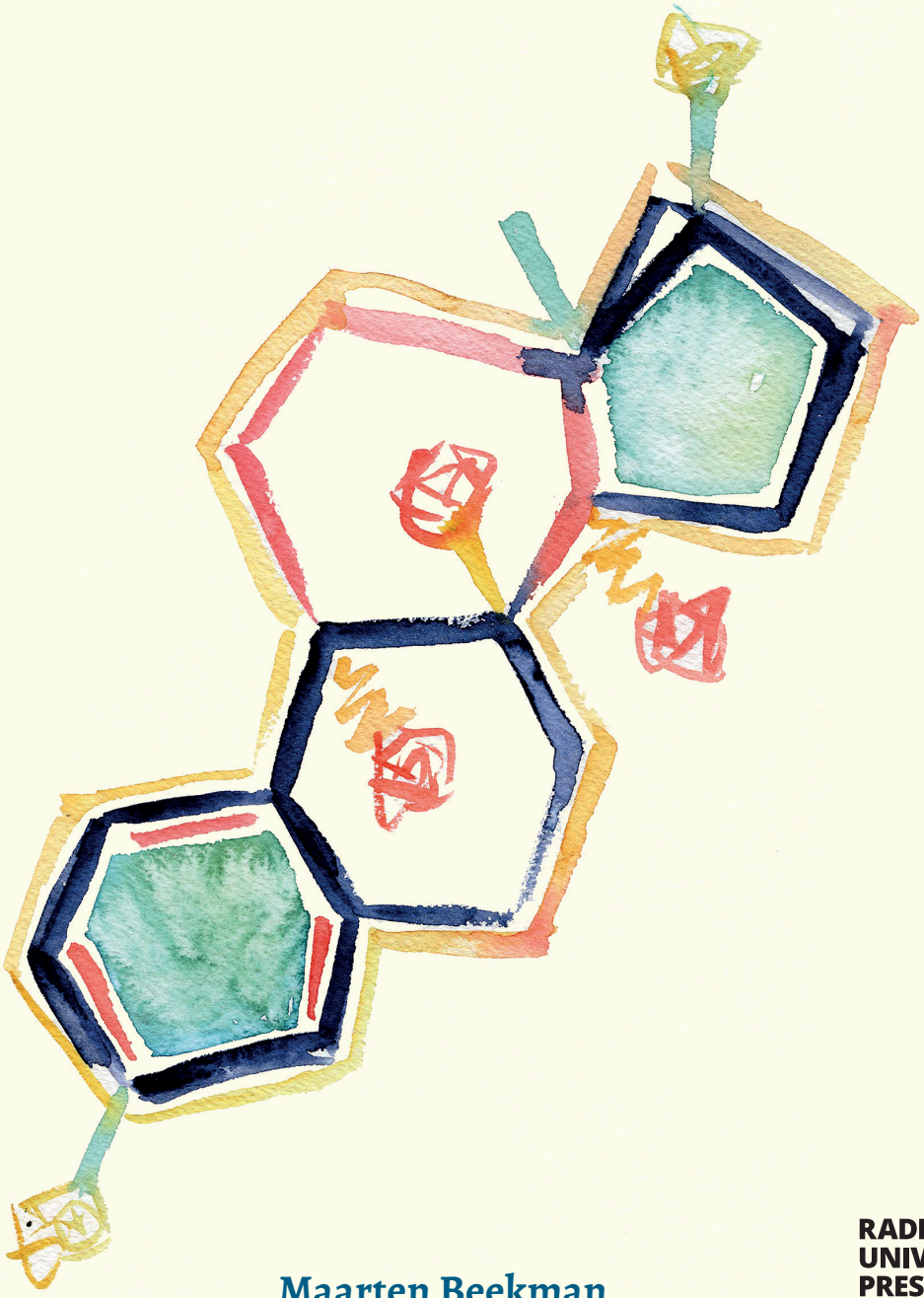
Long-term adverse effects of early surgical menopause due to risk-reducing salpingo-oophorectomy



Maarten Beekman

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Chapter 1

General introduction and thesis outline

Introduction

A growing number of women undergo premenopausal risk-reducing salpingo-oophorectomy (RRSO) because of familial risk of ovarian cancer. While RRSO leads to a substantial decrease of ovarian cancer risk, potential adverse effects of the associated earlier age at menopause remain largely unknown. This thesis aims to provide clarity about the long-term health effects of a premenopausal RRSO, to be able to better inform women and health care providers dealing with this impactful intervention.

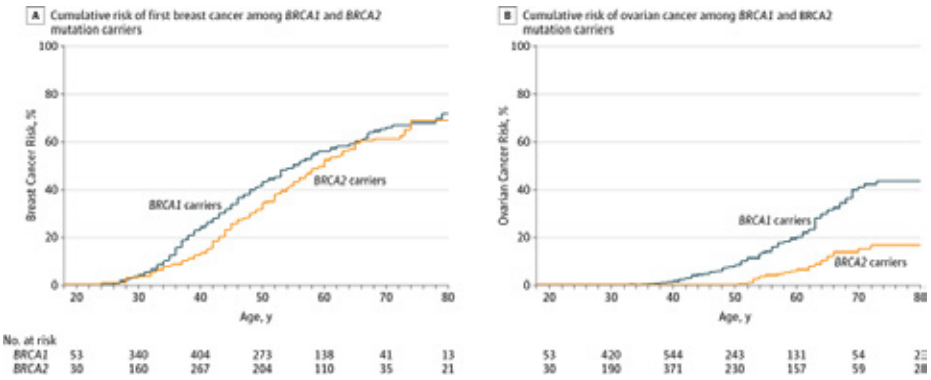

Figure 1. Lifetime risk of ovarian cancer in *BRCA1/2* germline pathogenic variant carriers.[1]

Premenopausal risk-reducing salpingo-oophorectomy (RRSO)

Women at high familial risk of breast and ovarian cancer, including *BRCA1/2* germline pathogenic variant (GPV) carriers, are recommended to undergo risk-reducing salpingo-oophorectomy (RRSO) to prevent ovarian cancer. Female *BRCA1* and *BRCA2* GPV carriers have lifetime risks of ovarian cancer of 44% and 17%, respectively (see Figure 1).[1] In the past women were advised to undergo 3-monthly transvaginal ultrasound and serum cancer antigen 125 (CA-125) screening to detect ovarian cancer early. However, studies have shown that screening for ovarian cancer is not effective, neither for hereditary nor for sporadic ovarian cancer.[2-3] Because of this difficulty to screen for ovarian cancer and diagnose the disease early, 60-70% of the women with ovarian cancer are being diagnosed with stage III/IV disease, leading to a poor overall 5-year survival of around 50%.[4-6] Therefore, a change of the guidelines in 2007 recommends women at high familial risk for ovarian cancer to undergo RRSO after completion of child bearing, preferably at ages 35 to 40 years for *BRCA1* GPV carriers and at ages 40 to 45 years for *BRCA2* GPV carriers.[7] This has led to an uptake of 81-99% of premenopausal RRSO (before the age of 45) in female *BRCA1/2* GPV in the Netherlands resulting in a subsequent reduction of ovarian cancer risk.[8-11] Figure 2

illustrates the increasing prevalence of premenopausal RRSO (≤45 years) over time in women participating in the Hereditary Breast and Ovarian cancer study Netherlands (HEBON study): a Dutch cohort of women at high familial risk of breast and/or ovarian cancer recruited from all genetic testing centers in the Netherlands (eight Dutch University Medical Centers and the Netherlands Cancer Institute. [12] Although RRSO performed at the recommended age is very effective in preventing ovarian cancer, it also leads to early surgical menopause, which can have a large impact on the life of these women.

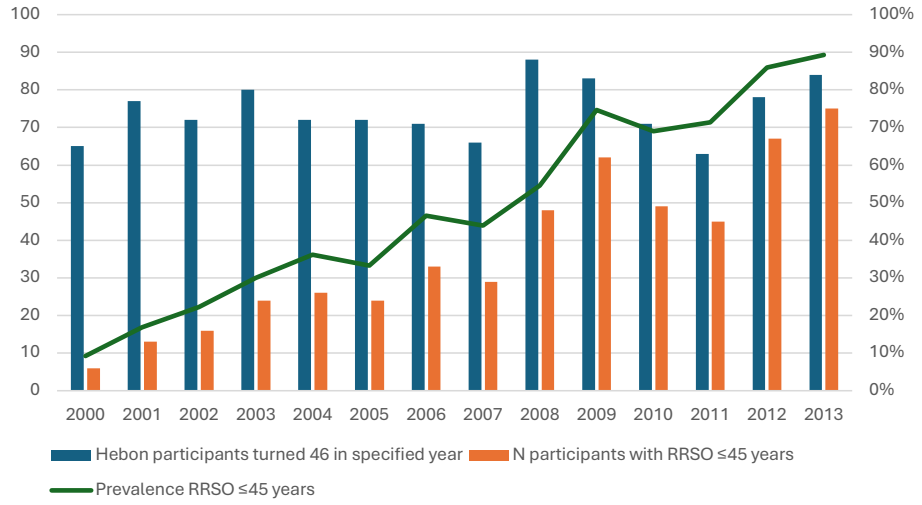

Figure 2. Prevalence of RRSO ≤45 years in participants of HEBON who turned 46 in a specific year.

Early menopause

In high income countries median age at menopause is between 50 and 52 years and approximately 9.7% of the women in these countries experience early menopause, defined as menopause before the age of 45. [13] Early natural menopause (menopause <45 years) is caused by decreasing endogenous estrogen production by the ovaries. However, specific groups of women experience an early iatrogenic menopause caused by chemotherapeutic agents, radiation treatment to the ovaries or bilateral oophorectomy. Early menopause has been associated with a wide variety of sequelae caused by short-term and long-term effects of estrogen deficiency. Menopausal symptoms are diverse and may consist of mood changes, vasomotor symptoms and urological, gynecological and sexual functioning changes that affect quality of life. Long-term effects attributed to early menopause include urogenital problems, osteoporosis, accelerated cognitive impairment, increased cardiovascular disease (CVD) risk and even premature mortality (see Figure 3). [14-15]

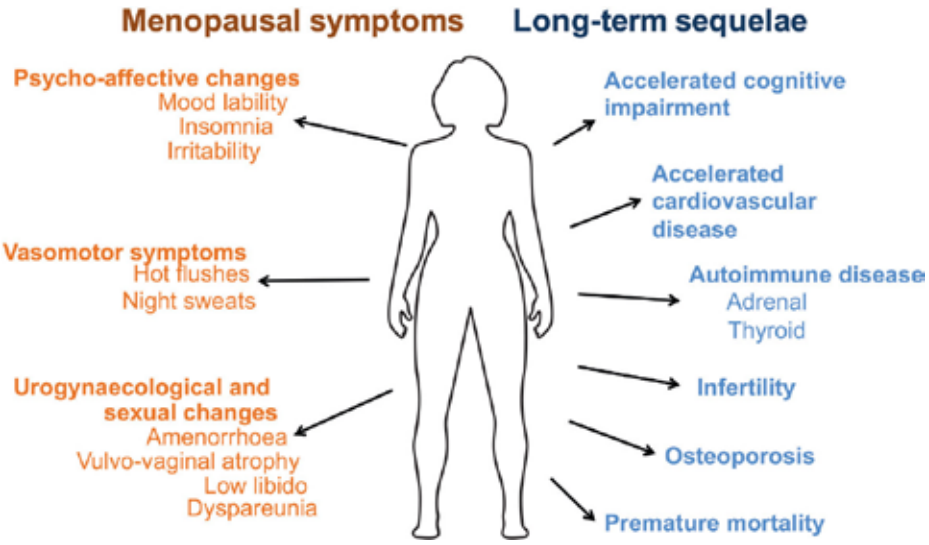

Figure 3. Short-term and long-term effects of early natural menopause.[14]

Early natural menopause versus surgical menopause

Results from studies investigating the effects of early menopause are not always consistent and the interpretation is still subject to debate. It is important to consider that the literature predominantly concerns studies on early *natural* menopause. It is important to note that the mechanism of estrogen deficiency due to early natural menopause and surgical menopause may be different. Women undergoing early natural menopause, such as premature ovarian insufficiency (POI, natural menopause <40 years), experience a gradual decline of estrogen levels, whereas women undergoing early surgical menopause, lose almost all estrogen production overnight. It is therefore not evident that early natural menopause leads to the same long-term effects as surgical menopause. In addition, because a substantial proportion of women use oral contraceptives in their forties, or undergo hysterectomy, the true age at menopause in studies investigating effects of natural menopause is often unclear for part of the study population.

Cardiovascular disease after early menopause

There is ample evidence that early natural menopause is associated with increased CVD risk in later life. Two recent large meta-analyses show especially increased risks of stroke and ischemic heart disease (IHD) in women with POI.[16-17] As estrogens are cardio-protective during the fertile years of life, women with an early menopause experience a longer period of decreased levels of estrogen at an earlier age, compared with women in the general population.[18] However, whether or not CVD risk is increased after surgical menopause has been investigated less frequently and with

inconsistent results.[19-22] The differences in results between studies investigating CVD risk after surgical menopause might be explained by methodological issues when comparing women who underwent surgical menopause with the general population, due to confounding by indication for bilateral oophorectomy. Such bias may arise because the indication for bilateral oophorectomy might be associated with increased CVD risk rather than the surgical procedure itself. Among the most frequent indications for surgical menopause are risk-reducing salpingo-oophorectomy, endometriosis and benign cysts, which have all been reported to be associated with increased CVD risk.[22] Endometriosis has been associated with an increased risk for CVD, irrespective of a history of surgical menopause, while CVD risk in women with cysts remains unclear.[23-24] Women who undergo RRSO are predominantly *BRCA1/2* GPV carriers and therefore many of them have a history of breast cancer. Breast cancer treatments such as chemotherapy and radiotherapy (especially of the internal mammary chain) are known to influence CVD risk.[25] Furthermore, some studies suggest *BRCA* genes play a cardio-protective role by downregulation of reactive oxygen species and apoptosis, potentially leading to increased CVD risk in *BRCA1/2* GPV carriers.[26] To restrict potential bias due to confounding, studies investigating long-term CVD risk after surgical menopause should therefore aim to compare subgroups restricted to women with the same indication for surgical menopause, with different ages at surgery. Previous studies investigating long-term CVD risk after surgical menopause did not perform subgroup analyses among women who underwent RRSO.

Reverse causality hypothesis about early menopause and increased CVD risk

Another possible hypothesis that might (partly) explain the observed association between early natural menopause and CVD risk, is the reverse causality hypothesis. Early loss of ovarian function may not be the (only) cause of the increased CVD risk in women with early natural menopause. It has been postulated that early menopause is the result of accelerated vascular ageing (also involving the ovaries), which may also explain a statistical (non-causal) association between earlier natural menopause and increased CVD risk (see Figure 3).[27] If this were true, no elevated CVD risk would be expected in women with an early surgical menopause. This hypothesis is supported by a small recent study in which no association was observed between time since RRSO and other measures of subclinical atherosclerosis, including pulse wave velocity (PWV) and carotid intima thickness (cIMT) in a cohort of women *BRCA1/2* GPV carriers.[28]

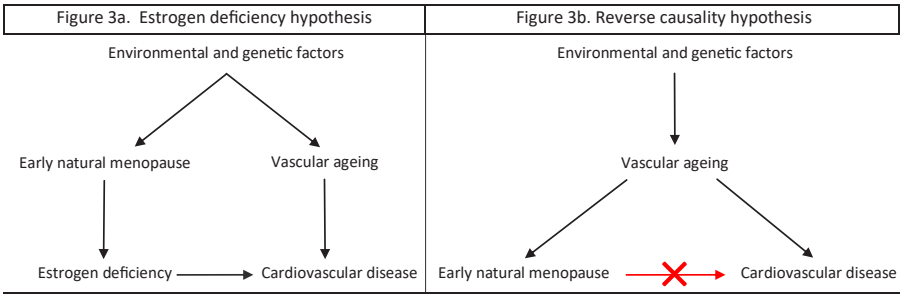

Figure 4. Explanations for observed associations between early natural menopause and increased CVD risk.

Bone mineral density after early menopause

Estrogen deficiency is the main cause of bone loss after menopause, which may lead to osteopenia or osteoporosis and an increased risk of fractures. [29] Studies suggest a perimenopausal peak of bone loss of especially the lumbar spine (LS), with a more gradual decline of bone mineral density (BMD) after 4-5 years. [30] Two recent longitudinal studies showed a significantly lower BMD in *BRCA1/2* GPV carriers who underwent a premenopausal RRSO compared with carriers who underwent a postmenopausal RRSO or no RRSO at all. [31-32] However, these studies had a median follow-up after RRSO of 3 years or less, so it remains unclear whether these effects persist over time. Cross-sectional studies investigating the effects of a premenopausal RRSO on BMD in *BRCA1/2* GPV carriers (median follow-up 4-6 years) show inconsistent results. [33-35] In addition, studies not restricted to *BRCA1/2* GPV carriers suggest that the long-term impact of early menopause on BMD might be attenuated at older age. [36] The long-term effects of premenopausal RRSO on bone health are particularly important because the risk of especially osteoporotic hip fractures and subsequent morbidity and mortality increase sharply with age (see Figure 4). Therefore, studies with longer follow-up are needed to better understand the impact of early menopause on bone health. [37]

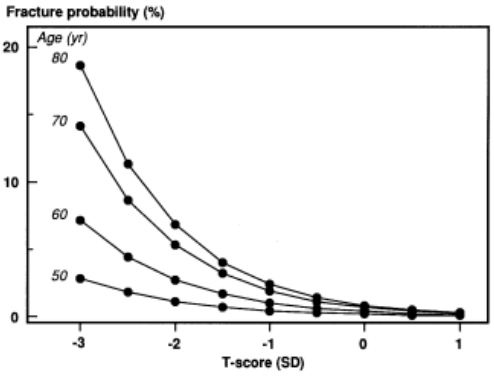

Figure 5. Relationship between BMD at the hip expressed as a T-score and hip fracture probability in women according to age. For any given T-score the risk is higher with increasing age. [37]

Sexual and urogenital problems after surgical menopause

Vulvovaginal atrophy caused by reduced circulating estrogen levels is common in postmenopausal women, and is associated with symptoms of the lower urinary tract and decreased sexual functioning.[38] Available literature on sexual functioning after RRSO is predominantly focusing on the short-term adverse effects, reporting a negative impact on especially sexual pleasure and comfort.[39-41] Furthermore, hypoactive sexual desire disorder is more prevalent among women who underwent surgical menopause compared with women with a natural menopause, leading to less sexual satisfaction.[42] Although there is ample evidence of short-term effects of surgical menopause on sexual functioning, it remains largely unknown whether these changes still exist on the long-term, or attenuate with ageing. Postmenopausal changes because of reduced estrogen levels not only lead to vulvovaginal atrophy but also affect other urogenital tissues resulting in symptoms of the lower urinary tract that can have a large impact on quality of life, including urgency and urinary incontinence.[43] However, the associations between surgical menopause after bilateral oophorectomy and these symptoms still need to be examined.

Health-related quality of life after surgical menopause

Several studies have investigated the short-term effects of RRSO on health-related quality of life (HRQOL).[44-45] Although RRSO is known to influence HRQOL related outcomes including more vasomotor/menopausal complaints and worsened sexual function, generic HRQOL appears to be unaffected.[41-46] A possible explanation is that RRSO has been shown to reduce short-term cancer-specific distress and women appear to be satisfied with their choice to undergo prophylactic surgery.[47-49] However, most studies do not specify for timing of RRSO (pre- vs postmenopausal), and have relatively short follow-up times (median up to 5 years).[44-45] Therefore, it remains unclear whether premenopausal RRSO influences long-term HRQOL.

The HARMOny study

The Health After Early Menopause Due to Oophorectomy (HARMOny) study aimed to investigate the long-term health effects of women at high familial risk of ovarian cancer who underwent RRSO at age 45 years or earlier.[50] To overcome the aforementioned challenges when researching the long-term effects of surgical menopause we performed a cross-sectional study in which we compared 500 women in the premenopausal RRSO group with 250 women with the same familial risk, who underwent such surgery after menopause, at age 54 years or later. This meant that we were able to minimize selection bias and confounding by indication. Women included in the study had to be 55-years or older, so all women in the premenopausal RRSO group had at least 10 years of follow-up after surgical menopause. We

performed a nationwide multi-center study nested in the Hereditary Breast and Ovarian cancer study Netherlands (HEBON study.[12] Participation in the HARMOny study consisted of completion of an extensive online questionnaire and a clinical visit. The clinical visit included a visit with the research physician at the outpatient clinic to obtain anthropometric measurements and determine blood pressure, pulse wave velocity (PWV) and advanced glycation end products (AGEs). During the clinical visit, non-fasting blood samples were taken to analyze levels of lipids, glucose, HbA1c, C-reactive protein (CRP), cardiac troponin, calcium, albumin, phosphate, creatinine, 25-hydroxyvitamin D (25OHD), beta-C-terminal collagen crosslink (β-CTX) and N-terminal procollagen type 1 (P1NP). Furthermore, dual-energy X-ray absorptiometry (DXA) of the lumbar spine (LS) and femoral neck (FN) was performed to calculate absolute BMD, T-scores and Z-scores of the L1-L4 vertebral bodies and femoral neck. In addition, CAC scores were assessed by computed tomography using standardized local scan protocols for Agatston scoring at the various participating medical centers. During the first years of my PhD trajectory, a large proportion of the time was used to plan and conduct the clinical visits of over 540 participants at the participating hospitals outpatient clinics, as well as communicating the results of the various outcomes to the participating women and their general practitioners.

Coronary artery calcium and pulse wave velocity as measure of cardiovascular disease risk

Coronary artery calcium (CAC) score, as a measure of the amount of calcification of the coronary arteries, is assessed on blank computed tomography (CT) by calculating the Agatston score. CAC is an established method to assess subclinical atherosclerosis and therefore individual CVD risk in asymptomatic individuals, even at relatively young ages.[51-55] In addition, a recent study showed that CAC is an excellent predictor of CVD in asymptomatic postmenopausal women, also those who experienced an early natural menopause (See Figure 5).[56] So far, there are no studies that assessed CAC scores in relation to the timing of surgical menopause. Increased aortic stiffness is a sign of vascular ageing and independent predictor for CVD in the general population.[57-58] Although the pathophysiology is different, some studies suggest that CAC and arterial stiffness are associated. While CAC assesses calcification of the tunica intima, arterial stiffness is primarily influenced by ageing and high blood pressure, leading to functional changes in the architecture throughout the arterial wall. The gold standard for aortic stiffness, brachial-ankle pulse wave velocity (PWV), is a relatively simple and noninvasive test that could provide additional information in clinical practice.[59] Studies show that PWV increases more strongly during the menopausal transition and that increased PWV is associated with the risk of major adverse cardiovascular events in postmenopausal women.[60-62] However, whether early

menopause leads to a long-term increase of PWV remains unknown. [63] The long-term effects of surgical menopause on both CAC and PWV could provide more insight into the development of CVD after early menopause.

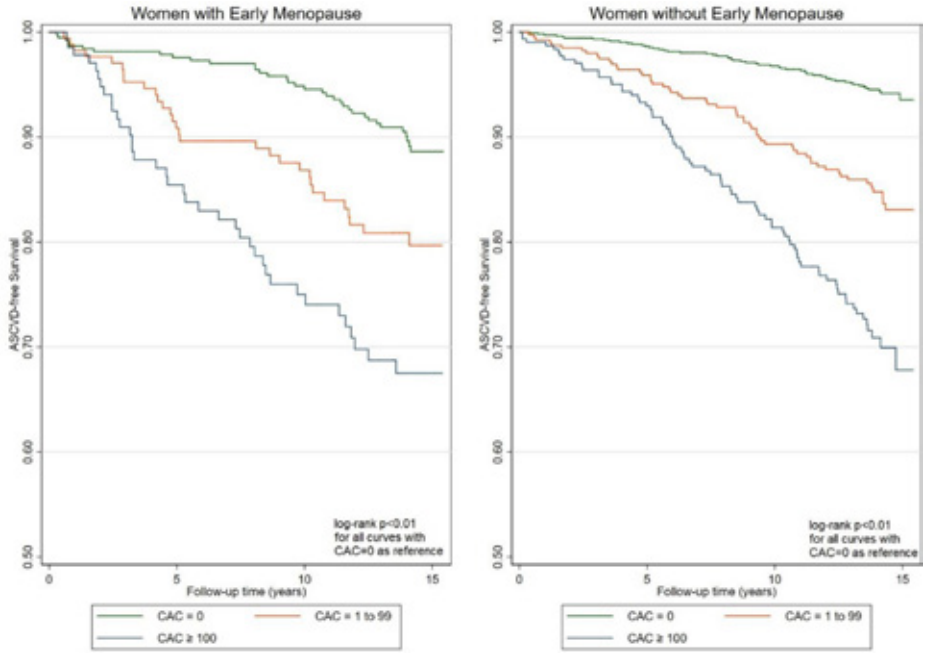

Figure 6. Kaplan-Meier curves of atherosclerotic cardiovascular disease (ASCVD)-free survival for women with and without early menopause stratified by baseline CAC score. The difference in survival was statistically significant (log-rank $p < 0.01$ for all comparisons). A calculated interaction term between EM and CAC was not statistically significant with $p = 0.09$. [56]

Measures of bone mineral density

Bone mineral density is commonly assessed using a dual-energy X-ray absorptiometry (DXA) scan of the lumbar spine (LS) and femoral neck (FN). Because BMD is gender and age related, the absolute BMD is usually converted to T- and Z-scores. T-scores compare the observed BMD with the mean BMD of a young adult reference population, by calculating the number of standard deviations by which the observed BMD differs from the BMD of a young adult reference population. Z-scores compare the observed BMD with the mean BMD of a reference population of the same age by calculating the number of standard deviations by which the observed BMD differs from the BMD of a reference population of the same age. The World Health Organization guidelines define presence of osteopenia as having a T-score between -1.0 to -2.5 and presence of osteoporosis is defined as having a T-score ≤-2.5. [64] Bone

turnover markers (BTM) may predict future fracture risk in postmenopausal women independent of BMD. [65] Studies show that serum beta-C-terminal collagen crosslink (β-CTX) as a marker for bone resorption and serum N-terminal procollagen type 1 (P1NP) as a marker for bone formation are the most sensitive BTM after surgical menopause. [66-67]

Outline of this thesis

All results presented in this thesis are based on data originating from the HARMOny study. In **Chapter 2** we report on CAC scores in women who underwent a premenopausal RRSO compared with women who underwent a postmenopausal RRSO as well as with a reference population from the ROBINSCA trial. **Chapter 3** shows the long-term effects of premenopausal RRSO compared to postmenopausal RRSO on PWV and describes the association between PWV and CAC in women with a premenopausal RRSO. **Chapter 4** evaluates the effect of a premenopausal compared with a postmenopausal RRSO on BMD. **Chapter 5** provides a comprehensive overview of all long-term adverse effects examined in the HARMOny study, including quality of life. **Chapter 6** evaluates the effect of a premenopausal RRSO on sexual functioning, compared to a postmenopausal RRSO. **Chapter 7** evaluates urogenital functioning, and urinary incontinence in particular, in women with a premenopausal RRSO compared with women with a postmenopausal RRSO. In **Chapter 8,** we summarize and discuss the content of this thesis and provide clinical implications and perspectives for future research.

References

1. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA. 2017;317(23):2402-16.
2. Henderson JT, Webber EM, Sawaya GF. Screening for Ovarian Cancer: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA. 2018;319(6):595-606.
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(4):919-23.
4. Gaitskell K, Hermon C, Barnes I, Pirie K, Floud S, Green J, et al. Ovarian cancer survival by stage, histotype, and pre-diagnostic lifestyle factors, in the prospective UK Million Women Study. Cancer Epidemiol. 2022;76:102074.
5. Heemskerk-Gerritsen BAM, Hollestelle A, van Asperen CJ, van den Beek I, van Driel WJ, van Engelen K, et al. Progression-free survival and overall survival after BRCA1/2-associated epithelial ovarian cancer: A matched cohort study. PLoS One. 2022;17(9):e0275015.
6. Bolton KL, Chenevix-Trench G, Goh C, Sadetzki S, Ramus SJ, Karlan BY, et al. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA. 2012;307(4):382-90.
7. Daly MB, Pal T, Berry MP, Buys SS, Dickson P, Domchek SM, et al. Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2021;19(1):77-102.
8. Domchek SM, Friebel TM, Singer CF, Evans DG, Lynch HT, Isaacs C, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA. 2010;304(9):967-75.
9. 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(2):80-7.
10. Harmsen MG, Arts-de Jong M, Horstik K, Manders P, Massuger L, Hermens R, et al. Very high uptake of risk-reducing salpingo-oophorectomy in BRCA1/2 mutation carriers: A single-center experience. Gynecol Oncol. 2016;143(1):113-9.
11. van der Aa JE, Hoogendam JP, Butter ES, Ausems MG, Verheijen RH, Zweemer RP. The effect of personal medical history and family history of cancer on the uptake of risk-reducing salpingo-oophorectomy. Fam Cancer. 2015;14(4):539-44.
12. Brohet RM, Velthuizen ME, Hogervorst FB, Meijers-Heijboer HE, Seynaeve C, Collee MJ, et al. Breast and ovarian cancer risks in a large series of clinically ascertained families with a high proportion of BRCA1 and BRCA2 Dutch founder mutations. J Med Genet. 2014;51(2):98-107.
13. Mishra GD, Pandeya N, Dobson AJ, Chung HF, Anderson D, Kuh D, et al. Early menarche, nulliparity and the risk for premature and early natural menopause. Hum Reprod. 2017;32(3):679-86.
14. Nguyen HH, Milat F, Vincent A. Premature ovarian insufficiency in general practice: Meeting the needs of women. Aust Fam Physician. 2017;46(6):360-6.
15. Shuster LT, Rhodes DJ, Gostout BS, Grossardt BR, Rocca WA. Premature menopause or early menopause: long-term health consequences. Maturitas. 2010;65(2):161-6.
16. Muka T, Oliver-Williams C, Kunutsor S, Laven JS, Fauser BC, Chowdhury R, et al. Association of Age at Onset of Menopause and Time Since Onset of Menopause With Cardiovascular Outcomes, Intermediate Vascular Traits, and All-Cause Mortality: A Systematic Review and Meta-analysis. JAMA Cardiol. 2016;1(7):767-76.

17. Roeters van Lennep JE, Heida KY, Bots ML, Hoek A, collaborators of the Dutch Multidisciplinary Guideline Development Group on Cardiovascular Risk Management after Reproductive D. Cardiovascular disease risk in women with premature ovarian insufficiency: A systematic review and meta-analysis. Eur J Prev Cardiol. 2016;23(2):178-86.
18. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. N Engl J Med. 1999;340(23):1801-11.
19. Dam V, van der Schouw YT, Onland-Moret NC, Groenwold RHH, Peters SAE, Burgess S, et al. Association of menopausal characteristics and risk of coronary heart disease: a pan-European case-cohort analysis. Int J Epidemiol. 2019;48(4):1275-85.
20. Honigberg MC, Zekavat SM, Aragam K, Finneran P, Klarin D, Bhatt DL, et al. Association of Premature Natural and Surgical Menopause With Incident Cardiovascular Disease. JAMA. 2019;322(24):2411-21.
21. Parker WH, Broder MS, Chang E, Feskanich D, Farquhar 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(5):1027-37.
22. Rivera CM, Grossardt BR, Rhodes DJ, Brown RD, Jr., Roger VL, Melton LJ, 3rd, et al. Increased cardiovascular mortality after early bilateral oophorectomy. Menopause. 2009;16(1):15-23.
23. Mu F, Rich-Edwards J, Rimm EB, Spiegelman D, Missmer SA. Endometriosis and Risk of Coronary Heart Disease. Circ Cardiovasc Qual Outcomes. 2016;9(3):257-64.
24. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2018;33(9):1602-18.
25. Boekel NB, Jacobse JN, Schaapveld M, Hooning MJ, Gietema JA, Duane FK, et al. Cardiovascular disease incidence after internal mammary chain irradiation and anthracycline-based chemotherapy for breast cancer. Br J Cancer. 2018;119(4):408-18.
26. van Westerop LL, Arts-de Jong M, Hoogerbrugge N, de Hullu JA, Maas AH. Cardiovascular risk of BRCA1/2 mutation carriers: A review. Maturitas. 2016;91:135-9.
27. Kok HS, van Asselt KM, van der Schouw YT, van der Tweel I, Peeters PH, Wilson PW, et al. Heart disease risk determines menopausal age rather than the reverse. J Am Coll Cardiol. 2006;47(10):1976-83.
28. van Bommel MHD, de Jong MA, Steenbeek MP, Bots ML, van Westerop LLM, Hopman MTE, et al. No signs of subclinical atherosclerosis after risk-reducing salpingo-oophorectomy in BRCA1/2 mutation carriers. J Cardiol. 2021;77(6):570-5.
29. Khosla S, Melton LJ, 3rd, Riggs BL. The unitary model for estrogen deficiency and the pathogenesis of osteoporosis: is a revision needed? J Bone Miner Res. 2011;26(3):441-51.
30. Gallagher JC. Effect of early menopause on bone mineral density and fractures. Menopause. 2007;14(3 Pt 2):567-71.
31. Kotsopoulos J, Hall E, Finch A, Hu H, Murphy J, Rosen B, et al. Changes in Bone Mineral Density After Prophylactic Bilateral Salpingo-Oophorectomy in Carriers of a BRCA Mutation. JAMA Netw Open. 2019;2(8):e198420.
32. Chan LN, Chen LM, Goldman M, Mak JS, Bauer DC, Boscardin J, et al. Changes in Bone Density in Carriers of BRCA1 and BRCA2 Pathogenic Variants After Salpingo-Oophorectomy. Obstet Gynecol. 2023;142(1):160-9.
33. Fakkert IE, Abma EM, Westrik IG, Lefrandt JD, Wolffenbuttel BH, Oosterwijk JC, et al. Bone mineral density and fractures after risk-reducing salpingo-oophorectomy in women at increased risk for breast and ovarian cancer. Eur J Cancer. 2015;51(3):400-8.

34. Powell CB, Alabaster A, Stoller N, Armstrong MA, Salyer C, Hamilton I, et al. Bone loss in women with BRCA1 and BRCA2 mutations. Gynecol Oncol. 2018;148(3):535-9.
35. 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(1):69-75.
36. Hadjidakis DJ, Kokkinakis EP, Sfakianakis ME, Raptis SA. Bone density patterns after normal and premature menopause. Maturitas. 2003;44(4):279-86.
37. Kanis JA, Johnell O, Oden A, Dawson A, De Laet C, Jonsson B. Ten year probabilities of osteoporotic fractures according to BMD and diagnostic thresholds. Osteoporos Int. 2001;12(12):989-95.
38. Mac Bride MB, Rhodes DJ, Shuster LT. Vulvovaginal atrophy. Mayo Clin Proc. 2010;85(1):87-94.
39. Kershaw V, Hickey I, Wyld L, Jha S. The impact of risk reducing bilateral salpingo-oophorectomy on sexual function in BRCA1/2 mutation carriers and women with Lynch syndrome: A systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2021;265:7-17.
40. Finch A, Metcalfe KA, Chiang JK, Elit L, McLaughlin J, Springate C, et al. The impact of prophylactic salpingo-oophorectomy on menopausal symptoms and sexual function in women who carry a BRCA mutation. Gynecol Oncol. 2011;121(1):163-8.
41. Hall E, Finch A, Jacobson M, Rosen B, Metcalfe K, Sun P, et al. Effects of bilateral salpingo-oophorectomy on menopausal symptoms and sexual functioning among women with a BRCA1 or BRCA2 mutation. Gynecol Oncol. 2019;152(1):145-50.
42. Dennerstein L, Koochaki P, Barton I, Graziottin A. Hypoactive sexual desire disorder in menopausal women: a survey of Western European women. J Sex Med. 2006;3(2):212-22.
43. Robinson D, Cardozo LD. The role of estrogens in female lower urinary tract dysfunction. Urology. 2003;62(4 Suppl 1):45-51.
44. Vermeulen RFM, Beurden MV, Korse CM, Kenter GG. Impact of risk-reducing salpingo-oophorectomy in premenopausal women. Climacteric. 2017;20(3):212-21.
45. Wei X, Oxley S, Sideris M, Kalra A, Brentnall A, Sun L, et al. Quality of life after risk-reducing surgery for breast and ovarian cancer prevention: a systematic review and meta-analysis. Am J Obstet Gynecol. 2023;229(4):388-409 e4.
46. Madalinska JB, Hollenstein J, Bleiker E, van Beurden M, Valdimarsdottir HB, Massuger LF, et al. Quality-of-life effects of prophylactic salpingo-oophorectomy versus gynecologic screening among women at increased risk of hereditary ovarian cancer. J Clin Oncol. 2005;23(28):6890-8.
47. Westin SN, Sun CC, Lu KH, Schmeler KM, Soliman PT, Lacour RA, et al. Satisfaction with ovarian carcinoma risk-reduction strategies among women at high risk for breast and ovarian carcinoma. Cancer. 2011;117(12):2659-67.
48. Hooker GW, King L, Vanhusen L, Graves K, Peshkin BN, Isaacs C, et al. Long-term satisfaction and quality of life following risk reducing surgery in BRCA1/2 mutation carriers. Hered Cancer Clin Pract. 2014;12(1):9.
49. Finch A, Metcalfe KA, Chiang J, Elit L, McLaughlin J, Springate C, et al. The impact of prophylactic salpingo-oophorectomy on quality of life and psychological distress in women with a BRCA mutation. Psychooncology. 2013;22(1):212-9.
50. Terra L, Hooning MJ, Heemskerk-Gerritsen BAM, van Beurden M, Roeters van Lennep JE, van Doorn HC, et al. Long-Term Morbidity and Health After Early Menopause Due to Oophorectomy in Women at Increased Risk of Ovarian Cancer: Protocol for a Nationwide Cross-Sectional Study With Prospective Follow-Up (HARMOny Study). JMIR Res Protoc. 2021;10(1):e24414.
51. Hoffmann U, Massaro JM, Fox CS, Manders E, O'Donnell CJ. Defining normal distributions of coronary artery calcium in women and men (from the Framingham Heart Study). Am J Cardiol. 2008;102(9):1136-41, 41 e1.

52. McClelland RL, Jorgensen NW, Budoff M, Blaha MJ, Post WS, Kronmal RA, et al. 10-Year Coronary Heart Disease Risk Prediction Using Coronary Artery Calcium and Traditional Risk Factors: Derivation in the MESA (Multi-Ethnic Study of Atherosclerosis) With Validation in the HNR (Heinz Nixdorf Recall) Study and the DHS (Dallas Heart Study). J Am Coll Cardiol. 2015;66(15):1643-53.

53. Oei HH, Vliegenthart R, Hak AE, Iglesias del Sol A, Hofman A, Oudkerk M, et al. The association between coronary calcification assessed by electron beam computed tomography and measures of extracoronary atherosclerosis: the Rotterdam Coronary Calcification Study. J Am Coll Cardiol. 2002;39(11):1745-51.

54. Schmermund A, Mohlenkamp S, Stang A, Gronemeyer D, Seibel R, Hirche H, et al. Assessment of clinically silent atherosclerotic disease and established and novel risk factors for predicting myocardial infarction and cardiac death in healthy middle-aged subjects: rationale and design of the Heinz Nixdorf RECALL Study. Risk Factors, Evaluation of Coronary Calcium and Lifestyle. Am Heart J. 2002;144(2):212-8.

55. Dzaye O, Razavi AC, Dardari ZA, Shaw LJ, Berman DS, Budoff MJ, et al. Modeling the Recommended Age for Initiating Coronary Artery Calcium Testing Among At-Risk Young Adults. J Am Coll Cardiol. 2021;78(16):1573-83.

56. Chu JH, Michos ED, Ouyang P, Vaidya D, Blumenthal RS, Budoff MJ, et al. Coronary artery calcium and atherosclerotic cardiovascular disease risk in women with early menopause: The Multi-Ethnic Study of Atherosclerosis (MESA). Am J Prev Cardiol. 2022;11:100362.

57. Cooper LL, Palmisano JN, Benjamin EJ, Larson MG, Vasan RS, Mitchell GF, et al. Microvascular Function Contributes to the Relation Between Aortic Stiffness and Cardiovascular Events: The Framingham Heart Study. Circ Cardiovasc Imaging. 2016;9(12).

58. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol. 2010;55(13):1318-27.

59. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur Heart J. 2016;37(29):2315-81.

60. Samargandy S, Matthews KA, Brooks MM, Barinas-Mitchell E, Magnani JW, Janssen I, et al. Arterial Stiffness Accelerates Within 1 Year of the Final Menstrual Period: The SWAN Heart Study. Arterioscler Thromb Vasc Biol. 2020;40(4):1001-8.

61. Khan ZA, Janssen I, Mazzarelli JK, Powell LH, Dumasius A, Everson-Rose SA, et al. Serial Studies in Subclinical Atherosclerosis During Menopausal Transition (from the Study of Women's Health Across the Nation). Am J Cardiol. 2018;122(7):1161-8.

62. Kim M, Kim HL, Cho Y, Lim WH, Seo JB, Kim SH, et al. Prognostic value of arterial stiffness in menopausal women. Menopause. 2022;29(5):573-9.

63. Gunning MN, Meun C, van Rijn BB, Daan NMP, Roeters van Lennep JE, Appelman Y, et al. The cardiovascular risk profile of middle age women previously diagnosed with premature ovarian insufficiency: A case-control study. PLoS One. 2020;15(3):e0229576.

64. Kanis JA, Melton LJ, 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res. 1994;9(8):1137-41.

65. Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. J Bone Miner Res. 2000;15(8):1526-36.

66. 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(2):391-420.

67. Peris P, Alvarez L, Monegal A, Guanabens N, Duran M, Pons F, et al. Biochemical markers of bone turnover after surgical menopause and hormone replacement therapy. Bone. 1999;25(3):349-53.

Chapter 2

Coronary Artery Calcium Scores after Prophylactic Premenopausal Bilateral Salpingo-Oophorectomy

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Abstract

Background

Premenopausal risk-reducing salpingo-oophorectomy (RRSO) in women at high familial risk of ovarian cancer leads to immediate menopause. While early natural menopause is associated with increased cardiovascular disease risk, evidence on long-term cardiovascular disease risk after early surgical menopause is scarce.

Objectives

To determine the long-term influence of the timing of RRSO on the development of coronary artery calcium (CAC), an established marker for cardiovascular disease risk.

Methods

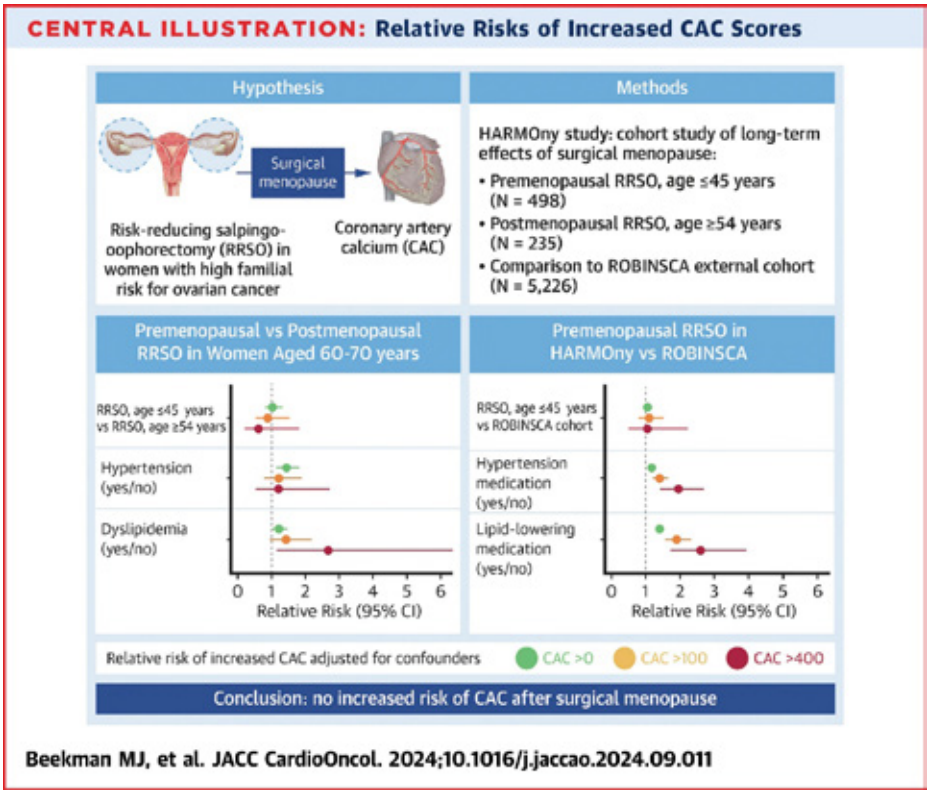
We conducted a cross-sectional study (n=733) nested in a nationwide cohort of women at high familial risk of ovarian cancer. In women aged 60-70 years (n=328), we compared CAC scores between women with a premenopausal RRSO (≤45 years) and women with a postmenopausal RRSO (≥54 years), using multivariable Poisson analyses. Within the premenopausal RRSO group (n=498), we also examined the effect of age at RRSO. In addition, we compared the premenopausal RRSO group with an external reference cohort (n=5226).

Results

Multivariable analyses showed that the prevalence rates of any CAC (CAC > 0), at least moderate CAC (CAC > 100), and severe CAC (CAC > 400) were comparable between the premenopausal and postmenopausal RRSO groups (relative risk [RR]: 0.93; 95% CI: 0.75-1.15 for any CAC; RR: 0.71; 95% CI: 0.43-1.17 for at least moderate CAC; RR: 0.81; 95% CI: 0.30-2.13 for severe CAC). There was no difference in CAC between the premenopausal RRSO group and a similar aged reference cohort. Timing of premenopausal RRSO (early premenopausal RRSO [<41 years] vs late premenopausal RRSO [41-45 years]) did not affect the outcomes.

Conclusion

Our results do not show a long-term adverse effect of surgical menopause on the development of CAC.



Abbreviations List

GPV = germline pathogenic variant
RRSO = risk-reducing salpingo-oophorectomy
IHD = ischemic heart disease
POI = premature ovarian insufficiency
CAC = coronary artery calcium
IMC = internal mammary chain
MHT = menopausal hormone therapy

Introduction

Available screening methods for early detection of ovarian cancer remain ineffective.[1] Therefore, current guidelines for women at high familial risk for ovarian cancer, such as carriers of *BRCA1/2* germline pathogenic variants (GPV), recommend risk-reducing salpingo-oophorectomy (RRSO) to prevent ovarian cancer. RRSO is advised after completion of childbearing, ideally between the ages of 35 and 40 years for *BRCA1* GPV carriers, and between 40 and 45 years for *BRCA2* GPV carriers.[2] While RRSO reduces the risk of ovarian cancer by 96%, it also induces early surgical menopause.[3-4]

Early menopause (≤45 years) has been associated with various long-term adverse effects, including an increased risk of cardiovascular disease, lowered bone mineral density, reduced quality of life, and cognitive impairment.[4] There is ample evidence that early natural menopause increases the risk of cardiovascular disease in later life. Recent studies show especially increased risks of stroke and ischemic heart disease (IHD) after early natural menopause due to premature ovarian insufficiency (POI).[5-8] This increased risk is commonly attributed to the decreased production of endogenous estrogens.[9] However, whether cardiovascular disease risk is similarly increased after *surgical* menopause has been less frequently investigated, with inconsistent results.[5-6-10]

Coronary artery calcium (CAC) measured by computed tomography is an established method for assessing individual cardiovascular disease risk in asymptomatic individuals, even at relatively young ages.[11-15] In addition, a recent study showed that CAC is an excellent predictor of cardiovascular disease in asymptomatic postmenopausal women who experienced an early natural menopause.[16] However, no studies have yet assessed CAC scores in relation to the timing of surgical menopause.

We aimed to investigate the long-term effect of a premenopausal RRSO (≤45yrs) on the presence of CAC in a cross-sectional study of 733 women at high familial risk for ovarian cancer. We compared women who underwent a premenopausal RRSO (≤45yrs) with women who underwent a postmenopausal RRSO (≥54 years), and we examined the effect of timing of RRSO within the premenopausal group. Additionally, we compared the premenopausal RRSO group with an external reference cohort.

Methods

Study cohort

The HARMOny study is a Dutch multicenter cross-sectional study investigating the long-term effects of RRSO on cardiovascular disease, bone health, cognition, and quality of life. The study design of the HARMOny study (ClinicalTrials.gov NCT03835793) has been described in detail previously and was approved in writing by the Medical Ethics Committee of the Antoni van Leeuwenhoek/Netherlands Cancer Institute (AVL/NKI).[18] Women were recruited from the Hereditary Breast and Ovarian cancer study Netherlands (HEBON), a nationwide cohort of women at high familial risk of breast and/or ovarian cancer recruited from all eight Dutch University Medical Centers and the Netherlands Cancer Institute.[19] Between 2018 and 2022, 1207 women were invited to participate in the study: 733 women who underwent a premenopausal RRSO (≤45yrs) and were at least 55 years old at inclusion, and 474 women who underwent a postmenopausal RRSO (≥54 years) (Figure 1). Exclusion criteria included a history of ovarian cancer, age older than 80 years, therapy-induced menopause more than five years before RRSO, metastatic disease, or a prior intervention interfering with the assessment of CAC, such as a percutaneous coronary intervention or mechanical cardiac valve. A history of cancer, other than ovarian cancer, was not a reason for exclusion.

