which might be explained by the influence
of many other factors that affect BMD. The effects of factors like chronological age, comorbidities, use of medication that either slows or increases bone loss, and lifestyle factors (e.g. smoking, alcohol consumption, exercise, exposure to sunlight) might mediate or overrule the effects of RRSO on BMD after the age of natural menopause. Women at hereditary increased risk for cancer opting for RRSO might adopt healthier behaviour than women from the general population, which may have a positive effect on bone health. This is supported by the finding from a study on cardiovascular disease after RRSO, in which women with RRSO reported to perform more physical activities and to smoke less (362). Although we have knowledge on physical activities and smoking in women with RRSO, we have no knowledge on these variables in the reference populations, thus we cannot evaluate this theory. In addition, falling is an important risk factor for fractures in postmenopausal women, but our study population is relatively young and falling is mainly a problem of the elderly (363).

Since the publication of Chapter 5, two studies on BMD after RRSO were published and concluded that osteoporosis risk after RRSO was increased (364,365). Hibler et al. described bone loss in the observational arm of an intervention study with zoledronic acid therapy after RRSO, sponsored by Novartis Pharmaceuticals (364). They describe outcomes of BMD measurements before RRSO and 9 and 18 months after RRSO. The 52 women with a lumbar spine BMD measurement 18 months after RRSO had a mean Z-score of -0.22, compared to 0.33 in 78 women measured at baseline, which was a significant decrease (364). Although statistic test results for comparisons with women without RRSO were not provided, lumbar spine BMD Z-scores after 18 months seem to be lower than a mean Z-score of 0 in the general population (364). These findings are in contrast with our results in Chapter 5, for which various explanations can be proposed. Firstly, follow-up the study of Hibler et al. is 18 months, while in Chapter 5 median time after RRSO is five years with a minimum of two years. An initial effect of RRSO on BMD might be attenuated after longer follow-up due to the effect of other risk factors. Also, Hibler et al. exclusively describe women not using HRT or anti-osteoporotic drugs, and exclude women with various comorbidities, which are all factors that might have attenuated the effect of RRSO on BMD in Chapter 5. Furthermore, they only describe BMD 18 months after RRSO for 67% of the women measured at baseline, which might have induced bias. Garcia et al. described results of BMD measurements in 99 out of 225 women with RRSO and conclude that incidence of osteopenia and osteoporosis are high (365). However, similar to the study of Hibler et al. and several older studies on bone health after RRSO, Garcia et al. did not measure BMD systematically in all women with RRSO.
and they provided no comparison to BMD in women without RRSO. These are both factors that we addressed in Chapters 5 and 6 by inviting all women with RRSO known at our centre and providing statistical analyses on BMD, fracture incidence and BTMs compared to population data.

Methodological considerations
Our studies on BMD, fractures and BTMs after RRSO addressed several limitations present in older literature on bone health after RRSO. In contrast to previous studies on bone health after RRSO, we included a large consecutive study population, performed a systematic prospective data collection on BMD and BTMs, and provided a comparison to population data for BMD, BTMs and fractures. As a consequence, our study was the first to study a large population of women after RRSO, was more representative and selection bias was reduced. However, also our design had limitations, some of which were in line with limitations of older studies on BMD and fractures after surgical menopause in the general population that were described in Chapter 4.

Age is an important determinant of bone health. Therefore, in Chapter 4, only studies that corrected for the effect of age were included. In Chapters 5 and 6, we used age-matched reference data for BMD, fractures and BTMs to correct for the effect of age. In Chapter 4 several studies matched for age at surgical and natural menopause, which seems incorrect, as surgical menopause is per definition before natural menopause. Because natural menopausal women also experience perimenopause with associated bone loss, this design might cause underestimation of the effect of surgical menopause. In Chapters 5 and 6, we use population based reference data, so age at natural menopause is likely to be higher than age at RRSO. A limitation of our project, and most studies included in Chapter 4, is that surgical menopause or RRSO status was assessed retrospectively. In Chapter 4, studies often excluded women using HRT or anti-osteoporotic drugs, which could result in exclusion of high risk women. Although in Chapters 5 and 6 we did not exclude women using HRT or anti-osteoporotic drugs, women might have refused BMD measurement because they were already under treatment for osteoporosis. In addition, a large proportion of the study population had used bone protective medication, such as HRT (47%), tamoxifen (8%) and anti-osteoporotic drugs (9%), which might have attenuated the effect of RRSO on BMD and fracture incidence.