External reference cohort ROBINSCA

We used an external reference cohort from the Risk or Benefit in Screening for Cardiovascular Disease (ROBINSCA) study, which was recruited from the Dutch general population in three different regions. Eligibility criteria required participants to have no history of cardiovascular disease but at least one cardiovascular disease risk factor.[20] In the ROBINSCA study, CAC scores and cardiovascular disease risk factors were available for 5226 women aged 55-70 years.

Study assessments

Participation in the HARMOny study involved completing an extensive online questionnaire and attending a clinical visit.[18] The questionnaire covered traditional and female-specific cardiovascular disease risk factors, medical history, and medication use, including menopausal hormone therapy (MHT). The clinical visit included a CAC score measurement by computed tomography, blood sampling, and an outpatient clinic visit with a research physician for anthropometric measurements (height, weight, heart rate, blood pressure, and waist and hip circumference). CAC scores were calculated by experienced cardiovascular radiologists at the participating medical centers using the standardized Agatston scoring method, which is known for

its excellent interscanner and interrater reliability. [21-23] Percentiles of the CAC score were determined using the Multi-Ethnic Study of Atherosclerosis (MESA) score. [24-25] Blood samples were taken to analyze non-fasting levels of lipids, glucose, HbA1c, high-sensitivity C-reactive protein (hs-CRP), and high-sensitivity cardiac troponin (hs-Troponin). If a participant had undergone radiotherapy for breast cancer, the radiotherapy records were evaluated for internal mammary chain (IMC) irradiation, a known risk factor for IHD. [26] According to the study protocol, the results of all measurements were shared with participants, and a letter detailing the results was sent to their general practitioners.

Statistical analyses

Continuous data were presented as means with standard deviation for normally distributed variables and as medians with 25th-75th percentiles (Q1-Q3) for skewed distribution. Categorical data were presented as counts with percentages. Characteristics of women in the premenopausal RRSO (≤45 years) and postmenopausal RRSO (≥54 years) groups were compared using the independent samples *t* test or Wilcoxon rank-sum test for continuous data, and the Fisher exact test or $\chi 2$ test for categorical data. A two-sided *P* value of less than 0.05 was considered statistically significant. Normality of data was assessed using the Shapiro-Wilk test. According to the HARMOny study protocol, we attempted to match the premenopausal RRSO (≤45 years) and postmenopausal RRSO (≥54 years) groups on age at the study visit. [18] However, during the inclusion period, we observed a substantial age difference (median 10.1 years) between the two groups. This age difference was attributed to the change in the 2007 guidelines for the management of ovarian cancer risk in *BRCA1/2* GPV carriers, which led to a significant increase in the prevalence of premenopausal RRSO. [2] Therefore, in the current study, we restricted the comparison of CAC scores between the premenopausal and postmenopausal RRSO groups to women who were between 60 and 70 years old at the time of the study visit (Figure 1). In addition, within the entire premenopausal RRSO group, we evaluated CAC scores in women who had an early premenopausal RRSO (≤41 years) and those who had a late premenopausal RRSO (41-45 years). We performed sensitivity analyses in women with and without MHT use and women without a history of breast cancer. Finally, we compared the CAC scores of women in our premenopausal RRSO group who met the eligibility criteria for ROBINSCA with the CAC scores of similarly aged women in the ROBINSCA study.

To evaluate whether the timing of premenopausal RRSO affects CAC scores later in life, we estimated relative risks (RRs) with 95% confidence intervals (CIs) for various CAC score cutoff points using multivariable Poisson regression analysis.

The assumptions of the Poisson model were assessed through the deviance and Pearson goodness-of-fit tests. The outcome categories analyzed were any CAC (CAC > 0), at least moderate CAC (CAC > 100) and severe CAC (CAC > 400). The variables assessed as possible confounders included age at study entry, current or ever smoking, alcohol use, use of menopausal MHT, history of breast cancer, history of IMC irradiation, body mass index (BMI), diabetes mellitus (defined as the use of antidiabetic medication for type 1 or 2 diabetes), hypertension (defined as the use of antihypertensive medication, a systolic blood pressure >140 mmHg, or a diastolic blood pressure >90 mmHg), and dyslipidemia (defined as the use of lipid-lowering medication or LDL cholesterol >4.0 mmol/L). A variable was considered a confounder if the RR estimate for the association of interest was changed by more than 10% when added to the model. All statistical analyses were performed using STATA version 15.1 (StataCorp LLC, College Station, TX).

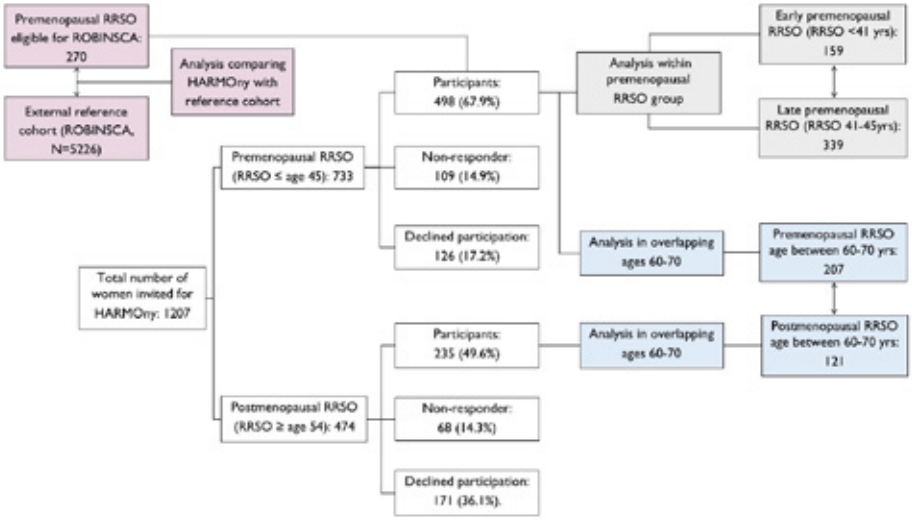


Figure 1. Flowchart of Participation in the HARMOny Study

The colors represent the 3 different statistical comparisons made: blue illustrates CAC scores of the premenopausal RRSO group vs the postmenopausal RRSO group (aged 60-70 years at the study visit); grey shows CAC scores of the early premenopausal RRSO group (≤41 years) vs the late premenopausal RRSO group (41-45 years); and pink compares CAC scores of the premenopausal RRSO group eligible for the ROBINSCA cohort vs the ROBINSCA external reference cohort (aged 55-70 years at the study visit).

Results

Participant characteristics of the entire HARMOny study population are provided in the supplemental material (Supplemental Table 1).

Participant characteristics of women aged 60-70 years at study visit

We included 328 women who were aged 60 to 70 years at the time of the study visit (207 in the premenopausal RRSO group [≤45 years] and 121 in the postmenopausal RRSO group [≥54 years]). The median age at the study visit was 64.5 years (61.9-67.0 years). The median time since RRSO was 21.0 years in the premenopausal group (18.3-23.3 years) and 10.7 years in the postmenopausal group (9.6-11.9 years) (Table 1). Both groups were comparable in terms of *BRCA1/2* GPV carrier status (overall, 66.7%) and history of breast cancer (overall, 61.8%). Compared with the postmenopausal RRSO group, the premenopausal RRSO group had significantly higher rates of IMC radiotherapy (8.7% vs 2.5%) and a more frequent history of MHT use (29.5% vs 6.6%). Hypertension was significantly less prevalent in the premenopausal RRSO group compared with the postmenopausal RRSO group (53.1% vs 65.3%).

Table 1. Participant characteristics.

	Age 60-70 y at study visit (n=328[a])			Premenopausal RRSO (n=498[a])		
	Premenopausal RRSO (≤45 y)	**Postmenopausal RRSO (≥54 y)**	***P* value**	**Early premenopausal RRSO (≤41 y)**	**Late premenopausal RRSO (41-45 y)**	***P* value**
N	207	121		159	339	
Age at study visit, y	62.4 (61.0-64.4)	67.2 (65.6-68.5)	<0.001	58.8 (57.2-61.6)	59.0 (57.8-62.3)	0.033
Time since RRSO, y	21.0 (18.3-23.3)	10.7 (9.6-11.9)	<0.001	20.9 (19.1-23.3)	16.6 (14.3-19.5)	<0.001
Age at menopause, y	42.0 (40.0-44.0)	51.0 (50.0-54.0)	<0.001	39.0 (37.0-40.0)	43.0 (42.0-44.0)	<0.001
BRCA* GPV *carrier status			<0.001			<0.001
BRCA1BRCA2	106 (51.2)	36 (29.8)		96 (60.4)	144 (42.5)	
	37 (17.9)	44 (36.4)		23 (14.5)	73 (21.5)	
Non-carrier	64 (30.9)	41 (33.9)		40 (25.2)	122 (36.0)	
MHT use	61 (29.5)	8 (6.6)	<0.001	74 (46.5)	68 (20.1)	<0.001
Breast cancer history	126 (60.9)	71 (58.7)	0.70	81 (50.9)	217 (64.0)	0.006
Chemotherapy	90 (43.5)	45 (37.2)	0.26	57 (37.1)	170 (50.2)	0.006
IMC radiotherapy	18 (8.7)	3 (2.5)	0.024	11 (6.9)	31 (9.1)	0.47
Endocrine therapy	41 (19.8)	26 (21.5)	0.72	19 (12.0)	97 (28.6)	<0.001
Smoking			0.16			0.77
Current smoker	18 (8.7)	6 (5.0)		15 (9.4)	34 (10.0)	
Former smoker	108 (52.2)	56 (46.3)		62 (39.0)	142 (41.9)	
Never	81 (39.1)	59 (48.8)		82 (51.6)	163 (48.1)	

Table 1. Continued

	Age 60-70 y at study visit (n=328[a])			Premenopausal RRSO (n=498[a])		
	Premenopausal RRSO (≤45 y)	**Postmenopausal RRSO (≥54 y)**	***P* value**	**Early premenopausal RRSO (≤41 y)**	**Late premenopausal RRSO (41-45 y)**	***P* value**
Alcohol >2 drinks daily	100 (48.3)	60 (49.6)	0.82	81 (50.9)	187 (55.2)	0.38
Family member with MI[b]	71 (34.3)	40 (33.1)	0.84	42 (26.4)	117 (34.5)	0.068
BMI, kg/m²	25.1 (22.7-28.8)	25.3 (23.2-28.7)	0.97	24.5 (22.5-29.1)	25.5 (22.8-29.0)	0.31
Systolic blood pressure, mm Hg	135.8 (17.6)	143.7 (15.9)	<0.001	132.7 (17.4)	134.1 (17.2)	0.40
Diastolic blood pressure, mm Hg	77.5 (12.1)	81.0 (11.5)	0.011	76.4 (11.5)	77.7 (12.3)	0.24
Total cholesterol, mmol/L	5.6 (1.1)	5.6 (1.4)	0.72	5.6 (1.1)	5.6 (1.0)	0.61
LDL cholesterol, mmol/L	3.3 (1.0)	3.4 (1.2)	0.47	3.3 (0.9)	3.3 (0.9)	0.99
HDL cholesterol, mmol/L	1.8 (0.4)	1.8 (0.6)	0.70	1.7 (0.6)	1.7 (0.4)	0.93
Antihypertensive medication	57 (27.5)	22.3%	0.30	32 (20.1)	65 (19.2)	0.80
Lipid lowering medication	37 (17.9)	23 (19.0)	0.80	18 (11.3)	41 (12.1)	0.80
Diabetes mellitus any type	20 (9.7)	10 (8.3)	0.66	9 (5.7)	25 (7.4)	0.47
Hypertension[c]	110 (53.1)	79 (65.3)	0.031	66 (41.5)	155 (45.7)	0.38
Dyslipidemia[d]	81 (39.1)	59 (48.8)	0.090	61 (38.4)	120 (35.4)	0.52
MESA 10-year CHD risk[e]	2.6 (1.6-6.6)	3.8 *1.9-7.4)	0.036	2.1 (1.3-4.7)	2.2 (1.5-4.9)	0.26
CAC score	1 (0-74)	13 (0-136)	0.088	0 (0-28)	0 (0-39)	0.26
CAC >0	107 (51.7)	71 (58.7)	0.22	65 (40.9)	155 (45.7)	0.31
CAC >100	40 (19.3)	32 (26.5)	0.13	17 (10.7)	54 (15.9)	0.12

Table 1. Continued

	Age 60-70 y at study visit (n=328[a])			Premenopausal RRSO (n=498[a])		
	Premenopausal RRSO (≤45 y)	**Postmenopausal RRSO (≥54 y)**	***P* value**	**Early premenopausal RRSO (≤41 y)**	**Late premenopausal RRSO (41-45 y)**	***P* value**
CAC >400	11 (5.3)	16 (13.2)	0.015	6 (3.8)	16 (4.7)	0.63
MESA score	57 (0-80)	58 (0-80)	0.65	0 (0-77)	0 (0-81)	0.30
MESA >75%	65 (31.4)	34 (28.1)	0.53	46 (28.9)	103 (30.4)	0.74

Values are numbers with percentages (No. (%)) for categorical variables, means with standard deviation for normally distributed variables, and medians with 25th-75th percentiles for skewed distributions. The P value was calculated using independent samples t test, X^2 test, and Wilcoxon rank-sum test.

RRSO, premenopausal risk-reducing salpingo-oophorectomy; GPV, pathogenic variant; MHT, menopausal hormone therapy; IMC, internal mammary chain; MI, myocardial infarction; BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; MESA, Multi-Ethnic Study of Atherosclerosis; CAC, coronary artery calcium.

[a]See Figure 1.

[b]Myocardial infarction first- or second-degree family member before the age of 65.

[c]Hypertension: defined as either the use of antihypertensive medication, a systolic blood pressure >140 mmHg, or a diastolic blood pressure >90 mmHg.

[d]Dyslipidemia: defined as either the use of lipid lowering medication or LDL cholesterol >4.0 mmol/L.

[e]MESA: estimated 10-year risk of coronary heart disease event, including CAC score.Participant characteristics of women with a premenopausal RRSO.

Within the entire premenopausal group (n=498), we compared women who had an early premenopausal RRSO (≤41years) (n=159) with those who had a late premenopausal RRSO (41-45 years) (n=339). Compared with the late premenopausal RRSO group, women in the early premenopausal RRSO group were significantly more likely to be *BRCA1/2* GPV carriers (74.8% vs 64.0%) and were less likely to have a history of breast cancer (50.9% vs 64.0%), chemotherapy (37.1% vs 50.2%), and endocrine therapy (12.0% vs 28.6%).

Table 2. RRs of increased CAC scores according to timing of RRSO in women aged 60-70 years.

	Adjusted RR (95% CI)			
	CAC > 0[a]	**CAC > 100[a]**	**CAC > 400[a]**	**MESA >75%[b]**
Timing of RRSO				
Postmenopausal RRSO (≥54 y)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Premenopausal RRSO (≤45 y)	1.07 (0.83-1.37)	0.89 (0.52-1.52)	0.61 (0.21-1.74)	1.13 (0.72-1.80)
Hypertension	1.55 (1.23-1.95)	1.36 (0.88-2.11)	1.19 (0.54-2.61)	1.51 (1.05-2.17)
Dyslipidemia	1.21 (0.99-1.46)	1.48 (0.98-2.24)	2.80 (1.20-6.52)	1.52 (1.09-2.11)

RR, relative risks; RRSO, premenopausal risk-reducing salpingo-oophorectomy, CAC, coronary artery calcium.

[a]Risk of having any, moderate, severe CAC. RRs were multivariably adjusted for age, hypertension, dyslipidemia, and timing of RRSO.

[b]Risk of having a MESA score above 75%. RRs were multivariably adjusted for hypertension, dyslipidemia, and timing of RRSO.

CAC scores after premenopausal versus postmenopausal RRSO in women aged 60-70 years at study visit

Univariable analyses showed a higher prevalence of increased CAC scores in the postmenopausal RRSO (≥54 years) group compared with the premenopausal RRSO (≤45 years) group. For instance, severe CAC (CAC > 400) was observed in 13.2% versus 5.3% of women, respectively. The MESA score was comparable between both groups (median 57; Q1-Q3: 0-80 vs median 58; Q1-Q3: 0-80). After adjustment for age, hypertension, and dyslipidemia, there was no statistically significant difference in CAC scores between the premenopausal and postmenopausal RRSO groups among women aged 60-70 years (Table 2 and Central Illustration). The prevalence rates of any CAC (CAC > 0), at least moderate CAC (CAC >100), and severe CAC (CAC > 400) were not higher in the premenopausal RRSO group compared with the postmenopausal RRSO group (RR: 1.07; 95% CI: 0.83-1.37for any CAC; RR: 0.89; 95% CI: 0.52-1.52for CAC > 100; RR: 0.61; 95% CI: 0.21-1.74for CAC > 400). The prevalence rates of participants with a MESA percentile score above 75% were also comparable

in both groups (RR: 1.13; 95% CI: 0.72-1.80). Participants with hypertension and/or dyslipidemia had significantly higher CAC scores and MESA percentiles compared with women without these risk factors. Including MHT use, current smoking, BMI, history of breast cancer, diabetes mellitus, and IMC radiotherapy in the analyses did not change the outcomes (Supplemental Table 2).

CAC scores according to timing of premenopausal RRSO

The prevalence rates of any CAC, at least moderate CAC, and severe CAC were comparable between the early and late premenopausal groups (RRs adjusted for age, hypertension, and dyslipidemia: RR: 0.93; 95% CI: 0.75-1.15 for any CAC; RR: 0.71; 95% CI: 0.43-1.17 for CAC > 100; RR: 0.81; 95% CI: 0.30-2.13 for CAC > 400 (Table 3). The prevalence rates of participants with a MESA score above 75% percentile were also comparable between the two groups (RR: 0.96; 95% CI: 0.72-1.28). Participants with hypertension and/or dyslipidemia had significantly higher CAC scores and MESA percentiles than those without these risk factors. Including MHT use, current smoking, BMI, history of breast cancer, diabetes mellitus, and IMC radiotherapy in the analyses did not affect the results.

Table 3. RRs of increased CAC scores according to timing of RRSO in women with a premenopausal RRSO.

	Adjusted RR (95% CI)			
	CAC > 0[a]	**CAC > 100[a]**	**CAC > 400[a]**	**MESA >75%[b]**
Timing of RRSO				
Late premenopausal RRSO (41-45 y)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Early premenopausal RRSO (<41 y)	0.93 (0.75-1.15)	0.71 (0.43-1.17)	0.81 (0.34-2.13)	0.96 (0.72-1.28)
Hypertension	1.43 (1.16-1.75)	1.33 (0.86-2.06)	1.30 (0.59-2.84)	1.42 (1.08-1.86)
Dyslipidemia	1.13 (0.93-1.37)	1.68 (1.10-2.56)	4.35 (1.81-10.45)	1.33 (1.02-1.73)

RR, relative risks; RRSO, premenopausal risk-reducing salpingo-oophorectomy, CAC, coronary artery calcium.

[a]Risk of having any, moderate, or severe CAC. RRs were multivariably adjusted for age, hypertension, dyslipidemia, and timing of RRSO.

[b]Risk of having a MESA score above 75%. RRs were multivariably adjusted for hypertension, dyslipidemia, and timing of RRSO.

Sensitivity analyses

Sensitivity analyses conducted in women with and without MHT use (Supplemental Tables 2, 3, and 4) and in women with and without breast cancer (Supplemental Tables 5 and 6) yielded similar results.

CAC scores in the premenopausal RRSO group compared with an external reference cohort

In total, 270 women in the premenopausal RRSO (≤45 years) group met the eligibility criteria for the ROBINSCA study (Supplemental Table 7). Women in the premenopausal RRSO group were significantly younger and had a significantly higher BMI and a higher prevalence of any type of diabetes mellitus compared with women in the ROBINSCA study in the same age group (55-70 years; n=5226). Other measured cardiovascular disease risk factors were comparable between the two groups. The prevalence rates of increased CAC scores were comparable between the two groups for any CAC, CAC > 100, and CAC > 400. Multivariable Poisson analyses showed no significant differences between the premenopausal RRSO group and the ROBINSCA reference group for the different CAC outcomes (analyses adjusted for age, hypertension medication, and lipid-lowering medication) (Table 4). Including BMI or the prevalence of diabetes mellitus in the analyses did not change the outcomes.

Table 4. RRs of increased CAC scores in the premenopausal RRSO group compared with the ROBINSCA cohort

	Adjusted RR (95% CI)		
	CAC > 0[a]	**CAC > 100[a]**	**CAC > 400[a]**
Timing of RRSO			
ROBINSCA age 55-70 y	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Premenopausal RRSO (≤45y)	1.05 (0.92-1.21)	1.11 (0.80-1.53)	1.05 (0.50-2.20)
Hypertension	1.18 (1.11-1.26)	1.43 (1.23-1.67)	1.92 (1.41-2.61)
Dyslipidemia	1.48 (1.37-1.59)	2.12 (1.76-2.55)	2.61 (1.77-3.85)

RR, relative risks; RRSO, premenopausal risk-reducing salpingo-oophorectomy, CAC, coronary artery calcium.

[a]Risk of having any, moderate, or severe CAC. RRs were multivariably adjusted for age, antihypertensive medication, lipid lowering medication, and timing of RRSO.

Discussion

Twenty-one years after surgical menopause, we did not observe increased CAC scores in women who underwent a premenopausal RRSO (≤45 years), either when compared with women who underwent postmenopausal RRSO (≥54 years) or with an external reference population. Furthermore, an early premenopausal RRSO (≤41 years), compared with a late premenopausal RRSO (41-45 years), was not associated with increased CAC scores.

Our nationwide study is the first to investigate CAC scores after premenopausal RRSO in women at high familial risk for ovarian cancer. Studies investigating cardiovascular disease risk after surgical menopause are scarce and inconclusive, primarily due to limited power and methodological issues, such as confounding by indication for surgical menopause.[5-6-10-27] The most frequently reported indications for surgical menopause include RRSO, endometriosis, and benign cysts. Endometriosis has been associated with an increased risk of cardiovascular disease, regardless of a history of surgical menopause, while cardiovascular disease risk in women with cysts remains unclear.[28-29] However, previous studies did not conduct subgroup analyses specifically among women with RRSO. Our findings in women who underwent surgical menopause are consistent with a recent smaller study by Van Bommel et al., which found no association between time since RRSO and other measures of subclinical atherosclerosis, including pulse wave velocity (PWV) and carotid intima thickness (cIMT), in a cohort of women *BRCA1/2* GPV carriers.[30]

Although surgical menopause does not appear to be associated with CAC, this does not entirely rule out the possibility of increased cardiovascular disease risk through other (less likely) pathways. Two recent studies did also did not show no differences in the prevalence of increased CAC levels after POI. However, the women included in these studies may have been too young (median ages 49.4 and 50 years, respectively) to detect differences in subclinical atherosclerosis.[31-33] In contrast, two recent large meta-analyses convincingly showed increased cardiovascular disease risk after early natural menopause.[7-8] Interestingly, a study by Krul et al. showed no increase in cardiovascular disease risk after early iatrogenic menopause caused by chemotherapy-induced POI in Hodgkin lymphoma survivors.[34] This supports our hypothesis that early natural menopause is associated with increased cardiovascular disease risk, whereas early surgical (or otherwise iatrogenic) menopause is not. This apparent discrepancy may be explained by the reverse causality hypothesis, which postulates that early natural menopause is the result of accelerated vascular aging, leading to a statistical (non-causal) association between early natural menopause and increased cardiovascular disease risk.[17]

It has been suggested that MHT may protect women against IHD after surgical menopause before the age of 45.[27-35] Therefore, we considered MHT as a potential confounder in our analyses. However, the prevalence of MHT use was relatively low in our study. Furthermore, we did not find a protective effect of MHT use, neither for ever use nor for the duration of use (Supplemental Tables 2, 3, and 4, and Supplemental Tables 8 and 9), and MHT use was not a confounder in our analyses.

Strengths and limitations

The strengths of our nationwide study include the large sample size, which provided sufficient power for subgroup analyses, the long-term follow-up after premenopausal RRSO (≤45 years) and the use of a comparison group of women who also underwent RRSO, but at a later age. By excluding women who underwent RRSO between the ages of 46 and 54 years, we were able to make a more distinct evaluation of the differences in cardiovascular disease risk between the premenopausal and postmenopausal RRSO groups. Unlike other studies, the comparisons in our study are not affected by confounding due to the indication for bilateral oophorectomy.

Since the current standard of care for women at high familial risk of ovarian cancer is to undergo premenopausal RRSO, almost all women have the surgery before reaching menopause.[2] Consequently, our study took advantage of a window of opportunity to recruit a large group of women who had undergone postmenopausal RRSO (≥54 years) years earlier. The participation rate of our study was strong (60.7%), considering the relatively long time since RRSO at the time of the study visit. In addition, our outcome measure, CAC, is an established predictor of cardiovascular disease risk in asymptomatic women, independent of other cardiovascular disease risk factors.[11-14] The CAC score has also been shown to be a reliable predictor of cardiovascular disease risk in women with an early menopause (before age 45).[16]

A limitation of our study is the age difference between the premenopausal RRSO (≤45 years) and postmenopausal RRSO (≥54 years) groups in the entire study population. However, we addressed this limitation by restricting our analysis to women aged 60-70 years at study enrollment. In addition, we used the entire premenopausal RRSO group to assess the association between timing of a premenopausal RRSO (≤41 years vs 41-45 years) and cardiovascular disease risk. Moreover, we had the unique opportunity to compare the CAC scores of our premenopausal RRSO group with those of similarly aged women in the ROBINSCA general population cohort, showing no differences. When interpreting our results, it is important to note that 98% of the participants were Caucasian. Another potential limitation of this study is survival bias, as death related to cardiovascular disease after RRSO may have occurred before recruitment into the HARMOny study. Since our study was nested within the HEBON cohort, we had the opportunity to obtain causes of death from Statistics Netherlands for all women who were otherwise eligible for our study but died before possible inclusion.[19] Only 1.9% of these women died from a cardiovascular event, while the most frequent cause of death was cancer (87.6%). Selection bias may also have occurred due to differences in response rates between the premenopausal (68.0%) and postmenopausal groups (50.8%). We addressed

this potential bias by using previously collected data from questionnaire surveys completed for the HEBON study.[19] In these questionnaires, current non-responders in the postmenopausal RRSO group did not report a lower or higher prevalence of cardiovascular disease than responders.

Conclusion

In conclusion, this study does not support a long-term adverse effect of surgical menopause on the development of CAC, a marker of cardiovascular disease risk. This is an important and reassuring message for women at high familial risk of ovarian cancer and may assist physicians in counseling these women. Our results may also have broader relevance for women who experience iatrogenic menopause after cancer treatment. Future studies should examine the risk of clinical cardiovascular disease after iatrogenic menopause.

References

1. Henderson JT, Webber EM, Sawaya GF. Screening for Ovarian Cancer: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA. 2018;319(6):595-606.
2. Daly MB, Pal T, Berry MP, Buys SS, Dickson P, Domchek SM, et al. Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2021;19(1):77-102.
3. Domchek SM, Friebel TM, Singer CF, Evans DG, Lynch HT, Isaacs C, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA. 2010;304(9):967-75.
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(2):80-7.
5. Dam V, van der Schouw YT, Onland-Moret NC, Groenwold RHH, Peters SAE, Burgess S, et al. Association of menopausal characteristics and risk of coronary heart disease: a pan-European case-cohort analysis. Int J Epidemiol. 2019;48(4):1275-85.
6. Honigberg MC, Zekavat SM, Aragam K, Finneran P, Klarin D, Bhatt DL, et al. Association of Premature Natural and Surgical Menopause With Incident Cardiovascular Disease. JAMA. 2019;322(24):2411-21.
7. Muka T, Oliver-Williams C, Kunutsor S, Laven JS, Fauser BC, Chowdhury R, et al. Association of Age at Onset of Menopause and Time Since Onset of Menopause With Cardiovascular Outcomes, Intermediate Vascular Traits, and All-Cause Mortality: A Systematic Review and Meta-analysis. JAMA Cardiol. 2016;1(7):767-76.
8. Roeters van Lennep JE, Heida KY, Bots ML, Hoek A, collaborators of the Dutch Multidisciplinary Guideline Development Group on Cardiovascular Risk Management after Reproductive D. Cardiovascular disease risk in women with premature ovarian insufficiency: A systematic review and meta-analysis. Eur J Prev Cardiol. 2016;23(2):178-86.
9. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. N Engl J Med. 1999;340(23):1801-11.
10. Parker WH, Broder MS, Chang E, Feskanich D, Farquhar 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(5):1027-37.
11. Hoffmann U, Massaro JM, Fox CS, Manders E, O'Donnell CJ. Defining normal distributions of coronary artery calcium in women and men (from the Framingham Heart Study). Am J Cardiol. 2008;102(9):1136-41, 41 e1.
12. McClelland RL, Jorgensen NW, Budoff M, Blaha MJ, Post WS, Kronmal RA, et al. 10-Year Coronary Heart Disease Risk Prediction Using Coronary Artery Calcium and Traditional Risk Factors: Derivation in the MESA (Multi-Ethnic Study of Atherosclerosis) With Validation in the HNR (Heinz Nixdorf Recall) Study and the DHS (Dallas Heart Study). J Am Coll Cardiol. 2015;66(15):1643-53.
13. Oei HH, Vliegenthart R, Hak AE, Iglesias del Sol A, Hofman A, Oudkerk M, et al. The association between coronary calcification assessed by electron beam computed tomography and measures of extracoronary atherosclerosis: the Rotterdam Coronary Calcification Study. J Am Coll Cardiol. 2002;39(11):1745-51.
14. Schmermund A, Mohlenkamp S, Stang A, Gronemeyer D, Seibel R, Hirche H, et al. Assessment of clinically silent atherosclerotic disease and established and novel risk factors for predicting myocardial infarction and cardiac death in healthy middle-aged subjects: rationale and design of the Heinz Nixdorf RECALL Study. Risk Factors, Evaluation of Coronary Calcium and Lifestyle. Am Heart J. 2002;144(2):212-8.

15. Dzaye O, Razavi AC, Dardari ZA, Shaw LJ, Berman DS, Budoff MJ, et al. Modeling the Recommended Age for Initiating Coronary Artery Calcium Testing Among At-Risk Young Adults. J Am Coll Cardiol. 2021;78(16):1573-83.
16. Chu JH, Michos ED, Ouyang P, Vaidya D, Blumenthal RS, Budoff MJ, et al. Coronary artery calcium and atherosclerotic cardiovascular disease risk in women with early menopause: The Multi-Ethnic Study of Atherosclerosis (MESA). Am J Prev Cardiol. 2022;11:100362.
17. Kok HS, van Asselt KM, van der Schouw YT, van der Tweel I, Peeters PH, Wilson PW, et al. Heart disease risk determines menopausal age rather than the reverse. J Am Coll Cardiol. 2006;47(10):1976-83.
18. Terra L, Hooning MJ, Heemskerk-Gerritsen BAM, van Beurden M, Roeters van Lennep JE, van Doorn HC, et al. Long-Term Morbidity and Health After Early Menopause Due to Oophorectomy in Women at Increased Risk of Ovarian Cancer: Protocol for a Nationwide Cross-Sectional Study With Prospective Follow-Up (HARMOny Study). JMIR Res Protoc. 2021;10(1):e24414.
19. Brohet RM, Velthuizen ME, Hogervorst FB, Meijers-Heijboer HE, Seynaeve C, Collee MJ, et al. Breast and ovarian cancer risks in a large series of clinically ascertained families with a high proportion of BRCA1 and BRCA2 Dutch founder mutations. J Med Genet. 2014;51(2):98-107.
20. van der Aalst CM, Denissen S, Vonder M, Gratama JWC, Adriaansen HJ, Kuijpers D, et al. Screening for cardiovascular disease risk using traditional risk factor assessment or coronary artery calcium scoring: the ROBINSCA trial. Eur Heart J Cardiovasc Imaging. 2020;21(11):1216-24.
21. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990;15(4):827-32.
22. Ghadri JR, Goetti R, Fiechter M, Pazhenkottil AP, Kuest SM, Nkoulou RN, et al. Inter-scan variability of coronary artery calcium scoring assessed on 64-multidetector computed tomography vs. dual-source computed tomography: a head-to-head comparison. Eur Heart J. 2011;32(15):1865-74.
23. Takx RA, de Jong PA, Leiner T, Oudkerk M, de Koning HJ, Mol CP, et al. Automated coronary artery calcification scoring in non-gated chest CT: agreement and reliability. PLoS One. 2014;9(3):e91239.
24. Joshi PH, Patel B, Blaha MJ, Berry JD, Blankstein R, Budoff MJ, et al. Coronary artery Calcium predicts Cardiovascular events in participants with a low lifetime risk of Cardiovascular disease: The Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis. 2016;246:367-73.
25. McClelland RL, Chung H, Detrano R, Post W, Kronmal RA. Distribution of coronary artery calcium by race, gender, and age: results from the Multi-Ethnic Study of Atherosclerosis (MESA). Circulation. 2006;113(1):30-7.
26. Boekel NB, Jacobse JN, Schaapveld M, Hooning MJ, Gietema JA, Duane FK, et al. Cardiovascular disease incidence after internal mammary chain irradiation and anthracycline-based chemotherapy for breast cancer. Br J Cancer. 2018;119(4):408-18.
27. Rivera CM, Grossardt BR, Rhodes DJ, Brown RD, Jr., Roger VL, Melton LJ, 3rd, et al. Increased cardiovascular mortality after early bilateral oophorectomy. Menopause. 2009;16(1):15-23.
28. Mu F, Rich-Edwards J, Rimm EB, Spiegelman D, Missmer SA. Endometriosis and Risk of Coronary Heart Disease. Circ Cardiovasc Qual Outcomes. 2016;9(3):257-64.
29. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2018;33(9):1602-18.

30. van Bommel MHD, de Jong MA, Steenbeek MP, Bots ML, van Westerop LLM, Hopman MTE, et al. No signs of subclinical atherosclerosis after risk-reducing salpingo-oophorectomy in BRCA1/2 mutation carriers. J Cardiol. 2021;77(6):570-5.

31. Gunning MN, Meun C, van Rijn BB, Maas A, Benschop L, Franx A, et al. Coronary artery calcification in middle-aged women with premature ovarian insufficiency. Clin Endocrinol (Oxf). 2019;91(2):314-22.

32. Freaney PM, Petito L, Colangelo LA, Lewis CE, Schreiner PJ, Terry JG, et al. Association of Premature Menopause With Coronary Artery Calcium: The CARDIA Study. Circ Cardiovasc Imaging. 2021;14(11):e012959.

33. Mortensen MB, Gaur S, Frimmer A, Botker HE, Sorensen HT, Kragholm KH, et al. Association of Age With the Diagnostic Value of Coronary Artery Calcium Score for Ruling Out Coronary Stenosis in Symptomatic Patients. JAMA Cardiol. 2022;7(1):36-44.

34. Krul IM, Opstal-van Winden AWJ, Janus CPM, Daniels LA, Appelman Y, Maas A, et al. Cardiovascular Disease Risk After Treatment-Induced Premature Ovarian Insufficiency in Female Survivors of Hodgkin Lymphoma. J Am Coll Cardiol. 2018;72(25):3374-5.

35. Lokkegaard E, Jovanovic Z, Heitmann BL, Keiding N, Ottesen B, Pedersen AT. The association between early menopause and risk of ischaemic heart disease: influence of Hormone Therapy. Maturitas. 2006;53(2):226-33.

Declarations

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Supplements

Table S1. Participant characteristics whole study population.

	Premenopausal RRSO	Postmenopausal RRSO	p-value
N	498	235	
Age at study visit, years	58.8 (56.8-61.8)	68.9 (65.8-72.5)	<0.001
Time since RRSO, years	18.1 (15.3-21.3)	11.6 (10.2-13.9)	<0.001
Age at menopause, years	42.0 (40.0-44.0)	51.0 (50.0-53.0)	<0.001
BRCA* gPV *carrier status			<0.001
BRCA 1	240 (48.2)	70 (29.8)	
BRCA 2	96 (19.3)	83 (35.3)	
Non-carrier	162 (32.5)	82 (34.9)	
MHT use	142 (28.5)	24 (10.2)	<0.001
Breast cancer history	298 (59.8)	155 (66.0)	0.11
Chemotherapy	229 (46.0)	83 (35.3)	0.007
Internal mammary chain radiotherapy	42 (8.4)	16 (6.8)	0.43
Endocrine therapy	116 (23.3)	50 (21.3)	0.60
Smoking			0.001
Current smoker	49 (9.8)	8 (3.4)	
Former smoker	204 (41.0)	121 (51.5)	
Never	245 (49.2)	106 (45.1)	
Alcohol >2 drinks daily	268 (53.8)	118 (50.2)	0.36
MI first degree family member[a]	159 (31.9)	75 (31.9)	0.87
BMI, kg/m²	25.3 (22.7-29.0)	25.1 (22.9-28.7)	0.42
Waist circumference, cm	88.0 (80.0-97.0)	89.0 (82.0-99.0)	0.42
Systolic blood pressure, mm Hg	133.7 (17.3)	143.9 (17.8)	<0.001
Diastolic blood pressure, mm Hg	77.3 (12.0)	79.6 (11.7)	0.017
Total cholesterol, mmol/L	5.6 (1.0)	5.6 (1.2)	0.55
LDL cholesterol, mmol/L	3.3 (0.9)	3.2 (1.1)	0.28
HDL cholesterol, mmol/L	1.7 (0.5)	1.8 (0.5)	0.024
Antihypertensive medication	97 (19.5)	69 (29.4)	0.003
Lipid lowering medication	59 (11.9)	51 (21.7)	<0.001

Table S1. Continued

	Premenopausal RRSO	Postmenopausal RRSO	p-value
Diabetes mellitus any type	34 (6.8)	17 (7.2)	0.82
Hypertension[b]	221 (44.4)	155 (66.0)	<0.001
Dyslipidemia[c]	181 (36.4)	106 (45.1)	0.023
MESA 10-year CHD risk[d]	2.2 (1.4-4.8)	5.1 (2.2-9.9)	<0.001
CAC score median	0 (0-37)	30 (0-175)	<0.001
CAC>0	220 (44.2)	153 (65.1)	<0.001
CAC>100	71 (14.3)	80 (34.0)	<0.001
CAC>400	22 (4.4)	33 (14.0)	<0.001
MESA score	0 (0-80)	54 (0-79)	<0.001
MESA percentile >75%	149 (29.9)	66 (28.1)	0.61

Values are numbers with percentages (No. (%)) for categorical variables, means with standard deviation for normal distributed variables, and medians with 25th-75th percentiles for variables with a skewed distribution. P-value was calculated using independent samples t-test, X^2test and Wilcoxon rank-sum test. Abbreviations: gPV, pathogenic variant; MHT, menopausal hormone therapy; MI, myocardial infarction; BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein.

[a]Myocardial infarction first degree family member before the age of 65.

[b]Hypertension: either antihypertensive medication, a systolic blood pressure >140mmHg or a diastolic blood pressure >90mmH.

[c]Dyslipidemia** either lipid lowering medication or a LDL cholesterol >4.0.

[d]MESA estimated 10-year risk of CHD event, including CAC score.

Table S2. RRs of increased CAC scores according to timing of RRSO in women aged 60-70 years.

	RR (95% CI)			
	CAC >0	**CAC >100**	**CAC >400**	**MESA >75%**
Univariate model				
Postmenopausal RRSO (RRSO≥54y)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Premenopausal RRSO (RRSO≤45y)	0.88 (0.72-1.08)	0.73 (0.49-1.10)	0.41 (0.21-1.14)	1.12 (0.79-1.59)
Model 1				
Postmenopausal RRSO (RRSO≥54y)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Premenopausal RRSO (RRSO≤45y)	1.04 (0.80-1.35)	0.88 (0.52-1.48)	0.61 (0.23-1.66)	1.12 (0.79-1.59)
Model 2				
Postmenopausal RRSO (RRSO≥54y)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Premenopausal RRSO (RRSO≤45y)	1.07 (0.83-1.37)	0.89 (0.52-1.52)	0.61 (0.21-1.74)	1.13 (0.72-1.80)
Model 3				
Postmenopausal RRSO (RRSO≥54y)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Premenopausal RRSO (RRSO≤45y)	1.09 (0.82-1.44)	0.94 (0.53-1.67)	0.78 (0.25-2.44)	1.25 (0.85-1.84)
Model 4				
Postmenopausal RRSO (RRSO≥54y)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Premenopausal RRSO (RRSO≤45y)	1.12 (0.84-1.50)	1.09 (0.62-1.92)	1.02 (0.28-3.66)	1.28 (0.86-1.90)

Model 1: Adjusted for age.Model 2: Adjusted for age, hypertension and dyslipidemiaModel 3: Adjusted for age, hypertension dyslipidemia, current smoking, MHT use, BMI and history of breast cancerModel 4: Adjusted for age, hypertension dyslipidemia, current smoking, MHT use, BMI, history of breast cancer, diabetes mellitus and IMC irradiation (DM and IMC irradiation have a relative low prevalence in our study population leading to wider 95% confidence intervals).

Table S3. RRs of increased CAC scores according to timing of RRSO in women aged 60-70 without a history of (any) MHT use.

	Adjusted RR (95% CI)			
	CAC >0[a]	**CAC >100[a]**	**CAC >400[a]**	**MESA> 75%[b]**
Timing of RRSO				
Postmenopausal RRSO (RRSO ≥54y)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Premenopausal RRSO (RRSO ≤45y)	0.99 (0.72-1.37)	0.79 (0.41-1.53)	0.55 (0.15-2.01)	0.96 (0.55-1.66)
Hypertension	1.51 (1.14-2.01)	1.26 (0.75-2.13)	1.38 (0.49-3.84)	1.60 (1.01-2.54)
Dyslipidemia	1.12 (0.89-1.40)	1.04 (0.64-1.69)	1.83 (0.67-5.01)	1.25 (0.84-1.86)

[a.] Risk of having any/moderate/severe coronary artery calcification (CAC), RRs were multivariably adjusted for all other factors in the model: age, hypertension, dyslipidemia and timing of RRSO.

[b.] Risk of having a MESA percentile above 75%, RRs were multivariably adjusted for all other factors in the model: hypertension, dyslipidemia and timing of RRSO.

Table S4. RRs of increased CAC scores according to timing of premenopausal RRSO a in women with a history of MHT use.

	Adjusted RR (95% CI)			
	CAC >0[a]	**CAC >100[a]**	**CAC >400[a]**	**MESA> 75%[b]**
Timing of RRSO				
Late premenopausal RRSO (41-45y)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Early premenopausal RRSO (<41y)	0.74 (0.52-1.05)	0.47 (0.19-1.13)	0.46 (0.13-1.72)	0.66 (0.40-1.09)
Hypertension	1.87 (1.26-2.78)	1.32 (0.52-3.31)	0.73 (0.19-2.83)	1.37 (0.91-2.72)
Dyslipidemia	1.28 (0.89-1.84)	2.65 (1.09-6.44)	6.98 (1.81-26.89)	2.21 (1.31-3.75)

[a.] Risk of having any/moderate/severe coronary artery calcification (CAC), RRs were multivariably adjusted for all other factors in the model: age, hypertension, dyslipidemia and timing of RRSO.

[b.] Risk of having a MESA percentile above 75%, RRs were multivariably adjusted for all other factors in the model: hypertension, dyslipidemia and timing of RRSO.

Table S5. RRs of increased CAC scores according to timing of premenopausal RRSO in women without a history of MHT use.

	Adjusted RR (95% CI)			
	CAC >0[a]	**CAC >100[a]**	**CAC >400[a]**	**MESA> 75%[b]**
Timing of RRSO				
Late premenopausal RRSO (41-45y)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Early premenopausal RRSO (<41y)	1.10 (0.83-1.45)	0.91 (0.46-1.79)	1.10 (0.32-3.81)	1.16 (0.80-1.67)
Hypertension	1.32 (1.02-1.69)	1.36 (0.79-2.34)	2.02 (0.68-5.97)	1.37 (0.99-1.91)
Dyslipidemia	1.04 (0.81-1.33)	1.26 (0.74-2.14)	3.37 (1.10-10.30)	1.08 (0.78-1.51)

[a.] Risk of having any/moderate/severe coronary artery calcification (CAC), RRs were multivariably adjusted for all other factors in the model: age, hypertension, dyslipidemia and timing of RRSO.
[b.] Risk of having a MESA percentile above 75%, RRs were multivariably adjusted for all other factors in the model: hypertension, dyslipidemia and timing of RRSO.

Table S6. RRs of increased CAC scores according to timing of RRSO in women without a history of breast cancer.

	Adjusted RR (95% CI)			
	CAC >0[a]	**CAC >100[a]**	**CAC >400[a]**	**MESA> 75%[b]**
Timing of RRSO				
Postmenopausal RRSO (RRSO ≥54y)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Premenopausal RRSO (RRSO ≤45y)	1.03 (0.73-1.47)	0.98 (0.41-2.36)	0.89 (0.14-5.50)	1.35 (0.81-2.27)
Hypertension	1.80 (1.26-2.58)	2.00 (0.86-4.68)	1.11 (0.29-4.20)	1.56 (0.87-2.80)
Dyslipidemia	1.10 (0.84-1.44)	2.09 (1.01-4.31)	2.34 (0.61-8.94)	1.92 (1.14-3.23)

[a.] Risk of having any/moderate/severe coronary artery calcification (CAC), RRs were multivariably adjusted for all other factors in the model: age, hypertension, dyslipidemia and timing of RRSO.
[b.] Risk of having a MESA percentile above 75%, RRs were multivariably adjusted for all other factors in the model: hypertension, dyslipidemia and timing of RRSO.

Table S7. RRs of increased CAC scores according to timing of premenopausal RRSO in women without a history of breast cancer.

	Adjusted RR (95% CI)			
	CAC >0[a]	**CAC >100[a]**	**CAC >400[a]**	**MESA> 75%[b]**
Timing of RRSO				
Late premenopausal RRSO (41-45y)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Early premenopausal RRSO (<41y)	0.67 (0.48-0.94)	0.58 (0.24-1.41)	0.47 (0.11-2.09)	0.81 (0.52-1.26)
Hypertension	1.65 (1.20-2.56)	2.29 (0.95-5.51)	1.40 (0.41-4.78)	1.67 (1.06-2.64)
Dyslipidemia	1.21 (0.91-1.61)	1.65 (0.76-3.57)	5.47 (1.40-21.41)	1.61 (1.05-2.47)

[a.] Risk of having any/moderate/severe coronary artery calcification (CAC), RRs were multivariably adjusted for all other factors in the model: age, hypertension, dyslipidemia and timing of RRSO.

[b.] Risk of having a MESA percentile above 75%, RRs were multivariably adjusted for all other factors in the model: hypertension, dyslipidemia and timing of RRSO.

Table S8. Participant characteristics ROBINSCA cohort and women after a premenopausal RRSO in HARMOny eligible for ROBINSCA.

	ROBINSCA (55-70 years)	**Premenopausal RRSO and eligible for ROBINSCA**	**p-value**
N	5226	270	
Age median	62.0 (58.0-65.0)	59.1 (57.6-61.6)	<0.001
Current smoker	784 (15.0)	43 (15.9)	0.68
BMI median	25.4 (23.3-28.3)	27.2 (24.4-30.5)	<0.001
Family member with MI[a]	2362 (45.2)	115 (42.6)	0.41
DM	146 (2.8)	17 (6.3)	0.001
Antihypertensive medication	962 (18.4)	41.0 (15.2)	0.19
Lipid lowering medication	355 (6.8)	12 (4.4)	0.14
CAC score median	0 (0-38)	0 (0-31)	0.21
CAC>0	2545 (48.7)	122 (45.2)	0.27
CAC>100	747 (14.3)	33 (12.2)	0.35
CAC>400	193 (3.7)	7 (2.6)	0.37

Values are numbers with percentages (No. (%)) for categorical variables and medians with 25th-75th percentiles for variables with a skewed distribution. P-value was calculated using independent samples t-test and Wilcoxon rank-sum test.

BMI, body mass index; MI, myocardial infarction; DM, diabetes mellitus; CAC, Coronary Artery Calcium

[a] Myocardial infarction first or second degree family member before the age of 65.

Table S9. Risks of increased CAC-scores according to different MHT durations in women aged 60-70.

	Adjusted RR and 95% (CI) for different CAC-scores (n=328)				
	MHT ever yes vs no	**MHT <3 vs 3+ years**	**MHT <5 vs 5+ years**	**MHT <10 vs 10+ years**	**MHT through age 45 yes/no**
CAC>0[a]	1.07 (0.83-1.39)	1.08 (0.85-1.43)	1.08 (0.83-1.41)	1.02 (0.75-1.39)	1.09 (0.81-1.48)
CAC>100[a]	1.13 (0.65-1.96)	1.06 (0.59-1.92)	1.11 (0.61-1.99)	1.07 (0.55-2.08)	1.02 (0.51-2.04)
CAC>400[a]	1.15 (0.39-3.34)	0.97 (0.29-3.22)	0.99 (0.30-3.31)	0.88 (0.21-3.69)	0.45 (0.06-3.27)
MESA>75%[b]	1.19 (0.79-1.79)	1.13 (0.73-1.75)	1.18 (0.77-1.82)	0.98 (0.58-1.68)	1.20 (0.75-1.93)

[a.] Risk of having any/moderate/severe coronary artery calcification (CAC), RRs were multivariably adjusted for all other factors in the model: age, hypertension, dyslipidemia and timing of RRSO.

[b.] Risk of having a MESA percentile above 75%, RRs were multivariably adjusted for all other factors in the model: hypertension, dyslipidemia and timing of RRSO.

Table S10. Risks of increased CAC-scores according to different MHT durations in women with a premenopausal RRSO.

	Adjusted RR and 95% (CI) for different CAC-scores according to MHT use yes/no [a] (n=498)				
	MHT ever yes vs no	**MHT <3 vs 3+ years**	**MHT <5 vs 5+ years**	**MHT <10 vs 10+ years**	**MHT through age 45 yes/no**
CAC>0[a]	1.05 (0.84-1.30)	1.05 (0.83-1.31)	1.00 (0.79-1.26)	0.99 (0.76-1.27)	1.12 (0.88-1.44)
CAC>100[a]	1.02 (0.62-1.70)	0.94 (0.56-1.60)	1.03 (0.61-1.75)	1.12 (0.37-3.34)	1.28 (0.75-2.20)
CAC>400[a]	1.41 (0.57-3.51)	1.27 (0.50-3.25)	1.39 (0.54-3.58)	1.04 (0.37-3.34)	1.38 (0.47-4.09)
MESA>75%[b]	1.06 (0.78-1.44)	1.05 (0.77-1.44)	1.05 (0.76-1.45)	1.03 (0.72-1.47)	1.15 (0.81-1.64)

[a.] Risk of having any/moderate/severe coronary artery calcification (CAC), RRs were multivariably adjusted for all other factors in the model: age, hypertension, dyslipidemia and timing of RRSO.

[b.] Risk of having a MESA percentile above 75%, RRs were multivariably adjusted for all other factors in the model: hypertension, dyslipidemia and timing of RRSO.

Chapter 3

No increased arterial stiffness after premenopausal risk-reducing salpingo-oophorectomy

Maturitas, 2025

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Abstract

Objective

Women at high familial risk of ovarian cancer are recommended to undergo premenopausal risk-reducing salpingo-oophorectomy (RRSO), leading to immediate surgical menopause. While early natural menopause is associated with increased cardiovascular disease (CVD) risk, evidence on CVD risk after surgical menopause is inconsistent.

Main outcome measures

To investigate long-term CVD risk after surgical menopause we conducted a cross-sectional study in women who underwent a premenopausal RRSO (≤45yrs) compared with a postmenopausal RRSO (≥54yrs). We assessed arterial stiffness, an established marker of CVD risk, measured by pulse wave velocity (PWV). Age differences between the pre- and postmenopausal RRSO groups were accounted for by restricting analyses to women aged 60-70 at study visit (n=307). Within the premenopausal RRSO group (n=461), we also examined the effect of timing of premenopausal RRSO on PWV (RRSO<41 vs RRSO 41-45 years). In addition, we assessed the association between PWV and coronary artery calcium (CAC) in women with a premenopausal RRSO.

Results

In women aged 60-70 at study visit, PWV levels were significantly lower in the premenopausal RRSO group compared with the postmenopausal RRSO group (β: -0.87, 95% CI, -1.45, -0.28 for PWV level; RR: 0.47, 95% CI, 0.24, 0.93 for being in the upper PWV quintile). Timing of premenopausal RRSO did not influence PWV. Among all women who underwent a premenopausal RRSO, having a PWV in the upper quintile was an independent predictor of the presence of CAC (RR 1.32, 95% CI, 1.04-1.68 for CAC>0).

Conclusion

Our study does not support a long-term adverse effect of premenopausal RRSO on arterial stiffness. Increased arterial stiffness is associated with presence of CAC in women after a premenopausal RRSO.

Introduction

Women at high familial risk of ovarian cancer, such as *BRCA1/2* germline pathogenic variant (GPV) carriers, are advised to undergo risk-reducing salpingo-oophorectomy (RRSO) at an early age before the risk of ovarian cancer increases. [1] Therefore, RRSO is recommended at ages 35 to 40 years for *BRCA1* GPV carriers and at ages 40 to 45 years for *BRCA2* GPV carriers, leading to surgical menopause at considerably earlier ages than in the general population. [2-3] Although early *natural* menopause has been associated with increased cardiovascular disease (CVD) risk, the long-term effect of *surgical* menopause on CVD risk is unclear. [4-6]

Increased arterial stiffness is a sign of vascular ageing and an independent predictor for CVD in the general population. [7-8] Arterial stiffness is primarily influenced by ageing and blood pressure, leading to functional changes in the vascular architecture throughout the arterial wall, especially in the tunica media. [9] The gold standard for arterial stiffness, brachial-ankle pulse wave velocity (PWV), is a relatively simple and noninvasive test, providing additional information next to conventional CVD risk assessment in clinical practice. [10-12] In addition, several studies have shown that PWV increases more rapidly during the menopausal transition and that increased PWV is associated with major adverse cardiovascular events in postmenopausal women. [13-16] However, it remains largely unknown whether early menopause leads to a long-term increase of arterial stiffness (and subsequently increased CVD risk), or whether it has a short-term influence that is nullified by the general effect of ageing on arterial stiffness. [17-19]

In clinical practice the use of PWV in assessment for CVD risk has been surpassed by measurement of the coronary artery calcification (CAC) on computed tomography, as this technique is highly reproducible with a validated assessment of individual risk. [20-22] While CAC and arterial stiffness are both influenced by age and hypertension as important risk factors, CAC is also influenced by conventional CVD risk factors which are less involved in arterial stiffness, such as smoking, diabetes and dyslipidemia. [23] In addition, the pathophysiology of CAC, atherosclerotic calcification because of plaque forming in the coronary arteries, differs from arterial stiffness. [24] Despite differences in etiology and pathophysiology, studies suggest that CAC and PWV measurements are correlated. [25-29]

We aimed to examine the long-term effect of a premenopausal RRSO compared to a postmenopausal RRSO on PWV measurements in a large cohort of women at high

familial risk of ovarian cancer. In addition, we investigated whether PWV measurements are associated with CAC scores in women who underwent premenopausal RRSO.

Methods

Study cohort

The study cohort consisted of participants of the HARMOny study, a Dutch multicenter cross-sectional study investigating long-term effects of RRSO on CVD, bone health, cognition and quality of life (ClinicalTrials.gov NCT03835793). [30] Women were invited from the Hereditary Breast and Ovarian cancer study Netherlands (HEBON), a nationwide cohort of women at high familial risk of breast and/or ovarian cancer recruited from all eight Dutch University Medical Centers and the Netherlands Cancer Institute. [31] Between 2018 and 2022, 1207 women were invited to join the study: 733 women who underwent a premenopausal RRSO (≤45yrs) and were ≥55 years old at inclusion and 474 women who underwent a postmenopausal RRSO (≥54 years) (see Figure 1). Exclusion criteria were a history of ovarian cancer, age >80 years, therapy-induced menopause >5 years before RRSO, metastatic disease or a prior cardiac interventions. A history of cancer, other than ovarian cancer was not a reason for exclusion. Women with unreliable PWV measurements were excluded.

Study assessments

The online patient questionnaire included traditional and female-specific risk factors, medical history and medication use. During the outpatient clinic visit ambiguities in the questionnaire were checked with the participant, or, if needed, with the general practitioner. Anthropometric measurements were performed including height, weight, heart rate, arterial blood pressure and aortic PWV to measure aortic stiffness. PWV was assessed by a oscillometric device (Arteriograph, TensioMed Kft), using an occlusive upper arm cuff to measure the time interval between the peak of the first systolic wave and the peak of the reflected systolic wave. [32] CAC scores of the heart were measured by computed tomography (CT,) using standardized local scan protocols for Agatston scoring at the various participating medical centers. Percentiles of the CAC score were calculated according to the Multi-Ethnic Study of Atherosclerosis (MESA) score. [33] A MESA percentile above 75% was considered high risk. Blood samples were taken to analyze non-fasting levels of lipids, glucose, HbA1c, high-sensitivity C-reactive protein (hs-CRP) and high-sensitivity cardiac troponin (hs-Troponin).

Statistical analyses

The premenopausal and postmenopausal RRSO groups were compared by using the independent samples t-test or Wilcoxon rank-sum test for continuous data and the Fisher exact test or $\chi 2$ test for categorical data; a two-sided $P < 0.05$ was considered significant. According to the study protocol we attempted to match the pre- and postmenopausal RRSO groups on age at study visit.[30] However, because of a substantial age difference between the premenopausal and postmenopausal RRSO groups it turned out this was not possible. This age difference was the result of a change in the 2007 guidelines for management of ovarian cancer risk in *BRCA1/2* GPV carriers, leading to a strongly increased uptake of premenopausal RRSO.[1] Therefore, in the current study, we restricted the comparison of PWV between the premenopausal and postmenopausal RRSO groups to women who were between 60-70 years old at study visit (see Figure 1). In addition, within the entire premenopausal RRSO group we evaluated PWV in women with an RRSO < 41 years (early premenopausal RRSO group) and women aged 41-45 years at RRSO (late premenopausal RRSO group). Finally, we investigated the association between PWV and CAC within the premenopausal RRSO group.

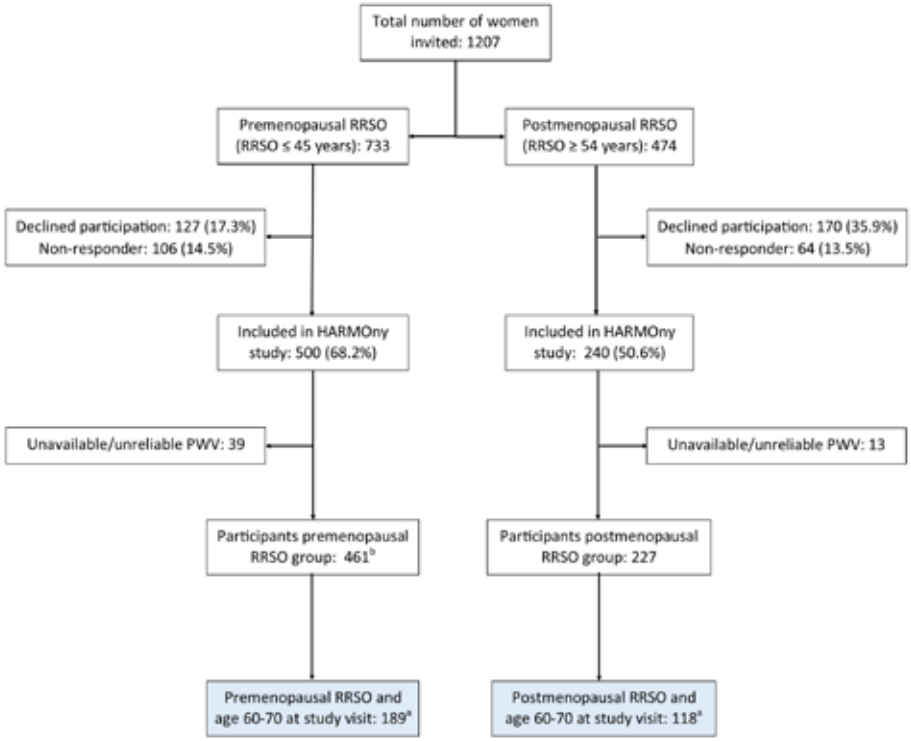

Figure 1. Flowchart participation HARMOny study.

[a.] Comparison PWV scores between premenopausal and postmenopausal RRSO group aged 60-70 at study visit. [b.] Entire premenopausal RRSO group used for assessment of the association between PWV and CAC.

We performed multivariable linear regression analyses to calculate regression coefficients for the increase of PWV associated with timing of RRSO. In addition, we used Poisson regression analysis to calculate relative risks (RRs) of having a high PWV (upper quintile of the study population). The cut-offs used for CAC related outcomes were presence of CAC (CAC>0) or having a MESA percentile above 75%. Variables assessed as possible confounders were: age at study visit, current or ever smoking, alcohol use, ever use of menopausal hormone therapy (MHT), history of breast cancer, history of internal mammary chain (IMC) irradiation, body mass index (BMI), diabetes mellitus (use of anti-diabetic medication), hypertension (either antihypertensive medication, a systolic blood pressure >140mmHg or a diastolic blood pressure >90mmHg) and dyslipidemia (either lipid-lowering medication or LDL cholesterol >4.0 mmol/L). A variable was considered a confounder if the coefficient estimate for the association of interest was affected by at least 10% when added to the model. All statistical analyses were performed using STATA version 15.1 (StataCorp LLC, College Station, TX).

Results

Participant characteristics of the entire HARMOny study population are provided in the supplements (Table S1).

Participant characteristics of women aged 60-70 at study visit

We included 307 women aged 60 to 70 at study visit (189 in the premenopausal RRSO group, 118 in the postmenopausal RRSO group, Table 1.). Median time since premenopausal RRSO was 21.1 years (IQR 18.3-23.3) and median time since menopause in the postmenopausal RRSO group was 15.5 years (IQR 12.7-18.2). The large majority carried a BRCA1/2 GPV (69.4% in the premenopausal RRSO group and 67% in the postmenopausal group). Women in the premenopausal RRSO group more often had a history of MHT use or internal mammary chain (IMC) radiotherapy, and less often had hypertension compared with the postmenopausal RRSO group (30.9% vs 6.4% for ever MHT use, 8.3% vs 2.6% for IMC radiotherapy, 51.9% vs 66.9% for hypertension). Other CVD risk factors were comparable between both groups.

Table 1. Patient characteristics of women aged 60-70 at study visit (n=307).

	Age 60-70 Premenopausal RRSO (RRSO ≤45)	Age 60-70 Postmenopausal RRSO (RRSO≥54)	p-value
	N=189	N=118	
Age at study visit, years	62.1 (60.7-64.3)	66.6 (65.0-68.5)	<0.001
Age at menopause, years	42.0 (40.0-44.0)	51.0 (50.0-54.0)	<0.001
BRCA GPV carrier status			<0.001
BRCA1	51.9%	29.7%	
BRCA2	17.5%	37.3%	
Non-carrier of BRCA1/2	30.7%	33.1%	
Ever MHT use	30.9%	6.4%	<0.001
Breast cancer	61.4%	58.5%	0.61
Radiotherapy	39.2%	37.3%	0.74
IMC radiotherapy	8.3%	2.6%	0.043
Chemotherapy	43.9%	36.4%	0.20
Endocrine therapy	20.1%	22.0%	0.69
Smoking			0.080
Active smoker	9.5%	5.1%	
Former smoker	52.4%	44.9%	
Never	38.1%	50.0%	
Alcohol use	58.2%	52.5%	0.33
Regular physical exercise	73.5%	78.8%	0.30
Family history of myocardial infarction <65yrs	33.0%	34.2%	0.83
BMI, kg/m²	25.1 (22.6-28.7)	25.4 (23.2-28.7)	0.66
Systolic blood pressure, mmHg	136.4 (17.4)	144.3 (15.3)	<0.001
Diastolic blood pressure, mmHg	77.5 (12.0)	81.1 (11.2)	0.009
Total cholesterol, mmol/L	5.6 (1.0)	5.6 (1.4)	0.83
LDL cholesterol, mmol/L	3.3 (0.9)	3.4 (1.2)	0.43
HDL cholesterol, mmol/L	1.8 (0.4)	1.7 (0.6)	0.40
Triglycerides, mmol/L	1.5 (1.1-2.1)	1.5 (1.1-1.9)	0.55
Glucose, mmol/L	6.2 (1.6)	6.2 (1.4)	0.98
Antihypertensive medication	28.0%	22.9%	0.32
Lipid lowering medication	18.0%	18.6%	0.89
Diabetes Mellitus	9.0%	8.5%	0.86
Hypertension*	51.9%	66.9%	0.009

Table 1. Continued

	Age 60-70 Premenopausal RRSO (RRSO ≤45)	Age 60-70 Postmenopausal RRSO (RRSO≥54)	p-value
Dyslipidemia**	40.2%	49.2%	0.12
Pulse Wave Velocity (PWV), m/s	10.2 (1.9)	11.1 (1.8)	<0.001
PWV > 80 percentile	14.8%	27.1%	0.009

Values are numbers with percentages (No. (%)) for categorical variables, means with standard deviation for normal distributed variables, and medians with interquartile range for variables with a skewed distribution. P-value was calculated using independent samples t-test, X2 test and Mann-Whitney U test.Abbreviations: GPV, pathogenic variant; MHT, menopausal hormone therapy; IMC, internal mammary chain; BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; PWV, pulse wave velocity.*Hypertension: either antihypertensive medication, a systolic blood pressure >140mmHg or a diastolic blood pressure >90mmHg.**Dyslipidemia: either lipid lowering medication or a LDL cholesterol >4.0.

Pulse wave velocity after premenopausal vs postmenopausal RRSO in women aged 60-70 years

In univariate analyses, women who underwent premenopausal RRSO had lower PWV levels compared with women who underwent postmenopausal RRSO (10.2 vs 11.1 m/s, respectively). Multivariable adjusted linear regression analyses showed that after adjustment for age, hypertension and ever MHT use, women in the premenopausal RRSO group had significantly lower PWV levels compared with the postmenopausal RRSO group (β: -0.87, 95% CI, -1.45, -0.28; Table 2). The RR of having a PWV in the upper quintile was significantly lower in the premenopausal RRSO group compared with the postmenopausal RRSO group (RR: 0.47, 95% CI, 0.24, 0.93). As expected, hypertension was a strong risk factor for higher PWV levels. Remarkably, in both analyses MHT use was an independent risk factor for higher PWV levels. Adding IMC radiotherapy to the model did not affect the outcomes (results not shown).

Table 2. Influence of premenopausal RRSO compared with postmenopausal RRSO on PWV (continuous and PWV in the upper quintile yes/no) in women aged 60-70 at study visit.

	β regression coefficient (95% CI)*	RR PWV upper quintile yes/no (95% CI)**
RRSO≤45 years vs RRSO≥54 years	-0.87 (-1.45, -0.28)	0.47 (0.24-0.93)
Hypertension	1.03 (0.62, 1.45)	2.64 (1.47-4.73)
Ever MHT use	0.61 (0.10, 1.13)	1.88 (1.09-3.22)

Abbreviations: CI, confidence interval; RRSO, risk-reducing salpingo-oophorectomy; PWV, pulse wave velocity; MHT, menopausal hormone therapy; RR, relative risk.*Results of linear regression analysis (adjusted for all factors in the model simultaneously: PWV, age, hypertension and MHT use).**Results of Poisson regression analysis (adjusted for all factors in the model simultaneously: PWV, age, hypertension and MHT use.

Pulse wave velocity according to timing of premenopausal RRSO

In multivariable regression analyses, timing of premenopausal RRSO (<41yrs vs 41-45yrs) did not significantly influence PWV levels (β: -0.16, 95% CI, -0.55, 0.23) or RR of having a PWV in the upper quintile (RR: 0.73, 95% CI, 0.45-1.19; Table 3). Again, hypertension and MHT use were associated with higher PWV levels.

Table 3. Influence of timing of premenopausal RRSO and other risk factors on PWV (continuous or PWV in the upper quintile (yes/no) in women with a premenopausal RRSO.

PWV	β regression coefficient (95% CI)*	RR PWV upper quintile yes/no (95% CI)**
RRSO<41 vs RRSO 41-45 years	-0.16 (-0.55, 0.23)	0.73 (0.45-1.19)
Hypertension	0.95 (0.59, 1.31)	1.87 (1.23-2.84)
Ever MHT use	0.44 (0.03, 0.84)	1.69 (1.08-2.26)

Abbreviations: CI, confidence interval; RRSO, risk-reducing salpingo-oophorectomy; PWV, pulse wave velocity; MHT, menopausal hormone therapy; RR, relative risk. *Results of linear regression analysis (adjusted for all factors in the model simultaneously: PWV, age, hypertension and MHT use).**Results of Poisson regression analysis (adjusted for all factors in the model simultaneously: PWV, age, hypertension and MHT use).

Pulse wave velocity and coronary artery calcium after a premenopausal RRSO

Among the 461 women in the entire premenopausal RRSO group, 460 had an available CAC score. Median age at study was 58.9 years. 43.5% of the women had presence of CAC (CAC>0), and 29.8% had a MESA score above 75% (Table S1). Among women with a premenopausal RRSO, presence of CAC was more prevalent in women with a PWV in higher quintiles (Figure 2). In multivariable regression analyses, we found that higher PWV levels were significantly associated with having any CAC; every 1.0 m/s increase in PWV led to a 7% increase in risk of presence of CAC (RR: 1.07, 95% CI, 1.02-1.13 for presence of CAC Table 4). Higher PWV levels were also associated with higher continuous CAC scores (β: 0.12 95% CI 0.01-0.23). We observed a non-significant association between higher PWV levels and a MESA score above 75% (RR: 1.07, 95% CI, 0.99-1.15). Women with a PWV level in the upper quintile had a 32% increased risk of presence of CAC compared with women with a PWV in the other quintiles (RR: 1.32, 95% CI, 1.04-1.68). However, we did not find an association between a PWV in the upper quintile and continuous CAC scores or a MESA score above 75%. Adding IMC radiotherapy or other conventional CVD risk factors to the model did not affect our risk estimates (Supplement Table S2-3).

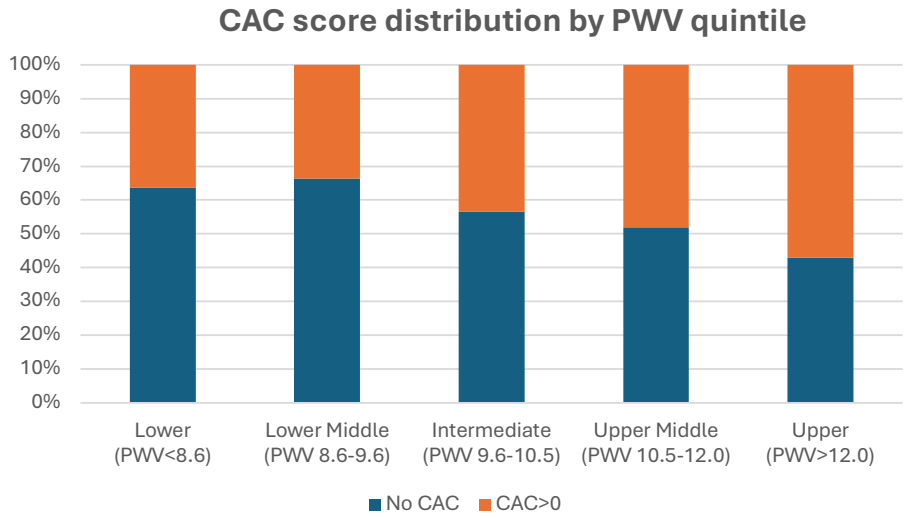

Figure 2. Distribution of CAC scores by PWV quintile in women with a premenopausal RRSO (N=460)
CAC: coronary artery calcification, PWV: pulse wave velocity.
PWV measured in m/s.

Table 4. Association between PWV level (continuous or PWV in upper quintile yes/no) and different measures of CAC in women with a premenopausal RRSO (N=460).

	Log (CAC+1)	CAC>0[b]	MESA score>75%[c]
n	**460**	**200**	**137**
	Adjusted β (95% CI)	**Adjusted RR (95% CI)**	
PWV continuous	0.12 (0.01-0.23)	1.07 (1.02-1.13)	1.07 (0.99-1.15)
PWV in upper quintile (yes/no)	0.37 (-0.16-0.91)	1.32 (1.04-1.68)	1.21 (0.84-1.72)

Abbreviations: CI, confidence interval; RR, relative risk; PWV, pulse wave velocity; CAC, coronary artery calcification, MESA, Multi-Ethnic Study of Atherosclerosis.

[a] Linear regression analyses adjusted for all factors in the model simultaneously (PWV, age, hypertension, dyslipidemia and ever menopausal hormone therapy).

[b] Poisson regression analyses adjusted for all factors in the model simultaneously (PWV, age, hypertension, dyslipidemia and ever menopausal hormone therapy).

[c] Poisson regression analyses adjusted for age for all factors in the model simultaneously (PWV, hypertension, dyslipidemia and ever menopausal hormone therapy). MESA percentile already corrects for age.

Discussion

In this cross-sectional study, we assessed arterial stiffness in women with a premenopausal RRSO compared with women who underwent a postmenopausal RRSO. After adjusting for age, hypertension and MHT use, we observed no increased PWV levels in women with a premenopausal RRSO compared with women who underwent postmenopausal RRSO. On the contrary, we found that women in the premenopausal RRSO group appeared to have lower PWV levels compared with the postmenopausal RRSO group. Furthermore, an early premenopausal RRSO before the age of 41 years, compared to an RRSO between ages 41 and 45 years was not associated with increased PWV levels.

PWV after surgical menopause

Although previous studies showed that women experience a short-term acceleration of the age-related increase of PWV after menopause, this effect might be attenuated by ageing when assessing long-term PWV after surgical menopause. [13-16-17] Our results are consistent with a recent study by Van Bommel et al, showing no increased PWV levels in women who underwent a premenopausal RRSO compared with a reference population. [34] That does not rule out the possibility that surgical menopause leads to increased CVD risk because of other pathways, such as atherosclerosis. However, previous analysis in our own cohort showed no increased CAC scores after a premenopausal RRSO, rendering this hypothesis less likely. [35] As a whole, studies investigating the long-term effects of surgical menopause on CVD are either inconclusive because of methodological limitations or show no difference (in predictors of) CVD risk. These results are in contrast with ample evidence that early *natural* menopause is associated with increased CVD risk. [36-37] The differences found in long-term effects of surgical vs early natural menopause on CVD risk could be explained by a reverse causality hypothesis, postulating that ovarian dysfunction causing early natural menopause is the result of the same process of accelerated vascular ageing found in CVD. [38] If this hypothesis were true, no increased risk of CVD would be expected after *surgical* menopause. The current study supports this hypothesis. For future studies it would be interesting to assess long-term effects of early natural menopause on arterial stiffness.

Remarkably, women who underwent postmenopausal RRSO had even higher PWV levels compared with women who underwent premenopausal RRSO. This surprising outcome might be explained by differences in lifestyle. Recent studies showed that *BRCA1/2* GPV carriers who underwent a premenopausal RRSO had a more favorable coronary heart disease risk profile, including less hypertension, abdominal

obesity, metabolic syndrome and more physical activity compared with reference populations, suggesting a more healthy lifestyle. [34-39] In our study, (history of) MHT use was associated with higher PWV levels, especially in the premenopausal RRSO group. If estrogen deficiency after early menopause would cause increased arterial stiffness, one would have expected lower PWV levels in (former) MHT users, instead of the higher PWV levels we found. Since data on duration and type of MHT use were largely missing, we were not able to investigate possible explanations in our data. Studies show that women with vasomotor symptoms, the most frequent reason for prescribing MHT, are at increased risk for (subclinical) CVD, therefore these findings are most likely caused by confounding by indication for MHT. [40-41] Omitting MHT as a confounder in our analyses, or sensitivity analyses in women without a history of MHT use did not change the outcomes (results not shown).

Associations between arterial stiffness and subclinical atherosclerosis

We found that higher PWV levels were associated with increased CAC scores in women who underwent a premenopausal RRSO. These results are consistent with current literature investigating this association in the general population. [27-29] Furthermore, adding other CVD risk factors to the model did not substantially influence the outcome, suggesting that PWV is an independent predictor for CAC (Supplement Table S2-3). Therefore, PWV measurement might be a viable non-invasive (cheap, quick and less radiation) option when screening asymptomatic women for CVD, including women with an early menopause.[11] Future studies should further investigate the health benefits and cost effectiveness of the use of arterial stiffness measurement in CVD risk assessment of postmenopausal women.

Strengths and Limitations

The strengths of our nationwide study include the long-term follow-up after premenopausal RRSO and the use of a comparison group of women who also underwent RRSO, but at a later age. By excluding women who underwent RRSO between the ages of 46 to 54 years we were able to make a more distinct evaluation of the differences in CVD risk between the premenopausal and postmenopausal RRSO groups. In contrast with other studies, the comparisons made in our study are not affected by confounding by indication for bilateral oophorectomy. Another strength of our study concerns the standardized PWV measurements, made during the same hospital visit as the CAC measurement. Other studies have a relatively long time (up to 1 year) between both measurements. The participation rate of our study was high (60.7%), considering the relatively long time since RRSO at study visit. A limitation of our study is the missing data about duration and type of MHT use, limiting our ability to investigate possible explanations for the observed association between

MHT use and increased PWV levels. Another limitation of our study is the difference in age between the premenopausal and postmenopausal RRSO groups in the entire study population. However, we could address this limitation by restricting our study population to women aged 60-70 years old at study enrolment. In addition, we could use the entire premenopausal RRSO group to assess the association between timing of a premenopausal RRSO (<41 years vs 41-45 years) and arterial stiffness. Another potential limitation of this study might be survival bias because CVD-related death after RRSO might have occurred before recruitment to the HARMOny study. As our study was nested in the HEBON cohort, we had the opportunity to obtain causes of death from Statistics Netherlands for all women who were otherwise eligible for our study but died before possible inclusion.[31] Only 1.9% of these women died because of a cardiovascular event. Unsurprisingly, the most frequent cause of death was cancer (87.6%).

Conclusion

Our study does not support a long-term adverse effect of surgical menopause on arterial stiffness. Arterial stiffness is associated with increased CAC scores independent of conventional risk factors and could be a useful tool in CVD risk assessment in women who underwent surgical menopause.

References

1. Daly MB, Pal T, Berry MP, Buys SS, Dickson P, Domchek SM, et al. Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2021;19(1):77-102.
2. Domchek SM, Friebel TM, Singer CF, Evans DG, Lynch HT, Isaacs C, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA. 2010;304(9):967-75.
3. 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(2):80-7.
4. Dam V, van der Schouw YT, Onland-Moret NC, Groenwold RHH, Peters SAE, Burgess S, et al. Association of menopausal characteristics and risk of coronary heart disease: a pan-European case-cohort analysis. Int J Epidemiol. 2019;48(4):1275-85.
5. Honigberg MC, Zekavat SM, Aragam K, Finneran P, Klarin D, Bhatt DL, et al. Association of Premature Natural and Surgical Menopause With Incident Cardiovascular Disease. JAMA. 2019;322(24):2411-21.
6. Parker WH, Broder MS, Chang E, Feskanich D, Farquhar 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(5):1027-37.
7. Cooper LL, Palmisano JN, Benjamin EJ, Larson MG, Vasan RS, Mitchell GF, Hamburg NM. Microvascular Function Contributes to the Relation Between Aortic Stiffness and Cardiovascular Events: The Framingham Heart Study. Circ Cardiovasc Imaging. 2016;9(12).
8. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol. 2010;55(13):1318-27.
9. O'Rourke MF, Nichols WW. Aortic diameter, aortic stiffness, and wave reflection increase with age and isolated systolic hypertension. Hypertension. 2005;45(4):652-8.
10. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur Heart J. 2016;37(29):2315-81.
11. Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, et al. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. J Am Coll Cardiol. 2014;63(7):636-46.
12. Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, et al. Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness: A Scientific Statement From the American Heart Association. Hypertension. 2015;66(3):698-722.
13. Samargandy S, Matthews KA, Brooks MM, Barinas-Mitchell E, Magnani JW, Janssen I, et al. Arterial Stiffness Accelerates Within 1 Year of the Final Menstrual Period: The SWAN Heart Study. Arterioscler Thromb Vasc Biol. 2020;40(4):1001-8.
14. Khan ZA, Janssen I, Mazzarelli JK, Powell LH, Dumasius A, Everson-Rose SA, et al. Serial Studies in Subclinical Atherosclerosis During Menopausal Transition (from the Study of Women's Health Across the Nation). Am J Cardiol. 2018;122(7):1161-8.

15. Kim M, Kim HL, Cho Y, Lim WH, Seo JB, Kim SH, et al. Prognostic value of arterial stiffness in menopausal women. Menopause. 2022;29(5):573-9.

16. Zaydun G, Tomiyama H, Hashimoto H, Arai T, Koji Y, Yambe M, et al. Menopause is an independent factor augmenting the age-related increase in arterial stiffness in the early postmenopausal phase. Atherosclerosis. 2006;184(1):137-42.

17. Laakkonen EK, Karppinen JE, Lehti S, Lee E, Pesonen E, Juppi HK, et al. Associations of Sex Hormones and Hormonal Status With Arterial Stiffness in a Female Sample From Reproductive Years to Menopause. Front Endocrinol (Lausanne). 2021;12:765916.

18. Gunning MN, Meun C, van Rijn BB, Daan NMP, Roeters van Lennep JE, Appelman Y, et al. The cardiovascular risk profile of middle age women previously diagnosed with premature ovarian insufficiency: A case-control study. PLoS One. 2020;15(3):e0229576.

19. Kilanowski-Doroh IM, McNally AB, Wong T, Visniauskas B, Blessinger SA, Imulinde Sugi A, et al. Ovariectomy-Induced Arterial Stiffening Differs from Vascular Aging and is Reversed by GPER Activation. bioRxiv. 2023.

20. Hoffmann U, Massaro JM, Fox CS, Manders E, O'Donnell CJ. Defining normal distributions of coronary artery calcium in women and men (from the Framingham Heart Study). Am J Cardiol. 2008;102(9):1136-41, 41 e1.

21. McClelland RL, Jorgensen NW, Budoff M, Blaha MJ, Post WS, Kronmal RA, et al. 10-Year Coronary Heart Disease Risk Prediction Using Coronary Artery Calcium and Traditional Risk Factors: Derivation in the MESA (Multi-Ethnic Study of Atherosclerosis) With Validation in the HNR (Heinz Nixdorf Recall) Study and the DHS (Dallas Heart Study). J Am Coll Cardiol. 2015;66(15):1643-53.

22. Schmermund A, Mohlenkamp S, Stang A, Gronemeyer D, Seibel R, Hirche H, et al. Assessment of clinically silent atherosclerotic disease and established and novel risk factors for predicting myocardial infarction and cardiac death in healthy middle-aged subjects: rationale and design of the Heinz Nixdorf RECALL Study. Risk Factors, Evaluation of Coronary Calcium and Lifestyle. Am Heart J. 2002;144(2):212-8.

23. Kronmal RA, McClelland RL, Detrano R, Shea S, Lima JA, Cushman M, et al. Risk factors for the progression of coronary artery calcification in asymptomatic subjects: results from the Multi-Ethnic Study of Atherosclerosis (MESA). Circulation. 2007;115(21):2722-30.

24. Wexler L, Brundage B, Crouse J, Detrano R, Fuster V, Maddahi J, et al. Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical implications. A statement for health professionals from the American Heart Association. Writing Group. Circulation. 1996;94(5):1175-92.

25. Muhammad IF, Engvall JE, Persson M, Borne Y, Nilsson PM, Ostgren CJ, Engstrom G. Association of arterial stiffness with coronary artery calcium score in the general-population: the Swedish CArdioPulmonary bioImage study. J Hypertens. 2022;40(5):933-9.

26. Ejiri K, Ding N, Kim E, Honda Y, Cainzos-Achirica M, Tanaka H, et al. Association of Segment-Specific Pulse Wave Velocity With Vascular Calcification: The ARIC (Atherosclerosis Risk in Communities) Study. J Am Heart Assoc. 2024;13(2):e031778.

27. van Popele NM, Mattace-Raso FU, Vliegenthart R, Grobbee DE, Asmar R, van der Kuip DA, et al. Aortic stiffness is associated with atherosclerosis of the coronary arteries in older adults: the Rotterdam Study. J Hypertens. 2006;24(12):2371-6.

28. Coutinho T, Turner ST, Kullo IJ. Aortic pulse wave velocity is associated with measures of subclinical target organ damage. JACC Cardiovasc Imaging. 2011;4(7):754-61.

29. Kullo IJ, Bielak LF, Turner ST, Sheedy PF, 2nd, Peyser PA. Aortic pulse wave velocity is associated with the presence and quantity of coronary artery calcium: a community-based study. Hypertension. 2006;47(2):174-9.

30. Terra L, Hooning MJ, Heemskerk-Gerritsen BAM, van Beurden M, Roeters van Lennep JE, van Doorn HC, et al. Long-Term Morbidity and Health After Early Menopause Due to Oophorectomy in Women at Increased Risk of Ovarian Cancer: Protocol for a Nationwide Cross-Sectional Study With Prospective Follow-Up (HARMOny Study). JMIR Res Protoc. 2021;10(1):e24414.

31. Brohet RM, Velthuizen ME, Hogervorst FB, Meijers-Heijboer HE, Seynaeve C, Collee MJ, et al. Breast and ovarian cancer risks in a large series of clinically ascertained families with a high proportion of BRCA1 and BRCA2 Dutch founder mutations. J Med Genet. 2014;51(2):98-107.

32. Horvath IG, Nemeth A, Lenkey Z, Alessandri N, Tufano F, Kis P, et al. Invasive validation of a new oscillometric device (Arteriograph) for measuring augmentation index, central blood pressure and aortic pulse wave velocity. J Hypertens. 2010;28(10):2068-75.

33. Joshi PH, Patel B, Blaha MJ, Berry JD, Blankstein R, Budoff MJ, et al. Coronary artery Calcium predicts Cardiovascular events in participants with a low lifetime risk of Cardiovascular disease: The Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis. 2016;246:367-73.

34. van Bommel MHD, de Jong MA, Steenbeek MP, Bots ML, van Westerop LLM, Hopman MTE, et al. No signs of subclinical atherosclerosis after risk-reducing salpingo-oophorectomy in BRCA1/2 mutation carriers. J Cardiol. 2021;77(6):570-5.

35. Beekman MJ, Terra L, Heemskerk-Gerritsen BAM, van der Aalst CM, Roeters van Lennep JE, van Beurden M, et al. Coronary Artery Calcium Scores after Prophylactic Premenopausal Bilateral Salpingo-Oophorectomy [In press]. JACC CardioOncol. 2024.

36. Muka T, Oliver-Williams C, Kunutsor S, Laven JS, Fauser BC, Chowdhury R, et al. Association of Age at Onset of Menopause and Time Since Onset of Menopause With Cardiovascular Outcomes, Intermediate Vascular Traits, and All-Cause Mortality: A Systematic Review and Meta-analysis. JAMA Cardiol. 2016;1(7):767-76.

37. Roeters van Lennep JE, Heida KY, Bots ML, Hoek A, collaborators of the Dutch Multidisciplinary Guideline Development Group on Cardiovascular Risk Management after Reproductive D. Cardiovascular disease risk in women with premature ovarian insufficiency: A systematic review and meta-analysis. Eur J Prev Cardiol. 2016;23(2):178-86.

38. Kok HS, van Asselt KM, van der Schouw YT, van der Tweel I, Peeters PH, Wilson PW, et al. Heart disease risk determines menopausal age rather than the reverse. J Am Coll Cardiol. 2006;47(10):1976-83.

39. Michelsen TM, Tonstad S, Pripp AH, Trope CG, Dorum A. Coronary heart disease risk profile in women who underwent salpingo-oophorectomy to prevent hereditary breast ovarian cancer. Int J Gynecol Cancer. 2010;20(2):233-9.

40. Thurston RC, Aslanidou Vlachos HE, Derby CA, Jackson EA, Brooks MM, Matthews KA, et al. Menopausal Vasomotor Symptoms and Risk of Incident Cardiovascular Disease Events in SWAN. J Am Heart Assoc. 2021;10(3):e017416.

41. Thurston RC, El Khoudary SR, Tepper PG, Jackson EA, Joffe H, Chen HY, et al. Trajectories of Vasomotor Symptoms and Carotid Intima Media Thickness in the Study of Women's Health Across the Nation. Stroke. 2016;47(1):12-7.

Declarations

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Conflict of interest disclosure

The authors report no conflicts of interest.

Data availability statement

With publication, de-identified data collected for the study, including participant data, will be made available to others upon reasonable request. Data can be requested with a proposal by sending an e-mail to the corresponding author.

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Ethics approval statement

This study was conducted according to the standards of Good Clinical Practice, in agreement with the principles of the Declaration of Helsinki and with the Dutch law as stated in the Medical Research Involving Human Subjects Act (WMO). The study has been approved in writing by the Institutional Review Board of the AVL/NKI to be conducted in all nine University Medical Centers and the Antoni van Leeuwenhoek.

Patient consent statement

All participants included in this study signed informed consent.

Clinical trial registration

The pre-registered clinical trial number is <NCT03835793>

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Supplements

Table S1. Participant characteristics of the premenopausal RRSO group in the entire study.

	Premenopausal RRSO (RRSO ≤45)
	N=460
Age at study visit, years	58.9 (57.0-61.7)
Age at menopause, years	42.0 (40.0-44.0)
BRCA PV carrier status	
BRCA1 germline mutation	48.2%
BRCA2 germline mutation	20.0%
Non-carrier of BRCA1/2	31.9%
MHT use	31.7%
Breast cancer	59.0%
Radiotherapy	37.3%
Parasternal radiotherapy	8.6%
Chemotherapy	45.1%
Endocrine therapy	22.6%
Smoking	
Active smoker	9.8%
Former smoker	40.6%
Never	49.7%
Alcohol use	47.5%
Regular physical exercise	73.8%
Family history of myocardial infarction <65yrs	31.1%
BMI, kg/m²	25.1 (22.7-28.9)
Systolic blood pressure, mmHg	134.1 (17.4)
Diastolic blood pressure, mmHg	77.3 (12.1)
Total cholesterol, mmol/L	5.6 (1.0)
LDL cholesterol, mmol/L	3.3 (0.9)
HDL cholesterol, mmol/L	1.7 (0.5)
Triglycerides, mmol/L	1.5 (1.1-2.1)
Glucose, mmol/L	6.0 (1.5)
Antihypertensive medication	19.7%

Table S1. Continued

	Premenopausal RRSO (RRSO ≤45)
	N=460
Lipid lowering medication	12.1%
Diabetes	6.1%
Hypertension*	44.5%
Dyslipidemia**	36.4%
Pulse Wave Velocity	10.2 (2.0)
CAC>0	43.5%
MESA score >75	29.8%

Values are numbers with percentages (No. (%)) for categorical variables, means with standard deviation for normal distributed variables, and medians with interquartile range for variables with a skewed distribution. P-value was calculated using independent samples t-test, X2 test and Mann-Whitney U test. Abbreviations: GPV, pathogenic variant; MHT, menopausal hormone therapy; IMC, internal mammary chain; BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; MESA, Multi-Ethnic Study of Atherosclerosis; CAC, Coronary Artery Calcium.*Hypertension: either antihypertensive medication, a systolic blood pressure >140mmHg or a diastolic blood pressure >90mmHg.**Dyslipidemia: either lipid lowering medication or a LDL cholesterol >4.0.

Table S2. Association between having a PWV in the upper quintile (yes/no) and different measures of CAC in women with a premenopausal RRSO.

	Log (CAC+1)	**CAC>0[b]**	**MESA score>75%[c]**
n	**460**	**200**	**137**
PWV in upper quintile (yes/no)	**Adjusted β (95% CI)**	**Adjusted RR (95% CI)**	
Age-adjusted model	0.15 (0.05-0.25)	1.63 (1.25-2.13)	1.79 (1.14-2.80)
Model 1	0.39 (-0.16-0.89)	1.29 (1.02-1.63)	1.21 (0.84-1.72)
Model 2	0.32 (-0.20-0.83)	1.25 (1.00-1.57)	1.18 (0.84-1.67)
Model 3	0.35 (-0.17-0.88)	1.25 (1.00-1.58)	1.20 (0.84-1.70)

Abbreviations: CI, confidence interval; RR, relative risk; PWV, pulse wave velocity; CAC, coronary artery calcification, MESA, Multi-Ethnic Study of Atherosclerosis.

Model 1. Adjusted for PWV in upper quintile, age, hypertension, dyslipidemia and history of IMC radiotherapy.

Model 2. Adjusted for PWV in upper quintile, age, hypertension, dyslipidemia, current smoking, BMI>30 and diabetes.

Model 3. Adjusted for PWV in upper quintile, age, hypertension, dyslipidemia, MHT use, history of IMC radiotherapy, current smoking, BMI>30 and diabetes.

Table S3. Association between PWV level (continuous) and different measures of CAC in women with a premenopausal RRSO.

	Log (CAC+1)	CAC>0[b]	MESA score>75%[c]
n	**460**	**200**	**137**
PWV continuous	**Adjusted β (95% CI)**	**Adjusted RR (95% CI)**	
Age-adjusted model	0.15 (0.05-0.25)	1.09 (1.04-1.14)	1.06 (0.99-1.14)
Model 1	0.10 (0.00-0.21)	1.07 (1.01-1.13)	1.06 (0.98-1.14)
Model 2	0.10 (0.00-0.21))	1.06 (1.01-1.12)	1.06 (0.98-1.14
Model 3	0.10 (0.00-0.21)	1.06 (1.01-1.12)	1.06 (0.98-1.14)

Abbreviations: CI, confidence interval; RR, relative risk; PWV, pulse wave velocity; CAC, coronary artery calcification, MESA, Multi-Ethnic Study of Atherosclerosis.
Model 1. Adjusted for PWV level, age, hypertension, dyslipidemia and history of IMC radiotherapy.
Model 2. Adjusted for PWV level, age, hypertension, dyslipidemia, current smoking, BMI>30 and diabetes.
Model 3. Adjusted for PWV level, age, hypertension, dyslipidemia, MHT use, history of IMC radiotherapy, current smoking, BMI>30 and diabetes.

Chapter 4

Long-term Effects of Premenopausal Risk-Reducing Salpingo-Oophorectomy on Bone Mineral Density

Submitted

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Abstract

Purpose

To prevent ovarian cancer, *BRCA1/2* germline pathogenic variant carriers are recommended to undergo premenopausal risk-reducing salpingo-oophorectomy (RRSO). Premenopausal RRSO leads to immediate menopause, which has been associated with an acute phase of rapid bone loss. However, data on long-term bone mineral density (BMD) is scarce and inconclusive. We aimed to investigate long-term BMD after premenopausal RRSO.

Methods

We conducted a cross-sectional study nested in a nationwide cohort of women at high familial risk of ovarian cancer. We compared 493 women who underwent premenopausal RRSO (≤45 years) with 228 women who underwent postmenopausal RRSO (≥54 years). BMD was assessed by Dual-Energy X-ray absorptiometry of the lumbar spine (LS) and femoral neck (FN). Age differences between the pre- and postmenopausal RRSO groups were accounted for using Z-scores.

Results

Median age at study visit was 59.2 years in the premenopausal RRSO group and 69.7 years in the postmenopausal RRSO group (P<0.001), median time since premenopausal RRSO was 18.1 years (IQR 15.3-21.3). In multivariable regression analyses the BMD Z-scores of the LS and FN were significantly lower for the premenopausal compared with the postmenopausal RRSO group (β -0.88, 95% CI, -1.10,-0.66 for LS; β -0.51, 95% CI, -0.71,-0.31 for FN). Relative risks (RRs) of having a Z-score ≤-1.0 were also higher in the premenopausal compared with the postmenopausal RRSO group (RR 2.35, 95% CI, 1.26-4.40 and RR 1.84, 95% CI, 1.08-3.13, respectively).

Conclusion

Premenopausal RRSO appears to be associated with long-term reduction of BMD Z-scores, emphasizing the importance of counseling women about bone health after premenopausal RRSO.

Introduction

To prevent ovarian cancer, current guidelines for *BRCA1/2* germline pathogenic variant (GPV) carriers recommend risk-reducing salpingo-oophorectomy (RRSO) before natural menopause.[1] Women are advised to undergo RRSO after completion of child bearing, preferably at ages 35 to 40 years for *BRCA1* GPV carriers and at ages 40 to 45 years for *BRCA2* GPV carriers. Although leading to a 96% reduction for ovarian cancer risk, RRSO also induces immediate surgical menopause at a considerably earlier age than in the general population.[2-3] Early *natural* menopause has been associated with various adverse long-term effects including decreased bone mineral density (BMD) and subsequently increased fracture risk.[4-5] However, studies investigating the effect of *surgical* menopause on BMD in *BRCA1/2* GPV carriers, are scarce and showed inconsistent results.

Estrogen deficiency is the main cause of bone loss after menopause, which may lead to osteopenia or osteoporosis and an increased risk of fractures.[6] Studies suggest a perimenopausal peak of bone loss of especially the lumbar spine (LS), with a more gradual decline of BMD after 4-5 years.[7] Two recent longitudinal studies showed a significantly lower BMD in *BRCA1/2* GPV carriers who underwent premenopausal RRSO compared with carriers who underwent postmenopausal RRSO or no RRSO at all. However, these studies had a median follow-up after RRSO of 3 years or less, so it remains unclear whether these effects persist over time.[8-9] Cross-sectional studies investigating the effects of premenopausal RRSO on BMD in *BRCA1/2* GPV carriers (median follow-up 4-6 years) showed inconsistent results.[10-12] In addition, studies not restricted to *BRCA1/2* GPV carriers suggest that the long-term impact of early menopause on BMD might be attenuated at older age.[13-14] The long-term effects of premenopausal RRSO on bone health are important because the risk of especially osteoporotic hip fractures and subsequent morbidity and mortality increases sharply with age.[15]

Because of the high morbidity and mortality after osteoporotic fractures, especially in the elderly, more insight into the *long-term* effects of surgical menopause on BMD and fracture risk is required. This study aimed to investigate the long-term effects of premenopausal RRSO in a large Dutch cohort of women at high familial risk for breast and ovarian cancer.