In addition, other choices made in the study design for Chapters 5 and 6, might have affected the possibility of finding a significant difference in bone health in women with and without RRSO. We selected women with RRSO before
age 53 and assumed they were premenopausal before RRSO. However, 13% of the included women reported to be postmenopausal before RRSO, either due to chemotherapy or natural menopause. Women who were postmenopausal before RRSO did not have differences in BMD Z-score, but they did have lower OC and PINP Z-scores than women who were premenopausal at the time of RRSO. Although the percentage of women who were postmenopausal before RRSO was small, including these women might have caused underestimation of the effect of surgical menopause on RRSO. Furthermore, we included women at least two years after RRSO, because it is generally assumed that a minimum of two years is needed to measure a reliable change in BMD (366). However, women in our study population were relatively young with a median age of 49. This might have been too early to see an effect of RRSO on fracture rate, because it is known that fracture incidence is relatively low at young age and increases with aging (295).

In Chapters 5 and 6, we compared results of our measurements of BMD, fracture rate and BTMs to existing reference data. Although this method provided us with the opportunity to compare our results to population based age-matched women, it also had several limitations. LS BMD reference data were retrieved from healthy women without oophorectomy, but unselected for age at menopause (316). Reference data for FN BMD were population based and were likely to include women with a normal range in age and duration of menopause, but might have also included women with surgical menopause (201). In several studies, observed BMD was higher than reference data retrieved from DXA scanners (364,367). If this was similar for BMD before RRSO in Chapter 5, this might have caused an underestimation of the effect of RRSO on BMD. Fracture incidence was assessed by questionnaires and VFA, while fracture incidence in the general population was assessed through GP registrations. Women retrospectively might forget to mention fractures in a questionnaire or mention fractures that were not confirmed with radiology. Offering VFA to all included women, might have caused overestimation of vertebral fractures compared to GP data. In GP registrations, data on fractures diagnosed by other physicians might be incomplete. However, we observed that fracture incidence before RRSO was comparable to fracture incidence from GP data. Although knowledge on clinical characteristics of the BTM reference cohort in Chapter 6 is limited, it is from the same geographical region as our study population, which makes them more likely to be similar than the international BMD cohorts. Menopausal status of women aged ≤ 50 years was not known and women aged > 50 were ≥ 5 years postmenopausal, so distribution of age at menopause in this reference cohort
might be different from the general population. Also, the percentage of women with surgical menopause was unknown. These difficulties can be prevented by using an age-matched reference cohorts from the same population with more baseline information available, so both baseline characteristics and study observations can be compared. This can be achieved by including a prospective age-matched reference cohort that can be followed together with the RRSO cohort.

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. Furthermore, as BTM levels are influenced by the use of several medications used to prevent osteoporosis and fractures (HRT, anti-osteoporotic drugs), medications used by women with breast cancer (aromatase inhibitors) and the occurrence of fractures, BTMs cannot be measured reliably in a relatively large proportion of women at risk for osteoporosis after RRSO.

We did not observe significant differences in BMD and fracture incidence after RRSO compared to population based reference data, however BTMs were significantly higher. Increased BTM levels might indicate elevated bone loss and increased fracture risk (232). It might be that after RRSO, 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, as represented by BTMs. 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). However, BTMs might also be elevated by other causes, of which bone metastases after breast cancer might be relevant in our study population (368). Although we were not aware of any bone metastases in women with elevated BTMs in our study population, long-term follow-up is needed to determine the meaning of elevated BTMs after RRSO, especially in women with a history of breast cancer.

**Clinical implications**

The findings in part II of this thesis do not support the hypothesis that overall BMD is decreased and fracture risk increased in women five years after surgical menopause due to RRSO compared to age-matched women with natural menopause. However, our results do indicate that never users of HRT and women
who were relatively young at time of RRSO have lower BMD and higher BTMs than age-matched women. HRT can be prescribed after RRSO up until age of natural menopause, but many women with RRSO will not be eligible for HRT, as a consequence of breast cancer in history. Age at natural menopause seems to have only small effects on fracture risk in older women, and it is unknown if these effects are stronger for surgical menopause (295,369). Offering BMD measurement to women with RRSO at young age without HRT can be considered. However, because the aim of BMD measurement is to select women at increased risk for fractures eligible for preventive therapy and there is no consistent proof of a significant effect of surgical menopause on fracture risk at older age, systematic screening should be offered with caution, to prevent overdiagnosis, associated anxiety and overtreatment.