Methods

Study cohort

This study is part of the HARMOny study (ClinicalTrials.gov NCT03835793): a Dutch multicenter cross-sectional study investigating long-term effects of RRSO on cardiovascular disease, bone health, cognition and quality of life. The study design has been described in detail previously.[16] Women were selected from the Hereditary Breast and Ovarian cancer study Netherlands (HEBON study): a nationwide cohort of women at high familial risk of breast and/or ovarian cancer recruited from all eight Dutch University Medical Centers and the Netherlands Cancer Institute (NKI).[17] Between 2018 and 2022 a total of 1207 women were invited to join the study: 733 women who underwent a premenopausal RRSO (≤45 years) and were older than 55 years at inclusion (premenopausal RRSO group) and 474 women who underwent a postmenopausal RRSO (≥54 years) and were older than 55 years at inclusion (postmenopausal RRSO group). Exclusion criteria were a history of ovarian cancer, age over 80 years old, therapy-induced menopause >5 years before RRSO, metastatic disease or inability to (accurately) assess BMD. A medical history of cancer, other than ovarian cancer, was not a reason for exclusion.

Study assessments

Participation in the HARMOny study consisted of a clinical visit and an extensive online questionnaire. Among the bone health related topics included in the online questionnaire were current alcohol use, smoking, physical activity, medical history (including cancer treatment history) and medication and/or hormone use. During the outpatient clinic visit the completed questionnaire was discussed with the participants and ambiguities were checked with the participant, in the medical file or with the general practitioner. The results of all measurements were discussed by phone with the individual participants and a letter with the results was sent to their general practitioners. The clinical visit included measurement of the BMD and vertebral fracture assessment (VFA), blood sampling and an outpatient clinic visit with the research physician for anthropometric measurements including height and weight to calculate body mass index (BMI). 10-year risk of major osteoporotic fracture and hip fracture based on risk factors (without BMD) were calculated using FRAX calibrated for the Dutch population.[18] A Dual-Energy X-ray absorptiometry (DXA) scan of the lumbar spine (LS) and femoral neck (FN) was used to calculate absolute BMD, T-scores and Z-scores of the L1-L4 vertebral bodies and femoral neck. BMD measurements from different DXA-scanners were converted using standard reference methods.[19] Presence of a low Z-score was defined as having a Z-score ≤-1.0. Presence of osteopenia was defined as having a T-score between -1.0 to -2.5 and presence of osteoporosis was

defined as having a T-score ≤-2.5, in accordance with the World Health Organization guidelines using the NHANES database. [20] Presence of decreased BMD was defined as having either osteopenia or osteoporosis (T-score ≤-1.0). A vertebral fracture was defined as having a vertebral fracture of grade 2 or higher. [21]

During the visit non-fasting blood samples were taken, that were analyzed for calcium, albumin, phosphate, creatinine and 25-hydroxyvitamin D (25OHD) levels. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. [22] Chronic kidney disease (CKD) was defined by estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m^2. [23] Serum calcium levels were corrected for albumin using the formula: serum calcium + 0.02(41 - serum albumin). Bone turnover markers (BTM) may predict future fracture risk in postmenopausal women independent of BMD. [24] Studies show that serum beta-C-terminal collagen crosslink (β-CTX) as a marker for bone resorption and serum N-terminal procollagen type 1 (P1NP) as a marker for bone formation are the most sensitive BTM after surgical menopause, therefore, we used these reference markers to measure bone turnover. [25-26]

Statistical analyses

The premenopausal and postmenopausal RRSO groups were compared by using the independent samples t-test or Wilcoxon rank-sum test for continuous data and the Fisher exact test or $\chi 2$ test for categorical data, a two-sided P <0.05 was considered significant. According to the study protocol we attempted to match the pre- and postmenopausal RRSO groups on age at study visit. [16] During the inclusion period it turned out this was not possible because of a substantial age difference between the premenopausal and postmenopausal RRSO groups. This age difference was caused by a change in the 2007 guidelines for management of ovarian cancer risk in *BRCA1/2* GPV carriers, leading to a strongly increased uptake of premenopausal RRSO. [1] Therefore, when evaluating the BMD of the entire study population, we only assessed the Z-scores of the LS and FN, because Z-scores are already adjusted for age. Using multivariable linear regression analysis we calculated regression coefficients of Z-scores, furthermore, we used Poisson regression analysis to calculate relative risks (RRs) of having a low Z-score (Z-score≤-1.0). To assess other outcomes not adjusted for age, including prevalence of osteopenia/osteoporosis (T-score ≤-1.0) and self-reported fractures in the last year, we performed subgroup analyses on women aged 60-70 years at study visit (the age range that overlapped between the pre- and postmenopausal RRSO groups). In addition, we performed analyses within the entire premenopausal RRSO group to evaluate the prevalence of osteopenia/osteoporosis (T-score ≤-1.0) and Z-scores in women with an RRSO before

age 41 (early premenopausal RRSO group) and women aged 41-45 years at RRSO (late premenopausal RRSO group). Variables assessed as potential confounders were: age at study, BMI, current smoking, current alcohol use, ever menopausal hormone therapy (MHT) use, history of breast cancer, history of chemotherapy, ever use of corticosteroids (both systemic and inhaled) >3 months, current antiresorptive medication (AR) use, current vitamin D and/or calcium supplements use, parent with hip fracture, self-reported rheumatoid arthritis, vitamin D deficiency and CKD. A variable was considered a confounder if the regression coefficient estimate for the association of interest was changed by more than 10% when adding the variable to the model.

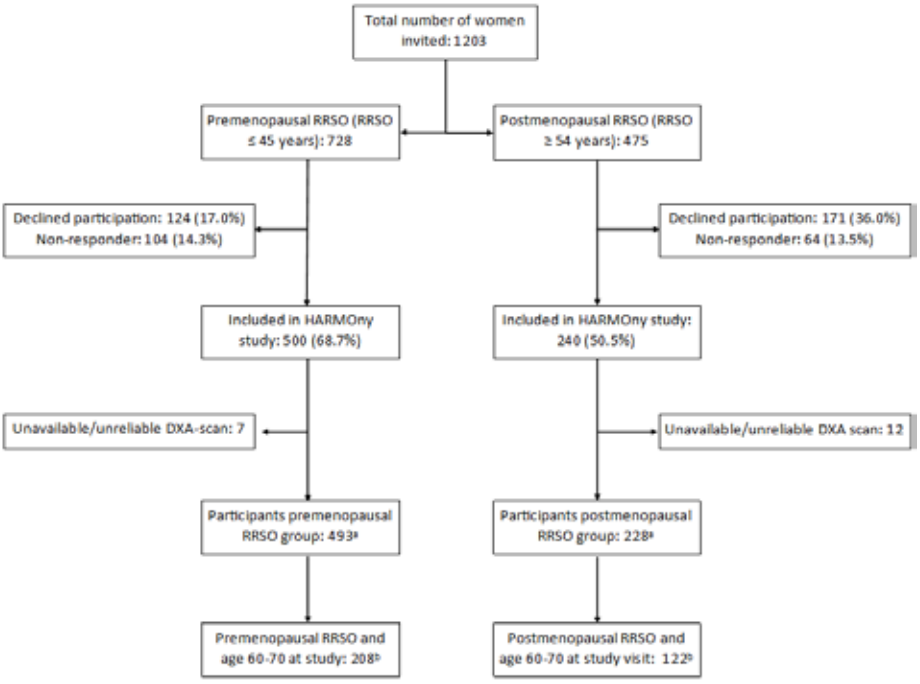

Figure 1. Flowchart participation HARMOny study.

[a.] Entire study population, used for analyses Z-scores only because of large age difference.

[b.] Overlapping age group 60-70 years at study, used for analyses into the prevalence of either osteopenia/osteoporosis (T-score≤-1.0).

Results

During the inclusion period 721 participants gave written informed consent (response 61.5%), of whom 493 in the premenopausal RRSO group and 228 in the postmenopausal RRSO group. (See Figure 1.) Median age was 59.2 years in the premenopausal RRSO group and 69.7 years in the postmenopausal RRSO group (See Table 1). Median time since premenopausal RRSO was 18.1 years (IQR 15.3-21.3). Both groups were comparable for *BRCA1/2* GPV carrier status, history of breast cancer, BMI and current AR use. Compared with the postmenopausal RRSO group, women in the premenopausal RRSO group were significantly more often current smokers (9.7% vs 3.5%, P=0.005) and more often had a history of MHT use (29.7% vs 10.5%, P<0.001) and chemotherapy (45.4% vs 34.8%, P=0.007). Because of the substantial age difference between both groups, the 10-year estimated risk of major osteoporotic and hip fractures based on risk factors was lower in the premenopausal compared with the postmenopausal RRSO group (both P<0.001).

Table 1. Participant characteristics of the premenopausal and postmenopausal RRSO groups.

	Premenopausal RRSO (RRSO ≤45)	**Postmenopausal RRSO (RRSO≥54)**	**p-value**
	N=493	**N=228**	
Age at study visit, years	59.2 (57.6-62.1)	69.7 (67.0-73.1)	<0.001
Age at menopause, years	42.0 (40.0-44.0)	51.0 (50.0-53.0)	<0.001
Time since RRSO, years	18.1 (15.3-21.3)	11.6 (10.1-13.8)	<0.001
***BRCA* GPV carrier status**			0.38
BRCA1 germline mutation	48.1%	29.4%	
BRCA2 germline mutation	19.3%	34.7%	
Non-carrier of *BRCA1/2*	32.6%	35.9%	
MHT use	29.7%	10.5%	<0.001
Smoking			0.86
Never	49.5%	45.2%	
Former smoker	40.8%	51.3%	
Current smoker	9.7%	3.5%	
Alcohol use (>1 drinks daily)	54.4%	50.0%	0.28
Daily sitting time, hours	6.1 (2.7)	5.7 (2.3)	0.08
Weekly sport time, hours	1.5 (0-3)	2.0 (0-3)	0.36

Table 1. Continued

	Premenopausal RRSO (RRSO ≤45)	Postmenopausal RRSO (RRSO≥54)	p-value
	N=493	**N=228**	
BMI, kg/m²	25.2 (22.7-28.9)	24.9 (22.8-28.4)	0.22
Breast cancer	59.6%	66.2%	0.090
Radiotherapy	37.9%	40.4%	0.54
Chemotherapy	45.4%	34.8%	0.007
Endocrine therapy	23.1%	21.8%	0.69
Parent with hip fracture	11.8%	15.0%	0.23
Self-reported rheumatoid arthritis	12.7%	15.3%	0.33
Current supplement use			
Vitamin D alone	30.2%	40.2%	0.009
Calcium alone	1.5%	0.5%	0.25
Calcium and vitamin D	37.1%	30.2%	0.087
Corticosteroids use >3 months	17.0%	17.7%	0.82
Current antiresorptive medication	7.1%	5.9%	0.54
Calcium, mmol/L	2.5 (0.1)	2.5 (0.1)	0.25
Albumin, g/L	46.9 (2.7)	46.1 (3.3)	<0.001
Phosphate, mmol/L	1.2 (0.2)	1.2 (0.2)	0.11
Creatinine, nmol/L	67.6 (12.0)	68.3 (12.3)	0.48
eGFR, ml/min/1.73m²	63.3 (5.9)	62.9 (6.2)	0.38
CKD*	15.0%	14.3%	0.80
10yr risk osteoporotic fracture, %	8.1 (6.7-11.0)	12.0 (10.0-17.0)	<0.001
10yr risk hip fracture, %	1.3 (0.9-2.4)	3.4 (2.1-5.7)	<0.001

Values are numbers with percentages for categorical variables, means with standard deviation for normal distributed variables, and medians with interquartile range for variables with a skewed distribution. P-value was calculated using independent samples t-test, X2test and Mann-Whitney U test. PV, pathogenic variant; MHT, menopausal hormone therapy; BMI, body mass index; BMD, bone mineral density; eGFR, estimated glomerular filtration rate; *CKD, chronic kidney disease (eGFR<60).

BMD after premenopausal vs postmenopausal RRSO

Table 2 shows univariate analyses of the main outcomes for the premenopausal and postmenopausal RRSO groups. Z-scores of the LS and FN were lower among women in the premenopausal RRSO group compared with the postmenopausal RRSO group. The prevalence of osteopenia/osteoporosis of either the LS or FN based on T-score ≤-1.0 was higher in the premenopausal RRSO group compared with the postmenopausal RRSO group; however this difference was not significant (70.8% vs 67.3%, P=0.34). In multivariable linear regression analyses, women who underwent premenopausal RRSO had significantly lower Z-scores of both the LS and the FN compared with women who underwent postmenopausal RRSO (β -0.88, 95% CI, -1.10, -0.66 for LS; β -0.51; 95% CI -0.71, -0.31 for FN, Table 3). A higher BMI and ever MHT use were associated with significantly higher Z-scores of both the LS and FN. Current smokers had significantly lowered Z-scores of the FN, compared with former or never smokers. Figure 2 shows that, in multivariable Poisson regression analyses, women who underwent premenopausal RRSO had increased RRs for having a low Z-score (≤-1.0) compared with women who underwent postmenopausal RRSO (RR 2.35, 95% CI, 1.26-4.40 for LS; RR 1.84 95% CI, 1.08-3.13 for FN; RR 2.05, 95% CI, 1.30-3.25 for LS or FN). Including history of chemotherapy in the analyses did not influence the outcome.

Table 2. Descriptive statistics of the BMD and BTM in the premenopausal and postmenopausal RRSO groups.

	Premenopausal RRSO (RRSO ≤45)	**Postmenopausal RRSO (RRSO≥54)**	**p-value**
	N=493	**N=228**	
Lumbar spine bone density, gr/cm²	0.947 (0.1)	0.956 (0.1)	0.44
Lumbar spine T-score	-0.9 (1.3)	-0.8 (1.3)	0.43
Normal BMD	48.1%	52.9%	0.23
Osteopenia (T-score -1 to -2.5)	42.2%	37.3%	0.22
Osteoporosis (T-score ≤ -2.5)	9.7%	9.8%	0.99
Lumbar spine Z-score	0.5 (1.3)	1.2 (1.4)	<0.001
Lumbar spine Z-score ≤ -1.0	11.0%	5.3%	0.017
Femoral neck bone density, gr/cm²	0.767 (0.1)	0.760 (0.1)	0.50
Femoral neck T-score	-1.0 (0.9)	-1.1 (1.0)	0.13
Normal BMD	44.2%	41.1%	0.42
Osteopenia (T-score -1 to -2.5)	51.2%	50.0%	0.76
Osteoporosis (T-score ≤ -2.5)	4.5%	8.9%	0.02

Table 2. Continued

	Premenopausal RRSO (RRSO ≤45)	Postmenopausal RRSO (RRSO≥54)	p-value
	N=493	N=228	
Femoral neck Z-score	0.1 (0.9)	0.6 (1.1)	<0.001
Femoral neck Z-score ≤ -1.0	12.6%	7.6%	0.049
Any osteopenia/osteoporosis*	70.8%	67.3%	0.34
Vertebral fracture at study visit	5.3%	7.5%	0.25
Vitamin D, µmol/L	68.3 (25.7)	71.8 (22.8)	0.089
β-CTX, ng/L	296.5 (175.9)	292.9 (147.7)	0.79
P1NP, µg/L	49.0 (19.8)	48.8 (17.7)	0.91

Values are numbers with percentages for categorical variables, means with standard deviation for normal distributed variables, and medians with interquartile range for variables with a skewed distribution. P-value was calculated using independent samples t-test, X2test and Mann-Whitney U test. BMD, bone mineral density. *Having a T-score ≤ -1.0 of either lumbar spine and/or femoral neck.

Table 3. Influence of premenopausal RRSO compared with postmenopausal RRSO on BMD Z-scores (clustered for center) of the lumbar spine and femoral neck adjusted for risk factors (n=721).

	β regression coefficient (95% CI)*	p-value
Z-score LS		
RRSO≤45 years vs RRSO≥54 years	-0.88 (-1.10, -0.66)	<0.001
BMI	0.09 (0.07- 0.10)	<0.001
Current smoker yes/no	-0.30 (-0.59, -0.18)	0.041
Ever MHT use yes/no	0.27 (-0.12, 0.66)	0.14
Current AR use yes/no	-0.57 (-1.17, 0.02)	0.056
Z-score FN		
RRSO≤45 years vs RRSO≥54 years	-0.51 (-0.71, -0.31)	0.001
BMI	0.06 (0.03, 0.09)	0.003
Current smoker yes/no	-0.28 (-0.45, -0.10)	0.008
Ever MHT use yes/no	0.24 (0.10, 0.38)	0.005
Current AR use yes/no	-0.43 (-0.7, -0.16)	0.009

CI, confidence interval; RRSO, risk-reducing salpingo-oophorectomy; LS, lumbar spine; FN, femoral neck; BMI, body mass index; MHT, menopausal hormone therapy; AR, antiresorptive medication.*Results of linear regression analysis (adjusted for all factors in the model simultaneously: Timing of RRSO, BMI, smoking and MHT use).

BMD in women aged 60-70 at study visit

For outcomes other than Z-scores, analyses comparing the pre- and postmenopausal RRSO groups need to be adjusted for age. Therefore we performed subgroup analyses in women aged 60-70 at study visit, the age range that overlapped between the pre- and postmenopausal RRSO groups. Participant characteristics and univariate analyses of the main outcomes in supplementary Tables S1-2). Univariately, the groups were comparable for 10-year estimated risk of major osteoporotic fractures and hip fractures, based on risk factors (P=0.37 and P=0.21, respectively). Figure 3 shows that, after adjustment for confounders (age, BMI, ever MHT use, current smoking and current AR use), there was no difference in the prevalence of osteopenia/osteoporosis (T-score ≤-1.0) in the premenopausal RRSO group compared with the postmenopausal RRSO group (RR 1.04, 95% CI, 0.74-1.48 for LS; RR 1.09, 95% CI, 0.81-1.46 for FN; RR 1.01, 95% CI, 0.79-1.29 for either LS or FN; RR 1.19, 95% CI, 0.77-1.84 for both LS and FN). We found no difference in age-adjusted prevalence of self-reported fractures in the 12 months before study visit between the premenopausal and postmenopausal RRSO groups (RR 0.24, 95% CI, 0.05-1.22, data not shown). As in the entire study population women who were 60-70 years old at study visit and underwent premenopausal RRSO had significantly lower Z-scores of the LS compared with women who underwent postmenopausal RRSO (β -0.49, 95% CI, -0.86, -0.12, supplementary Table S3).

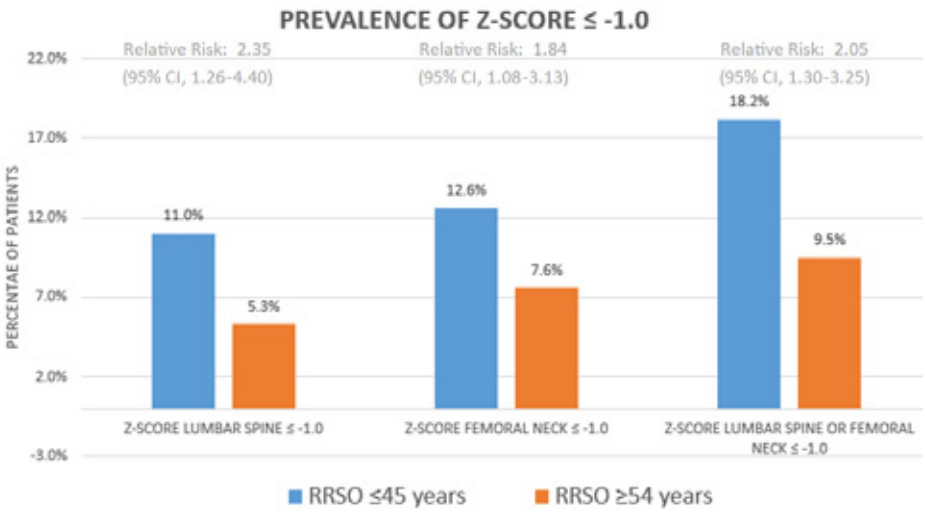

Figure 2. Prevalence of having Z-score ≤-1.0 of the LS and FN in women with a premenopausal and postmenopausal RRSO. Relative risks calculated using Poisson regression analyses and adjusted for body mass index, current smoking, current antiresorptive medication use and ever menopausal hormone therapy use.

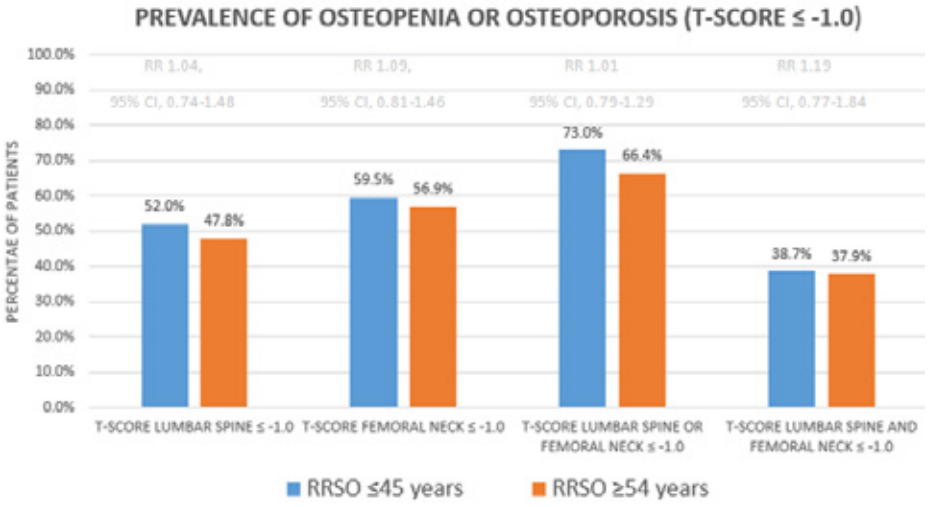

Figure 3. Prevalence of either osteopenia or osteoporosis (T-score (≤ -1.0) of the LS and/or the FN in women with a premenopausal and postmenopausal RRSO aged 60-70 at study visit. Relative risks calculated using Poisson regression analyses and adjusted for body mass index, current smoking, current antiresorptive medication use and ever menopausal hormone therapy use.

Bone Turnover Markers

Among women aged 60-70 at study visit we found no significant difference for both β-CTX and P1NP levels when comparing the premenopausal and postmenopausal RRSO groups (median 276 vs 290 for β-CTX; median 48.0 vs 47.5 for P1NP see Supplementary table S4). In the entire study population, AR users had significantly lower BTM levels (P<0.001 for P1NP; P<0.001 for β-CTX). Women with osteopenia and osteoporosis had significantly higher levels of β-CTX (P=0.002 for LS; P=0.003 for FN) and P1NP (P=0.004 for LS; P=0.002 for FN). Subgroup analyses in women without current AR use yielded similar results (Supplementary table S5).

BMD according to timing of premenopausal RRSO

Within the entire premenopausal RRSO group (n=493) we compared women with an RRSO before the age of 41 (early premenopausal RRSO group, n=158) with women with an RRSO between the age of 41 and 45 years old (late premenopausal RRSO group, n=335). There were no significant differences between the early and late premenopausal RRSO groups, neither for BMD Z-scores nor for prevalence of osteopenia/osteoporosis based on T-score≤-1.0 (see Supplementary tables S6-7).

Discussion

Eighteen years after RRSO, we observed lower Z-scores of both the lumbar spine and femoral neck in women with a premenopausal RRSO (≤45 years), compared with women who underwent postmenopausal RRSO (≥54 years). In addition, the RR of having a Z-score ≤-1.0 was significantly higher after a premenopausal RRSO. The prevalence of osteopenia/osteoporosis (T-score ≤-1.0) could only be compared in a subgroup with limited sample size (women aged 60-70 years at study), showing no difference between the pre- and postmenopausal RRSO groups.

BMD after premenopausal RRSO

To our knowledge, this nationwide study is the first to investigate long-term BMD after a premenopausal RRSO in women at high familial risk for ovarian cancer. Two recent studies showed a sharp loss of absolute BMD shortly after premenopausal RRSO compared with postmenopausal RRSO, however both studies were limited by short follow-up times (21.3 and 36 months).[8-9] Although these results show that a premenopausal RRSO leads to decreased BMD in the short-term, they provide no information on whether this effect persist in later life. After natural menopause, the rapid bone loss phase in the first 4-5 years is followed by a less steep but stable decline.[7] Therefore, the short-term results on bone loss observed in *BRCA1/2* GPV carriers who underwent premenopausal RRSO might reflect a temporary difference in BMD that disappears over time, as women who retain their ovaries will also experience a rapid bone loss phase around their natural menopause at a later age. To draw sound conclusions when investigating possible long-term differences in BMD after surgical menopause and natural menopause, all women should be at least 5 years postmenopausal (beyond the period of rapid bone loss) at measurement of BMD.

Studies with longer follow-up not restricted to women at high familial risk of ovarian cancer reported short-term decreased BMD after early surgical menopause before the age of 45 compared with menopause after 45 years. However, this difference gradually disappeared after the age of 55, suggesting that the long-term effects of early menopause might be attenuated by factors related to ageing.[13-14] Remarkably, in the current study we demonstrated that the effect of premenopausal RRSO on BMD as assessed by Z-scores was still measurable a median of 18.1 years after premenopausal RRSO, therefore rendering it less likely that these effects are temporary. These results are consistent with a recent study investigating long-term bone health after early *natural* menopause showing increased risk of osteoporosis and fractures 23 years after menopause.[5] In contrast to our study, Fakkert et al found no differences in Z-scores, neither for the LS nor for the FN, in *BRCA1/2* GPV carriers

who underwent RRSO before age 52 (median follow-up 5.0 years) compared with a local reference population. [10] However, in multivariate analyses younger age at RRSO was associated with a lower Z-score of the LS. Other studies investigating the effects of RRSO on BMD in BRCA1/2 carriers show inconsistent results. [11-12]

Since the guidelines for prevention and treatment of decreased BMD in postmeno_ pausal women are based on the prevalence of either osteopenia or osteoporosis (T-score≤-1.0) and/or fracture risk , most studies focus on these parameters. In the current study it was not possible to multivariably assess T-scores and fracture risk in the entire population of the HARMOny study because of the substantial age difference between the premenopausal and postmenopausal RRSO groups. Therefore, we compared T-scores of the premenopausal and postmenopausal RRSO groups in the overlapping age group of women aged 60-70 years at study visit. Somewhat surprisingly, multivariable regression analyses showed no difference in the prevalence of osteopenia/osteoporosis (T-score ≤ -1.0) between both groups (RR 1.04, 95% CI, 0.74-1.48 for LS; RR 1.09, 95% CI, 0.81-1.46 for FN; RR 1.01, 95% CI, 0.79-1.29 for LS or FN). These results are in contrast with the results we found for the Z-scores in the entire study population. This difference might partially be explained by the smaller sample size of this subgroup of women aged 60-70 at study visit (n=330). Another explanation could be that although a premenopausal RRSO has long-term effects on BMD, the increased risk of either osteopenia or osteoporosis (T-score ≤ -1.0) might be attenuated by competing risk factors such as ageing. On the basis of the results of this study, we do not suggest to actively screen women who underwent premenopausal RRSO to prevent future fractures. However, we do support the incorporation of surgical menopause (≤45years) as a potential cause of secondary osteoporosis, which is consistent with the currently available tools assessing future fracture risk.

The current study demonstrates that women who underwent premenopausal RRSO still had lowered Z-scores of the LS and FN compared with women who underwent postmenopausal RRSO. This is even more important when considering that age itself is a risk factor for fracture risk, independent of BMD.[27] A Swedish study showed that for any given T-score, the risk of hip fracture is increased when comparing women at age 80 with women at age 50, and this difference increases with lower T-scores.[15] Because morbidity and mortality after fractures are known to be substantially higher in later life, long-term effects of premenopausal RRSO on bone health may very well have a greater impact than short-term effects.

Studies show that both bone resorption markers (osteoclast activity) and bone formation markers (osteoblast activity) are elevated in the first year after surgical

menopause. [26-28] A recent study among *BRCA1/2* GPV carriers showed increased BTM ≥2 years after RRSO compared with age-matched reference values. [29] This study observed no long-term differences in BTM levels between the premenopausal and postmenopausal RRSO groups. Consistent with the current literature, our results showed that women with osteopenia and osteoporosis had significantly higher levels of both β-CTX and P1NP and that current AR users had strongly decreased BTM levels. [25] Although BTM can be used to assess risk of decreased BMD after RRSO, in our study we did not observe long-term effects of surgical menopause on BTM. This may indicate that the long-term decreased Z-scores after surgical menopause are unlikely to be caused by higher bone loss at time of study. However, these results should be interpreted with caution because they concern subgroup analyses based on non-fasting blood samples.

Strengths and limitations

Strengths of our study include the long follow-up time after RRSO (median 18.1 years after premenopausal RRSO, median 19.0 years after menopause in the postmenopausal RRSO group. To our knowledge, this follow-up time is substantially longer than in any other study investigating the effect of premenopausal RRSO on BMD. [8-12] Furthermore, because our study was designed to have a substantial difference in age at menopause between women in the premenopausal and postmenopausal RRSO groups (42.0 vs 51.0 years, respectively), we were better able to detect possible differences in BMD between both groups compared to similar studies in which the difference in age at menopause was less pronounced. A limitation of our study is the difference in age at study visit between the pre- and postmenopausal RRSO groups. We were able to account for this difference using Z-scores, which are by definition adjusted for age. In addition, we performed subgroup analyses in women who were 60-70 years of age at study visit, enabling comparison of T-scores, albeit with somewhat reduced statistical power. Another potential limitation might be selection bias due to differences in participation rates between the premenopausal group (68.7%) and the postmenopausal group (50.8%). A likely explanation is that women in the postmenopausal RRSO group felt less inclined to participate in our study as our research hypotheses focused on the consequences of premenopausal RRSO. Another limitation is the cross-sectional design of this study; unfortunately we had no longitudinal data on BMD in our study population. However, our study was nested in a large nationwide cohort of women at high familial risk of ovarian cancer. [17] Finally, current AR use turned out to be a confounder by indication for low Z-scores; women with current AR use had lower Z-scores compared to women without AR use. However, risk estimates for decreased BMD associated with premenopausal RRSO did not change after adding current AR use to the model. Furthermore, sensitivity

analyses with current AR use considered as an additional outcome of decreased BMD showed the same results as our main analyses (data not shown).

Conclusion

Premenopausal RRSO appears to be associated with reduced BMD Z-scores more than 18 years later. Future studies with larger sample sizes are needed to provide more insight into the long-term risk of surgical menopause on osteopenia and osteoporosis and fracture risk.

References

1. Daly MB, Pal T, Berry MP, Buys SS, Dickson P, Domchek SM, et al. Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2021;19(1):77-102.
2. Domchek SM, Friebel TM, Singer CF, Evans DG, Lynch HT, Isaacs C, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA. 2010;304(9):967-75.
3. McKinlay SM. The normal menopause transition: an overview. Maturitas. 1996;23(2):137-45.
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(2):80-7.
5. Jones AR, Enticott J, Ebeling PR, Mishra GD, Teede HT, Vincent AJ. Bone health in women with premature ovarian insufficiency/early menopause: a 23-year longitudinal analysis. Hum Reprod. 2024;39(5):1013-22.
6. Khosla S, Melton LJ, 3rd, Riggs BL. The unitary model for estrogen deficiency and the pathogenesis of osteoporosis: is a revision needed? J Bone Miner Res. 2011;26(3):441-51.
7. Gallagher JC. Effect of early menopause on bone mineral density and fractures. Menopause. 2007;14(3 Pt 2):567-71.
8. Kotsopoulos J, Hall E, Finch A, Hu H, Murphy J, Rosen B, et al. Changes in Bone Mineral Density After Prophylactic Bilateral Salpingo-Oophorectomy in Carriers of a BRCA Mutation. JAMA Netw Open. 2019;2(8):e198420.
9. Chan LN, Chen LM, Goldman M, Mak JS, Bauer DC, Boscardin J, et al. Changes in Bone Density in Carriers of BRCA1 and BRCA2 Pathogenic Variants After Salpingo-Oophorectomy. Obstet Gynecol. 2023;142(1):160-9.
10. Fakkert IE, Abma EM, Westrik IG, Lefrandt JD, Wolffenbuttel BH, Oosterwijk JC, et al. Bone mineral density and fractures after risk-reducing salpingo-oophorectomy in women at increased risk for breast and ovarian cancer. Eur J Cancer. 2015;51(3):400-8.
11. Powell CB, Alabaster A, Stoller N, Armstrong MA, Salyer C, Hamilton I, et al. Bone loss in women with BRCA1 and BRCA2 mutations. Gynecol Oncol. 2018;148(3):535-9.
12. 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(1):69-75.
13. Hadjidakis DJ, Kokkinakis EP, Sfakianakis ME, Raptis SA. Bone density patterns after normal and premature menopause. Maturitas. 2003;44(4):279-86.
14. Francucci CM, Romagni P, Camilletti A, Fiscaletti P, Amoroso L, Cenci G, et al. Effect of natural early menopause on bone mineral density. Maturitas. 2008;59(4):323-8.
15. Kanis JA, Johnell O, Oden A, Dawson A, De Laet C, Jonsson B. Ten year probabilities of osteoporotic fractures according to BMD and diagnostic thresholds. Osteoporos Int. 2001;12(12):989-95.
16. Terra L, Hooning MJ, Heemskerk-Gerritsen BAM, van Beurden M, Roeters van Lennep JE, van Doorn HC, et al. Long-Term Morbidity and Health After Early Menopause Due to Oophorectomy in Women at Increased Risk of Ovarian Cancer: Protocol for a Nationwide Cross-Sectional Study With Prospective Follow-Up (HARMOny Study). JMIR Res Protoc. 2021;10(1):e24414.
17. Brohet RM, Velthuizen ME, Hogervorst FB, Meijers-Heijboer HE, Seynaeve C, Collee MJ, et al. Breast and ovarian cancer risks in a large series of clinically ascertained families with a high proportion of BRCA1 and BRCA2 Dutch founder mutations. J Med Genet. 2014;51(2):98-107.

18. Lalmohamed A, Welsing PM, Lems WF, Jacobs JW, Kanis JA, Johansson H, et al. Calibration of FRAX (R) 3.1 to the Dutch population with data on the epidemiology of hip fractures. Osteoporos Int. 2012;23(3):861-9.
19. Lu Y, Fuerst T, Hui S, Genant HK. Standardization of bone mineral density at femoral neck, trochanter and Ward's triangle. Osteoporos Int. 2001;12(6):438-44.
20. Kanis JA, Melton LJ, 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res. 1994;9(8):1137-41.
21. Genant HK, Li J, Wu CY, Shepherd JA. Vertebral fractures in osteoporosis: a new method for clinical assessment. J Clin Densitom. 2000;3(3):281-90.
22. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.
23. Kidney Disease: Improving Global Outcomes CKDMBDWG. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). Kidney Int Suppl. 2009(113):S1-130.
24. Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. J Bone Miner Res. 2000;15(8):1526-36.
25. 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(2):391-420.
26. Peris P, Alvarez L, Monegal A, Guanabens N, Duran M, Pons F, et al. Biochemical markers of bone turnover after surgical menopause and hormone replacement therapy. Bone. 1999;25(3):349-53.
27. Hui SL, Slemenda CW, Johnston CC, Jr. Age and bone mass as predictors of fracture in a prospective study. J Clin Invest. 1988;81(6):1804-9.
28. Vatrasresth J, Suwan A, Panyakhamlerd K. Effects of early estradiol valerate administration on bone turnover markers in surgically induced menopausal women. BMC Womens Health. 2021;21(1):363.
29. Fakkert IE, van der Veer E, Abma EM, Lefrandt JD, Wolffenbuttel BH, Oosterwijk JC, et al. Elevated Bone Turnover Markers after Risk-Reducing Salpingo-Oophorectomy in Women at Increased Risk for Breast and Ovarian Cancer. PLoS One. 2017;12(1):e0169673.

Declarations

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Conflict of interest disclosure

The authors report no conflict of interest.

Data availability statement

With publication, de-identified data collected for the study, including participant data, will be made available to others upon reasonable request. Data can be requested with a proposal by sending an e-mail to the corresponding author.

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Ethics approval statement

This study was conducted according to the standards of Good Clinical Practice, in agreement with the principles of the Declaration of Helsinki and with the Dutch law as stated in the Medical Research Involving Human Subjects Act (WMO). The study has been approved in writing by the Institutional Review Board of the AVL/NKI to be conducted in all nine University Medical Centers and the Antoni van Leeuwenhoek.

Patient consent statement

All participants included in this study signed informed consent.

Clinical trial registration

The pre-registered clinical trial number is <NCT03835793>

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Supplements

Table S1. Participant characteristics of the premenopausal and postmenopausal RRSO groups in women aged 60-70 years at study visit.

	Age 60-70 Premenopausal RRSO (RRSO ≤45)	**Age 60-70 Postmenopausal RRSO (RRSO≥54)**	**p-value**
	N=208	**N=122**	
Age at study visit, years	62.5 (61.0-64.4)	67.2 (65.6-68.5)	<0.001
Age at menopause, years	42.0 (40.0-44.0)	51.0 (50.0-54.0)	<0.001
Time since RRSO, years	21.1 (18.3-23.3)	10.7 (9.6-11.8)	<0.001
BRCA PV carrier status			0.36
BRCA1 germline mutation	51.5%	30.8%	
BRCA2 germline mutation	17.6%	33.3%	
Non-carrier of BRCA1/2	30.9%	35.9%	
MHT use	31.8%	6.5%	<0.001
Smoking			0.15
Never	40.2%	50.4%	
Former smoker	51.5%	44.4%	
Current smoker	8.3%	5.1%	
Alcohol use	49.0%	49.6%	0.92
Daily sitting time, hours	6.2 (2.7)	5.8 (2.4)	0.20
Weekly sport time, hours	1.5 (0-3)	2.0 (0-9)	0.86
BMI, kg/m²	25.1 (22.7-28.8)	25.2 (23.2-28.7)	0.94
Breast cancer	60.3%	59.8%	0.78
Radiotherapy	39.2%	38.5%	0.89
Chemotherapy	42.6%	37.6%	0.38
Endocrine therapy	19.6%	21.4%	0.71
Parent with hip fracture	10.8%	17.4%	0.091
Self-reported rheumatoid arthritis	13.0%	11.6%	0.70
Supplement use			
Vitamin D alone	30.2%	38.6%	0.13
Calcium alone	1.0%	0.9%	0.89
Calcium and vitamin D	35.8%	28.0%	0.17

Table S1. Continued

	Age 60-70 Premenopausal RRSO (RRSO ≤45)	Age 60-70 Postmenopausal RRSO (RRSO≥54)	p-value
	N=208	N=122	
Corticosteroids use > 3 months	19.1%	16.8%	0.62
Current antiresorptive medication	5.7%	6.1%	0.88
Calcium	2.5 (0.2)	2.5 (0.1)	0.51
Albumin	46.8 (2.9)	46.7 (3.4)	0.86
Phosphate	1.2 (0.2)	1.2 (0.1)	0.27
Creatinine	67.9 (12.3)	68.6 (12.0)	0.65
eGFR	63.2 (6.1)	62.8 (6.0)	0.61
CKD*	15.0%	14.2%	0.84
10-year risk major osteoporotic fracture, %	10.0 (8.4-13.0)	11.0 (8.5-15.0)	0.37
10-year risk hip fracture,%	2.1 (1.4-3.1)	2.3 (1.4-3.5)	0.21

Values are numbers with percentages for categorical variables, means with standard deviation for normal distributed variables, and medians with interquartile range for variables with a skewed distribution. P-value was calculated using independent samples t-test, X2test and Mann-Whitney U test.PV, pathogenic variant; MHT, menopausal hormone therapy; BMI, body mass index; BMD, bone mineral density; eGFR, estimated glomerular filtration rate; *CKD, chronic kidney disease (eGFR<60).

Table S2. Descriptive statistics of BMD and BTM in the premenopausal and postmenopausal RRSO groups in women aged 60-70 years at study visit.

	Age 60-70 Premenopausal RRSO (RRSO ≤45)	**Age 60-70 Postmenopausal RRSO (RRSO≥54)**	**p-value**
	N=208	**N=122**	
Lumbar spine bone density, gr/cm²	0.955 (0.2)	0.967 (0.2)	0.55
Lumbar spine T-score	-0.9 (1.4)	-0.7 (1.0)	0.47
Normal BMD	49.0%	54.2%	0.37
Osteopenia (T-score -1 to -2.5)	40.4%	35.0%	0.34
Osteoporosis (T-score ≤ -2.5)	10.6%	10.8%	0.94
Lumbar spine Z-score	0.8 (1.5)	1.1 (1.7)	0.061
Lumbar spine Z-score ≤ -1.0	10.1%	8.2%	0.57
Femoral neck bone density, gr/cm²	0.766 (0.1)	0.775 (0.1)	0.55
Femoral neck T-score	-1.1 (0.9)	-1.0 (1.1)	0.69
Normal BMD	41.2%	44.6%	0.54
Osteopenia (T-score -1 to -2.5)	52.0%	47.1%	0.40
Osteoporosis (T-score ≤ -2.5)	6.9%	8.3%	0.64
Femoral neck Z-score	0.2 (0.9)	0.4 (1.2)	0.18
Femoral neck Z-score ≤ -1.0	10.1%	12.3%	0.54
Any osteopenia/osteoporosis*	72.1%	64.5%	0.15
Vertebral fracture at study visit	5.8%	7.4%	0.58
Vitamin D, µmol/L	70.0 (25.3)	71.7 (21.2)	0.54
β-CTX, ng/L	276 (173-387)	290 (199-384)	0.94
P1NP, µg/L	47.5 (36-62)	48 (39-61.0)	0.88

BMD, bone mineral density.*Having a T-score ≤ -1.0 of either lumbar spine and/or femoral neck.

Table S3. Regression coefficients of Z-scores of the lumbar spine and femoral neck (clustered for center) according to timing of RRSO and other for risk factors in a subgroup of women aged 60-70 years at study visit (n=330).

	Multivariably adjusted β regression coefficient (95% CI)*	
	Z-score LS	**Z-score FN**
RRSO≤45 years vs RRSO≥54 years	-0.49 (-0.86, -0.12)	-0.32 (-0.71, 0.07)
BMI	0.07 (0.06, 0.08)	0.05 (0.03, 0.08)
Current smoker yes/no	-0.46 (-1.07, 0.15)	-0.46 (-1.08, 0.16)
Ever MHT use yes/no	0.31 (-0.56, 1.18)	0.34 (0.01, 0.67)
Current AR use yes/no	-0.36 (-1.24, 0.52)	-0.57 (-1.17, 0.03)

CI, confidence interval; RRSO, risk-reducing salpingo-oophorectomy; LS, lumbar spine; FN, femoral neck; BMI, body mass index; MHT, menopausal hormone therapy; AR, antiresorptive medication.
* Results of linear regression analysis in women aged 60-70 at time of study (adjusted for all factors in the model simultaneously: Timing of RRSO, BMI, smoking, current AR use and ever MHT use).

Table S4. Descriptive statistics of Bone Turnover Markers (BTM) according to different possible confounders/ outcomes of BMD.

Univariate analyses BTM	**P1NP level, Median (IQR)**	**p-value**	**Beta-CTX level, Median (IQR)**	**p-value**
Age study visit		**0.94**		**0.88**
55-59 years	47 (35-59)		264 (180-352)	
60-64 years	47 (36-62)		262 (170-384)	
65-69 years	48 (38-61)		289 (202-384)	
70-74 years	43.5 (33.5-54)		239 (166-362)	
75-79 years	47 (35-56)		275 (218-437)	
Timing of RRSO		**0.99**		**0.48**
Premenopausal RRSO	47 (36-60)		264 (174-367)	
Postmenopausal RRSO	46 (37-58)		273.5 (193-379)	
Timing RRSO in women aged 60-70		**0.57**		**0.46**
Premenopausal RRSO	47 (36-62)		275 (173-376)	
Postmenopausal RRSO	48 (38-60)		292.5 (202-384)	
Timing of premenopausal RRSO		**0.09**		**0.53**
RRSO <41 years	45 (34-57)		263 (160-378)	
RRSO 41-45 years	47 (36-61)		264.5 (181-366)	

Table S4. Continued

Univariate analyses BTM	P1NP level, Median (IQR)	p-value	Beta-CTX level, Median (IQR)	p-value
Smoking		**0.82**		**0.07**
Current smoker	47 (35-62)		321.5 (187-462)	
Former smoker	46 (37-60)		273.5 (178-397)	
Never smoker	47 (35-58)		261 (178-356)	
MHT use		**0.07**		**0.39**
Ever MHT use	48 (38-64)		283 (162-404)	
Never MHT use	46 (35-57.5)		259 (184-362)	
Antiresorptive medication use		**<0.001**		**<0.001**
Current antiresorptive medication	25 (18-37)		140 (72-218)	
Former antiresorptive medication	44 (33-57)		282.5 (162-372)	
Never antiresorptive medication	48 (38-60.5)		277 (191-378)	
Lumbar spine		**0.004**		**0.002**
Normal BMD	45 (34-57)		241 (162-347)	
Osteopenia	48 (39-60)		292 (197-397)	
Osteoporosis	54 (36-70)		279.5 (200-425.5)	
Z-score lumbar spine		**<0.001**		**0.014**
LS Z-score ≤-1.0	55 (39-78)		312 (202-445)	
LS Z-score >-1.0	46 (36-58)		263 (177.5-365)	
Femoral neck		**0.002**		**0.003**
Normal BMD	45 (35-57)		261 (162-356)	
Osteopenia	48 (37-61)		271 (187-376)	
Osteoporosis	58 (46-70)		364 (230-501)	
Z-score femoral neck		**0.065**		**0.24**
FN Z-score ≤-1.0	51 (36-68)		278 (192-425)	
FN Z-score >-1.0	46 (36-58)		265.5 (179-366)	

Values are medians with interquartile range. P-value was calculated using Mann-Whitney U test or Kruskal-Wallis test. BMD, bone mineral density; IQR, interquartile range; RRSO, risk-reducing salpingo-oophorectomy; MHT, menopausal hormone therapy; LS, lumbar spine; FN, femoral neck.

Table S5. Descriptive statistics of bone turnover markers in women without current antiresorptive medication use (n=676).

Univariate analyses BTM	P1NP level, median (IQR)	p-value	Beta-CTX level, median (IQR)	p-value
Age study visit		**0.53**		**0.82**
55-59 years	47 (37-60)		273 (191-359)	
60-64 years	48 (37-63)		266 (173-388)	
65-69 years	50 (39-63)		306 (208-397)	
70-74 years	44 (35-55)		264 (175-364)	
75-79 years	47 (35-56)		280 (206-442)	
Timing of RRSO		**0.99**		**0.73**
Premenopausal RRSO	47 (37-60)		275 (187-374)	
Postmenopausal RRSO	47 (38-58)		281.5 (200-384)	
Timing of RRSO in women aged 60-70		**0.57**		**0.36**
Premenopausal RRSO	48 (37-62.5)		276 (181-387)	
Postmenopausal RRSO	48 (41-61)		303 (207-384)	
Timing of premenopausal RRSO		**0.12**		**0.94**
RRSO <41 years	47 (36-57.5)		283 (187-394)	
RRSO 41-45 years	48 (38-62)		273 (187-368)	
Smoking		**0.83**		**0.060**
Current smoker	48 (36-63)		310 (187-484)	
Former smoker	47 (38-61.5)		294 (194.5-404)	
Never smoker	48 (37-58)		267.5 (187-358)	
MHT use		**0.19**		**0.39**
Ever MHT use	48 (38-64)		283 (162-404)	
Never MHT use	47 (37-59)		259 (184-362)	
Lumbar spine		**<0.001**		**<0.001**
Normal BMD	45 (35-57)		251 (173-340)	
Osteopenia	49 (41-61.5)		304 (218-405)	
Osteoporosis	55 (44-70)		296 (201.5-424.5)	
Z-score lumbar spine		**<0.001**		**0.029**
LS Z-score ≤-1.0	56 (44-81)		331.5 (221-445)	
LS Z-score >-1.0	47 (37-59)		274 (188-371)	
Femoral neck		**<0.001**		**0.011**
Normal BMD	45 (36-57)		265 (180-358)	
Osteopenia	49 (39-62)		284 (196-389)	

Table S5. Continued

Univariate analyses BTM	P1NP level, median (IQR)	p-value	Beta-CTX level, median (IQR)	p-value
Osteoporosis	58 (47-70)		364 (254-501)	
Z-score femoral neck		**0.021**		**0.25**
FN Z-score ≤-1.0	52 (40-68)		287 (200-425)	
FN Z-score >-1.0	47 (38-59)		278.5 (190.5-374.5)	

Values are medians with interquartile range. P-value was calculated using Mann-Whitney U test or Kruskal-Wallis test. BMD, bone mineral density; IQR, interquartile range; RRSO, risk-reducing salpingo-oophorectomy; MHT, menopausal hormone therapy; LS, lumbar spine; FN, femoral neck.

Table S6. Regression coefficients of Z-scores (clustered for center) of the lumbar spine and femoral neck according to timing of RRSO and other for risk factors in women with a premenopausal RRSO (n=445).

	Multivariably adjusted β regression coefficient (95% CI)*	
	Z-score LS	**Z-score FN**
RRSO <41 years vs RRSO 41-45 years	-0.09 (-0.33, 0.16)	-0.01 (-0.02, 0.26)
BMI	0.08 (0.06, 0.11)	0.05 (0.02, 0.08)
Current smoking yes/no	-0.30 (-0.58, -0.02)	-0.24 (-0.40, -0.08)
Ever MHT use yes/no	0.21 (-0.23, 0.66)	0.14 (-0.06, 0.34)
Current AR use yes/no	-0.57 (-1.28, 0.14)	-0.40 (-0.71, -0.10)

CI, confidence interval; RRSO, risk-reducing salpingo-oophorectomy; LS, lumbar spine; FN, femoral neck; BMI, body mass index; MHT, menopausal hormone therapy; AR, antiresorptive medication.
*Results of linear regression analysis (adjusted for all factors in the model simultaneously: Timing of RRSO, BMI, current smoking, current AR use and ever MHT use).

Table S7. Relative risk of decreased BMD (T-score ≤-1.0) of the lumbar spine and femoral neck according to timing of RRSO and other risk factors in a women with a premenopausal RRSO (n=445).

	Multivariably adjusted relative risk (95% CI) for decreased BMD*			
	LS	**FN**	**LS or FN**	**LS and FN**
Timing of RRSO				
RRSO 41-45 years	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
RRSO <41 years	0.96 (0.79, 1.18)	0.87 (0.72, 1.06)	0.92 (0.80, 1.05)	0.92 (0.71, 1.21)
BMI	0.94 (0.92, 0.96)	0.97 (0.95, 0.99)	0.96 (0.95, 0.98)	0.93 (0.90, 0.96)
Current smoker yes/no	1.16 (0.93, 1.45)	1.15 (0.91, 1.45)	1.14 (0.99, 1.32)	1.19 (0.87, 1.62)
Ever MHT use yes/no	0.93 (0.76, 1.15)	0.98 (0.80, 1.19)	1.00 (0.87, 1.15)	0.85 (0.64, 1.14)
Current AR use yes/no	1.46 (1.20, 1.79)	1.34 (1.08, 1.67)	1.24 (1.09, 1.42)	1.70 (1.28, 2.27)

CI, confidence interval; RRSO, risk-reducing salpingo-oophorectomy; LS, lumbar spine; FN, femoral neck; BMI, body mass index; MHT, menopausal hormone therapy; AR, antiresorptive medication.
*Results of Poisson regression analysis in women with a premenopausal RRSO (adjusted for all factors in the model simultaneously: Timing of RRSO, age, BMI, current smoking, current AR use and ever MHT use).

Chapter 5

Long-term outcomes of surgical menopause after risk-reducing salpingo-oophorectomy; results of the HARMOny study

Submitted

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Abstract

Introduction

Premenopausal risk-reducing salpingo-oophorectomy (RRSO) in women at high familial risk of ovarian cancer induces immediate menopause. Evidence about long-term effects of surgical menopause is scarce.

Methods

We conducted a cross-sectional study (n=740) nested in a nationwide cohort of women at high familial risk of ovarian cancer. Participants completed a cognition test and a questionnaire on lifestyle, sexual functioning, urinary incontinence, HRQOL (SF-36) and cancer worries (CWS). Cardiovascular disease (CVD) risk and bone mineral density (BMD) were assessed during a clinical visit. In women aged 60-70 years at study visit (n=330), we compared potential long-term health effects between women who underwent a premenopausal RRSO (≤45 years) and women with a postmenopausal RRSO (≥54 years).

Results

Median age at study was 64.3 years, median time since premenopausal RRSO was 21 years. A comprehensive overview of our (partially published) results showed that a premenopausal RRSO compared with a postmenopausal RRSO was not associated with long-term coronary artery calcification, objective cognitive functioning, urinary incontinence, impaired quality of life or fear of cancer. However, women in the premenopausal RRSO group had lowered BMD and reported more vaginal dryness and sexual discomfort compared with the postmenopausal RRSO group.

Conclusion

Premenopausal RRSO does not appear to be associated with long-term cardiovascular disease risk, cognition or HRQOL. However, it negatively influences bone mineral density and sexual discomfort.

Introduction

Female *BRCA1* and *BRCA2* germline pathogenic variant (GPV) carriers have a lifetime risk of ovarian cancer of 44% and 17%, respectively.[1] Studies have shown that screening for ovarian cancer is not effective, neither for hereditary nor for sporadic ovarian cancer.[2-3] Therefore women at high familial risk for ovarian cancer are recommended to undergo risk-reducing salpingo-oophorectomy (RRSO) after completion of child bearing, preferably at ages 35 to 40 years for *BRCA1* GPV carriers and at ages 40 to 45 years for *BRCA2* GPV carriers.[4] While RRSO performed at the recommended age is very effective in preventing ovarian cancer, it also leads to early surgical menopause due to acute estrogen deficiency.[5-6]

Early *natural* menopause (≤45 years) has been associated with various long-term effects, including increased risk of cardiovascular disease (CVD), lowered bone mineral density (BMD) and cognitive impairment, which are all known to influence health-related quality of life (HRQOL). However, whether these effects are also present after *surgical* menopause has less often been investigated with inconsistent results.[7] In the HARMOny study, we recently investigated long-term effects of a premenopausal RRSO on coronary artery calcification (CAC), BMD, cognition, sexual functioning and urinary incontinence.[8-11]

Several studies have investigated the short-term effects of RRSO on HRQOL.[12-13] While RRSO is associated with HRQOL-related outcomes, including more vasomotor/menopausal complaints and worsened sexual function, generic HRQOL appears to be unaffected.[14-15] A potential explanation is that RRSO reduces short-term cancer specific distress; women who underwent RRSO appear to be satisfied with their choice to undergo prophylactic surgery.[16-18] However, most studies do not stratify for timing of RRSO (pre- vs postmenopausal), and have relatively short follow-up times (median up to 5 years).[12-13] Therefore, it remains unclear whether a premenopausal RRSO influences long-term HRQOL.

The aim of the current study was to provide a comprehensive overview of published reports on individual outcomes of the HARMOny study, including (subclinical) CVD risk, BMD, cognitive functioning, sexual functioning and urogenital complaints.[8-11-19] In addition, we report on unpublished data concerning the long-term effects of premenopausal (≤45 years) compared with postmenopausal (≥54 years) RRSO on HRQOL and cancer worries.

Methods

Study cohort

The HARMOny study is a Dutch multicenter cross-sectional study investigating long-term effects of RRSO on CVD risk, bone health, cognition, quality of life and urogenital problems. The study design of the HARMOny study (ClinicalTrials.gov NCT03835793) has been described in detail previously and was approved by the Medical Ethics Committee of the Antoni van Leeuwenhoek/Netherlands Cancer Institute (AVL/NKI). [19] Women were recruited from the Hereditary Breast and Ovarian cancer study Netherlands (HEBON), a nationwide cohort of women at high familial risk of breast and/or ovarian cancer recruited from all eight Dutch University Medical Centers and the Netherlands Cancer Institute. [20] Between 2018 and 2022, 1207 women were invited to join the study: 733 women who underwent a premenopausal RRSO (≤45yrs) and were ≥55 years old at inclusion and 474 women who underwent a postmenopausal RRSO (≥54 years) (see Figure 1). Exclusion criteria were a history of ovarian cancer, age over 80 years and therapy-induced menopause more than five years before RRSO. A history of cancer other than ovarian cancer, was not a reason for exclusion.

Study assessments

Participation in the HARMOny study consisted of an extensive online questionnaire and a clinical visit. [19] Among the topics included in the online questionnaire were lifestyle, medical history, menopausal hormone therapy (MHT) use, cardiovascular risk factors, bone health, cognitive functioning, sexual problems, urological complaints, HRQOL and cancer worries. HRQOL was assessed using the eight subscales of the SF-36 health survey (physical functioning, role limitations physical health, role limitations emotional health, pain, general health, social functioning, emotional well-being and energy/fatigue). [21-22] The SF-36 domain scores were linearly converted to a 0 to 100 scale, with higher scores indicating higher levels of HRQOL. In addition, the SF-36 physical component score (SF36-PCS) and mental component score (SF36-MCS) were calculated. [23] We calculated the relative risks (RRs) of having a low SF36-PCS or SF36-MCS in the premenopausal versus the postmenopausal RRSO groups. The cut-offs used for a low component scores were ≤50.0 for SF36-PCS and ≤42.0 for SF36-MCS. [24] Cancer worries were measured with the eight item Cancer Worry Scale (CWS). [25-27] In addition, we calculated the RRs of having fear of cancer (CWS≥14) in the premenopausal compared with the postmenopausal RRSO group. [26] Experience with the choice to undergo RRSO was measured in three statements (i.e. (1) the choice to undergo RRSO was difficult (2) undergoing RRSO was a well-informed decision (3) RRSO reduced my fear of cancer) using a 5-point Likert scale, varying

from completely disagree to completely agree. Women were asked to indicate their level of agreement or disagreement with the statements. Completely agree and agree were considered as agreement with the statement, disagree and completely disagree were considered as disagreement. The clinical program for the study consisted of a series of non-invasive measurements to assess subclinical atherosclerosis and BMD: height, weight, blood pressure, coronary artery calcium score (CAC score), pulse wave velocity (PWV), DEXA scan of lumbar spine (LS) and femoral neck (FN) and blood levels of lipid spectrum, random glucose, HbA1c, vitamin D, two bone turnover markers (P1NP and β-CTX) and two cardiac biomarkers (High-sensitivity cardiac troponin and CRP). According to the study protocol, the results of all measurements were shared with the individual participants and a letter with results was sent to their general practitioners. [19]

Statistical analyses

Characteristics of women in the premenopausal and postmenopausal RRSO groups were compared by using the independent samples t-test or Wilcoxon rank-sum test for continuous data and the Fisher exact test or $\chi 2$ test for categorical data, a two-sided $P < 0.05$ was considered significant. According to the HARMOny study protocol, we attempted to match the pre- and postmenopausal RRSO groups on age at study visit. [19] However, during the inclusion period we observed a substantial age difference between the premenopausal and postmenopausal RRSO groups (postmenopausal RRSO group median 10.1 years older). This age difference was caused by a change in the 2007 guidelines for management of ovarian cancer risk in *BRCA1/2* GPV carriers, leading to a strongly increased prevalence of premenopausal RRSO. [4] Therefore, we restricted the comparisons between the premenopausal and postmenopausal RRSO groups to women who were between 60-70 years old at study visit (see Figure 1). In addition, within the entire premenopausal RRSO group we evaluated long-term effects of RRSO before age 41 (early premenopausal RRSO group) and women aged 41-45 years at RRSO (late premenopausal RRSO group). Results of multivariable analyses estimating associations between timing of RRSO and CAC scores, bone mineral density, cognition, sexual functioning and urinary incontinence were described previously. [8-10-28]

SF-36 subdomains and component scores were compared with a cohort from the Dutch general population of women of similar age. [29-30] To be able to multivariably adjust for possible confounders, we used Poisson regression analysis to calculate RRs of having a low SF36-PCS and SF36-MCS associated with timing of RRSO. Variables assessed as possible confounders of the associations of premenopausal RRSO with HRQOL and cancer worries were: age at study visit, education, work,

BRCA status, parity daily sitting hours, current or ever smoking, alcohol use, ever use of MHT, history of breast cancer, breast cancer treatment, body mass index (BMI), hypertension (either antihypertensive medication, a systolic blood pressure >140mmHg or a diastolic blood pressure >90mmHg), dyslipidemia (either lipid-lowering medication or LDL cholesterol >4.0mmol/L), depression and chronic disease. A variable was considered a confounder if the coefficient estimate for the association of interest was affected by more than 10% when added to the model.

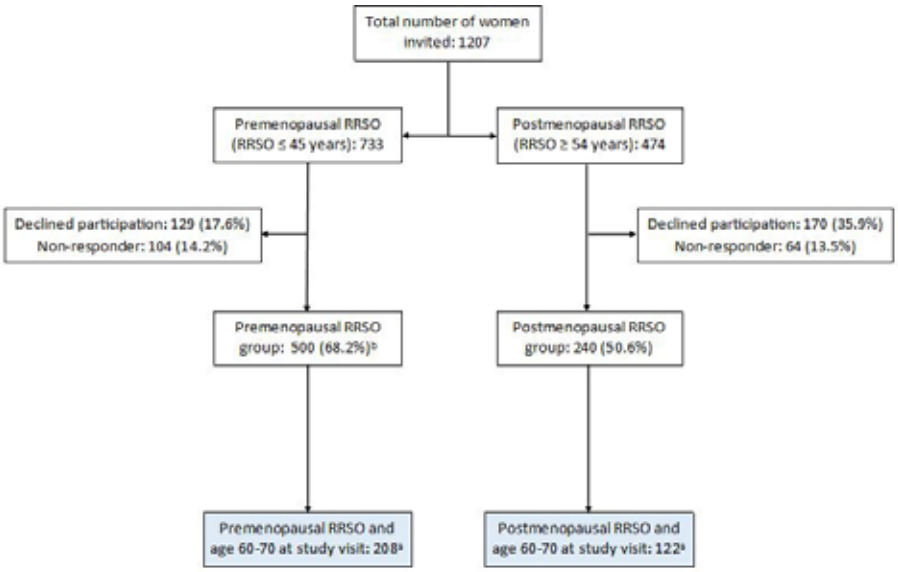

Figure 1. Flowchart participation HARMOny study [a.] Comparison between the pre- and postmenopausal RRSO groups aged 60-70 at study visit (in blue). [b.] Entire premenopausal RRSO group used for analyses on timing of premenopausal RRSO (<41 vs 41-45 years).

Results

During the inclusion period, 740 participants gave written informed consent (overall response 61.3%), of whom 500 in the premenopausal RRSO group and 240 in the postmenopausal RRSO group (See figure 1). Participant characteristics of the entire HARMOny study population are provided in the supplements (See Table S1).

Participant characteristics of women aged 60-70 at study visit

We included 330 women who were aged 60 to 70 years at study visit (208 in the premenopausal RRSO group, 122 in the postmenopausal RRSO group). Median age at study was 64.3 years; median time since RRSO was 21.0 years in the premenopausal

RRSO group and 10.7 years in the postmenopausal RRSO group (see Table 1). Both groups were comparable for *BRCA1/2* GPV carrier status (overall, 67.9%) and history of breast cancer (overall, 60.0%). Compared with the postmenopausal RRSO group, women in the premenopausal RRSO group more often had a history of MHT use (31.6% vs 6.2%).

Table 1. Patient characteristics women age 60-70 at study visit of the HARMOny study (n=330)*.

	Age 60-70 Premenopausal RRSO (RRSO ≤45)	**Age 60-70 Postmenopausal RRSO (RRSO>55)**	**p-value**
	N=208	**N=122**	
Age at study, years	62.4 (61.0-64.4)	67.2 (65.6-68.5)	<0.001
Age at menopause, years	42.0 (40.0-44.0)	51.0 (50.0-54.0)	<0.001
Time since RRSO years	21.0 (18.3-23.3)	10.7 (9.6-11.9)	<0.001
***BRCA* GPV carrier status**			<0.001
BRCA1 germline mutation	51.4%	29.5%	
BRCA2 germline mutation	17.8%	36.1%	
Non-carrier of *BRCA1/2*	30.8%	34.4%	
Education			0.28
Primary school/lower level high school	33.0%	42.1%	
Middle level high school	32.0%	25.6%	
Advanced vocational/ university	35.0%	33.3%	
Work status			<0.001
Full-time/part-time job	54.9%	16.1%	
Retired	14.4%	58.5%	
Housewife/voluntary work	14.4%	17.0%	
Completely/partially incapacitated	12.3%	8.5%	
Unemployed	4.1%	0.0%	
Children, one or more	84.1%	86.1%	0.64
Ever MHT use	31.6%	6.2%	<0.001
History of breast cancer	60.6%	59.0%	0.78
Radiotherapy	39.4%	38.5%	0.87
Chemotherapy	43.3%	37.7%	0.32
Endocrine therapy	19.7%	22.1%	0.60
No hours sport, weekly	1.5 (0.0-3.0)	2.0 (0.0-3.0)	0.87
No hours sitting, daily	6.0 (5.0-8.0)	6.0 (4.0-8.0)	0.045

Table 1. Continued

	Age 60-70 Premenopausal RRSO (RRSO ≤45)	Age 60-70 Postmenopausal RRSO (RRSO>55)	p-value
	N=208	**N=122**	
BMI, kg/m2	25.1 (22.7-28.8)	25.3 (23.2-28.7)	0.99
Smoking			0.15
Active smoker	8.7%	4.9%	
Former smoker	51.9%	45.9%	
Never	39.4%	49.2%	
Alcohol >1 drinks daily	48.6%	50.0%	0.80
Hypertension**	53.4%	65.6%	0.31
Dyslipidemia***	38.9%	48.4%	0.095
Depression, ever	14.5%	11.6%	0.46
Chronic disease, ever any	48.3%	45.5%	0.62

Values are percentages(%) for categorical variables, means with standard deviation for normal distributed variables, and medians with interquartile range for variables with a skewed distribution. P-value was calculated using independent samples t-test, X2 test and Mann-Whitney U test.

GPV, germline pathogenic variant; MHT, menopausal hormone therapy;; BMI, body mass index; LDL, low density lipoprotein.

*Due to COVID -19 some of the participants of the questionnaires were not able to participate in the clinical visit. Data shown is of the participants who completed the clinical visit.

**Hypertension: either antihypertensive medication, a systolic blood pressure >140mmHg or a diastolic blood pressure >90mmHg.

***dDyslipidemia: either lipid lowering medication or a LDL cholesterol >4.0 mmol/L.

Comprehensive overview of previously published potential adverse health outcomes after a premenopausal RRSO compared with a postmenopausal RRSO in women aged 60-70 at study visit

With regard to subclinical atherosclerosis, the premenopausal RRSO group showed no evidence of increased risk of CAC scores compared with the postmenopausal RRSO group (see Table 2) [28]. MHT use turned out not to be a confounder in our CAC analyses and adding MHT use to our model did not influence the outcomes. With regard to arterial stiffness, we did not observe increased PWV levels in women in the premenopausal RRSO group compared with women in the postmenopausal RRSO group. Regarding BMD: Z-scores of the lumbar spine were significantly lower in women who underwent a premenopausal RRSO compared with women who underwent a postmenopausal RRSO (β -0.49, 95% CI, -0.86, -0.12). However, there was no difference in the prevalence of osteopenia/osteoporosis (T-score ≤-1.0) in the premenopausal RRSO group compared with the postmenopausal RRSO group.

MHT use was included in these models as it was a confounder in our analyses. Regarding cognitive functioning, we found no differences in objective cognition between the premenopausal and postmenopausal RRSO groups. However, women in the premenopausal RRSO group more often reported (subjective) problems with reasoning (RR: 1.19, 95% CI, 1.01–3.60).[10] Regarding sexual functioning: women who underwent a premenopausal RRSO experienced more vaginal dryness and reported more sexual discomfort compared with women who underwent a postmenopausal RRSO (odds ratio (OR): 2.6, 95% CI 1.4-4.7 for vaginal dryness; OR: 3.1, 95% CI 1.04-9.4 for sexual discomfort), however, this did not lead to differences in sexual pleasure.[8] Regarding urinary incontinence: overall symptomatic urinary incontinence and urge urinary incontinence was not significantly different between the pre- and postmenopausal RRSO groups (OR: 2.1, 95% CI 0.93-4.78 for overall urinary incontinence).[9] A premenopausal RRSO was associated with increased risk of stress urinary incontinence (OR: 3.5, 95% CI 1.2-10.0).

Table 2. Influence of premenopausal RRSO versus postmenopausal RRSO on main outcomes of the HARMOny study in women aged 60-70 years at study visit.

	Premenopausal RRSO (RRSO≤45) N=208	**Postmenopausal RRSO (RRSO≥54) N=122**	**Multivariably adjusted risk of outcomes associated with a pre- vs postmenopausal RRSO (95% CI)**
Coronary artery calcification			**Relative risk[1]**
CAC > 0, (%)	40.9%	45.7%	1.07 (0.83-1.37)
CAC > 100, (%)	10.7%	15.9%	0.89 (0.52-1.52)
CAC > 400, (%)	3.8%	4.7%	0.61 (0.21-1.74)
MESA percentile > 75%, (%)	28.9%	30.4%	1.13 (0.72-1.80)
Bone mineral density			**Beta coefficient[2]**
Z-score lumbar spine (LS), mean (sd)	0.8 (1.5)	1.1 (1.7)	-0.49 (-0.86, -0.12)
Z-score femoral neck (FN), mean (sd)	0.2 (0.9)	0.4 (1.2)	-0.32 (-0.71, 0.07)
			Relative risk[3]
Any osteopenia/osteoporosis*, (%)	72.1%	64.5%	1.01 (0.79-1.29)
Osteopenia/osteoporosis* LS and FN, (%)	38.7%	37.9%	1.19 (0.77-1.84)
Objective cognitive functioning ACS			**Beta coefficient[4]**
Z-score verbal memory, mean (sd)	-0.17 (0.94)	-0.23 (0.88)	0.04 (-0.25, 0.34)
Z-score speed, mean (sd)	0.18 (0.89)	-0.02 (0.90)	-0.02 (-0.31, 0.27)
Z-score executive functioning, mean (sd)	0.30 (0.78)	0.10 (0.81)	0.03 (-0.26, 0.31)

Table 2. Continued

	Premenopausal RRSO (RRSO≤45) N=208	Postmenopausal RRSO (RRSO≥54) N=122	Multivariably adjusted risk of outcomes associated with a pre- vs postmenopausal RRSO (95% CI)
Z-score attention, mean (sd)	0.10 (0.79)	0.01 (0.69)	0.02 (-0.22, 0.26)
Subjective cognitive functioning ACS			**Odds ratio[4]**
Problems with reasoning, (%)	10.9%	8.1%	1.91 (1.01, 3.60)
Problems with memory, (%)	13.2%	11.7%	1.23 (0.65, 2.35)
Problems with attention, (%)	13.3%	7.3%	1.10 (0.58, 2.08)
Problems with concentration, (%)	9.2%	8.2%	1.53 (0.81, 2.87)
Problems with multitasking, (%)	5.2%	3.6%	1.66 (0.82, 3.37)
Problems with slow thinking, (%)	2.9%	2.7%	1.18 (0.60, 2.31)
Sexual functioning			**Beta coefficient[5]**
SAQ sexual pleasure score, mean (sd)	8.6 (3.7)	8.6 (3.0)	0.4 (-1.6-2.4)
			Odds ratio[5]
Substantial sexual discomfort, (%)	35.6%	20.9%	3.1 (1.04-9.36)
Substantial vaginal dryness, (%)	47.0%	31.1%	2.56 (1.40-4.68)
Urinary incontinence			**Odds ratio[6]**
Symptomatic urinary incontinence, (%)	23.6%	18.9%	2.1 (0.9-4.8)
Urge urinary incontinence, (%)	19.6%	22.7%	1.1 (0.5-2.4)
Stress urinary incontinence, (%)	13.0%	8.0%	3.5 (1.2-10.0)
HRQOL SF-36 scale			**Relative risk[7]**
SF-36 PCS ≤50.0, (%)	51.3%	49.5%	0.96 (0.69-1.33)
SF-36 MCS ≤42.0, (%)	12.8%	7.9%	1.14 (0.35-3.77)
Cancer Worry scale			**Relative risk[8]**
Fear of cancer (score ≥14), (%)	51.8%	50.0%	0.81 (0.61-1.09)

Values are percentages (%) for categorical variables and means with standard deviation for normal distributed variables. PCS: physical component score, MCS; mental component score; SAQ: sexual activity questionnaire; sd: standard deviation; LS: lumbar spine; FN: femoral neck; CAC: coronary artery calcium; ACS: Amsterdam cognition scale.

[1.] Adjusted for age, hypertension and dyslipidemia. [2.] Adjusted for MHT, BMI and AR use. [3.] Adjusted for age, MHT, BMI and AR use. [4.] Adjusted for breast cancer, MHT, depression, educational level. [5.] Adjusted for age, breast cancer, MHT, BMI and body image. [6.] Adjusted for age, breast cancer, BMI parity and delivery mode. [7.] Adjusted for age, parity, work status and daily sitting hours. [8.] Adjusted for age. 1-6 previously published results, 7-8 unpublished data.

* Osteopenia/osteoporosis is defined as T-score ≤-1.0.

** % often/most of the time/always problems.

Health-related quality of life after premenopausal vs postmenopausal RRSO in women aged 60-70 at study visit

Univariate analyses showed no differences in reported quality of life between the pre- and postmenopausal RRSO groups, neither on the physical nor on the mental subscales (effect size <0.50 for all domains Table S2). HRQOL of the premenopausal RRSO group measured on all domains was at least as good as the reference data women in the Dutch general population in the same age group (see Figure 2). In addition, multivariable analyses showed no difference in risk of having a PCS or MCS score below the cutoff in the premenopausal RRSO group compared with the postmenopausal RRSO group (RR: 1.14, 95% CI 0.35-3.77 for MCS ≤ 42.0; RR 0.99, 95% CI 0.72-1.37 for PCS ≤ 50.0, see Table 2). Adding MHT use to the model did not change the outcome.

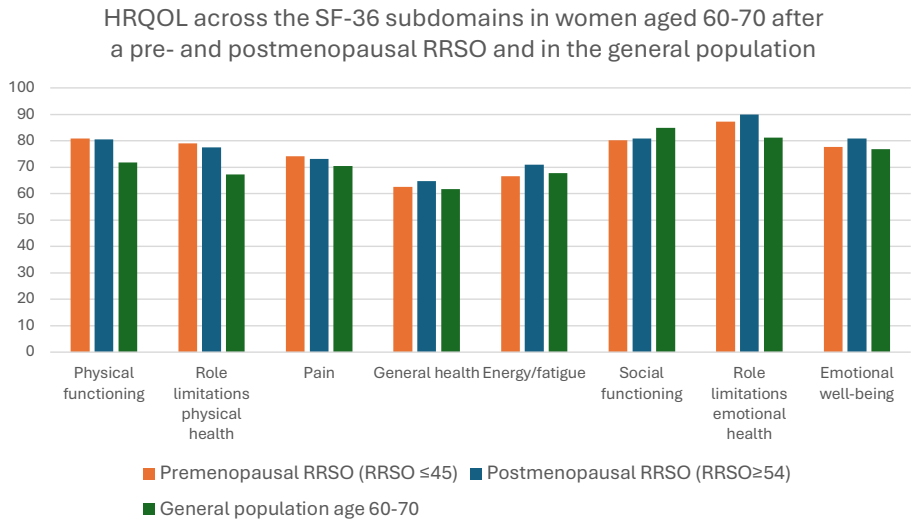

Figure 2. Descriptive statistics quality of life outcomes HARMOny study in women aged 60-70 at study visit and SF-36 norm data from women in the Dutch general population aged 60-70 years.[29]

The SF-36 domain scores linearly converted to a 0 to 100 scale, with higher scores indicating higher levels of HRQOL. Abbreviations: RRSO: risk-reducing salpingo-oophorectomy.

Cancer worries after premenopausal vs postmenopausal RRSO in women aged 60-70 years at study visit

In univariate analyses, there were no differences in CWS score or prevalence of fear of cancer between the premenopausal and postmenopausal RRSO groups (51.8% vs 50.0% reported fear of cancer, see Table 2). In addition, multivariable analyses showed no difference in fear of cancer (RR: 0.81, 95% CI 0.61-1.09, see Table 2).

Subgroup analyses in women who underwent a premenopausal RRSO

Within the entire premenopausal RRSO group (n=500), we compared women with an RRSO before the age of 41 (early premenopausal RRSO group, n=159) with women with an RRSO between the age of 41 and 45 years old (late premenopausal RRSO group, n=341). Participant characteristics are provided in the supplements (Table S3).

Potential long-term adverse health outcomes according to of timing of premenopausal RRSO (<41 years vs 41-45 years, previously published results)

Among women with a premenopausal RRSO, timing of premenopausal RRSO (<41yrs vs 41-45yrs) did not significantly influence CAC scores, PWV levels, BMD, sexual functioning or urinary incontinence risk (see Table 3). Although we did not observe significant differences in objective cognitive functioning, women in the early premenopausal RRSO group more often reported problems with reasoning and slow thinking (RR: 0.60, 95% CI, 0.39–0.94; RR: 0.56, 95% CI 0.36–0.89, respectively).

HRQOL and cancer worries according to timing of premenopausal RRSO (<41 years vs 41-45 years)

Women who underwent an early premenopausal RRSO reported comparable HRQOL on all subdomains of the SF-36 compared with the late premenopausal RRSO group. In multivariable analyses, we found no association between timing of premenopausal RRSO and a low SF36-PCS or SF36-MCS (RR: 0.99, 95% CI 0.81-1.22 for PCS ≤ 50.0; RR: 0.83, 95% CI 0.51-1.36 for MCS ≤ 42.0; see Table 3). Including MHT use or breast cancer history did not change the outcomes. In univariate analyses, there were no differences in CWS score or prevalence of fear of cancer between the early- and late premenopausal RRSO groups (51.8% vs 50.0% reported fear of cancer, see Table S4). In addition, multivariable analyses showed no difference in fear of cancer (RR: 0.81, 95% CI 0.61-1.09, see Table 4).

Table 3. Influence of timing of RRSO (<41 vs 41-45yrs) on main outcomes of the HARMOny study in women with a premenopausal RRSO.

	RRSO <41 years N=159	RRSO 41-45 years N=341	Multivariably adjusted risk of outcomes (95% CI)
Coronary artery calcification			**Relative risk[1]**
CAC > 0, (%)	40.9%	45.7%	0.93 (0.75-1.15)
CAC > 100, (%)	10.7%	15.9%	0.71 (0.43-1.17)
CAC > 400, (%)	3.8%	4.7%	0.81 (0.34-2.13)
MESA percentile > 75%, (%)	28.9%	30.4%	0.96 (0.72-1.28)
Bone mineral density			**Beta coefficient[2]**
Z-score lumbar spine (LS), mean (sd)	0.4 (1.4)	0.6 (1.4)	-0.09 (-0.33, 0.16)
Z-score femoral neck (FN), mean (sd)	0.1 (1.0)	0.2 (0.9)	-0.01 (-0.02, 0.26)
			Relative risk[3]
Any osteopenia/osteoporosis*, (%)	68.6%	70.7%	0.92 (0.80, 1.05)
Osteopenia/osteoporosis* LS and FN, (%)	35.9%	36.4%	0.92 (0.71, 1.21)
Objective cognitive functioning ACS			**Beta coefficient[4]**
Z-score verbal memory, mean (sd)	-0.22 (0.98)	-0.22 (0.97)	0.07 (-0.17-0.31)
Z-score speed, mean (sd)	0.34 (0.80)	0.14 (0.81)	0.13 (-0.05-0.31)
Z-score executive functioning, mean (sd)	0.44 (0.72)	0.29 (0.80)(	0.18 (-0.01-0.38)
Z-score attention, mean (sd)	0.20 (0.72)	0.07 (0.77)	0.13 (-0.04-0.31)
Subjective cognitive functioning ACS			**Odds ratio[4]**
Problems with reasoning, (%)	14.0%	13.9%	0.76 (0.38-1.51)
Problems with memory, (%)	19.2%	21.9%	0.97 (0.55-0.99)
Problems with attention, (%)	16.2%	19.0%	0.97 (0.53-1.77)
Problems with concentration, (%)	16.2%	19.7%	0.90 (0.48-1.66)
Problems with multitasking, (%)	12.3%	10.2%	0.62 (0.28-1.39)
Problems with slow thinking, (%)	8.1%	8.0%	0.67 (0.25-1.79)
Sexual functioning			**Beta coefficient[5]**
SAQ sexual pleasure score, mean (sd)	9.1	8.1	-1.01 (-1.97;-0.04)
			Odds ratio[5]
Substantial sexual discomfort, (%)	37.5%	41.5%	1.03 (0.59-1.79)
Substantial vaginal dryness, (%)	41.8%	49.0%	0.87 (0.57-1.33)
Urinary incontinence			**Odds ratio[6]**
Symptomatic urinary incontinence, (%)	22.6%	20.5%	1.00 (0.95-1.04)
Urge urinary incontinence, (%)	12.5%	16.6%	0.54 (0.28-1.04)

Table 3. Continued

	RRSO <41 years N=159	RRSO 41-45 years N=341	Multivariably adjusted risk of outcomes (95% CI)
Stress urinary incontinence, (%)	11.8%	12.5%	1.00 (0.52-1.92)
HRQOL SF-36 scale			**Relative risk[7]**
SF-36 PCS ≤50.0, (%)	48.0%	50.2%	0.99 (0.81-1.22)
SF-36 MCS ≤42.0, (%)	13.3%	17.4%	0.83 (0.51-1.36)
Cancer Worry scale			**Relative risk[8]**
Fear of cancer (score ≥14), (%)	51.1%	52.1%	0.98 (0.81-1.19)

Values are percentages (%) for categorical variables and means with standard deviation for normal distributed variables. PCS: physical component score, MCS; mental component score; SAQ: sexual activity questionnaire; sd: standard deviation; LS: lumbar spine; FN: femoral neck; CAC: coronary artery calcium; ACS: Amsterdam cognition scale.

[1.] Adjusted for age, hypertension and dyslipidemia. [2.] Adjusted for MHT, BMI and AR use. [3.] Adjusted for age, MHT, BMI and AR use. [4.] Adjusted for breast cancer, MHT, depression, educational level. [5.] Adjusted for age, breast cancer, MHT, BMI and body image. [6.] Adjusted for age, breast cancer, BMI parity and delivery mode. [7.] Adjusted for age, parity, work status and daily sitting hours. [8.] Adjusted for age. 1-6 previously published results, 7-8 unpublished data.

* Osteopenia/osteoporosis is defined as T-score ≤-1.0.

** % often/most of the time/always problems.

Perception of the decision to undergo RRSO

In the entire premenopausal RRSO group, a substantial proportion of women experienced the choice whether or not to undergo RRSO as difficult (21.2% difficult; 7.5% neutral; 71.7% not difficult; see Table 4). The large majority (75.9%) considered themselves to be well-informed when deciding to undergo RRSO (16.1% disagreed). In general, the decision to undergo RRSO lead to less fear of cancer (70.9% in the premenopausal RRSO group, 70.5% in the postmenopausal RRSO group).

Table 4. Decision to undergo RRSO: perception in retrospect in the entire HARMOny study (n=740).

	Premenopausal RRSO	Postmenopausal RRSO	Total
	N=500	N=240	
The decision was difficult			
Totally disagree	50.6%	61.4%	53.7%
Disagree	20.6%	19.0%	20.2%
Neutral	7.5%	6.5%	7.2%
Agree	13.7%	3.8%	10.9%
Totally agree	7.5%	9.2%	8.0%

Table 4. Continued

	Premenopausal RRSO	Postmenopausal RRSO	Total
	N=500	N=240	
I was well informed when making my decision			
Totally disagree	10.5%	13.5%	11.4%
Disagree	5.8%	2.1%	4.7%
Neutral	7.9%	3.7%	6.7%
Agree	31.0%	18.8%	27.4%
Totally agree	44.9%	62.0%	49.9%
This decision has reduced my fear of cancer			
Totally disagree	6.4%	10.0%	7.5%
Disagree	7.9%	3.2%	6.5%
Neutral	14.8%	16.3%	15.2%
Agree	32.6%	22.6%	29.7%
Totally agree	38.3%	47.9%	41.1%

Discussion

The HARMOny study is the first to investigate the long-term health effects of a premenopausal RRSO (15-25 years after surgery) on a number of outcomes, including HRQOL. Overall, the results are quite reassuring for women undergoing premenopausal RRSO.

Regarding adverse health outcomes, we did not find any evidence for a possibly increased risk of (subclinical) CVD in the premenopausal compared with the postmenopausal RRSO group. Although women who underwent a premenopausal RRSO had lower bone mineral density, they did not have increased risk of osteopenia or osteoporosis. While women in the premenopausal RRSO group reported more (subjective) problems with reasoning, we found no differences in objectively measured cognition using a validated online cognition test. Furthermore, we found no significant differences in sexual pleasure or urge urinary incontinence between the pre- and postmenopausal RRSO groups. We did observe, however, more vaginal dryness, sexual discomfort and more stress urinary incontinence in the premenopausal compared with the postmenopausal RRSO group. While these differences were statistically significant, they were rather small and their clinical relevance is debatable. We observed no differences in our outcomes of interest according to timing of premenopausal RRSO (RRSO<41 years vs RRSO 41-45 years).

Rather few women in our premenopausal RRSO group had used MHT, this may be related to the relative low frequency of MHT use in the Netherlands in general.[31] MHT use turned out not to be a confounder in our CAC analyses and we did not find a protective effect of MHT use, neither for ever use nor for the duration of use.[11] In contrast, MHT was a confounder for the association between premenopausal RRSO and both BMD and sexual functioning. Women who ever used MHT were at increased risk of experiencing symptomatic urinary incontinence, possibly because they were prescribed MHT more often for this very reason. Since data on the type of MHT use were largely missing, we were not able to investigate possible explanations regarding specific treatments further.

Regarding HRQOL, we observed no differences between women in the premenopausal and postmenopausal RRSO groups in women who were 60-70 years at study visit. In addition, the SF-36 scores of the premenopausal RRSO group were at as good as scores in the postmenopausal RRSO group and the general population on both the mental and physical health subdomains.[29] Our findings are consistent with studies with relatively shorter follow-up times (median up to 5 years), showing no clinically significant differences in HRQOL after RRSO compared with the general population or with women at high familial risk of ovarian cancer who underwent screening.[12-13] However, most studies did not account for timing of the RRSO or MHT use, obscuring possible effects of estrogen deficiency on HRQOL. In our study, we found no influence of ever MHT use on HRQOL in women aged 60-70 at study. In addition, two recent prospective trials that compared RRSO with salpingectomy with delayed oophorectomy (and therefore delayed estrogen deficiency) found no differences in HRQOL 1 year after surgery.[32-33]

Unsurprisingly, we observed no long-term influence of the timing of RRSO on fear of cancer, neither for pre vs postmenopausal RRSO, nor for RRSO < 41 years vs RRSO between 41-45 years (prevalence of fear of cancer ≈ 51% in all groups (see Table 3 and 4). Importantly, a large majority of the women in our study reported that the decision to undergo RRSO substantially reduced their fear of cancer (70.9% in the premenopausal RRSO group; 70.5% in the postmenopausal RRSO group, see Table 5). Studies suggest that women who underwent an RRSO had less short-term fear of cancer compared to women who underwent screening for familial ovarian cancer.[14-18] Because all women in our study underwent RRSO, it was not feasible to investigate the long-term effects of undergoing an RRSO compared to no RRSO on the fear of cancer. In the premenopausal RRSO group, 16.1% of the women reported they were not well informed when making the decision to undergo RRSO, this is important information for health care professionals involved in counseling these women.

Strengths and limitations

The HARMOny study has several strengths such as its large sample size, a good participation rate of 61.8%, the fact that is was nested in an established cohort, and the use of a comparison group of women with a postmenopausal RRSO selected from the same cohort. By directly comparing women at high familial risk of ovarian cancer with a pre- or postmenopausal RRSO, potential confounding by indication for surgical menopause and selection bias was strongly reduced. Such biases affected the results of most other studies in this research field because these reports included women with indications for oophorectomy other than RRSO in the intervention group and (premenopausal) women from the general population in the comparison group.

A limitation of our study is the difference in age between the premenopausal RRSO (≤45 years) and postmenopausal RRSO (≥54 years) groups in the entire study population. However, we addressed this limitation by restricting our analyses to women aged 60-70 years at study enrollment. In addition, we used the entire premenopausal RRSO group to assess the association between timing of a premenopausal RRSO (≤41 years vs 41-45 years) and potential long-term adverse outcomes. When interpreting our results, it is important to note that 98% of the participants were Caucasian. Selection bias may also have occurred due to differences in response rates between the premenopausal (68.0%) and postmenopausal groups (50.8%). A likely explanation is that women in the postmenopausal RRSO group felt less inclined to participate as our research hypotheses focused on the consequences of premenopausal surgical menopause. However, there is a possibility that the relatively older women eligible for the postmenopausal RRSO group did not participate in our study because of morbidity or mortality directly caused by outcomes in our study, especially CVD-related outcomes. We addressed this potential bias by using previously collected data from questionnaire surveys completed for the HEBON cohort in which our study was nested. [20] In these questionnaires, current non-responders in the postmenopausal RRSO group did not report a lower or higher prevalence of cardiovascular disease than responders.

Conclusion

A premenopausal RRSO does not appear to be associated with long-term cardiovascular disease risk, cognition, HRQOL or fear of cancer. However, it is associated with sexual functioning and bone mineral density. Overall, the results are quite reassuring for women undergoing premenopausal RRSO.

References

1. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA. 2017;317(23):2402-16.
2. Henderson JT, Webber EM, Sawaya GF. Screening for Ovarian Cancer: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA. 2018;319(6):595-606.
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(4):919-23.
4. Daly MB, Pal T, Berry MP, Buys SS, Dickson P, Domchek SM, et al. Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2021;19(1):77-102.
5. Domchek SM, Friebel TM, Singer CF, Evans DG, Lynch HT, Isaacs C, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA. 2010;304(9):967-75.
6. 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(2):80-7.
7. Eleje GU, Eke AC, Ezebialu IU, Ikechebelu JI, Ugwu EO, Okonkwo OO. Risk-reducing bilateral salpingo-oophorectomy in women with BRCA1 or BRCA2 mutations. Cochrane Database Syst Rev. 2018;8(8):CD012464.
8. Terra L, Beekman MJ, Engelhardt EG, Heemskerk-Gerritsen BAM, van Beurden M, Roeters van Lennep JE, et al. Sexual functioning more than 15 years after premenopausal risk-reducing salpingo-oophorectomy. Am J Obstet Gynecol. 2023;228(4):440 e1- e20.
9. Terra L, Heemskerk-Gerritsen BAM, Beekman MJ, Engelhardt E, Mourits MJE, van Doorn HC, et al. Urinary incontinence more than 15 years after premenopausal risk-reducing salpingo-oophorectomy: a multicentre cross-sectional study. BJOG. 2024;131(1):99-108.
10. Terra L, Lee Meeuw Kjoe PR, Agelink van Rentergem JA, Beekman MJ, Heemskerk-Gerritsen BAM, van Beurden M, et al. Long-term effects of premenopausal risk-reducing salpingo-oophorectomy on cognition in women with high familial risk of ovarian cancer: A cross-sectional study. BJOG. 2023;130(8):968-77.
11. Beekman MJ, Terra L, Heemskerk-Gerritsen BAM, Aalst CMvd, Lennep JERv, Beurden Mv, et al. Coronary Artery Calcium Scores After Prophylactic Premenopausal Bilateral Salpingo-Oophorectomy. JACC: CardioOncology. 2024;6(6):922-31.
12. Vermeulen RFM, Beurden MV, Korse CM, Kenter GG. Impact of risk-reducing salpingo-oophorectomy in premenopausal women. Climacteric. 2017;20(3):212-21.
13. Wei X, Oxley S, Sideris M, Kalra A, Brentnall A, Sun L, et al. Quality of life after risk-reducing surgery for breast and ovarian cancer prevention: a systematic review and meta-analysis. Am J Obstet Gynecol. 2023;229(4):388-409 e4.
14. Madalinska JB, Hollenstein J, Bleiker E, van Beurden M, Valdimarsdottir HB, Massuger LF, et al. Quality-of-life effects of prophylactic salpingo-oophorectomy versus gynecologic screening among women at increased risk of hereditary ovarian cancer. J Clin Oncol. 2005;23(28):6890-8.
15. Hall E, Finch A, Jacobson M, Rosen B, Metcalfe K, Sun P, et al. Effects of bilateral salpingo-oophorectomy on menopausal symptoms and sexual functioning among women with a BRCA1 or BRCA2 mutation. Gynecol Oncol. 2019;152(1):145-50.

16. Westin SN, Sun CC, Lu KH, Schmeler KM, Soliman PT, Lacour RA, et al. Satisfaction with ovarian carcinoma risk-reduction strategies among women at high risk for breast and ovarian carcinoma. Cancer. 2011;117(12):2659-67.
17. Hooker GW, King L, Vanhusen L, Graves K, Peshkin BN, Isaacs C, et al. Long-term satisfaction and quality of life following risk reducing surgery in BRCA1/2 mutation carriers. Hered Cancer Clin Pract. 2014;12(1):9.
18. Finch A, Metcalfe KA, Chiang J, Elit L, McLaughlin J, Springate C, et al. The impact of prophylactic salpingo-oophorectomy on quality of life and psychological distress in women with a BRCA mutation. Psychooncology. 2013;22(1):212-9.
19. Terra L, Hooning MJ, Heemskerk-Gerritsen BAM, van Beurden M, Roeters van Lennep JE, van Doorn HC, et al. Long-Term Morbidity and Health After Early Menopause Due to Oophorectomy in Women at Increased Risk of Ovarian Cancer: Protocol for a Nationwide Cross-Sectional Study With Prospective Follow-Up (HARMOny Study). JMIR Res Protoc. 2021;10(1):e24414.
20. Brohet RM, Velthuizen ME, Hogervorst FB, Meijers-Heijboer HE, Seynaeve C, Collee MJ, et al. Breast and ovarian cancer risks in a large series of clinically ascertained families with a high proportion of BRCA1 and BRCA2 Dutch founder mutations. J Med Genet. 2014;51(2):98-107.
21. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992;30(6):473-83.
22. Aaronson NK, Ahmedzai S, Bergman B, Bullinger M, Cull A, Duez NJ, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. J Natl Cancer Inst. 1993;85(5):365-76.
23. Ware JE, Kosinski M. SF-36 physical & mental health summary scales: a manual for users of version 1: Quality Metric; 2001.
24. Ware JE, Jr. SF-36 health survey update. Spine (Phila Pa 1976). 2000;25(24):3130-9.
25. Lerman C, Daly M, Masny A, Balshem A. Attitudes about genetic testing for breast-ovarian cancer susceptibility. J Clin Oncol. 1994;12(4):843-50.
26. Custers JA, van den Berg SW, van Laarhoven HW, Bleiker EM, Gielissen MF, Prins JB. The Cancer Worry Scale: detecting fear of recurrence in breast cancer survivors. Cancer Nurs. 2014;37(1):E44-50.
27. Douma KF, Aaronson NK, Vasen HF, Gerritsma MA, Gundy CM, Janssen EP, et al. Psychological distress and use of psychosocial support in familial adenomatous polyposis. Psychooncology. 2010;19(3):289-98.
28. Beekman MJ, Terra L, Heemskerk-Gerritsen BAM, van der Aalst CM, Roeters van Lennep JE, van Beurden M, et al. Coronary Artery Calcium Scores after Prophylactic Premenopausal Bilateral Salpingo-Oophorectomy [In press]. JACC CardioOncol. 2024.
29. Aaronson NK, Muller M, Cohen PD, Essink-Bot ML, Fekkes M, Sanderman R, et al. Translation, validation, and norming of the Dutch language version of the SF-36 Health Survey in community and chronic disease populations. J Clin Epidemiol. 1998;51(11):1055-68.
30. Ware JE, Jr., Gandek B, Kosinski M, Aaronson NK, Apolone G, Brazier J, et al. The equivalence of SF-36 summary health scores estimated using standard and country-specific algorithms in 10 countries: results from the IQOLA Project. International Quality of Life Assessment. J Clin Epidemiol. 1998;51(11):1167-70.

31. Faber A, Bouvy ML, Loskamp L, van de Berg PB, Egberts TC, de Jong-van den Berg LT. Dramatic change in prescribing of hormone replacement therapy in The Netherlands after publication of the Million Women Study: a follow-up study. Br J Clin Pharmacol. 2005;60(6):641-7.
32. Nebgen DR, Hurteau J, Holman LL, Bradford A, Munsell MF, Soletsky BR, et al. Bilateral salpingectomy with delayed oophorectomy for ovarian cancer risk reduction: A pilot study in women with BRCA1/2 mutations. Gynecol Oncol. 2018;150(1):79-84.
33. Steenbeek MP, Harmsen MG, Hoogerbrugge N, de Jong MA, Maas A, Prins JB, et al. Association of Salpingectomy With Delayed Oophorectomy Versus Salpingo-oophorectomy With Quality of Life in BRCA1/2 Pathogenic Variant Carriers: A Nonrandomized Controlled Trial. JAMA Oncol. 2021;7(8):1203-12.

Declarations

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Conflict of interest disclosure

The authors report no conflicts of interest.

Data availability statement

With publication, de-identified data collected for the study, including participant data, will be made available to others upon reasonable request. Data can be requested with a proposal by sending an e-mail to the corresponding author.

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Ethics approval statement

This study was conducted according to the standards of Good Clinical Practice, in agreement with the principles of the Declaration of Helsinki and with the Dutch law as stated in the Medical Research Involving Human Subjects Act (WMO). The study has been approved in writing by the Institutional Review Board of the AVL/NKI to be conducted in all nine University Medical Centers and the Antoni van Leeuwenhoek.

Patient consent statement

All participants included in this study signed informed consent.