During the osteoporosis screening visit at our outpatient clinics, many women asked practical questions considering HRT use and discontinuation, peritoneal cancer screening and preventive measures for long-term effects of RRSO. In addition, several women stated that follow-up care after RRSO was limited. This leaded to the implementation of a RRSO outpatient clinic, to counsel women before and after RRSO and to provide long-term follow-up if necessary.

**Future perspectives**

Fracture incidence increases with age, with a peak incidence of hip fractures between age 75 and 79 (207,208,370). Women in our study were relatively young with a median age of 49 and sample sizes might have been too small to detect a significant difference in fracture incidence. Furthermore, the median follow-up of five years after RRSO was relatively short. The remaining question is if premenopausal RRSO will be an important risk factor for fractures at older age or if other risk factors including chronological age will attenuate the effects. To obtain reliable estimates of the effect of premenopausal RRSO on bone, prospective inclusion of women with RRSO and longitudinal follow-up of BMD and fracture incidence is preferred. An age-matched reference group of women with RRSO from the same geographical region allows to correct for the effect of age on bone. Furthermore, such a reference group provides the opportunity to compare comorbidities and lifestyle factors that might influence on bone health in women with and without RRSO. HRT can be used until age of natural menopause, and although it is known that fracture risk remains decreased several years after discontinuation, it is unknown if this effects lasts with longer time after discontinuation (219).The long-term protective effects of HRT can be evaluated in a longitudinal study. The relevance of other potential risk factors
for fractures that are specific for BRCA1/2 mutation carriers, including breast cancer treatment, can be studied in more detail in a larger study population with longer follow-up. Because surgical menopause is by definition before age of natural menopause, follow-up in the reference group starts at premenopausal age. No restrictions for fracture history or use of medication affecting the bone should be applied. A time-to-event approach (e.g. time to fracture or to osteoporosis), or repeated measures for BMD, with a standardised DXA procedure could be adopted. Furthermore, other measures to assess bone health, such as BTM measurement and QCT can be included, to identify the most sensitive measure to identify women at risk for fractures. Other long-term health effects of RRSO, such as cardiovascular disease, menopausal symptoms and quality of life should also be evaluated.

Furthermore, BTMs remain elevated after a median follow-up of five years after RRSO in our study population. For these women, re-evaluation in a research setting after several years should be warranted, to explore if these elevated BTMs indeed indicate elevated fracture risk later on, or if they will normalise after age of natural menopause.

**Concluding remarks**

RRSO is currently the only proven effective option to reduce ovarian cancer risk in BRCA1/2 mutation carriers. The procedure improves life expectancy, but introduces several unwanted side effects. Although previous studies indicate a strong breast cancer risk reduction after RRSO, this thesis, together with other very recent studies, show that the observed risk reduction is likely the effect of bias. It is currently unsure if and how strong breast cancer risk after RRSO is reduced. Although a breast cancer risk reduction was suggested in BRCA2, but not BRCA1 mutation carriers, this is not supported by the hypotheses on the molecular effects of oestrogens on tumorigenesis of breast cancer. Thus, intensive breast cancer screening remains warranted after RRSO as long as the effect of RRSO on breast cancer risk is unclear. However, further research in large multinational cohorts with longer follow-up might indicate particular subgroups of BRCA1 or BRCA2 mutation carriers that do benefit from a breast cancer risk reduction after RRSO.

The results of our clinical studies on BMD, fractures and BTMs after RRSO were reassuring and did not indicate a need for intensive clinical follow-up or intervention. These findings were supported by the conclusions from our systematic review and meta-analysis on BMD and fractures after surgical
menopause in the general population. However, because long-term consequences of RRSO on bone health are unknown and BTMs remained elevated five years after RRSO compared to premenopausal controls, longitudinal long-term follow-up in a research setting is advised. Knowledge gained by long-term follow-up studies can be used to better inform and guide women in the difficult choices of choosing and timing RRSO and guide clinicians in counselling and advising for the long-term consequences.