Clinical trial registration

The pre-registered clinical trial number is <NCT03835793>

Supplements

Table S1. Patient characteristics women in the HARMOny study (n=740)*.

	Premenopausal RRSO (RRSO ≤45)	**Postmenopausal RRSO (RRSO>55)**	**p-value**
	N=500	**N=240**	
Age at study, years	59.2 (57.6-62.1)	69.8 (67.0-73.2)	<0.001
Age at menopause, years	42.0 (40.0-44.0)	51.0 (50.0-53.)	<0.001
Time since RRSO years	18.1 (15.3-21.3)	11.6 (10.2-13.9)	<0.001
***BRCA* GPV carrier status**			<0.001
BRCA1 germline mutation	48.2%	29.6%	
BRCA2 germline mutation	19.2%	35.8%	
Non-carrier of *BRCA1/2*	32.6%	34.6%	
Education			<0.001
Primary school/lower level high school	31.3%	44.6%	
Middle level high school	35.8%	17.7%	
Advanced vocational/ university	32.9%	37.7%	
Work status			<0.001
Full-time/part-time job	64.1%	9.8%	
Retired	8.2%	72.9%	
Housewife/voluntary work	12.3%	13.1%	
Completely/partially incapacitated	12.9%	4.2%	
Unemployed	2.6%	0.0%	
Children, one or more	83.1%	84.5%	0.65
Ever MHT use	29.9%	10.8%	<0.001
History of breast cancer	59.8%	65.4%	0.14
Radiotherapy	38.0%	40.0%	0.60
Chemotherapy	45.8%	35.2%	0.006
Endocrine therapy	23.2%	21.9%	0.70
No hours sport, weekly	1.5 (0.0-3.0)	2.0 (0.0-3.0)	0.45
No hours sitting, daily	6.0 (4.0-8.0)	6.0 (4.0-8.0)	0.23

Table S1. Continued

	Premenopausal RRSO (RRSO ≤45)	**Postmenopausal RRSO (RRSO>55)**	**p-value**
	N=500	**N=240**	
BMI, kg/m2	25.3 (22.8-29.0)	25.0 (22.9-28.7)	0.34
Smoking			0.71
Active smoker	9.8%	3.3%	
Former smoker	41.0%	52.1%	
Never	49.2%	44.6%	
Alcohol >1 drinks daily	53.8%	50.4%	0.39
Hypertension**	44.6%	66.7%	<0.001
Dyslipidemia***	36.2%	45.8%	0.012
Depression, ever	15.5%	11.4%	0.15
Chronic disease, ever any	47.2%	47.9%	0.86

Values are percentages(%) for categorical variables, means with standard deviation for normal distributed variables, and medians with interquartile range for variables with a skewed distribution. P-value was calculated using independent samples t-test, X2 test and Mann-Whitney U test.

GPV, germline pathogenic variant; MHT, menopausal hormone therapy; BMI, body mass index; LDL, low density lipoprotein.

*Due to COVID -19 some of the participants of the questionnaires were not able to participate in the clinical visit. Data shown is of the participants that completed the clinical visit.

**Hypertension: either antihypertensive medication, a systolic blood pressure >140mmHg or a diastolic blood pressure >90mmHg.

***Dyslipidemia: either lipid lowering medication or a LDL cholesterol >4.0 mmol/L.

Table S2. Descriptive statistics quality of life outcomes HARMOny study in women aged 60-70 at study visit and SF-36 data from the Dutch general population in women aged 60-70 years.

	Premenopausal RRSO (RRSO ≤45)	Postmenopausal RRSO (RRSO≥54)	Univariate analyses	Effect size	General population age 60-70[29-30]
	Score	Score	p-value	Cohen's d	Score
SF-36 scale					
Physical functioning	80.8 (21.2)	80.5 (19.6)	0.89	-0.02	70.4 (25.5)
Role limitations physical health	79.0 (35.9)	77.5 (36.5)	0.72	-0.04	64.4 (43.9)
Role limitations emotional health	87.2 (29.7)	90.0 (26.9)	0.40	0.10	79.4 (36.4)
Pain	74.2 (22.4)	73.2 (21.4)	0.71	-0.04	67.9 (24.8)
General health	62.6 (20.8)	64.7 (19.2)	0.38	0.10	62.8 (18.5)
Social functioning	80.2 (23.8)	80.8 (22.9)	0.59	0.03	82.3 (24.5)
Emotional well-being	77.6 (15.0)	80.8 (14.7)	0.066	0.22	73.4 (19.4)
Energy/fatigue	66.6 (18.7)	70.9 (18.1)	**0.048**	0.23	65.5 (20.1)
SF-36 component scores					
Physical component score (PCS)	49.7 (43.3-54.4)	50.2 (43.4-53.4)	0.51	-0.07	43.9 (11.2)
Mental component score (MCS)	54.7 (47.9-58.7)	56.8 (53.1-59.5)	**0.019**	0.27	52.2 (10.5)
Lerman Cancer Worry scale					
Total 8-point scale	14.1 (4.4)	14.7 (4.4)	0.31	0.13	
Fear of cancer (score ≥14)	51.8%	50.0%	0.69		

Values are means with standard deviation. P-value was calculated using independent samples t-test.
The SF-36 domain scores linearly converted to a 0 to 100 scale, with higher scores indicating higher levels of HRQOL.

Table S3. Descriptive statistics of women with a premenopausal RRSO (n=500).

	RRSO<41yr	RRSO 41-45yr	p-value
	N=159	N=341	
Age at study visit, years	58.8 (57.2-61.6)	59.5 (57.8-62.3)	0.020
Age at menopause, years	39.0 (37.0-40.0)	43.0 (42.0-44.0)	<0.001
Time since RRSO years	20.9 (19.1-23.3)	16.6 (14.3-19.5)	<0.001
BRCA PV carrier status			<0.001
BRCA1 germline mutation	60.4%	42.5%	
BRCA2 germline mutation	14.5%	21.4%	
Non-carrier of BRCA1/2	25.2%	36.1%	
Educational level			0.36
Primary school/lower level high school	31.5%	31.1%	
Middle level high school	30.2%	38.6%	
Advanced vocational/university	38.3%	30.5%	
Employment status			0.56
Completely/partially incapacitated for work	12.9%	12.9%	
Full-time job/part-time job	65.3%	63.5%	
Housewife/voluntary work	14.3%	11.3%	
Retired	5.4%	9.4%	
Unemployed	2.0%	2.8%	
Children, one or more			
MHT use, ever any	48.4%	21.2%	<0.001
Breast cancer	50.9%	63.9%	0.006
Radiotherapy	32.1%	40.8%	0.062
Parasternal radiotherapy	7.4%	9.3%	0.48
Chemotherapy	37.1%	49.9%	0.008
Endocrine therapy	11.9%	28.4%	<0.001
No hours sport, weekly	1.5 (0.0-3.0)	1.0 (0.0-3.0)	0.80
No hours sitting, daily	6.0 (5.0-9.0)	6.0 (4.0-8.0)	0.37
BMI, kg/m2	24.5 (22.5-29.1)	25.6 (22.9-29.0)	0.28
Smoking			0.77
Active smoker	9.4%	10.0%	
Former smoker	39.0%	41.9%	
Never	51.6%	48.1%	

Table S3. Continued

	RRSO<41yr	RRSO 41-45yr	p-value
	N=159	N=341	
Alcohol >1 drinks daily	50.9%	55.1%	0.38
Hypertension*	41.5%	46.0%	0.34
Dyslipidemia**	38.4%	35.2%	0.49
Depression, ever	17.1%	14.7%	0.49
Chronic disease, ever any***	50.0%	45.9%	0.39

Values are percentages(%) for categorical variables, means with standard deviation for normal distributed variables, and medians with interquartile range for variables with a skewed distribution. P-value was calculated using independent samples t-test, X2 test and Mann-Whitney U test.

GPV, germline pathogenic variant; MHT, menopausal hormone therapy; BMI, body mass index; LDL, low density lipoprotein.

*Due to COVID -19 some of the participants of the questionnaires were not able to participate in the clinical visit. Data shown is of the participants that completed the clinical visit.

**Hypertension: either antihypertensive medication, a systolic blood pressure >140mmHg or a diastolic blood pressure >90mmHg.

***Dyslipidemia: either lipid lowering medication or a LDL cholesterol >4.0 mmol/L.

Table S4. Descriptive statistics quality of life outcomes HARMOny study in women with a premenopausal RRSO.

	RRSO <41 years	RRSO 41-45 years	Univariate analyses	Effect size
	Score	**Score**	**p-value**	**Cohen's d**
SF-36 scale				
Physical functioning	83.1 (20.4)	82.3 (20.5)	0.71	-0.04
Role limitations physical health	76.8 (36.6)	74.9 (38.2)	0.61	-0.05
Role limitations emotional health	87.5 (28.4)	85.0 (32.8)	0.42	-0.08
Pain	74.4 (22.7)	72.4 (23.2)	0.39	-0.08
General health	62.3 (21.3)	63.4 (20.7)	0.57	0.05
Social functioning	79.9 (25.3)	76.7 (25.8)	0.19	-0.13
Emotional well-being	79.1 (14.8)	76.0 (15.9)	**0.040**	-0.20
Energy/fatigue	66.3 (19.0)	63.4 (20.4)	0.13	-0.15
SF-36 component scores				
Physical component score (PCS)	47.3 (9.9)	47.1 (10.5)	0.84	-0.02
Mental component score (MCS)	52.7 (9.2)	50.8 (10.1)	0.051	-0.19
Lerman Cancer Worry scale				
Total 8-point scale	14.0 (4.6)	13.8 (4.3)	0.63	-0.05
Fear of cancer (score ≥14)	51.1%	52.1%	0.84	

Values are means with standard deviation. P-value was calculated using independent samples t-test. The SF-36 domain scores linearly converted to a 0 to 100 scale, with higher scores indicating higher levels of HRQOL.

Chapter 6

Sexual functioning more than 15 years after premenopausal risk-reducing salpingo-oophorectomy

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Abstract

Background

Women with a *BRCA1/2* pathogenic variant are advised to undergo premenopausal risk-reducing salpingo-oophorectomy after completion of childbearing, to reduce their risk of ovarian cancer. Several studies reported less sexual pleasure one to three years after a premenopausal oophorectomy. However, the long-term effects of a premenopausal oophorectomy on sexual functioning are unknown.

Objective

Our aim was to study long-term sexual functioning in women at increased familial risk of breast/ovarian cancer who underwent a risk-reducing salpingo-oophorectomy either before the age of 46 years (premenopausal group), or after the age of 54 years (postmenopausal group). We performed subgroup analyses in the premenopausal group, comparing early (before the age of 41 years) and later (at ages 41-45 years) premenopausal risk-reducing salpingo-oophorectomy.

Study design

Between 2018 and 2021, we invited 817 women with a high familial risk of breast/ovarian cancer from an ongoing cohort study to participate in our study. Due to a large difference in age at study between the premenopausal and postmenopausal salpingo-oophorectomy groups, we restricted the comparison of sexual functioning between the groups to 368 women who were 60-70 years old at completion of the questionnaire (premenopausal group, n=226, postmenopausal group, n=142). In 496 women with a premenopausal risk-reducing salpingo-oophorectomy we compared sexual functioning between women in the early premenopausal group (n=151) and the later premenopausal group (n=345). Differences between groups were analyzed using multiple regression analyses adjusting for current age, breast cancer history, use of hormone replacement therapy, body mass index, chronic medication use (yes/no) and body image.

Results

Mean time since risk-reducing salpingo-oophorectomy was 20.6 years in the premenopausal group and 10.6 years in the postmenopausal group (p-value <.001). In the premenopausal group, mean age at questionnaire completion was 62.7 years, versus 67.0 years in the postmenopausal group (p<.001). In the premenopausal group, 47.4% was still sexually active, compared to 48.9% of the postmenopausal group (p-value: .80). Current sexual pleasure scores were the same for women in the premenopausal group and the postmenopausal group (mean pleasure score 8.6, p-value .99). However, women in the premenopausal group more often reported

substantial discomfort than women in the postmenopausal group (35.6% compared with 20.9%, p-value .04). After adjusting for confounders, premenopausal risk-reducing salpingo-oophorectomy was associated with substantially more discomfort during sexual intercourse, compared to postmenopausal risk-reducing salpingo-oophorectomy (odds ratio 3.1, 95% confidence interval 1.04; 9.4). Moreover, following premenopausal risk-reducing salpingo-oophorectomy, more severe complaints of vaginal dryness were observed (odds ratio 2.6, 95% confidence interval 1.4; 4.7). Women with a risk-reducing salpingo-oophorectomy before age 41 reported similar pleasure and discomfort scores as women with a risk-reducing salpingo-oophorectomy between ages 41 and 45.

Conclusion

More than 15 years after premenopausal risk-reducing salpingo-oophorectomy, the proportion of sexually active women was comparable to that among women with a postmenopausal risk-reducing salpingo-oophorectomy. However, after a premenopausal risk-reducing salpingo-oophorectomy, women experienced more vaginal dryness and more often had substantial sexual discomfort during sexual intercourse. This did not lead to less pleasure with sexual activity.

Introduction

Risk-reducing salpingo-oophorectomy (RRSO) is performed to prevent ovarian/tubal cancer in women with a high familial risk, such as *BRCA1*/2 pathogenic variant (PV) carriers. RRSO is advised after completion of childbearing, preferably at ages 35-40 years for *BRCA1* PV carriers, and at ages 40-45 years for *BRCA2* PV carriers.[1] RRSO induces an immediate menopause which may result in short-term and long-term morbidity such as decreased psychosexual functioning.

Reduced circulating estrogen levels due to menopause result in vulvovaginal atrophy, which may predispose to micro-traumata when vaginal penetration occurs.[2] Up to 69% of postmenopausal women report vulvovaginal atrophy, with an increasing prevalence with a longer duration of menopause.[3-8] Hormone replacement therapy (HRT) may not alleviate symptoms and is often not recommended in *BRCA* PV carriers due to the risk of breast cancer.[9]

Several studies have examined the effect of RRSO on sexual functioning.[10] Most showed that, shortly after RRSO, women experienced more discomfort and less pleasure with sexual activity.[11-14] However, this difference was not observed six years after RRSO.[15] It is possible that women developed coping mechanisms or explored practical solutions, in the years following RRSO, to be able to still be sexually active. Previous studies had several methodological limitations; age at study inclusion and age at RRSO varied widely and adjustment for confounding factors, i.e. breast cancer history and HRT use, was done inconsistently. Also, there are no long-term data on the impact of duration of menopause on sexual functioning.

The aim of this study was to investigate the impact of a premenopausal RRSO on sexual functioning after at least 10 years. To overcome limitations in previous research we selected a large study cohort of women currently aged 55 years or older with a high familial risk of breast/ovarian cancer. We compared women who underwent a premenopausal RRSO (≤45 years) with women who underwent a postmenopausal RRSO (>54 years), and we performed subgroup analyses according to age at premenopausal RRSO, breast cancer history and HRT use.

Materials and Methods

Patient selection and recruitment

Participants were Dutch women participating in the HARMOny study[16] (ClinicalTrials.gov NCT03835793): a multicenter cross-sectional study, nested in a cohort of women at high familial risk of breast/ovarian cancer. [17,18] Study design and procedures have been described previously. [16] Briefly, between 2018 and 2021, we invited women to participate in a study assessing the long-term effects of RRSO on cardiovascular disease, bone health, cognition and quality of life. Eligibility criteria included a high familial risk of breast/ovarian cancer, current age of ≥55 years and having undergone RRSO either before age 45 or after age 54. Exclusion criteria were ovarian cancer, metastatic disease and therapy-induced menopause >5 years before RRSO. Breast cancer was not an exclusion criterion. Women were recruited from all Dutch university medical centers and the Netherlands Cancer Institute (NKI). The study has been approved by the Institutional Review Board of the NKI

Study assessments

Women were asked to complete a questionnaire on general health, cancer-specific outcomes, and medical treatments, including use of HRT (never, former, current use) and alternatives for HRT (e.g. herbal supplements, cognitive behavioral therapy, exercise). The questionnaire extensively addressed menopausal symptoms, including vaginal dryness, and body image (Supplementary Table 1).[19]

Sexual Activity Questionnaire (SAQ)

We assessed sexual functioning using the Sexual Activity Questionnaire (SAQ) (Supplementary Table 1).[20] The SAQ is a validated questionnaire and consists of three parts.[21-22] The first part assesses whether a woman is currently sexually active; those who are not sexually active complete the second part on reasons for sexual inactivity (Supplementary Table 3). Sexually active women complete the third part, which assesses several aspects of sexual function: pleasure, desire, satisfaction, vaginal dryness, penetration pain and frequency of intercourse. We specifically asked women to report on non-coital intercourse and masturbation. The questionnaire employs a 4-point Likert scale ('very much', 'somewhat', 'a little', 'not at all'). A composite score was calculated for 'pleasure' (range 0-18), 'discomfort' (range 0-6) and 'habit' (i.e. frequency of habitual sexual activity, range 0-3).[20,22]

Statistical analyses

Differences in characteristics between the premenopausal and the postmenopausal RRSO groups were evaluated using the χ^2 test or Fisher's exact test for categorical

data, and independent samples t-test for continuous data. The association between timing of RRSO and the various endpoints was analyzed using multiple linear regression for the SAQ pleasure score and multiple logistic regression for the SAQ discomfort score, the SAQ habit score, vaginal dryness and pain with intercourse, yielding regression coefficients and odds ratios (ORs) with accompanying 95% confidence intervals (95%CI). We created dichotomous variables for the discomfort score and the severity of vaginal dryness, comparing no/some discomfort (discomfort score ≤2) with substantial discomfort (discomfort score ≥3), and no/somewhat vaginal dryness (score ≤3) with substantial vaginal dryness (score ≥4). The postmenopausal RRSO group was used as the reference group. We adjusted for age at questionnaire completion and breast cancer history as potential confounders. Last, we included HRT, BMI, hysterectomy (yes/no), preventive mastectomy (yes/no), chronic medication use (yes/no) and body image in our multiple regression analyses. A variable was removed from the model if the p-value for its association with the outcome in the multivariate model was >.10. Due to collinearity between the variable 'timing of RRSO' (premenopausal or postmenopausal RRSO) and 'years since RRSO', we performed regression analyses with 'timing of RRSO' as an independent variable. Subsequently, we performed sensitivity analyses with 'years since RRSO'. We also performed stratified analyses by breast cancer history and, within the premenopausal RRSO group, by age at RRSO (≤40 years vs 41-45 years), breast cancer history and HRT use. For all statistical analyses, Stata, version 15.0 (StataCorp LLC) was used. P-values <.05 were considered statistically significant.

Results

Participation

In total, 787 women gave informed consent (response rate 60.0%), of whom 525 were in the premenopausal RRSO group (RRSO ≤ 45 years of age) and 262 women in the postmenopausal RRSO group (RRSO ≥55 years of age) (Figure 1). In the premenopausal RRSO group 15.6% declined participation compared to 33.8% in the postmenopausal RRSO group.

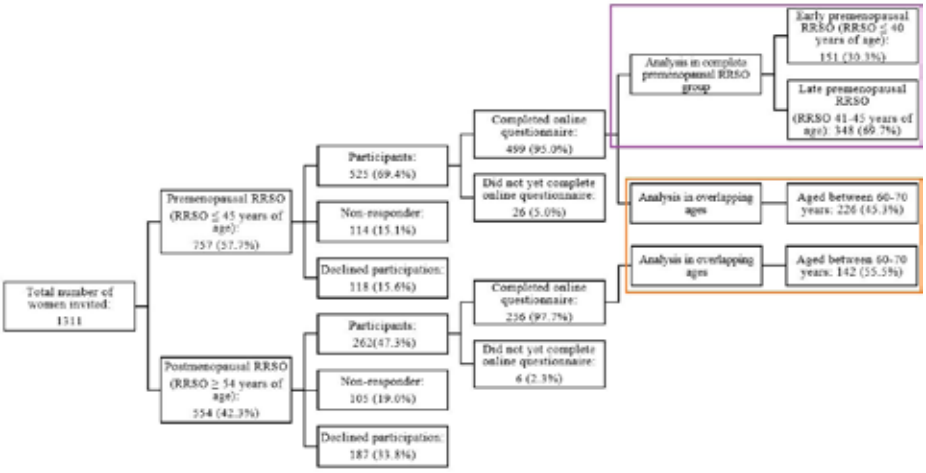

Figure 1. Participant Flowchart.

Number of participants enrolled, non-responders and number of women who declined participation. We have sent out regular reminders to women to complete the online questionnaire. We compare women with a premenopausal RRSO with women with a postmenopausal RRSO and secondly we compare within the premenopausal RRSO group women with an early premenopausal RRSO with women with a later premenopausal RRSO.

Participant characteristics

In the complete study population, mean age at questionnaire completion was 60.0 years in the premenopausal group, compared to 70.2 years in the postmenopausal group (p-value <.001) (Table 1). Compared to the postmenopausal RRSO group, women in the premenopausal group more often had a partner (83.7% versus 72.9%, p-value .001) and were more often sexually active (57.6% versus 39.3%, p-value <.001). These differences could be largely explained by the older age of the postmenopausal RRSO group at questionnaire completion; with advancing age, the percentage of sexually active women decreased (Figure 2). Because women in the premenopausal RRSO group were substantially younger than women in the postmenopausal RRSO group, we restricted the comparison of sexual functioning between these groups to 368 women who were 60-70 years old at completion of the questionnaire (premenopausal group, n=226, postmenopausal group, n=142). Within all 496 women with a premenopausal risk-reducing salpingo-oophorectomy we compared sexual functioning between women in the early premenopausal group (n=151) and the later premenopausal group (n=345). Results from analyses of the complete study population are provided in supplementary tables 5, 6 and Figure S1.

Among women aged 60-70 years at study, mean time since RRSO was 20.6 years in the premenopausal group and 10.6 years in the postmenopausal group (Table 1). This difference is inherent to the inclusion criteria for the study. In the premenopausal group, mean age at questionnaire completion was 62.7 years, compared to 67.0 years in the postmenopausal group (p-value <.001). Sixty-nine percent of women in the premenopausal-RRSO group carried a *BRCA1/2* PV versus 63.8% in the postmenopausal RRSO group (p-value .40). In the premenopausal RRSO group, 59.7% of women had a history of breast cancer, compared to 58.2% in the postmenopausal group (p-value .73). Breast cancer treatment did not differ between the groups. HRT was more often prescribed to women in the premenopausal RRSO group (29.1%, versus 9.2% in the postmenopausal RRSO group; p-value <.001). The duration of HRT use was similar in both groups (mean 1.9 years).

Table 1. Characteristics of study participants.

	Entire study population		Women aged 60-70 years	
Patient characteristics	**Premenopausal RRSO (n=499)**	**Postmenopausal RRSO (n=256)**	**Premenopausal RRSO (n=226)**	**Postmenopausal RRSO (n=142)**
Age at questionnaire completion (mean, SD)	60.0 (3.5)	70.2 (4.3) *	62.7 (2.5)	67.0 (2.1) *
Age at RRSO (mean, SD)	41.7 (2.8)	58.4 (3.6) *	42.1 (2.5)	56.5 (1.9) *
Time since RRSO (mean, SD)	18.3 (4.1)	11.9 (3.0) *	20.6 (3.3)	10.6 (1.9) *
Pathogenic genetic variants†				
BRCA1 germline mutation	241 (49.2%)	75 (29.4%) *	112 (49.6%)	39 (27.5%) *
BRCA2 germline mutation	96 (19.6%)	95 (37.3%) *	43 (19.0%)	51 (28.9%) *
Established non-carrier	153 (31.2%)	96 (33.3%)	70 (31.0%)	51 (28.9%)
Breast cancer (yes)	293 (59.0%)	166 (65.1%)	135 (59.7%)	82 (58.2%)
Breast cancer before RRSO	235 (84.8%)	146 (91.3%) *	104 (80.6%)	72 (91.1%) *
Breast cancer after RRSO	42 (15.2%)	14 (8.8%) *	25 (19.4%)	7 (8.9%) *
Treatment of breast cancer				
Surgery	284 (97.6%)	159 (98.8%)	132 (97.1%)	80 (98.8%)
Chemotherapy	222 (76.3%)	86 (52.4%) *	97 (48.7%)	51 (42.9%)
Radiotherapy	182 (62.5%)	95 (59.0%)	86 (63.2%)	54 (66.7%)
Endocrine therapy	106 (36.4%)	53 (32.9%)	41 (30.2%)	29 (35.8%)
Prophylactic mastectomy (yes) ‡	300 (62.1%)	84 (34.6%) *	140 (61.9%)	48 (33.8%) *
HRT use				
Current user	26 (5.2%)	2 (.8%) *	14 (6.2%)	1 (.7%) *

Table 1. Continued

	Entire study population		Women aged 60-70 years	
Past user	101 (20.0%)	27 (10.5%) *	46 (20.4%)	11 (7.7%) *
Never user	332 (66.5%)	210 (82.0%) *	146 (64.6%)	118 (83.1%) *
HRT duration in years (mean (SD))	2.2 (4.5)	1.4 (3.3)	2.1 (4.4)	1.6 (3.9)
Type of HRT				
Tibolone	37 (29.1%)	2 (6.9%)	1 (.4%)	0 (0.0%)
Estradiol/progestogen	30 (23.6%)	0 (0.0%)	13 (5.8%)	0 (0.0%)
Estradiol only	11 (8.7%)	2 (6.9%)	7 (3.1%)	1 (.7%)
Vaginal estrogen	2 (1.6%)	0 (0.0%)	1 (.4%)	0 (0.0%)
Unknown	47 (37.0%)	25 (86.2%)	204 (90.3%)	141 (99.3%)
BMI (mean, SD)	26.5 (5.0)	25.8 (4.5)	26.6 (5.2)	26.2 (5.0)
Hysterectomy (Yes) [§]	69 (16.2%)	53 (28.5%) *	43 (19.3%)	28 (19.7%)
Body Image (EORTC-BR23) (mean, sd) [||]	13.5 (18.3)	7.2 (11.3) *	19.6 (17.0)	9.0 (13.1) *
Chronic medication (yes) [¶]	217 (43.5%)	139 (54.3%) *	124 (54.9%)	49.3%)

*P-value < .05 Groups compared using independent samples t-test, Chi-squared test or Fishers exact test.† All participants had a high familial risk of ovarian cancer. All women were tested for pathogenic variants, not all had a BRCA1/2 mutation. Established non-carriers include women from BRCA1/2 families who tested negative as well as women from a breast/ovarian cancer family who tested negative for the pathogenic variants tested in the Netherlands.

[‡] Prophylactic mastectomy: bilateral or contralateral.

[§] In the Netherlands a hysterectomy is not standard of care when performing RRSO.

[||] European Organization for Research and Treatment of Cancer Breast Cancer-Specific Quality of Life Questionnaire19 (questions 9-12) with higher scores indicating more problems with body image (range 0-100).

[¶] Chronic medication use: any medication taken daily for cardiovascular risk factors, cardiovascular disease or chronic disease.

Abbreviations: RRSO: risk-reducing salpingo-oophorectomy; SD: standard deviation; BMI: body mass index; HRT: hormone replacement therapy in sexually active women Additional characteristics of the study population are provided in supplementary table 2.

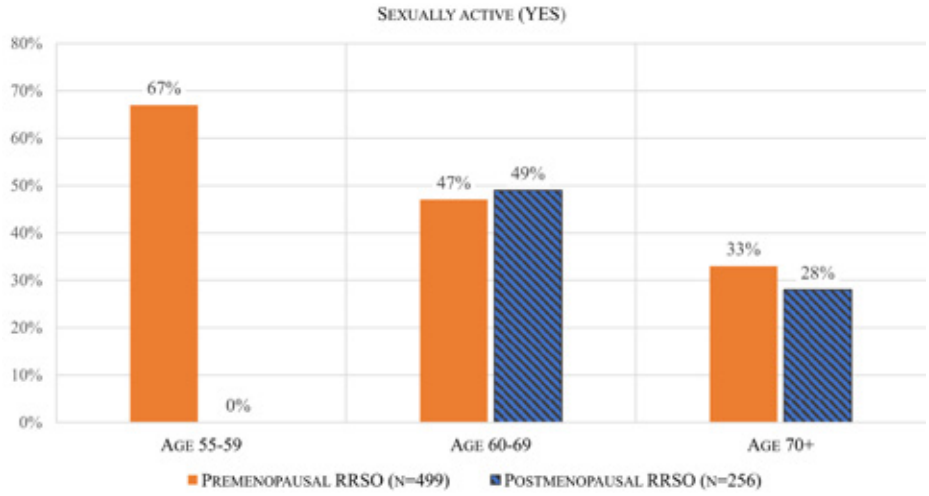

Figure 2. Proportion of sexually active women by age category at completion of questionnaire.

Age 55-59 years: Premenopausal RRSO n=267 of whom 180 sexually active, postmenopausal RRSO n= 0; Age 60-70 years: premenopausal RRSO n=226 of which 107 sexually active, postmenopausal RRSO n=142 of whom 70 sexually active; Age 71+ years: premenopausal RRSO n=6 of whom 2 sexually active, postmenopausal RRSO n= 114 of whom 32 sexually active.

Sexual activity and sexual functioning in women aged 60-70 years

In women aged 60-70 years, there was no difference in sexual activity between the groups (premenopausal RRSO 47.4% versus postmenopausal RRSO 48.9%, p-value .80). Among women who were sexually active (n=176), mean pleasure score in the premenopausal RRSO group was 8.6 (SD 3.7), versus 8.6 (SD 3.0) in the postmenopausal group (Figure 3a, p-value .80) (Answers to individual questions of the pleasure score are in Supplementary Figure 3). Sexually active women with a premenopausal RRSO had slightly higher discomfort scores than sexually active women with a postmenopausal RRSO (2.0 (SD 1.9) and 1.5 (SD 1.6, respectively p-value: .07) and women with a premenopausal RRSO more often had substantial discomfort than women with a postmenopausal RRSO (35.6% versus 20.9%, respectively, p-value .04) (Figure 3a, distribution of discomfort score Figure 3b). After adjustment for confounders, premenopausal RRSO was significantly associated with substantial discomfort during sexual intercourse (OR 3.1, 95%CI 1.04;9.4) (Table 2). The association between the mean pleasure score and the different discomfort scores can be found in Supplementary figure 4.

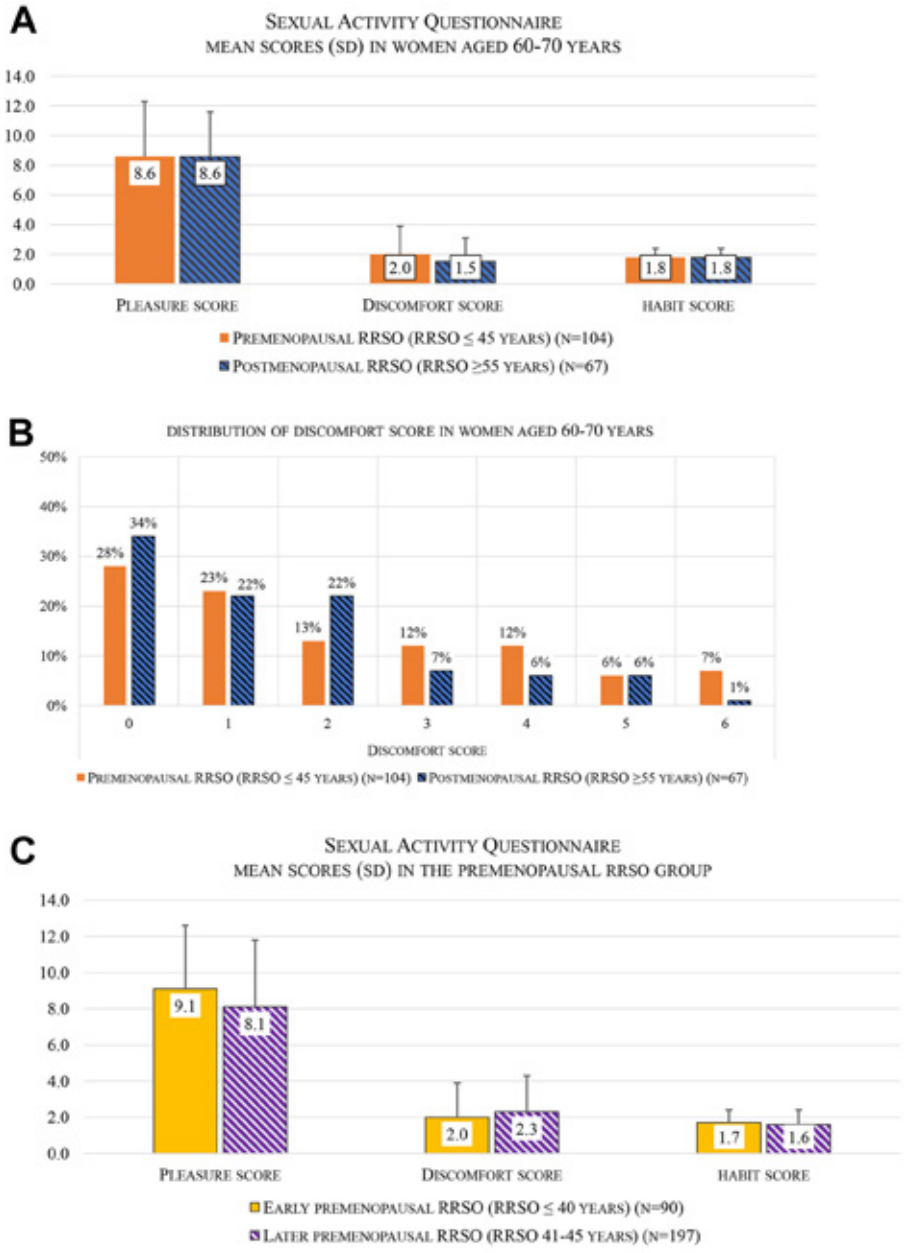

Figure 3 Mean sexual activity subscale scores and standard deviation

(A) Mean pleasure, discomfort and habit scores in women aged 60-70 years comparing premenopausal RRSO with postmenopausal RRSO. Range pleasure score 0 – 18. Range discomfort score 0 – 6. Range habit score 0-3 (**B**) Distribution of discomfort score in women aged 60-70 years comparing premenopausal RRSO with postmenopausal RRSO (**C)** Sexual activity questionnaire function subscales for women in the premenopausal RRSO group comparing early premenopausal RRSO with later premenopausal RRSO.

Table 2. Associations between various patient characteristics and the presence of substantial discomfort during sexual intercourse in sexually active women.

	Total sexually active women aged 60-70 years (n= 171)			Total sexually active women in the premenopausal RRSO group (n= 276)		
	Substantial discomfort* (n (%))	OR (95% CI) for substantial discomfort			Substantial discomfort* (n (%))	OR (95% CI) for substantial discomfort	
Timing of RRSO				**Timing of RRSO**			
Postmenopausal (RRSO ≥ 54 years)	14 (20.9%)	1.00	(REF)	Early premenopausal (RRSO ≤ 40 years)	33 (37.5%)	1.00	(REF)
Premenopausal (RRSO ≤ 45 years)	37 (35.6%)	3.13	(1.04;9.36)	Later premenopausal (RRSO 41-45 years)	78 (41.5%)	0.97	(0.56;1.69)
Age		1.15	(.98;1.35)	**Age**		1.00	(.92;1.08)
History of breast cancer				**History of breast cancer**			
No	21 (29.2%)	1.00		No	44 (35.8%)	1.00	(REF)
Yes	30 (30.3%)	1.02		Yes	67 (43.2%)	1.32	(0.79;2.21)
BMI (continuous, per 1 kg/m² increase)		1.08	(1.00;1.16)	**BMI (continuous, per 1 kg/m² increase)**		NS	
BR23-body image (continuous, per 1 point more)		NS		**BR23-body image (continuous, per 1 point more)**		1.01	(1.00;1.03)
Constant		0.38*10^-5	(0.55*10^-10;0.27)	**Constant**		0.64	(0.00;85.23)

The discomfort score from the sexual activity questionnaire ranges from 0-6, with higher scores indicating more discomfort.

* Substantial discomfort was defined as a discomfort score of 3 or higher (i.e. 3, 4, 5, 6).

Abbreviations: OR: odds ratio; CI: confidence interval; RRSO: risk-reducing salpingo-oophorectomy; BMI: body mass index; BR23-body image: body image score from the European Organization for Research and Treatment of Cancer Breast Cancer-Specific Quality of Life Questionnaire, score range 0-100; NA: not applicable; NS: significance level >.10, variable not in multivariate model.

Vaginal dryness was assessed among women who were and were not sexually active. Women with a premenopausal RRSO reported more severe complaints of vaginal dryness, with 47.0% of women in the premenopausal group reporting substantial vaginal dryness compared to 31.1% in the postmenopausal RRSO group (p-value <.001) (Figure 4b). Also after adjustment for confounders, a premenopausal RRSO was associated with substantial complaints of vaginal dryness (OR 2.6, 95%CI 1.4; 4.7). (Table 3). Within the sexually active group, results were similar: among women with a premenopausal RRSO 46.1% reported substantial complaints of vaginal dryness compared to 24.2% of women with a postmenopausal RRSO (p-value <.01) (Figure 4a).

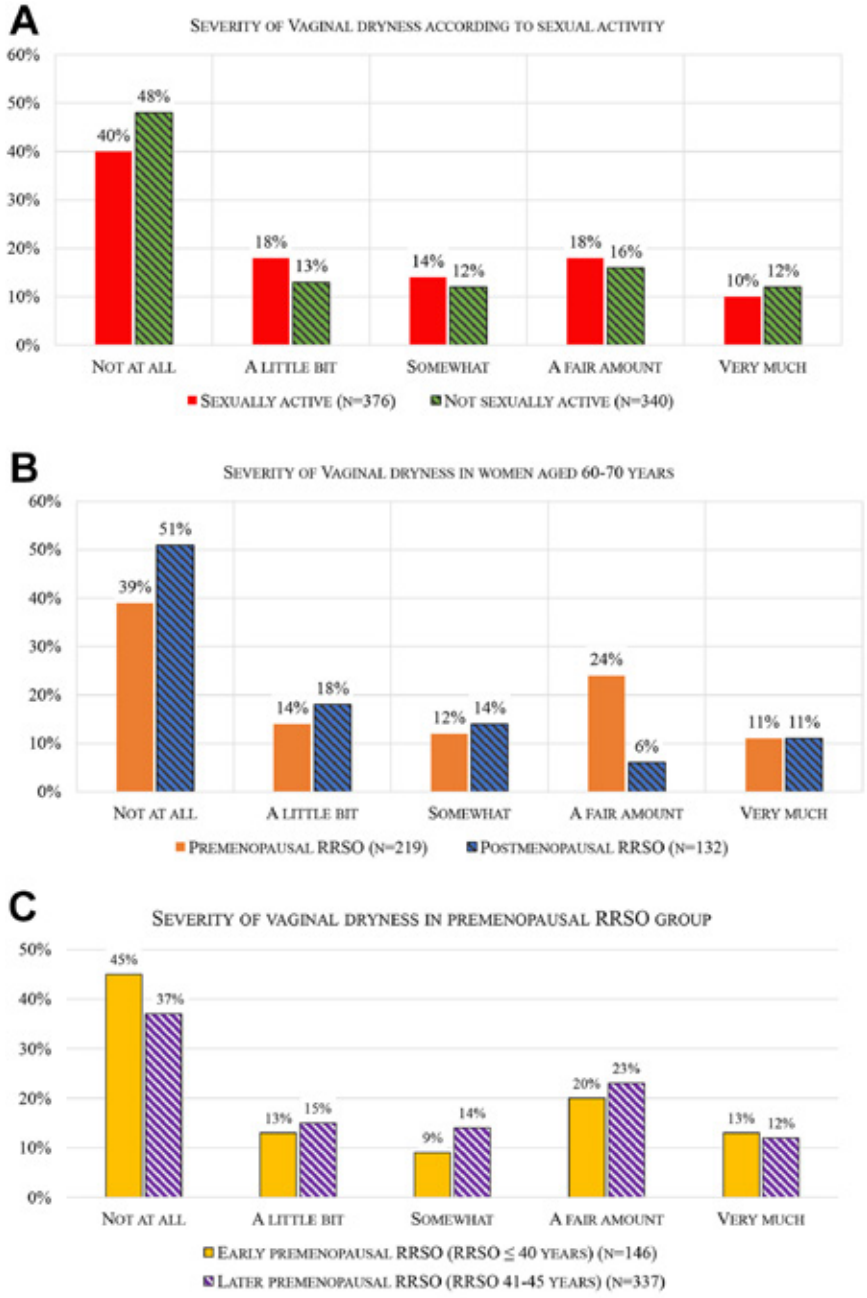

Figure 4. Severity of complaints of vaginal dryness

(**A**) in total study population comparing women who were sexually active and women who were not sexually active (**B**) in women aged 60-70 years comparing premenopausal RRSO with postmenopausal RRSO (**C**) in women with a premenopausal RRSO comparing women with a RRSO before age 41 and women with a RRSO at ages 41-45 years.

Table 3. Association between various patient characteristics and the presence of substantial vaginal dryness for all women (sexually active and not sexually active).

	Vaginal dryness in women aged 60-70 years (n=351)				Vaginal dryness in women with a premenopausal RRSO (n=483)		
	Substantial vaginal dryness* (n (%))	OR (95% CI) for substantial vaginal dryness			Substantial vaginal dryness* (n (%))	OR (95% CI) for substantial vaginal dryness	
Timing of RRSO				**Timing of RRSO**		N.A.	
Postmenopausal (RRSO ≥ 54 years)	41 (31.1%)	1.00	(REF)	Early premenopausal (RRSO ≤ 40 years)	61 (41.8%)	1.00	(REF)
Premenopausal (RRSO ≤ 45 years)	103 (47.0%)	2.56	(1.40;4.68)	Later premenopausal (RRSO 41-45 years)	165 (49.0%)	1.15	(0.75;1.77)
Age		1.06	(.97;1.16)	**Age**		1.02	(.96;1.08)
History of breast cancer				**History of breast cancer**			
No	57 (40.1%)	1.00	(REF)	No	84 (42.6%)	1.00	(REF)
Yes	87 (41.6%)	1.04	(0.67;1.62)	Yes	142 (49.7%)	1.24	(0.83;1.85)
Use of chronic medication†		NS		**Use of chronic medication‡**		NS	
No	63 (39.1%)			No	122 (44.9%)		
Yes	81 (42.6%)			Yes	104 (49.3%)		
BMI (continuous, per 1 kg/m² increase)		NS		**BMI (continuous, per 1 kg/m² increase)**		0.96	(0.92;1.00)
Constant		0.01	(0.00;4.07)	**Constant**		0.81	(0.2;27.16)

Vaginal dryness was assessed on a 5-point likert scale with higher scores indicating more vaginal dryness (FACT-ES)* Substantial vaginal dryness was defined as having somewhat – quite a bit or very much complaints regarding vaginal dryness.

† Chronic medication: any medication taken daily for cardiovascular risk factors, cardiovascular disease or chronic disease. Abbreviations: OR: odds ratio; CI: confidence interval; RRSO: risk-reducing salpingo-oophorectomy; BMI: body mass index; NA: not applicable; NS: significance level >.10.

Subgroup analyses in the entire premenopausal RRSO group

Timing of RRSO (before age 41 versus at ages 41-45 years)
Among women with an early premenopausal RRSO (before age 41, n=151), 56.0% were still sexually active at time of questionnaire completion, compared with 60.9% in the late premenopausal RRSO group (RRSO at ages 41-45 years, n=348) (p-value .34). Women with an early premenopausal RRSO did not differ from women with a late premenopausal RRSO with respect to sexual pleasure or discomfort scores (Figure 3c). Complaints about vaginal dryness were also similar (Figure 4c); 42% of women with an early premenopausal RRSO reported substantial vaginal dryness compared to 49% in the late premenopausal RRSO group (p-value .27).

Ever HRT-use versus never HRT-use in the premenopausal RRSO group
Women with a premenopausal RRSO who never used HRT did not differ from ever HRT users regarding sexual pleasure scores (mean pleasure score ever HRT-users 8.6 (SD 3.7), mean pleasure score never HRT-users 8.1 (SD 3.4) (p-value .32) or discomfort scores (mean discomfort score HRT-users 2.0 (SD: 1.9), mean discomfort score never HRT-users 2.6 (SD: 1.9, p-value .06). (Supplementary table 3)). However, women who used HRT at time of study experienced less discomfort than never users (proportions with substantial discomfort of 15.0% and 38.8%, respectively, p-value .04) and they also reported less vaginal dryness (current users 20.8%, never users 47.9%, p-value .01). However, this comparison was based on only 26 current users.

Women with a premenopausal RRSO with and without a history of breast cancer
Within the premenopausal RRSO group we compared women with (n=297) and without a history of breast cancer (n=220). The proportions of women who were sexually active, and the mean pleasure and discomfort scores were similar between the groups (detailed results in supplementary table 3).

Comment

Principal findings

In this large cross-sectional study we assessed long-term sexual functioning (>15 years) in women with a premenopausal RRSO (before age 46), compared to women with a postmenopausal RRSO (after age 54). After adjustment for age and breast cancer history, the proportion of sexually active women did not differ between the groups; at the age of 60-70 years 48% of women in the premenopausal RRSO group were still sexually active versus 45% in the postmenopausal RRSO group. Regarding

sexual pleasure; the premenopausal and postmenopausal RRSO groups scored similarly, indicating equal pleasure with sexual activity. However, after adjustment for confounders such as age and breast cancer history, women with a premenopausal RRSO more often experienced substantial discomfort during sexual intercourse, due to more severe complaints of vaginal dryness. When comparing women with RRSO before age 41 and RRSO at ages 41-45, there was no difference in mean discomfort scores or in severity of vaginal dryness. Longer time since RRSO was not associated with the amount of discomfort. Noteworthy, more vaginal dryness was not associated with less pleasure with sexual intercourse. We propose several possible explanations. Firstly, it is possible that women in our study experience discomfort with sexual intercourse, and therefore no longer engage in sex with penile penetration. However, they may be sexually active in other ways, from which they derive sexual pleasure without being bothered by discomfort from vaginal dryness. Secondly, it could be that women for whom sex is important are more proactive when it comes to coping mechanisms and exploring practical solutions, such as lubricants, to be able to be sexually active. Thirdly, it is possible that we experienced a so-called "floor" effect in the scoring of the pleasure domain because the majority of respondent do not consider sex a very important part of their life. Lastly, it is possible that the high scores in sexual satisfaction and the lower scores in arousal have attenuated respondents' overall pleasure score. In line with previous literature, sexual pleasure, sexual discomfort and/or the severity of vaginal dryness were not influenced by ever use of HRT.[14] However, women who used HRT at time of study experienced less discomfort and less vaginal dryness. As only 5.2% of women were current users, these results must be interpreted with caution.

Result in the Context of What is Known

To the best of our knowledge, the only study with normative data for the SAQ is a Norwegian study by Vistad et al.[22] Compared with this study, our subscale scores were lower, indicating less sexual pleasure, but also less discomfort. The frequency of sexual activity was comparable. In a study on sexual activity in a Dutch general population sample, 52% of the 60-70 year old participants were not sexually active, which is comparable to the 54% in our sample in the same age category.[23] As they used the Female Sexual Function Index rather than the SAQ, other comparisons with our results are not possible.

Previous studies on sexual functioning after RRSO had short follow-up (range 3-6 years) and reported that, shortly after RRSO, women experienced more discomfort and less pleasure when engaging in sexual activity. Our study, with a mean follow-up of 18.3 years after RRSO, is the first to assess the long-term effects

of a premenopausal RRSO on sexual functioning, and shows that, in the long run, pleasure with sexual activity is similar to that in women with a postmenopausal RRSO. However, women with a premenopausal RRSO more often experienced substantial discomfort during sexual intercourse and had more severe complaints of vaginal dryness. Comparison of our study with other reports is difficult as there were many differences in study populations and methods of analysis. Age at RRSO varied widely across studies, as well as the comparison groups used; e.g. in some analyses women with a premenopausal RRSO were combined with women with a postmenopausal RRSO. Moreover, in previous reports mean age at study (40-57 years) was younger than in ours, rendering comparisons of sexual functioning between studies difficult. Furthermore, earlier studies did not always account for the confounding and potential modifying effects of a breast cancer history and HRT use. In our study, the majority of women (77.8%) never used HRT, this is likely due to the high prevalence of previous breast cancer and conflicting reports regarding the safety of HRT in the period when our study population underwent RRSO.[24]

Clinical Implications

Our study provides important information for clinicians counselling women who are considering risk-reducing surgery. It is crucial to give a complete overview of possible clinical and psychological sequelae and to set realistic expectations. Integrating our results with studies evaluating short-term effects of RRSO, women can be informed that shortly after a premenopausal RRSO, they can expect less pleasure and more discomfort when engaging in sexual activity; in the long run, pleasure in sexual activity will not be different from that of women with RRSO after menopause. However, they can expect more discomfort with sexual intercourse and more vaginal dryness. Treating physicians should proactively discuss sexual functioning with their patients, and provide advice, including treatment options, in case of complaints.

Strengths and Limitations

A limitation of our study, although inherent to the inclusion criterion regarding age at RRSO, is the difference in mean age at questionnaire completion between the premenopausal and the postmenopausal RRSO groups. During recruitment, it became clear that frequency-matching on current age was not possible, because, from 2007 onwards, the national guideline for familial ovarian cancer strongly recommended RRSO for all women with *BRCA* PV, at the age of 35-40 years for *BRCA1* PV and at ages 41-45 for *BRCA2* PV carriers.[25] Consequently, the majority of women (94.5%) with a postmenopausal RRSO was tested and underwent RRSO before 2007. To overcome this limitation, we performed analyses for women in the overlapping age range, 60-70 at questionnaire completion. Another concern may be the difference

in response rates between the premenopausal group (70.3%) and the postmenopausal group (48.0%). A likely explanation is that women in the postmenopausal RRSO group felt less inclined to participate as our research hypotheses were focused on early surgical menopause. However, we do not think this has affected our results, as it seems unlikely that current sexual activity would have affected study participation differently in women with a premenopausal or postmenopausal RRSO. The HARMOny study invitation letter focused on potential effects of premenopausal RRSO on cardiovascular disease and bone health. A last concern may be that, despite the fact that we defined sexual activity to include non-coital sex and masturbation in the instructions for completing the SAQ, we cannot exclude the possibility that some women may have interpreted the questions as referring only to sexual intercourse. However, it is unlikely that such an interpretation would differ between the pre- and postmenopausal RRSO groups.

Strengths of our study include the large sample size, providing sufficient power to perform several subgroup analyses. Additionally, by excluding women with RRSO at ages 46-54, we were able to make a more distinct evaluation of the differences in sexual health between women who underwent RRSO prior to the onset of natural menopause and thereafter. Our participation rate was acceptable (59%), given the nature and focus of the study, and we employed validated questionnaires that are widely used. Moreover, all women in our study completed questions on vaginal dryness; not only women who were sexually active. Also, and more generally, our study is one of the first to assess sexual functioning in a large group of women aged 60 or older.

Conclusion

In conclusion, more than 15 years after premenopausal RRSO, women experienced more severe complaints of vaginal dryness and more discomfort with sexual intercourse than women with a postmenopausal RRSO. However, this did not result in less pleasure with sexual activity. This knowledge can be integrated into pre-surgery counseling regarding expected sexual functioning after premenopausal RRSO.

References

1. Kuchenbaecker KB, Hopper JL, Barnes DR, et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. Jama 2017;317(23):2402-2416. (In eng). DOI: 10.1001/jama.2017.7112.
2. Nappi RE, Martini E, Cucinella L, et al. Addressing Vulvovaginal Atrophy (VVA)/Genitourinary Syndrome of Menopause (GSM) for Healthy Aging in Women. Frontiers in endocrinology 2019;10:561. (In eng). DOI: 10.3389/fendo.2019.00561.
3. Mac Bride MB, Rhodes DJ, Shuster LT. Vulvovaginal atrophy. Mayo Clinic proceedings 2010;85(1):87-94. (In eng). DOI: 10.4065/mcp.2009.0413.
4. Robinson D, Cardozo LD. The role of estrogens in female lower urinary tract dysfunction. Urology 2003;62(4 Suppl 1):45-51. (In eng). DOI: 10.1016/s0090-4295(03)00676-9.
5. Sturdee DW, Panay N, International Menopause Society Writing G. Recommendations for the management of postmenopausal vaginal atrophy. Climacteric : the journal of the International Menopause Society 2010;13(6):509-22. (In eng). DOI: 10.3109/13697137.2010.522875.
6. Waetjen LE, Crawford SL, Chang PY, et al. Factors associated with developing vaginal dryness symptoms in women transitioning through menopause: a longitudinal study. Menopause (New York, NY) 2018;25(10):1094-1104. (In eng). DOI: 10.1097/gme.0000000000001130.
7. Stuursma A, van Driel CMG, Wessels NJ, de Bock GH, Mourits MJE. Severity and duration of menopausal symptoms after risk-reducing salpingo-oophorectomy. Maturitas 2018;111:69-76. (In eng). DOI: 10.1016/j.maturitas.2018.01.012.
8. Faubion SS, Kuhle CL, Shuster LT, Rocca WA. Long-term health consequences of premature or early menopause and considerations for management. Climacteric : the journal of the International Menopause Society 2015;18(4):483-91. (In eng). DOI: 10.3109/13697137.2015.1020484.
9. Madalinska JB, van Beurden M, Bleiker EM, et al. The impact of hormone replacement therapy on menopausal symptoms in younger high-risk women after prophylactic salpingo-oophorectomy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2006;24(22):3576-82. (In eng). DOI: 10.1200/JCO.2005.05.1896.
10. Kershaw V, Hickey I, Wyld L, Jha S. The impact of risk reducing bilateral salpingo-oophorectomy on sexual function in BRCA1/2 mutation carriers and women with Lynch syndrome: A systematic review and meta-analysis. European journal of obstetrics, gynecology, and reproductive biology 2021;265:7-17. (In eng). DOI: 10.1016/j.ejogrb.2021.08.001.
11. Tucker PE, Bulsara MK, Salfinger SG, Tan JJ, Green H, Cohen PA. Prevalence of sexual dysfunction after risk-reducing salpingo-oophorectomy. Gynecologic oncology 2016;140(1):95-100. (In eng). DOI: 10.1016/j.ygyno.2015.11.002.
12. Robson M, Hensley M, Barakat R, et al. Quality of life in women at risk for ovarian cancer who have undergone risk-reducing oophorectomy. Gynecologic oncology 2003;89(2):281-7. (In eng). DOI: 10.1016/s0090-8258(03)00072-6.
13. Finch A, Metcalfe KA, Chiang JK, et al. The impact of prophylactic salpingo-oophorectomy on menopausal symptoms and sexual function in women who carry a BRCA mutation. Gynecologic oncology 2011;121(1):163-8. (In eng). DOI: 10.1016/j.ygyno.2010.12.326.
14. Hall E, Finch A, Jacobson M, et al. Effects of bilateral salpingo-oophorectomy on menopausal symptoms and sexual functioning among women with a BRCA1 or BRCA2 mutation. Gynecologic oncology 2019;152(1):145-150. (In eng). DOI: 10.1016/j.ygyno.2018.10.040.

15. Mai PL, Huang HQ, Wenzel LB, et al. Prospective follow-up of quality of life for participants undergoing risk-reducing salpingo-oophorectomy or ovarian cancer screening in GOG-0199: An NRG Oncology/GOG study. Gynecologic oncology 2020;156(1):131-139. (In eng). DOI: 10.1016/j.ygyno.2019.10.026.

16. Terra L, Hooning MJ, Heemskerk-Gerritsen BAM, et al. Long-Term Morbidity and Health After Early Menopause Due to Oophorectomy in Women at Increased Risk of Ovarian Cancer: Protocol for a Nationwide Cross-Sectional Study With Prospective Follow-Up (HARMOny Study). JMIR research protocols 2021;10(1):e24414. (In eng). DOI: 10.2196/24414.

17. Schrijver LH, Olsson H, Phillips KA, et al. Oral Contraceptive Use and Breast Cancer Risk: Retrospective and Prospective Analyses From a BRCA1 and BRCA2 Mutation Carrier Cohort Study. JNCI Cancer Spectr 2018;2(2):pky023. DOI: 10.1093/jncics/pky023.

18. Wevers MR, Schmidt MK, Engelhardt EG, et al. Timing of risk reducing mastectomy in breast cancer patients carrying a BRCA1/2 mutation: retrospective data from the Dutch HEBON study. Familial cancer 2015;14(3):355-63. (In eng). DOI: 10.1007/s10689-015-9788-x.

19. Sprangers MA, Groenvold M, Arraras JI, et al. The European Organization for Research and Treatment of Cancer breast cancer-specific quality-of-life questionnaire module: first results from a three-country field study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1996;14(10):2756-68. (In eng). DOI: 10.1200/JCO.1996.14.10.2756.

20. Thirlaway K, Fallowfield L, Cuzick J. The Sexual Activity Questionnaire: a measure of women's sexual functioning. Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation 1996;5(1):81-90. (In eng).

21. Madalinska JB, Hollenstein J, Bleiker E, et al. Quality-of-life effects of prophylactic salpingo-oophorectomy versus gynecologic screening among women at increased risk of hereditary ovarian cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2005;23(28):6890-8. (In eng). DOI: 10.1200/JCO.2005.02.626.

22. Vistad I, Fosså SD, Kristensen GB, Mykletun A, Dahl AA. The sexual activity questionnaire: pychometric properties and normative data in a norwegian population sample. Journal of women's health (2002) 2007;16(1):139-48. (In eng). DOI: 10.1089/jwh.2006.0052.

23. Lammerink EAG, de Bock GH, Pascal A, et al. A Survey of Female Sexual Functioning in the General Dutch Population. The journal of sexual medicine 2017;14(7):937-949. (In eng). DOI: 10.1016/j.jsxm.2017.04.676.

24. Ameye L, Antoine C, Paesmans M, de Azambuja E, Rozenberg S. Menopausal hormone therapy use in 17 European countries during the last decade. Maturitas 2014;79(3):287-91. (In eng). DOI: 10.1016/j.maturitas.2014.07.002.

25. Hermsen BB, Olivier RI, Verheijen RH, et al. No efficacy of annual gynaecological screening in BRCA1/2 mutation carriers; an observational follow-up study. British journal of cancer 2007;96(9):1335-42. (In eng). DOI: 10.1038/sj.bjc.6603725

Declarations

Ethics approval and consent to participate

This study will be conducted according to the standards of Good Clinical Practice, in agreement with the principles of the Declaration of Helsinki and with the Dutch law as stated in the Medical Research Involving Human Subjects Act (WMO). The study has been approved in writing by the Institutional Review Board of the AVL/NKI to be conducted in all 9 University Medical Centers and the Antoni van Leeuwenhoek and has been registered at "CCMO Toetsingonline" from the Dutch Central Committee on Research involving Human Subjects (file number NL63554.031.17) and on clinicaltrials.gov, M18HAR. Results will be disseminated through peer-reviewed publications and will be incorporated in follow-up guidelines.

Authors contributions

were involved in the conception and design of the study. LT, FvL, MB, EE, MM, MH and BHG drafted the manuscript. MB, JR, HvD, JdH, EvD, CM, BS, KG, LvdK, JC, MW, MA, KvE, IvdB, LB, CvA, EG, NA and AM were involved in the final version of the manuscript.

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Supplements

Supplementary Table 1. Sexual activity questionnaire.

Questionnaire	Questions	Scoring system
Sexual activity questionnaire (SAQ) [1]	Sexual active / not I am not sexually active at the moment because*: • I do not have a partner • I am too tired • My partner is too tired • I am not interested in sex • My partner is not interested in sex • I have a physical problem which makes sexual relations difficult or uncomfortable • My partner has a physical problem which makes sexual relations difficult or uncomfortable *multiple reasons per person possible Domains: • Pleasure 1. Was having sex an important part of your life 2. Did you enjoy sexual activity 3. Did you desire to have sex with your partner? 4. In general were you satisfied after sexual activity 5. How often did you engage in sexual activity 6. Were you satisfied with the frequency of sex • Discomfort 1. Did you notice dryness of your vagina this month during sexual intercourse 2. Did you feel pain or discomfort with sexual intercourse this month? • Habit 1. How did the frequency of sexual behavior compare with what is usual for you?	9 items with a 4-point Likert scale. 0 = not at all, 1 = slightly, 2 = moderately, 3 = greatly. Domain scores were obtained by adding together the weighted loadings for each question that contributed to each factor. Subscale scores: Pleasure 0-18; higher scores indicate higher level of pleasure. Discomfort 0-6; higher scores indicate higher levels of discomfort. Habit 0-3; single item. 0 = less sexual activity than usual to 3=much more sexual activity than usual.
European Organization for Research and Treatment of Cancer Breast Cancer-Specific Quality of Life Questionnaire (EORTC QLQ-BR23) [2] body image questions	Did you feel yourself physically less attractive as a consequence from your illness or treatment? Did you feel less feminine as a consequence from your illness or treatment? Did you found it difficult to see yourself naked? Were you unhappy with your body?	Assessed on a 4-point Likert scale. 1 = not at all, 2 = slightly, 3 = moderately, 4 = greatly The scale is linearly transformed to a score range 0 – 100 with higher scores represents higher levels of functioning.

Supplementary Table 1. Continued

Questionnaire	Questions	Scoring system
Functional Assessment of Cancer Therapy - Endocrine Symptoms (FACT-ES)[2]	I have vaginal discharge I have vaginal itching/irritation I have vaginal bleeding/spotting I have vaginal dryness I have pain or discomfort with intercourse	Assessed on a 5-point Likert scale. 0 = not at all, 1 = a little bit, 2 = somewhat, 3 = quite a bit, 4 = very much

Supplementary Table 2. Additional characteristics of all study participants.

	Entire study population		Women aged 60-70 years	
	Premenopausal RRSO (n=499)	**Postmenopausal RRSO (n=256)**	**Premenopausal RRSO (n=226)**	**Postmenopausal RRSO (n=142)**
Psychological interventions taken for menopausal complaints (yes)	45 (9.0%)	15 (5.9%)	12 (5.3%)	7 (4.9%)
Dietary intervention for menopausal complaints (yes)	43 (8.6%)	9 (3.5%)	14 (6.2%)	6 (4.2%)
Physical exercise for menopausal complaints (yes)	27 (5.4%)	12 (4.7%)	13 (5.8%)	6 (4.2%)
BMI (mean, SD)	26.5 (5.0)	25.8 (4.5)	26.6 (5.2)	26.2 (5.0)
Smoking status at study questionnaire				
Non-smoker	250 (50.1%)	112 (43.8%)*	94 (41.6%)	64 (45.1%)
Former smoker	211 (42.3%)	134 (52.3%)*	106 (46.9%)	64 (45.1%)
Current smoker	36 (7.2%)	9 (3.5%)*	18 (8.0%)	7 (4.9%)
Pack-years smoked (mean, SD)	14.5 (11.4)	17.0 (15.4)	23.9 (14.4)	23.8 (15.1)
Educational level				
Primary school/lower level high school	138 (27.6%)	109 (42.6%)*	66 (29.2%)	51 (35.9%)
Middle level high school	165 (33.1%)	45 (17.6%)*	68 (30.1%)	34 (23.9%)
Advanced vocational/ university	158 (31.7%)	81 (31.6%)*	77 (34.1%)	44 (31.0%)
Employment status at study questionnaire				
Full-time job/part-time job	282 (56.5%)	29 (11.3%)*	109 (48.2%)	25 (17.6%)*
Retired	35 (7.0%)	159 (62.1%)*	29 (12.8%)	71 (50.0%)*
Housewife/voluntary work	42 (8.4%)	23 (9.0%)*	25 (11.1%)	14 (9.9%)*
Completely/partially incapacitated for work	51 (10.2%)	7 (2.7%)*	21 (9.3%)	7 (4.9%)*
(temporary) Unemployed	44 (8.8%)	11 (4.3%)*	23 (10.2%)	10 (7.0%)*

* P-value <.05. Groups compared using independent samples t-test, Chi-squared test or Fishers exact test. Abbreviations: RRSO: risk-reducing salpingo-oophorectomy; SD: standard deviation; BMI: body mass index; HRT: hormone replacement therapy in sexually active women.

Reasons for sexual inactivity

Among women who were not sexually active (n=355), not having a partner and arousal problems were the reasons reported most frequently in the premenopausal RRSO group (Supplementary Table 2). An arousal problem was the reason for sexual inactivity for 31.4% of women with a premenopausal RRSO and, for 23.4 % of women with a postmenopausal RRSO (p-value .04). Women in the premenopausal RRSO group reported more often fatigue as a reason for sexual inactivity (13.3% in the premenopausal group versus 4.1% in the postmenopausal RRSO group, p-value <.01). Women in the postmenopausal RRSO group more often reported that their partner had a physical problem interfering with sexual activity (premenopausal RRSO group 15.7%, postmenopausal RRSO group 27.6%, p-value .02).

Supplementary Table 3. Reasons for sexual inactivity in women who are not sexually active (multiple reasons per person possible).

	Premenopausal RRSO (n=210)	**Postmenopausal RRSO (n=145)**	**p-value**
No partner	60 (28.6%)	47 (32.4%)	.75
Arousal problem	66 (31.4%)	34 (23.4%)	.04
Fatigue	28 (13.3%)	6 (4.1%)	<.01
Physical problem	48 (22.9%)	29 (20.0%)	.32
Partner fatigue	12 (5.7%)	6 (4.1%)	.41
Partner physical problem	33 (15.7%)	40 (27.6%)	.02
No reason given	39 (18.6%)	30 (20.7%)	.83

Supplementary Table 4. SAQ function scores per analysis performed: Premenopausal RRSO group compared with the postmenopausal RRSO group in: (1) entire study population, (2) women aged 60-70 years at questionnaire completion, (3) women with a history of breast cancer, and (4) women without a history of breast cancer. Within the premenopausal RRSO group we compared (5) early premenopausal RRSO with later premenopausal RRSO, (6) Current or former HRT-users with never HRT users and (7) women with and without a history of breast cancer.

	Total study population									
		Number	**Sexually active (YES)**	**p-value**	**SAQ pleasure score (mean (SD))**	**p-value**	**SAQ discomfort score (mean (SD))**	**p-value**	**SAQ habit score (mean (SD))**	**p-value**
1	Premenopausal RRSO (RRSO ≤ 45 years)	499	57.9%	<.001	8.5 (3.6)	.75	2.2 (2.0)	.01	1.7 (.7)	.21
	Postmenopausal RRSO (RRSO ≥ 54 years)	256	39.8%		8.3 (3.1)		1.6 (1.6)		1.8 (.6)	
2	Premenopausal RRSO & ages 60-70 years at questionnaire completion	226	47.4%	.09	8.6 (3.7)	.99	2.0 (1.9)	.09	1.8 (.6)	.78
	Postmenopausal RRSO & ages 60-70 years at questionnaire completion	142	48.9%		8.6 (3.0)		1.5 (1.6)		1.8 (.6)	
3	Premenopausal RRSO & history of breast cancer (YES)	293	54.6%	<.01	8.3 (3.6)	.68	2.3 (1.9)	.02	1.7 (.7)	.17
	Postmenopausal RRSO & history of breast cancer (YES)	166	41.0%		8.1 (3.1)		1.7 (1.6)		1.8 (.5)	
4	Premenopausal RRSO & history of breast cancer (NO)	204	62.3%	<.001	8.7 (3.7)	.98	2.0 (2.0)	.25	1.6 (.7)	.99
	Postmenopausal RRSO & history of breast cancer (NO)	89	37.1%		8.7 (3.1)		1.6 (1.7)		1.6 (.8)	

Supplementary Table 4. Continued

	Premenopausal RRSO (RRSO ≤ 45 years)									
		Number	**Sexually active (YES)**	**p-value**	**SAQ pleasure score (mean (SD))**	**p-value**	**SAQ discomfort score (mean (SD))**	**p-value**	**SAQ habit score (mean (SD))**	**p-value**
5	Early premenopausal RRSO (RRSO ≤ 40 years)	151	59.6%	.55	9.1 (3.5)	.05	2.0 (1.9)	.41	1.7 (.7)	.90
	Later premenopausal RRSO (RRSO 41-45 years)	348	56.7%		8.1 (3.7)		2.3 (2.0)		1.6 (.8)	
6	Current HRT-users	26	72.9%	.09	9.6 (4.5)	.16	1.1 (1.5)	.01	1.4 (.9)	.01
	Former HRT-users	101	59.4%		8.1 (3.3)		2.5 (2.1)		1.5 (.8)	
	Never HRT-users	332	55.1%		8.5 (3.6)		2.2 (1.9)		1.7 (.7)	
7	History of breast cancer (YES)	293	54.6%	.09	8.3 (3.6)	.42	2.3 (1.9)	.20	1.7 (.7)	.20
	History of breast cancer (NO)	204	62.3%		8.7 (3.7)		2.0 (2.0)		1.6 (.7)	

Supplementary Table 5. Association between various patient characteristics and the presence of substantial discomfort during sexual intercourse for sexually active women.

	All sexually active women (n= 378)		
	Substantial discomfort* (n (%))	**OR (95% CI) for substantial discomfort**	
Timing of RRSO			
Postmenopausal (RRSO ≥ 54 years)	24 (24.5%)	1.00	(REF)
Premenopausal (RRSO ≤ 45 years)	111 (39.6%)	3.41	(1.29;9.03)
Age			
55-59 years		1.00	(REF)
60-64 years		.60	(.34;1.07)
65-70 years		1.34	(.56;3.25)
71+ years		1.98	(.55;7.12)
History of breast cancer			
No	51 (32.7%)	1.00	(REF)
Yes	84 (38.4%)	1.28	(0.81;2.04)
BMI (continuous, per 1 kg/m² increase)		NS	
BR23-body image (continuous, per 1 point more)		1.01	(1.00;1.03)
Constant		0.17	(0.06;0.49)

The discomfort score from the sexual activity questionnaire ranges from 0-6, with higher scores indicating more discomfort.

* Substantial discomfort was defined as a discomfort score of 3 or higher (i.e. 3, 4, 5, 6).

Abbreviations: OR: odds ratio; CI: confidence interval; RRSO: risk-reducing salpingo-oophorectomy; BMI: body mass index; BR23-body image: body image score from the European Organization for Research and Treatment of Cancer Breast Cancer-Specific Quality of Life Questionnaire, score range 0-100; NA: not applicable; NS: significance level >.10, variable not in multivariate model.

Supplementary Table 6. Association between various patient characteristics and the presence of substantial vaginal dryness for all women (sexually active and not sexually active).

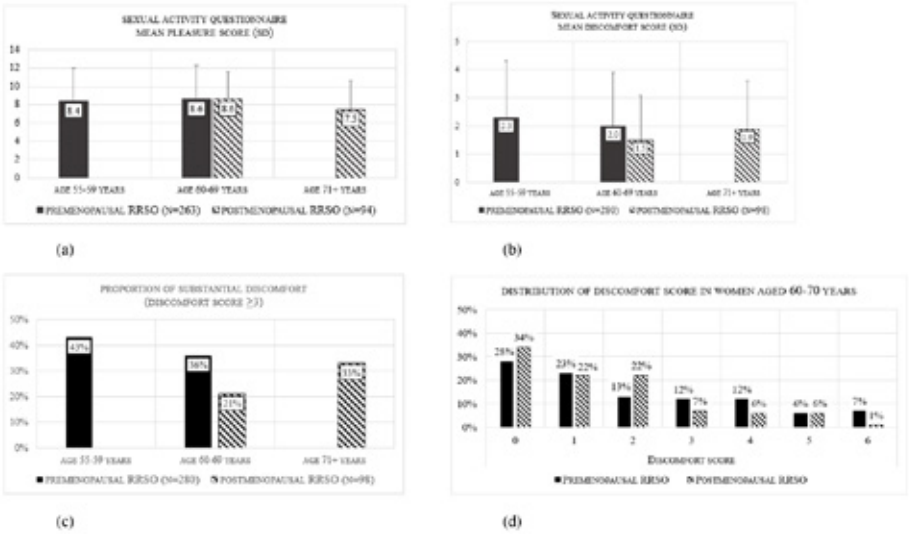
	Vaginal dryness all women (n=716)		
	Substantial vaginal dryness* **(n (%))**	**OR (95% CI) for substantial vaginal dryness**	
Timing of RRSO			
Postmenopausal (RRSO ≥ 54 years)	64 (27.5%)	1.00	(REF)
Premenopausal (RRSO ≤ 45 years)	226 (46.8%)	2.28	(1.25;4.16)
Age			
55-59 years		1.00	(REF)
60-64 years		.87	(.57;1.33)
65-70 years		1.25	(.68;2.29)
71+ years		.72	(.32;1.61)
History of breast cancer			
No	105 (37.8%)	1.00	(REF)
Yes	185 (42.2%)	1.25	(0.89;1.77)
Use of chronic medication†			
No	145 (38.8%)	1.00	(REF)
Yes	145 (42.4%)	1.44	(1.01;2.05)
BMI (continuous, per 1 kg/m² increase)		0.97	(0.93;1.00)
Constant		0.65	(0.21;2.00)

Vaginal dryness was assessed on a 5-point Likert scale with higher scores indicating more vaginal dryness (FACT-ES).

* Substantial vaginal dryness was defined as having somewhat – quite a bit or very much complaints regarding vaginal dryness.

†Chronic medication: any medication taken daily for cardiovascular risk factors, cardiovascular disease or chronic disease.

Abbreviations: OR: odds ratio; CI: confidence interval; RRSO: risk-reducing salpingo-oophorectomy; BMI: body mass index; NA: not applicable; NS: significance level >.10.

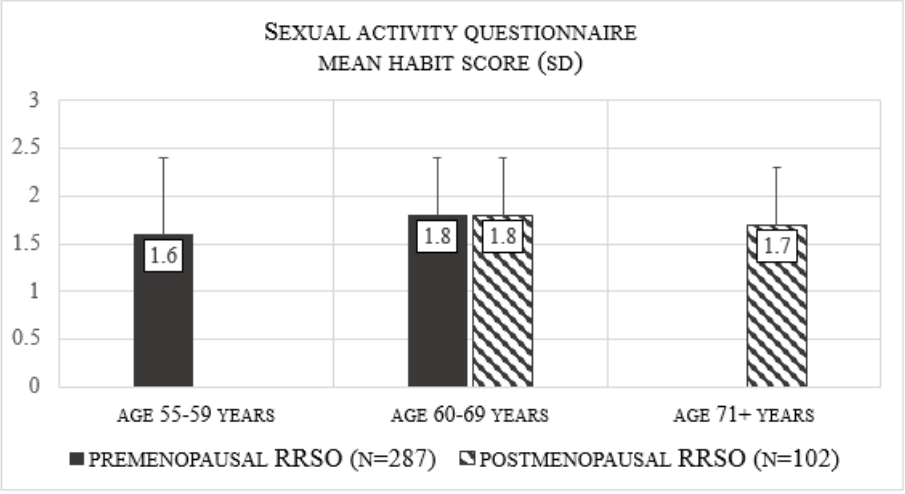


Supplementary Figure 1. Mean sexual activity subscale scores and standard deviation by age category at completion of questionnaire.

(a) Mean pleasure scores. Range pleasure score 0 – 18. Age 55-59 years: Premenopausal RRSO n=162, postmenopausal RRSO n= 0; Age 60-70 years: premenopausal RRSO n=99, postmenopausal RRSO n=65; Age 71+ years: premenopausal RRSO n=0, postmenopausal RRSO n= 28. **(b)** Mean discomfort scores. Range discomfort score 0 – 6. Age 55-59 years: Premenopausal RRSO n=173, postmenopausal RRSO n= 0; Age 60-70 years: premenopausal RRSO n=104, postmenopausal RRSO n=67; Age 71+ years: premenopausal RRSO n=1, postmenopausal RRSO n= 30. **(c)** Proportion of women with a substantial discomfort score (discomfort score ≥ 3). **(d)** The distribution of women aged 60-70 years per discomfort score.

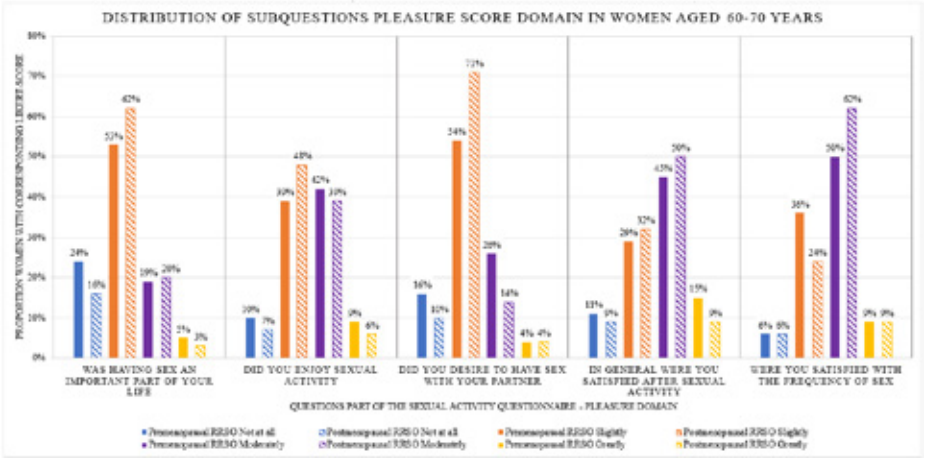
Sexual Activity Questionnaire - Habit score

The SAQ-habit score was comparable between the premenopausal RRSO and the postmenopausal RRSO groups (1.6 versus 1.8, p-value .16). This was also true for women aged 60-70 years; 1.8 (SD 0.6) in the premenopausal RRSO group and 1.8 (SD 0.7) in the postmenopausal RRSO group (supplementary Figure 1).

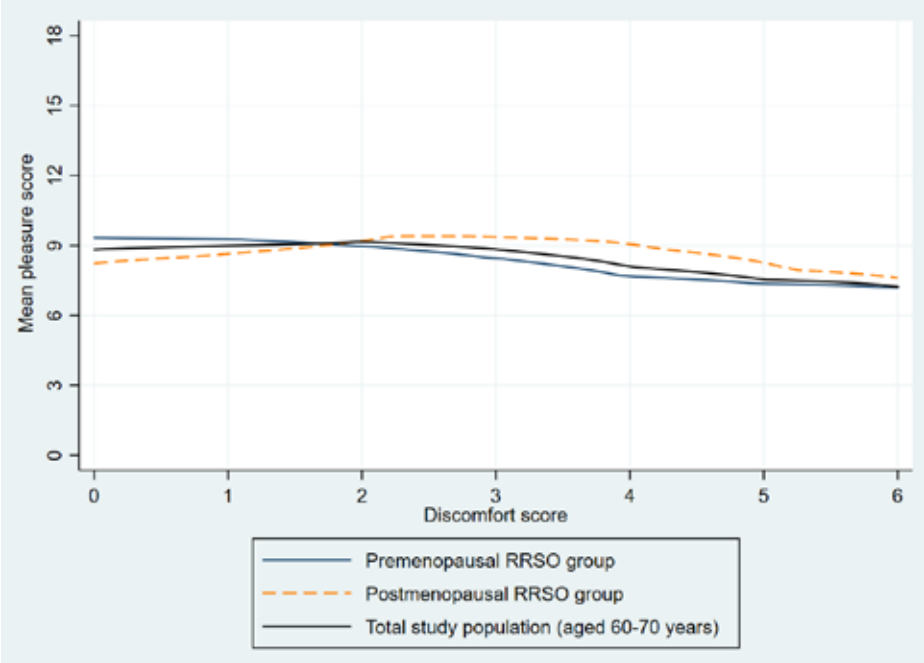


Supplemental Figure 2. Mean habit score and the corresponding standard deviation, in the premenopausal and postmenopausal RRSO groups per age group.

Range habit score 0 – 3. Age 55-59 years: Premenopausal RRSO n=178, postmenopausal RRSO n= 0; Age 60-70 years: premenopausal RRSO n=108, postmenopausal RRSO n=70; Age 71+ years: premenopausal RRSO n=1, postmenopausal RRSO n= 32.



Supplementary Figure 3. Distribution of answers given to the questions that cover the pleasure scale.



Supplementary Figure 4. Mean pleasure score in relation to the discomfort score in women aged 60-70 years at study comparing women with a premenopausal RRSO with women with a postmenopausal RRSO.

Supplementary references

1. Thirlaway K, Fallowfield L, Cuzick J. The Sexual Activity Questionnaire: a measure of women's sexual functioning. *Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation*. Feb 1996;5(1):81-90.
2. Sprangers MA, Groenvold M, Arraras JI, et al. The European Organization for Research and Treatment of Cancer breast cancer-specific quality-of-life questionnaire module: first results from a three-country field study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. Oct 1996;14(10):2756-68. doi:10.1200/JCO.1996.14.10.2756

Chapter 7

Urinary incontinence more than 15 years after premenopausal risk-reducing salpingo-oophorectomy: a multicenter cross-sectional study

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Abstract

Objective
To study the impact of premenopausal risk-reducing salpingo-oophorectomy (RRSO), compared to postmenopausal RRSO, on urinary incontinence (UI) ≥ 10 years later.

Design
Cross-sectional study, nested in a nationwide cohort.

Setting
Multicenter in the Netherlands.

Population
750 women (68% *BRCA1/2* pathogenic variant carriers) who underwent either premenopausal RRSO (≤45 years, n=496) or postmenopausal RRSO (≥54 years, n=254). All participants were ≥55 years at study.

Methods
UI was assessed by the urinary distress inventory-6 (UDI-6); a score ≥33.3 indicated symptomatic UI. The incontinence impact questionnaire short form (IIQ-SF) was used to assess the impact on women's health-related quality of life (HR-QoL). Differences between groups were analyzed using regression analyses adjusting for current age and other confounders.

Main Outcome Measures
Differences in UDI-6 scores and IIQ-SF scores between women with a premenopausal and a postmenopausal RRSO.

Results
Women in the premenopausal RRSO group had slightly higher UDI-6 score compared with women in the postmenopausal RRSO group (p-value 0.053), and their risk of symptomatic UI was non-significantly increased (odds ratio (OR) 2.1, 95% confidence interval (95%CI) 0.93;4.78). A premenopausal RRSO was associated with a higher risk of stress UI (OR 3.5 95%CI 1.2;10.0), and not with urge UI. The proportions of women with a significant impact of UI on HR-QoL were similar in the premenopausal and postmenopausal RRSO groups (10.4% and 13.0%, respectively (p-value .46)).

Conclusions

More than 15 years after premenopausal RRSO, there were no significant differences in overall symptomatic UI between women with a premenopausal and postmenopausal RRSO.

Introduction

Women carrying a *BRCA1/2* germline pathogenic variant (*BRCA1/2*pv) are advised to undergo risk-reducing salpingo-oophorectomy (RRSO) to prevent ovarian cancer. *BRCA1*pv carriers are advised to undergo RRSO at ages 35-40 and *BRCA2*pv carriers at ages 40-45, after completion of childbearing. The consequence of this procedure is an immediate menopause, at a considerably younger age than in women from the general population. This may induce long-term morbidity and reduced health-related quality of life (HR-QoL), due to menopause-related vulvovaginal atrophy and urinary tract symptoms. Reduced circulating estrogen levels due to menopause result in reduced collagen content, urethral shortening, thinning of urethral mucosa and vaginal epithelium, decreased urinary sphincter contractility and reduced bladder compliance.[1] These postmenopausal changes in the urogenital tissues may result in lower urinary tract symptoms such as urgency, recurrent urinary tract infections, and urinary incontinence (UI).[2,3]

The prevalence of UI in postmenopausal women aged over 60 years varies between 38-55%.[4] Up to 70% of women relate the onset of UI to their final menstrual period,[1] which is consistent with a peak in UI prevalence at ages 45-55, suggesting that menopause-associated anatomical and functional changes of the urogenital tissues are important contributors to UI.[5,6] Stress urinary incontinence (SUI, involuntary loss of urine due to abdominal pressure, such as during exercise or coughing) shows a peak prevalence around menopause, and declines afterwards. In contrast, urge urinary incontinence (UUI, the sudden need to pass urine that is difficult to postpone), shows an increasing prevalence with a longer duration after menopause possibly due to progressive atrophy.[4,7,8] The prevalence of UI rises with age.[9] Therefore, when examining risk factors for UI, it is difficult to discriminate effects of menopause from general aging effects. Other established risk factors for UI in women include higher body mass index (BMI), parity and vaginal delivery. Besides menopause, other potential risk factors include i.e. diabetes, and hysterectomy.[1,10,11] Systemic menopausal hormone therapy (MHT) does not appear to reduce UI risk, but may do so when administered vaginally.[12]

While hysterectomy appears to increase the risk of urinary incontinence, studies are inconsistent as to whether a bilateral salpingo-oophorectomy has an additional negative effect on UI.[13-15] The effect of a premenopausal salpingo-oophorectomy has not been examined. Therefore, we aimed to examine the impact of a premenopausal RRSO on the prevalence of UI at least 10 years later. We hypothesized that women with a premenopausal RRSO, compared to equally old women with a postmenopausal RRSO, would more often experience UI due to their longer postmenopausal period.

Materials and Methods

Patient selection and recruitment

Participants were Dutch women participating in the HARMOny study[16] (ClinicalTrials.gov NCT03835793): a multicenter cross-sectional study, nested in a nationwide cohort of women at high familial risk of breast/ovarian cancer.[17,18] Study design and procedures have been described previously.[16] Briefly, between 2018 and 2021, we invited women from this cohort to a study assessing the long-term effects of RRSO on cardiovascular disease, bone health, cognition and HR-QoL. Eligibility criteria included a high familial risk of breast/ovarian cancer, current age of ≥55 years and having undergone RRSO either before age 45 or after age 54. Exclusion criteria were ovarian cancer, metastatic disease and therapy-induced menopause >5 years before RRSO. Breast cancer was not an exclusion criterion. The study has been approved by the Institutional Review Board of the NKI.

Study assessments

Women were asked to complete an online questionnaire on general health, cancer-specific outcomes, cardiovascular health, reproductive history and medical treatments, including use of MHT.

Assessment of urinary incontinence

We assessed urogenital problems with the Urogenital Distress Inventory (UDI-6) and Incontinence Impact Questionnaire short-form (IIQ-SF),[19,20] two validated questionnaires designed to assess UI and the impact of UI on HR-QoL. The UDI-6 is a six-item symptom inventory to assess symptoms associated with lower urinary tract dysfunction. The IIQ-SF is an eight-item instrument to assess impact of UI on physical activity, travel, work, social activities, and emotional health. Responses are scored on a four-point Likert scale. Higher scores indicate more symptom distress (UDI-6) or more impact on daily life (IIQ-SF) (see Table S1 for detailed information). Based on literature, a UDI-6 score of 33.3 is the optimal cut-off for distinguishing women with symptomatic and asymptomatic UI. With a IIQ-SF score of 9.5 or higher, UI has a significant impact on a woman's HR-QoL.[21]

Statistical analyses

Characteristics between the premenopausal RRSO group (RRSO ≤45 years of age) and the postmenopausal RRSO group (≥55 years of age) were compared using the χ^2 test or Fisher's exact test for categorical data, and independent samples t-test for continuous data. We created several dichotomous variables; first for symptomatic UI (UDI-6 score ≥33), second for a significant impact of UI on the HR-QoL (IIQ-SF

score ≥9.5), and last for substantial UUI and SUI by combining the categories 'moderately' and 'greatly' for scoring complaints of UUI and SUI. To examine associations between timing of RRSO and various endpoints, we used multivariable linear regression for the UDI-6 score and the IIQ-SF score, and multivariable logistic regression analyses for the presence of symptomatic UI, UI affecting HR-QoL, UUI, and SUI, yielding regression coefficients and odds ratios (OR) with accompanying 95% confidence intervals (95%CI). We used a Directed Acyclic Graph (DAG) to visualize confounding factors, mediating factors and competing exposures (Figure S1).

In all regression analyses, we explored the confounding effects of age at questionnaire completion, breast cancer history, MHT, BMI, parity, diabetes, and hysterectomy. A variable was removed from the model if the association between the exposure (RRSO) and the outcome (UI) did not change significantly (>10%), except for age and breast cancer which always remained in our model. Because the question on type of delivery was added later in the study questionnaire, this variable was missing for 54.8% of women. Among 335 women who filled out their delivery mode, 88.1% delivered only vaginally, 5.4% had both a vaginal delivery and a caesarean section, and 6.6% delivered by caesarean section. Among women with known delivery mode we explored if delivery mode was a confounding variable. As this was not the case, we did not include delivery mode in our models. We also performed several stratified analyses. Because of the recommendation for *BRCA1*pv carriers to undergo a RRSO between ages 35-40 and for *BRCA2*pv carriers to undergo a RRSO between ages 40-45, we compared prevalence of UI between women with RRSO before age 41 (the early premenopausal group) and between ages 41 and 45 (the later premenopausal group). Additionally, we examined whether the effect of RRSO on UI differed by MHT use (current, former, never), delivery mode and by parity (Supplementary results I, Table S3-S12). For all statistical analyses, Stata, version 15.0 (StataCorp LLC) was used. P-values <.05 were considered statistically significant.

Results

In total, 817 women gave informed consent (response rate 62.3%), of whom 529 were in the premenopausal RRSO group (RRSO ≤45 years of age) and 288 women in the postmenopausal RRSO group (RRSO ≥55 years of age) (Figure 1).

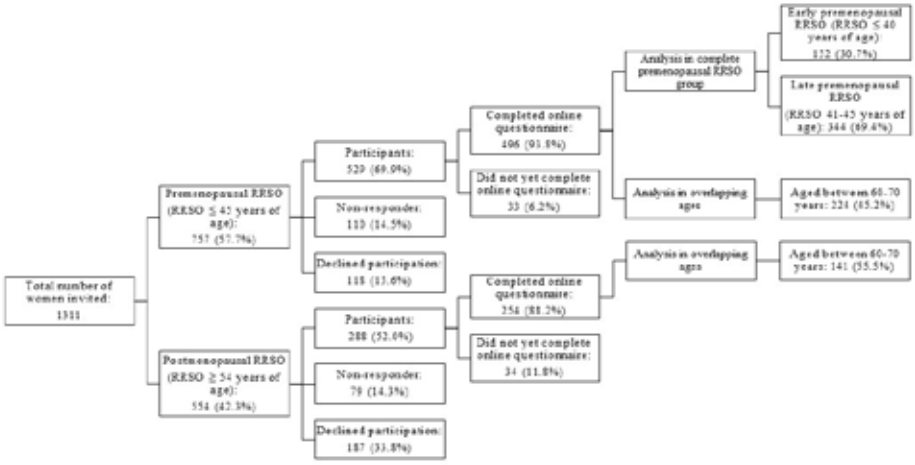

Figure 1. Participant Flowchart. Number of participants enrolled, non-responders and number of women who declined participation. We have sent out regular reminders to women to complete the online questionnaire.

Study participant characteristics

Mean age at questionnaire completion was 60.0 years in the premenopausal group, compared to 70.2 years in the postmenopausal group (p-value <.001) (Table 1). Because women in the premenopausal RRSO group were substantially younger than women in the postmenopausal RRSO group, we restricted the comparison of UI between these groups to 365 women in the overlapping age range, i.e., 60-70 years old at completion of the questionnaire (premenopausal group, n=224, postmenopausal group, n=141). Within all 496 women with a premenopausal RRSO we compared UI between women in the early premenopausal group (n=152) and the later premenopausal group (n=344).

Among women aged 60-70 years at study, mean time since RRSO was 20.6 years in the premenopausal group and 10.6 years in the postmenopausal group (Table 1). In the premenopausal group, mean age at questionnaire completion was 62.7 years, compared to 67.0 years in the postmenopausal group (p-value <.001). Mean time since menopause was 20.6 years in the premenopausal group and 16.7 years in the postmenopausal group (p-value <.001). Sixty-eight percent of women in the premenopausal-RRSO group carried a *BRCA1/2*pv versus 63.1% in the postmenopausal

RRSO group (p-value .32). In the premenopausal RRSO group, 60.3% of women had a history of breast cancer, compared to 58.9% in the postmenopausal group (p-value .79). MHT was more often prescribed to women in the premenopausal RRSO group (29.1%, versus 9.2% in the postmenopausal RRSO group; p-value <.001). Parity and mode of delivery were comparable between the two groups. In the premenopausal RRSO group 18.6% of women had no children compared with 23.0% of women in the postmenopausal RRSO group (p-value .31).

Table 1. Baseline sociodemographic and clinical characteristics of study participants.

	Entire study population		Women aged 60-70 years	
	Premenopausal RRSO (RRSO ≤45 years, n=496)	**Postmenopausal RRSO (RRSO ≥54 years, n=254)**	**Premenopausal RRSO (RRSO ≤45 years, n=224)**	**Postmenopausal RRSO (RRSO ≥54 years, n=141)**
Age at questionnaire completion (mean, SD)	60.0 (3.5)	70.2 (4.3) *	62.7 (2.5)	67.0 (2.1) *
Age at RRSO (mean, SD)	41.7 (2.8)	58.4 (3.6) *	42.1 (2.5)	56.5 (1.9) *
Time since RRSO (mean, SD)	18.3 (4.1)	11.9 (3.0) *	20.6 (3.3)	10.6 (1.9) *
Time since menopause (mean, SD)	18.3 (4.2)	19.9 (6.5) *	20.6 (3.4)	16.7 (5.5) *
Pathogenic genetic variants†				
BRCA1 germline mutation	243 (49.0%)	74 (29.1%) *	109 (48.9%)	39 (27.7%) *
BRCA2 germline mutation	97 (19.6%)	94 (37.0%)	43 (19.3%)	50 (35.5%)
Established non-carrier	156 (31.5%)	86 (33.9%)	71 (31.8%)	52 (36.9%)
Breast cancer (yes)	293 (59.0%)	164 (64.6%)	135 (60.3%)	83 (58.9%)
Breast cancer before RRSO	237 (84.3%)	148 (91.4%) *	105 (80.8%)	73 (91.3%) *
Breast cancer after RRSO	44 (15.7%)	14 (8.6%) *	25 (19.3%)	7 (8.8%) *
Treatment of breast cancer				
Surgery	284 (97.6%)	159 (98.8%)	132 (97.1%)	80 (98.8%)
Chemotherapy	222 (76.3%)	86 (52.4%) *	97 (48.7%)	51 (42.9%)
Radiotherapy	182 (62.5%)	95 (59.0%)	86 (63.2%)	54 (66.7%)
Endocrine therapy	106 (36.4%)	53 (32.9%)	41 (30.2%)	29 (35.8%)
Prophylactic mastectomy (yes) ‡	300 (62.1%)	84 (34.6%) *	140 (61.9%)	48 (33.8%) *
MHT use				
Current user	26 (5.2%)	2 (.8%) *	14 (6.3%)	1 (.7%) *
Past user	101 (20.4%)	28 (11.0%) *	46 (20.5%)	11 (7.8%) *
Never user	337 (67.9%)	213 (83.9%) *	147 (65.6%)	119 (84.4%) *

Table 1. Continued

	Entire study population		Women aged 60-70 years	
	Premenopausal RRSO (RRSO ≤45 years, n=496)	**Postmenopausal RRSO (RRSO ≥54 years, n=254)**	**Premenopausal RRSO (RRSO ≤45 years, n=224)**	**Postmenopausal RRSO (RRSO ≥54 years, n=141)**
Unknown	32 (6.5%)	11 (4.3%)	17 (7.6%)	10 (7.1%)
MHT duration in years (mean (SD))	2.2 (4.5)	1.4 (3.3)	2.1 (4.4)	1.6 (3.9)
BMI (mean, SD)	26.5 (5.0)	25.8 (4.5)	26.6 (5.2)	26.2 (5.0)
Hysterectomy (Yes) [§]	69 (16.2%)	53 (28.5%) *	43 (19.3%)	28 (19.7%)
Mode of delivery				
Vaginal delivery	202 (85.2%)	94 (95.0%) *	101 (90.2%)	52 (96.3%)
Vaginal delivery and Caesarean section	14 (5.9%)	4 (4.0%) *	6 (6.4%)	1 (1.9%)
Caesarean section	21 (8.9%)	1 (1.0%) *	5 (4.5%)	1 (1.9%)
Missing	*259 (52.2%)*	*156 (61.4%)*	*112 (50.0%)*	*88 (62.4%)*
Parity				
0	93 (18.8%)	40 (15.7%)	44 (19.6%)	21 (14.9%)
1-2	278 (56.0%)	151 (59.4%)	134 (59.8%)	79 (56.0%)
3-4	113 (22.8%)	57 (22.4%)	45 (20.1%)	36 (25.5%)
≥ 5	7 (1.4%)	3 (1.2%)	1 (.5%)	2 (14.9%)

* P-value <.05. Groups compared using independent samples t-test, Chi-squared test or Fishers exact test.

[†] All participants had a high familial risk of ovarian cancer. All women were tested for pathogenic variants, not all had a *BRCA1/2* mutation. Established non-carriers include women from BRCA1/2 families who tested negative as well as women from a breast/ovarian cancer family who tested negative for the pathogenic variants tested in the Netherlands.

[‡] Prophylactic mastectomy: bilateral or contralateral.

[§] In the Netherlands a hysterectomy is not standard of care when performing RRSO.

Abbreviations: RRSO: risk-reducing salpingo-oophorectomy; SD: standard deviation; BMI: body mass index; MHT: menopausal hormone therapy.

Additional characteristics of the study population are provided in supplementary table 2.

Urinary incontinence and its impact on HR-QoL at ages 60-70 years in women with a premenopausal or postmenopausal RRSO

Unadjusted mean UDI-6 scores were 20.4 (SD 17.7) and 18.8 (SD 16.2) (p-value .39) in the premenopausal RRSO group and in the postmenopausal RRSO group, respectively (Figure 2). After adjustment for confounders in a linear regression analysis, a premenopausal RRSO was associated with a slightly higher UDI-6 score, but this difference was not statistically significant (β-coefficient 5.0, 95%CI -0.1;10.1).

The proportion of women with a premenopausal RRSO who had symptomatic UI according to the cutoff of 33.33 points was 23.6% compared with 18.9% of women with a postmenopausal RRSO (p-value .31). After adjustment for confounders in a logistic regression analysis, an association between premenopausal RRSO and symptomatic urinary incontinence (UDI-6 score ≥ 33.33) was borderline statistically significant (OR 2.1, 95% CI 0.93;4.78).

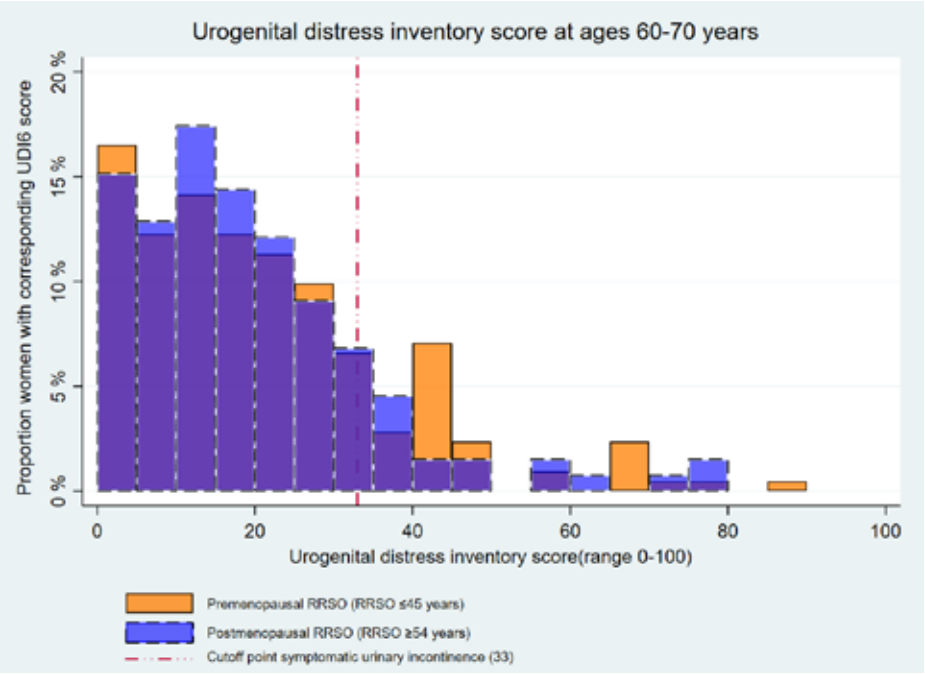

Figure 2. Distribution of UDI-6 scores of women in the premenopausal RRSO group and the postmenopausal RRSO group. Twenty-four percent of women with a premenopausal RRSO experience symptomatic urinary incontinence (UDI-6 score ≥33.33) compared with 19% in the postmenopausal RRSO group.

Assessing the impact of UI using the IIQ-SF, mean scores in the premenopausal and postmenopausal RRSO groups were 3.2 (SD 8.4) and 3.8 (SD 8.9), respectively (p-value .53) (Figure 3). After adjustment for confounders, linear regression analysis did also not show a difference between the groups (β-coefficient -1.0, 95%CI -3.6;1.5). The proportion of women with an IIQ-SF score ≥9.5 was 10.4% in women with a premenopausal RRSO and 13.0% in women with a postmenopausal RRSO (p-value .46). After adjustment for confounders in a logistic regression analysis, a premenopausal RRSO was also not associated with a significant impact of UI on the HR-QoL (OR 0.7, 95%CI 0.3;2.0).

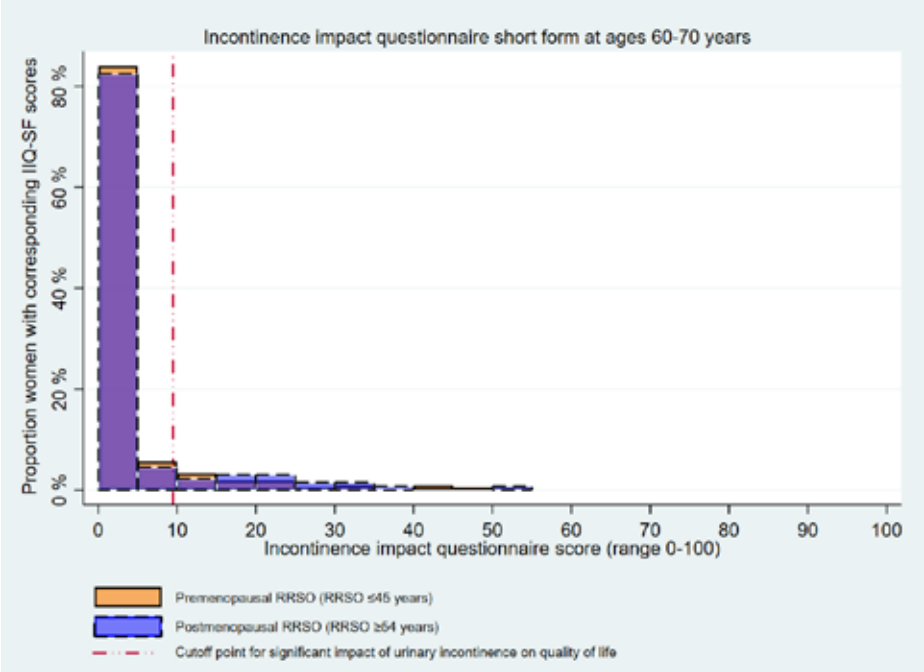

Figure 3. Distribution of IIQ-SF scores of women in the premenopausal RRSO group and the postmenopausal RRSO group. Ten percent of women in the premenopausal RRSO group experience a significant influence of urinary incontinence on the quality of life (IIQ-SF score ≥9.5) compared with 13% in the postmenopausal RRSO group.

Urge and stress urinary incontinence at ages 60-70 years in women with a premenopausal or postmenopausal RRSO

Substantial UUI was reported by 19.6% of women with a premenopausal RRSO (Figure 4), compared with 22.7% in the postmenopausal RRSO group (p-value .48). After adjustment for age, breast cancer and BMI, a premenopausal RRSO was not associated with substantial UUI (OR 1.1, 95% CI 0.5;2.4). Substantial complaints with regard to SUI were experienced by 13% of women with a premenopausal RRSO, compared to eight percent in the postmenopausal RRSO group (p-value .15, Figure 4). After adjustment for age, breast cancer history and BMI, a premenopausal RRSO was associated with a higher risk of substantial SUI complaints (OR 3.5, 95%CI 1.2;10.0). In a regression analysis with 'time since RRSO' as continuous variable, the risk of having substantial SUI complaints increased with 10% with every year since RRSO (OR 1.1, 95%CI 1.01;1.2).

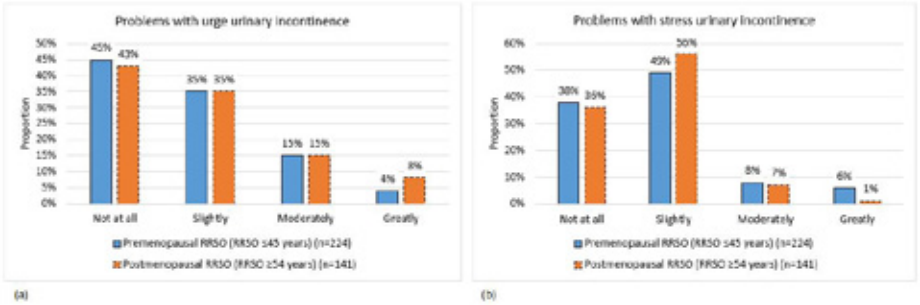

Figure 4. Prevalence of problems with (a) urge urinary incontinence and (b) stress urinary incontinence per RRSO group for women aged 60-70 years.

Urinary incontinence by age at RRSO among women with a premenopausal RRSO, comparing an early premenopausal RRSO (RRSO ≤40 years of age) with later premenopausal RRSO (RRSO at 41-45 years of age)

Mean UDI-6 scores in the early and later premenopausal RRSO groups were 18.2 (SD 17.1) and 18.8 (SD 17.3), respectively (p-value .74) (Figure 5). After adjustment for confounders in a linear regression analysis, an early premenopausal RRSO was not associated with a higher UDI-6 score (95%CI -4.2;2.9). The proportions of women with symptomatic UI were 22.6% and 20.5% in the early and later premenopausal RRSO groups, respectively (p-value .61). Multivariable logistic regression analysis also showed that an early premenopausal RRSO was not associated with symptomatic UI (OR1.0, 95%CI 0.95;1.04).

When we stratified the premenopausal RRSO group according to MHT use, mean UDI-6 scores in women who still used MHT (n=25), previously used MHT (n=97) and never used MHT (n=320) were 18.9 (SD 20.2), 20.2 (SD 18.3), and 17.6 (16.2) (p-value .40), respectively. In women who ever used MHT or who still used MHT at time of study, 28.1% had symptomatic UI, compared with 18.1% in women who never used MHT (p-value .02). After adjustment for age, breast cancer and BMI, former MHT use was significantly associated with symptomatic UI (OR1.9, 95%CI 1.1;3.3), but current MHT use was not (OR1.9, 95%CI 0.7;5.2).

Results from the IIQ-SF score by age at RRSO show no clear differences in UI impact between the early and later premenopausal RRSO groups (Supplementary Results II).

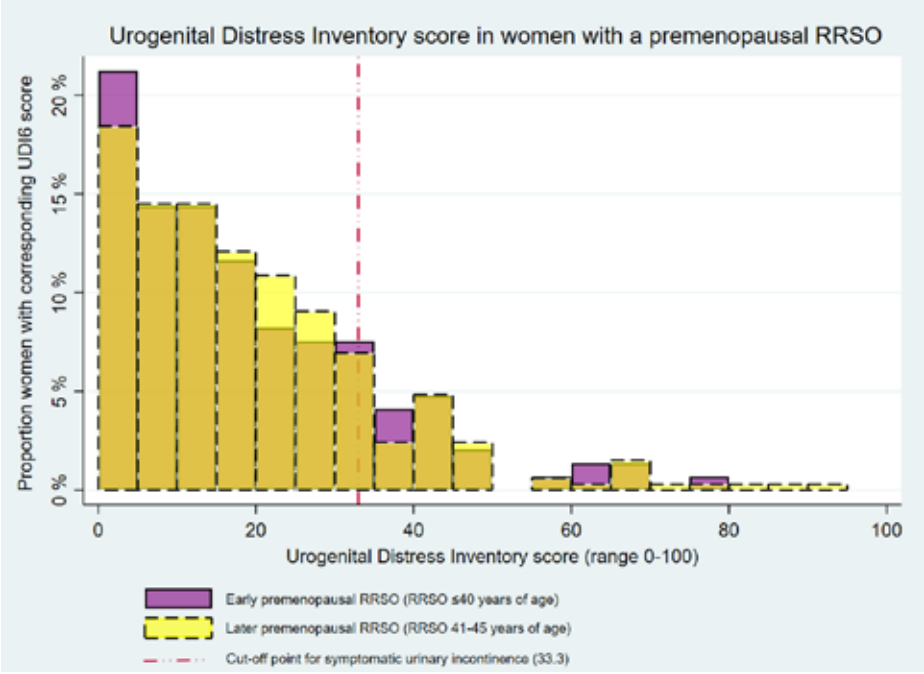

Figure 5. Distribution of urogenital distress inventory scores of women in the early premenopausal RRSO group and the later premenopausal RRSO group.

Urge and stress urinary incontinence in women with a premenopausal RRSO, comparing an early premenopausal RRSO with later premenopausal RRSO

Substantial UUI was reported by 12.5% of women with an early premenopausal RRSO, compared with 16.6% in the later premenopausal RRSO group (p-value .25) (Figure S3a). After adjustment for age, breast cancer and BMI, an early premenopausal RRSO was not associated with substantial UUI (OR 0.54, 95% CI 0.28;1.04). Regarding SUI, 11.8% of women with an early premenopausal RRSO experienced substantial complaints, compared with 12.5% of women in the later premenopausal RRSO group (p-value .84) (Figure S3b). After adjustment for age, breast cancer history and BMI, an early premenopausal RRSO was not associated with a higher risk of substantial SUI complaints (OR 0.998, 95%CI 0.52;1.92).

Discussion

Main findings

Our study is the first one to assess UI more than 15 years after a premenopausal RRSO (≤ age 45) compared with women with a postmenopausal RRSO (≥ age 54). At the age of 60-70 years, women with a premenopausal RRSO had a slightly higher UDI-6 score, but the difference with the postmenopausal group was not statistically significant. The risk of symptomatic UI associated with a premenopausal RRSO was also somewhat increased, but not statistically significantly so. There was no difference between the two groups regarding impact of UI on HR-QoL. However, a premenopausal RRSO was associated with substantial complaints of SUI: women with a premenopausal RRSO had a 3.5-fold increased risk of substantial SUI compared with women with a postmenopausal RRSO. Regarding UUI we found no difference between the two RRSO groups. When we examined UI within the premenopausal group and compared women with a very early RRSO (before age 41) and a later premenopausal RRSO (age 41-45), we found no differences in symptoms of UI and the impact of UI on HR-QoL. We did find that women who had ever used MHT (current and former users) more often experienced symptomatic UI according to the UDI-6, and their incontinence was more often of influence on the HR-QoL.

Within the premenopausal RRSO group we performed stratified analysis according to age at RRSO and MHT use. Based on our hypothesis and the results in the 60-70 year old group, we would have expected more UI in the early premenopausal group. However, we did not find an association between timing of premenopausal RRSO and UI. This might be explained by the rather small difference in time since RRSO between the two groups; women with an early premenopausal RRSO were on average 21.1 years since oophorectomy, women with a later premenopausal RRSO were on average 17.0 years since oophorectomy. Remarkably, both past users of MHT and women who currently used MHT more often experienced symptomatic UI and UI impacting HR-QoL. This association might be explained by confounding by indication, considering that women with more substantial complaints of UI may have been prescribed MHT more frequently.

Strengths and Limitations

One of the limitations of our study is the difference in age distributions of the premenopausal and postmenopausal RRSO groups at time of study participation. This age difference was largely due to the strongly increasing prevalence of premenopausal RRSO after 2007.[22] To overcome this limitation, we compared UI between women with a premenopausal and a postmenopausal RRSO in the overlapping age range, 60-70 years at questionnaire completion, and corrected for age in all analyses.

Furthermore, as this is a cross-sectional study >15 years after RRSO, we do not have data on UI shortly after RRSO. As we are the first to assess UI after a premenopausal bilateral oophorectomy, there are no available data on UI prevalence directly after RRSO. Future research should focus more on the development of UI in the years after RRSO to see how many women experience SUI and UUI.

Strengths of our study include the large sample size, providing sufficient power to perform subgroup analyses. Additionally, by excluding women with RRSO between ages 46 and 54, we were able to make a more distinct evaluation of the differences in UI between women who had undergone RRSO prior to the onset of natural menopause and women with a postmenopausal RRSO. The participation rate was good (62.3%) and we employed validated questionnaires that are widely used.

Interpretation

We can compare our results with UDI-6 and IIQ-SF scores reported for the Dutch general population.[19] The mean UDI-6 scores in our study (20.4 and 18.8.in the premenopausal and postmenopausal RRSO groups, respectively) were higher than the mean UDI-6 score of 12.2 (SD 12.7) in the Dutch reference data, but this difference may not be clinically relevant.[19] The mean IIQ-SF scores in our study (3.2 and 3.8 for the pre- and postmenopausal groups, respectively) are comparable with the Dutch reference data,[19] which show a mean IIQ-SF score of 4.2 (SD 11.2). Comparing our results on UI prevalence with the prevalence in the general population of other Western countries is difficult, as the questionnaires and definitions of UI used in the literature differ substantially. The prevalence of substantial UUI (19.6% and 22.7% in the premenopausal and postmenopausal RRSO groups, respectively) in our study was higher than reported by Linde et al. (7.9%), while the prevalence of substantial SUI in our population was lower (13.4% and 8.5% in the premenopausal and postmenopausal RRSO groups, respectively) compared to the prevalence found by Linde et al. (25.4%).[10]

We calculated that, based on a two-sided α of 0.05 and 350 women in the study, we had 80% power to detect a difference in UDI-6 score of 3.8 between the two groups. We observed a nonsignificant difference of 1.6 in women aged 60-70 years. Based on our effect size calculations, we cannot exclude the possibility that the number of women included in this analysis was not large enough to identify this difference as statistically significant. However, one could also argue that this difference is not clinically relevant. Our findings are generally reassuring for women who underwent a premenopausal. RRSO. Our results regarding SUI are remarkable as, in general, the peak prevalence of SUI occurs postpartum and around menopause, and the prevalence of UUI increases after menopause. As our study participants had been

postmenopausal for a substantial period, we had expected a higher prevalence of UUI rather than SUI in women with a premenopausal RRSO. It is possible that the peak prevalence of SUI is higher after surgical menopause than after natural menopause. Unfortunately, we could not find any literature regarding the prevalence of SUI after early surgical menopause.

Future studies should focus on the short- and long-term consequences of a RRSO on urinary incontinence as it can have a significant impact on the HR-QoL. Future researchers should specifically take into account use of MHT, as the low proportion of MHT users in our study precluded subgroup analyses in MHT users.

Conclusions

To conclude, at the age of 60-70 years, more than 15 years after premenopausal RRSO, women reported slightly higher UI scores and slightly more symptomatic UI than women of similar age with a RRSO after natural menopause. However, these differences were not statistically significant and did not to lead to a lower HR-QOL in women with a premenopausal RRSO. Unexpectedly, we found an association between a premenopausal RRSO and SUI, which deserves further study. This study highlights the importance of addressing UI when counseling this special population of BRCA pathogenic variant carriers, as many women do not bring this subject up spontaneously.[23]

References

1. Calleja-Agius J, Brincat MP. The urogenital system and the menopause. Climacteric : the journal of the International Menopause Society 2015;18 Suppl 1:18-22. (In eng). DOI: 10.3109/13697137.2015.1078206.
2. Robinson D, Cardozo LD. The role of estrogens in female lower urinary tract dysfunction. Urology 2003;62(4 Suppl 1):45-51. (In eng). DOI: 10.1016/s0090-4295(03)00676-9.
3. Sturdee DW, Panay N, International Menopause Society Writing G. Recommendations for the management of postmenopausal vaginal atrophy. Climacteric : the journal of the International Menopause Society 2010;13(6):509-22. (In eng). DOI: 10.3109/13697137.2010.522875.
4. Russo E, Caretto M, Giannini A, et al. Management of urinary incontinence in postmenopausal women: An EMAS clinical guide. Maturitas 2021;143:223-230. (In eng). DOI: 10.1016/j.maturitas.2020.09.005.
5. Ebbesen MH, Hunskaar S, Rortveit G, Hannestad YS. Prevalence, incidence and remission of urinary incontinence in women: longitudinal data from the Norwegian HUNT study (EPINCONT). BMC urology 2013;13:27. (In eng). DOI: 10.1186/1471-2490-13-27.
6. Hannestad YS, Rortveit G, Sandvik H, Hunskaar S. A community-based epidemiological survey of female urinary incontinence: the Norwegian EPINCONT study. Epidemiology of Incontinence in the County of Nord-Trøndelag. Journal of clinical epidemiology 2000;53(11):1150-7. (In eng). DOI: 10.1016/s0895-4356(00)00232-8.
7. Haylen BT, de Ridder D, Freeman RM, et al. An International Urogynecological Association (IUGA)/International Continence Society (ICS) joint report on the terminology for female pelvic floor dysfunction. Neurourology and urodynamics 2010;29(1):4-20. (In eng). DOI: 10.1002/nau.20798.
8. Mishra GD, Cardozo L, Kuh D. Menopausal transition and the risk of urinary incontinence: results from a British prospective cohort. BJU international 2010;106(8):1170-5. (In eng). DOI: 10.1111/j.1464-410X.2010.09321.x.
9. Chen YC, Chen GD, Hu SW, Lin TL, Lin LY. Is the occurrence of storage and voiding dysfunction affected by menopausal transition or associated with the normal aging process? Menopause (New York, NY) 2003;10(3):203-8. (In eng). DOI: 10.1097/00042192-200310030-00005.
10. Linde JM, Nijman RJM, Trzpis M, Broens PMA. Urinary incontinence in the Netherlands: Prevalence and associated risk factors in adults. Neurourology and urodynamics 2017;36(6):1519-1528. (In eng). DOI: 10.1002/nau.23121.
11. Alvaro R, Araco F, Gravante G, et al. Epidemiological aspects of urinary incontinence in a female population of an Italian region. International urogynecology journal 2010;21(7):873-83. (In eng). DOI: 10.1007/s00192-010-1112-6.
12. Quinn SD, Domoney C. The effects of hormones on urinary incontinence in postmenopausal women. Climacteric : the journal of the International Menopause Society 2009;12(2):106-13. (In eng). DOI: 10.1080/13697130802630083.
13. Walsh K, Stone AR. How is the lower urinary tract affected by gynaecological surgery? BJU international 2004;94(3):272-5. (In eng). DOI: 10.1111/j.1464-410X.2003.04961.x.
14. Kudish BI, Shveiky D, Gutman RE, et al. Hysterectomy and urinary incontinence in postmenopausal women. International urogynecology journal 2014;25(11):1523-31. (In eng). DOI: 10.1007/s00192-014-2422-x.
15. Juliato CR, Baccaro LF, Pedro AO, Gabiatti JR, Lui-Filho JF, Costa-Paiva L. Factors associated with urinary incontinence in middle-aged women: a population-based household survey. International urogynecology journal 2017;28(3):423-429. (In eng). DOI: 10.1007/s00192-016-3139-9.

16. Terra L, Hooning MJ, Heemskerk-Gerritsen BAM, et al. Long-Term Morbidity and Health After Early Menopause Due to Oophorectomy in Women at Increased Risk of Ovarian Cancer: Protocol for a Nationwide Cross-Sectional Study With Prospective Follow-Up (HARMOny Study). JMIR research protocols 2021;10(1):e24414. (In eng). DOI: 10.2196/24414.
17. Schrijver LH, Olsson H, Phillips KA, et al. Oral Contraceptive Use and Breast Cancer Risk: Retrospective and Prospective Analyses From a BRCA1 and BRCA2 Mutation Carrier Cohort Study. JNCI Cancer Spectr 2018;2(2):pky023. DOI: 10.1093/jncics/pky023.
18. Wevers MR, Schmidt MK, Engelhardt EG, et al. Timing of risk reducing mastectomy in breast cancer patients carrying a BRCA1/2 mutation: retrospective data from the Dutch HEBON study. Familial cancer 2015;14(3):355-63. (In eng). DOI: 10.1007/s10689-015-9788-x.
19. Utomo E, Korfage IJ, Wildhagen MF, Steensma AB, Bangma CH, Blok BF. Validation of the Urogenital Distress Inventory (UDI-6) and Incontinence Impact Questionnaire (IIQ-7) in a Dutch population. Neurourology and urodynamics 2015;34(1):24-31. (In eng). DOI: 10.1002/nau.22496.
20. Uebersax JS, Wyman JF, Shumaker SA, McClish DK, Fantl JA. Short forms to assess life quality and symptom distress for urinary incontinence in women: the Incontinence Impact Questionnaire and the Urogenital Distress Inventory. Continence Program for Women Research Group. Neurourology and urodynamics 1995;14(2):131-9. (In eng). DOI: 10.1002/nau.1930140206.
21. Skorupska K, Grzybowska ME, Kubik-Komar A, Rechberger T, Miotla P. Identification of the Urogenital Distress Inventory-6 and the Incontinence Impact Questionnaire-7 cutoff scores in urinary incontinent women. Health Qual Life Outcomes 2021;19(1):87. (In eng). DOI: 10.1186/s12955-021-01721-z.
22. Hermsen BB, Olivier RI, Verheijen RH, et al. No efficacy of annual gynaecological screening in BRCA1/2 mutation carriers; an observational follow-up study. British journal of cancer 2007;96(9):1335-42. (In eng). DOI: 10.1038/sj.bjc.6603725.
23. Sullivan R, McNicholas T, Claffey P, Kenny RA, Briggs R. Unreported urinary incontinence: population-based prevalence and factors associated with non-reporting of symptoms in community-dwelling people ≥ 50 years. Eur Geriatr Med 2021;12(2):405-412. (In eng). DOI: 10.1007/s41999-020-00412-0.

Declarations

Ethics approval and consent to participate

This study will be conducted according to the standards of Good Clinical Practice, in agreement with the principles of the Declaration of Helsinki and with the Dutch law as stated in the Medical Research Involving Human Subjects Act (WMO). The study has been approved in writing by the Institutional Review Board of the AVL/NKI to be conducted in all 9 University Medical Centers and the Antoni van Leeuwenhoek and has been registered at "CCMO Toetsingonline" from the Dutch Central Committee on Research involving Human Subjects (file number NL63554.031.17) and on clinicaltrials.gov, M18HAR. Results will be disseminated through peer-reviewed publications and will be incorporated in follow-up guidelines.

Authors' contributions

FvL, MH, AM and LT were involved in the conception and design of the study. LT, FvL, MB, EE, MM, MH and BHG drafted the manuscript. MB, JR, HvD, JdH, EvD, CM, BS, KG, LvdK, JC, MW, MA, KvE, IvdB, LB, CvA, EG, AS and AM were involved in the final version of the manuscript.

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Conflicts of interest

The authors declare that they have no conflicts of interests.

Data sharing

With publication, de-identified data collected for the study, including participant data, will be made available to others upon reasonable request. Data can be requested with a proposal by sending an e-mail to the corresponding author. Study protocol and statistical analysis plan are available on clinicaltrials.gov, file number NCT03835793.

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Supplementary Material

Supplementary Methods: Imputation of type of delivery

Because the question on type of delivery was added later in the study questionnaire, this variable is missing for 54.8% of women. Based on previous literature, we expected that 92% of women would have delivered vaginally and 8% by caesarean section, as this was the standard in the Netherlands around 1990. [1,2] Based on the 335 women who filled out their mode of delivery, 88.1% delivered only vaginally, 5.4% had both a vaginal delivery and a caesarean section, and 6.6% delivered by caesarean section. In our main analysis we imputed the data on delivery mode by assuming all missing values were vaginal deliveries. In sensitivity analyses we repeated the analyses with random assignment of missings to vaginal deliveries (92%) and caesarean sections (8%).

Supplementary Results I

Sensitivity analyses in women aged 60-70 years comparing women with a premenopausal RRSO with women with a postmenopausal RRSO on prevalence of stress urinary incontinence

In sensitivity analyses in which different imputation strategies were used for women with missing mode of delivery, results were the same, showing an association between premenopausal RRSO and an increased OR for SUI. Additionally, we performed a sensitivity analysis in which women with at least one child but missing mode of delivery were assigned to a vaginal delivery. The results remained the same: a premenopausal RRSO was associated with a higher risk of substantial SUI. Subsequently we performed a stratified analysis according to mode of delivery in which a premenopausal RRSO was even more strongly associated with SUI with an OR of 6.9 (95%CI 1.9;24.7) in women with a vaginal delivery.

Supplementary Results II

Incontinence impact questionnaire score by age at RRSO among women with a premenopausal RRSO

Mean IIQ-SF score in the early premenopausal RRSO group was 3.0 (SD 8.8) compared with 3.1 (SD 8.2) in the later premenopausal RRSO group (p-value .89) (Figure S1). After adjusting for age, breast cancer, BMI and modus of delivery, an early premenopausal RRSO was not associated with a higher IIQ-SF score (95%CI -3.3;1.6). Eight percent of women with an early premenopausal RRSO had UI with a significant impact on the HR-QoL compared with 10.9% of women with a later premenopausal

RRSO (p-value .29). After adjusting for age, breast cancer, MHT use and BMI an early premenopausal RRSO was also not significantly associated with UI with a significant impact on the HR-QoL (OR .44, 95%CI 0.2;1.1).

Mean IIQ-SF scores in women who still used MHT, formerly used MHT and never used MHT were 4.8 (SD 10.5), 4.6 (SD 11.5), and 2.5 (SD 7.2) (p-value .06), respectively. Of women who currently used MHT, 20% had UI with a significant impact on the HR-QoL. In women with a premenopausal RRSO who never used MHT this was 8.2% and in women with a premenopausal RRSO who formerly used MHT 14.3% had UI with a significant impact on the HR-QoL (p-value .04). After adjustment for age, breast cancer and BMI, current and former MHT use were borderline significantly associated with a higher odds for impact of UI on HR-QoL (current MHT use OR 3.3, 95%CI 0.997;11.2, former MHT use OR 2.0, 95%CI 0.96;4.2).

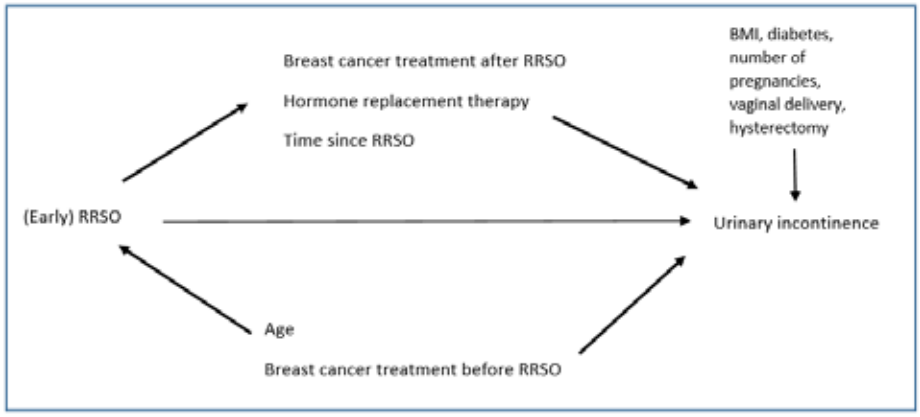

Figure S1. Directed Acyclic Graph.

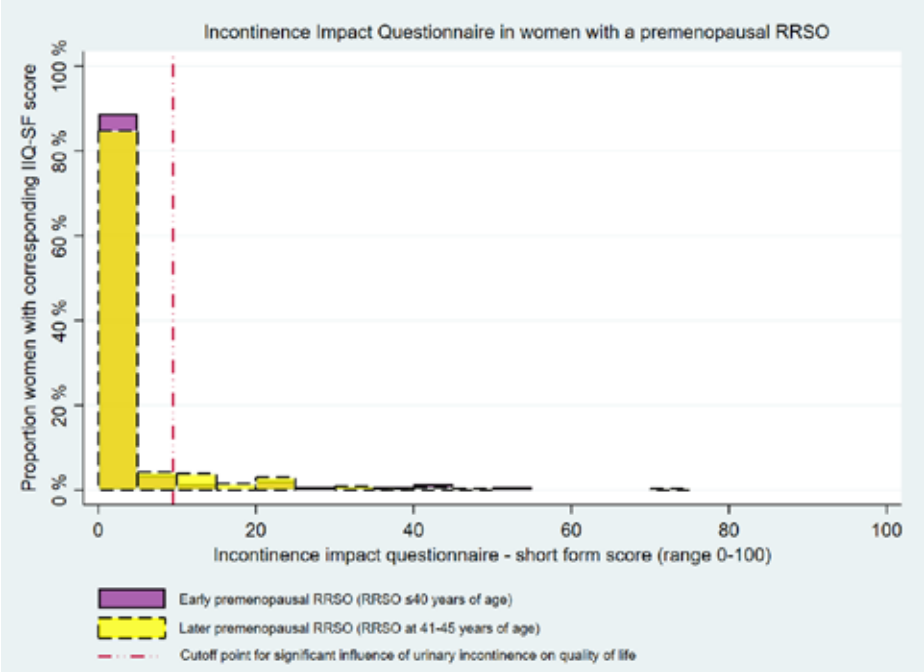

Figure S2. Distribution of incontinence impact questionnaire short form scores of women in the early premenopausal RRSO group and the later premenopausal RRSO group.

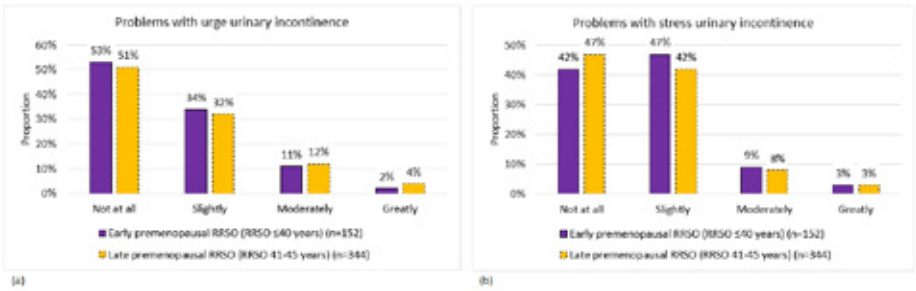

Figure S3. Prevalence of problems with (a) urge urinary incontinence and (b) stress urinary incontinence per premenopausal RRSO group.

Table S1. Study outcome measures and corresponding questionnaires.

Questionnaire	Variable	Questions	Scoring system
Urogenital distress inventory (UDI-6) [3]	Lower urinary tract	Frequent urination Leakage related to feeling of urgency Leakage related to activity, coughing or sneezing Small amounts of leakage (drops) Difficulty emptying bladder Pain or discomfort in lower abdomen or genital area	6 items, 4 point Likert-scale. 0 = not at all – 1 = slightly – 2 = moderately – 3 = greatly. Higher score indicate more symptoms associated with the lower urinary tract. If more than 2 items are missing, a total score is not to be calculated.
Incontinence Impact Questionnaire (IIQ7) [3]	life-impact assessment instrument specific to urinary incontinence	Has urinary leakage affected your: Ability to do household chores Physical recreation such as walking, swimming or other exercise Entertainment activities Ability to travel by car or bus more than 30 minutes from home Employment outside the house Participation in social activities outside your home Emotional health Feeling of frustration	7 items, 4 point Likert-scale. 0 = not at all – 1 = slightly – 2 = moderately – 3 = greatly. Higher score indicate more impact of urinary incontinence on daily life. If more than 2 items are missing, a total score is not to be calculated.

Table S2. Additional characteristics of all study participants.

	Entire study population		Women aged 60-70 years	
	Premenopausal RRSO (n=499)	**Postmenopausal RRSO (n=256)**	**Premenopausal RRSO (n=226)**	**Postmenopausal RRSO (n=142)**
Psychological interventions taken for menopausal complaints (yes)	45 (9.0%)	15 (5.9%)	12 (5.3%)	7 (4.9%)
Dietary intervention for menopausal complaints (yes)	43 (8.6%)	9 (3.5%)	14 (6.2%)	6 (4.2%)
Physical exercise for menopausal complaints (yes)	27 (5.4%)	12 (4.7%)	13 (5.8%)	6 (4.2%)
Smoking status at study questionnaire				
Non-smoker	250 (50.1%)	112 (43.8%)*	94 (41.6%)	64 (45.1%)
Former smoker	211 (42.3%)	134 (52.3%)*	106 (46.9%)	64 (45.1%)
Current smoker	36 (7.2%)	9 (3.5%)*	18 (8.0%)	7 (4.9%)
Pack-years smoked (mean, SD)	14.5 (11.4)	17.0 (15.4)	23.9 (14.4)	23.8 (15.1)
Educational level				
Primary school/lower level high school	138 (27.6%)	109 (42.6%)*	66 (29.2%)	51 (35.9%)
Middle level high school	165 (33.1%)	45 (17.6%)*	68 (30.1%)	34 (23.9%)
Advanced vocational/ university	158 (31.7%)	81 (31.6%)*	77 (34.1%)	44 (31.0%)
Employment status at study questionnaire				
Full-time job/ part-time job	282 (56.5%)	29 (11.3%)*	109 (48.2%)	25 (17.6%)*
Retired	35 (7.0%)	159 (62.1%)*	29 (12.8%)	71 (50.0%)*
Housewife/voluntary work	42 (8.4%)	23 (9.0%)*	25 (11.1%)	14 (9.9%)*
Completely/partially incapacitated for work	51 (10.2%)	7 (2.7%)*	21 (9.3%)	7 (4.9%)*
(temporary) Unemployed	44 (8.8%)	11 (4.3%)*	23 (10.2%)	10 (7.0%)*

* P-value <.05. Groups compared using independent samples t-test, Chi-squared test or Fishers exact test. Abbreviations: RRSO: risk-reducing salpingo-oophorectomy; SD: standard deviation; BMI: body mass index; HRT: hormone replacement therapy in sexually active women.

Table S3. Results of the Urogenital Distress Inventory (UDI-6) per RRSO group.

Urogenital Distress Inventory (UDI-6)	Premenopausal RRSO (RRSO ≤45 years, n=224) (n, %)	Postmenopausal RRSO (RRSO ≥54 years, n=141) (n, %)	p-value
How much are you bothered by frequent urination			**.62**
Not at all	101 (45.1%)	72 (51.1%)	
Slightly	70 (31.3%)	37 (26.2%)	
Moderately	42 (18.8%)	27 (19.2%)	
Greatly	11 (4.9%)	5 (3.6%)	
How much are you bothered by leakage related to feeling of urgency			**.61**
Not at all	101 (45.1%)	60 (42.6%)	
Slightly	79 (35.3%)	49 (34.8%)	
Moderately	34 (15.2%)	21 (14.9%)	
Greatly	10 (4.5%)	11 (7.8%)	
How much are you bothered by leakage related to activity, coughing or sneezing			**.21**
Not at all	92 (41.1%)	54 (38.0%)	
Slightly	105 (46.9%)	78 (54.9%)	
Moderately	16 (7.1%)	8 (5.6%)	
Greatly	11 (4.9%)	2 (1.4%)	
How much are you bothered by small amounts of leakage (drops)			**.72**
Not at all	159 (72.0%)	105 (75.5%)	
Slightly	50 (22.6%)	26 (18.7%)	
Moderately	8 (3.6%)	4 (2.9%)	
Greatly	4 (1.8%)	4 (2.9%)	
How much are you bothered by difficulty emptying bladder			**.54**
Not at all	134 (60.6%)	91 (65.0%)	
Slightly	61 (27.6%)	39 (27.9%)	
Moderately	20 (9.1%)	7 (5.0%)	
Greatly	6 (2.7%)	3 (2.1%)	
How much are you bothered by pain or discomfort in lower abdominal or genital area			**.68**
Not at all	75.9%)	114 (80.3%)	
Slightly	45 (20.1%)	25 (17.6%)	
Moderately	7 (3.1%)	3 (2.1%)	
Greatly	2 (0.9%)	0 (0.0%)	

Table S4. Results of the Incontinence Impact Questionnaire Short Form (IIQ-SF) per RRSO group.

Incontinence Impact Questionnaire Short Form (IIQ-SF)	Premenopausal RRSO (RRSO ≤45 years, n=224)(n, %)	Postmenopausal RRSO (RRSO ≥54 years, n=141)(n, %)	p-value
Has urine leakage affected your ability to do household chores (cooking, housecleaning, laundry)?			**1.00**
Not at all	210 (93.8%)	132 (93.6%)	
Slightly	12 (5.4%)	8 (5.7%)	
Moderately	2 (.9%)	1 (.7%)	
Greatly	0 (0.0%)	0 (0.0%)	
Has urine leakage affected your physical recreation such as walking swimming, or other exercise?			**.82**
Not at all	187 (83.5%)	115 (81.6%)	
Slightly	30 (13.4%)	20 (14.2%)	
Moderately	6 (2.7%)	6 (4.3%)	
Greatly	1 (.5%)	0 (0.0%)	
Has urine leakage affected your entertainment activities (movies, concerts, etc.)?			**.92**
Not at all	208 (92.4%)	129 (91.5%)	
Slightly	16 (7.1%)	11 (7.8%)	
Moderately	1 (.4%)	1 (.7%)	
Greatly	0 (0.0%)	0 (0.0%)	
Has urine leakage affected your ability to travel by car or bus more than 30 minutes from home?			**.34**
Not at all	209 (92.9%)	125 (89.9%)	
Slightly	11 (4.9%)	12 (8.6%)	
Moderately	5 (2.2%)	2 (1.4%)	
Greatly	0 (0.0%)	0 (0.0%)	
Has urine leakage affected your employment (work) outside the home?			**.19**
Not at all	211 (94.6%)	122 (90.4%)	
Slightly	10 (4.5%)	12 (8.9%)	
Moderately	2 (.9%)	1 (.7%)	
Greatly	0 (0.0%)	0 (0.0%)	

Table S4. Continued

Incontinence Impact Questionnaire Short Form (IIQ-SF)	Premenopausal RRSO (RRSO ≤45 years, n=224)(n, %)	Postmenopausal RRSO (RRSO ≥54 years, n=141)(n, %)	p-value
Has urine leakage affected your participation in social activities outside your home?			**.38**
Not at all	201 (91.8%)	118 (88.1%)	
Slightly	15 (6.9%)	15 (11.2%)	
Moderately	3 (1.4%)	1 (.8%)	
Greatly	0 (0.0%)	0 (0.0%)	
Has urine leakage affected your emotional health (nervousness, depression, etc.)?			**.76**
Not at all	199 (91.7%)	125 (94.0%)	
Slightly	15 (6.9%)	7 (5.3%)	
Moderately	3 (1.4%)	1 (.8%)	
Greatly	0 (0.0%)	0 (0.0%)	
Has urine leakage affected your feeling frustrated?			**.32**
Not at all	187 (87.0%)	116 (87.2%)	
Slightly	19 (8.8%)	15 (11.3%)	
Moderately	9 (4.2%)	2 (1.5%)	
Greatly	0 (0.0%)	0 (0.0%)	

Table S5. Mean scores of the urogenital distress inventory – 6 per stratified analysis.

Urogenital Distress Inventory – 6	Mean score	Standard deviation
Age 60-70		
Premenopausal RRSO (RRSO ≤45 years of age)	20.4	17.7
Postmenopausal RRSO (RRSO ≥ 54 years of age)	18.8	16.2
Premenopausal RRSO& 0 children	16.7	15.9
Postmenopausal RRSO & 0 children	14.7	17.1
Premenopausal RRSO & ≥ 1 child	21.3	18.0
Postmenopausal RRSO & ≥ 1 child	19.5	16.0
Premenopausal RRSO (RRSO ≤45 years of age)		
Early premenopausal RRSO (RRSO ≤40 years of age)	18.2	17.1
Later premenopausal RRSO (RRSO at 41-45 years of age)	18.8	17.3
Current users MHT	18.9	20.2
Former users MHT	20.2	18.3
Never users MHT	17.6	16.2

Table S6. Proportion of women with symptomatic urinary incontinence according to the cut-off score of ≥ 33.33 on the urogenital distress inventory -6 per stratified analysis.

Urogenital Distress Inventory – 6 cut-off for symptomatic urinary incontinence (score ≥ 33.33)	n	%
Age 60-70		
Premenopausal RRSO (RRSO ≤45 years of age)	50	23.6%
Postmenopausal RRSO (RRSO ≥ 54 years of age)	25	18.9%
Premenopausal RRSO& 0 children	7	16.7%
Postmenopausal RRSO & 0 children	2	10.0%
Premenopausal RRSO & ≥ 1 child	43	25.3%
Postmenopausal RRSO & ≥ 1 child	23	20.5%
Premenopausal RRSO (RRSO ≤45 years of age)		
Early premenopausal RRSO (RRSO ≤40 years of age)	33	22.6%
Later premenopausal RRSO (RRSO at 41-45 years of age)	68	20.5%
Current users MHT	6	24.0%
Former users MHT	27	27.8%
Never users MHT	58	18.1%

Table S7. Mean scores of the incontinence impact questionnaire – short form per stratified analysis.

Incontinence Impact Questionnaire short form	Mean score	Standard deviation
Age 60-70		
Premenopausal RRSO (RRSO ≤45 years of age)	3.2	8.4
Postmenopausal RRSO (RRSO ≥ 54 years of age)	3.8	8.9
Premenopausal RRSO& 0 children	2.3	6.0
Postmenopausal RRSO & 0 children	0.7	2.1
Premenopausal RRSO & ≥ 1 child	3.4	8.8
Postmenopausal RRSO & ≥ 1 child	4.4	9.5
Premenopausal RRSO (RRSO ≤45 years of age)		
Early premenopausal RRSO (RRSO ≤40 years of age)	3.0	8.8
Later premenopausal RRSO (RRSO at 41-45 years of age)	3.1	8.2
Current users MHT	4.8	10.5
Former users MHT	4.6	11.5
Never users MHT	2.5	7.2

Table S8. Proportion of women with symptomatic urinary incontinence according to the cut-off score of ≥9 on the incontinence impact questionnaire – short form per stratified analysis.

Incontinence Impact Questionnaire short form – cut-off for significant impact on quality of life (score ≥ 9)	n	%
Age 60-70		
Premenopausal RRSO (RRSO ≤45 years of age)	22	10.4%
Postmenopausal RRSO (RRSO ≥ 54 years of age)	17	13.0%
Premenopausal RRSO& 0 children	4	10.0%
Postmenopausal RRSO & 0 children	0	0.0%
Premenopausal RRSO & ≥ 1 child	18	10.5%
Postmenopausal RRSO & ≥ 1 child	17	15.2%
Premenopausal RRSO (RRSO ≤45 years of age)		
Early premenopausal RRSO (RRSO ≤40 years of age)	11	7.8%
Later premenopausal RRSO (RRSO at 41-45 years of age)	25	10.9%
Current users MHT	5	20.0%
Former users MHT	13	14.3%
Never users MHT	26	8.2%

Table S9. Regression coefficients on the urogenital distress inventory – 6 of variables and their corresponding 95% confidence interval.

Urogenital Distress Inventory – 6	B-coefficient	95% confidence interval	
Age 60-70			
Premenopausal RRSO (RRSO ≤45 years of age)	4.91	-0.22	10.05
Postmenopausal RRSO (RRSO ≥ 54 years of age)	0.00		
Age (per year increase)	0.70	-0.09	1.49
No history of breast cancer	0.00		
History of breast cancer	-3.22	-7.01	0.57
0 children	0.00		
≥ 1 child	2.50	-2.22	7.22
BMI (per 1kg/m^2 increase)	0.56	0.49	1.22
Premenopausal RRSO (RRSO ≤45 years of age)			
Early premenopausal RRSO (RRSO ≤40 years of age)	0.00		
Later premenopausal RRSO (RRSO at 41-45 years of age)	0.57	-3.26	4.40
Age (per year increase)	0.51	0.01	1.01
No history of breast cancer	0.00		
History of breast cancer	-0.96	-4.88	2.96
0 children	0.00		
≥ 1 child	0.35	-3.76	4.45
BMI (per 1kg/m^2 increase)	0.74	0.40	1.07
Current users MHT	0.25	-7.36	7.85
Former users MHT	2.36	-2.43	7.14
Never users MHT	0.00		

Table S10. Odd ratios for symptomatic urinary incontinence according to the urogenital distress inventory and their corresponding 95% confidence interval.

Urogenital Distress Inventory – 6 cut-off for symptomatic urinary incontinence (score ≥ 33.33)	Odds Ratio	95% confidence interval	
Age 60-70			
Premenopausal RRSO (RRSO ≤45 years of age)	2.06	0.90	4.71
Postmenopausal RRSO (RRSO ≥ 54 years of age)	1.00		
Age (per year increase)	1.09	0.96	1.23
No history of breast cancer	1.00		
History of breast cancer	0.58	0.32	1.04
0 children	1.00		
≥ 1 child	1.79	0.76	4.20
BMI (per 1kg/m^2 increase)	1.13	1.07	1.19
Premenopausal RRSO (RRSO ≤45 years of age)			
Early premenopausal RRSO (RRSO ≤40 years of age)	1.00		
Later premenopausal RRSO (RRSO at 41-45 years of age)	1.14	0.64	2.05
Age (per year increase)	1.03	0.96	1.11
No history of breast cancer	1.00		
History of breast cancer	1.06	0.58	1.94
0 children	1.00		
≥ 1 child	0.83	0.45	1.53
BMI (per 1kg/m^2 increase)	1.11	1.05	1.16
Current users MHT	1.69	0.55	5.20
Former users MHT	2.04	1.01	4.12
Never users MHT	1.00		

Table S11. Regression coefficients on incontinence impact questionnaire – short form of variables and their corresponding 95% confidence interval.

Incontinence Impact Questionnaire short form	B-coefficient	95% confidence interval	
Age 60-70			
Premenopausal RRSO (RRSO ≤45 years of age)	-1.07	-3.68	1.54
Postmenopausal RRSO (RRSO ≥ 54 years of age)	0.00		
Age (per year increase)	0.11	-0.29	0.52
No history of breast cancer	0.00		
History of breast cancer	0.61	-1.35	2.57
0 children	0.00		
≥ 1 child	1.49	-1.03	4.01
BMI (per 1kg/m² increase)	0.29	0.09	0.48
Premenopausal RRSO (RRSO ≤45 years of age)			
Early premenopausal RRSO (RRSO ≤40 years of age)	0.00		
Later premenopausal RRSO (RRSO at 41-45 years of age)	0.94	-1.12	2.99
Age (per year increase)	-0.03	-0.30	0.23
No history of breast cancer	0.00		
History of breast cancer	0.76	-1.33	2.85
BMI (per 1kg/m² increase)	0.28	0.10	0.47
0 children	0.00		
≥ 1 child	-1.89	-4.11	0.34
Current users MHT	2.00	-2.02	6.02
Former users MHT	3.33	0.74	5.92
Never users MHT	0.00		

Table S12. odd ratios for symptomatic urinary incontinence according to the incontinence impact questionnaire – short form of and their corresponding 95% confidence interval.

Incontinence Impact Questionnaire short form – cut-off for significant impact on quality of life (score ≥ 9)	Odds Ratio	95% confidence interval	
Age 60-70			
Premenopausal RRSO (RRSO ≤45 years of age)	0.73	0.27	1.98
Postmenopausal RRSO (RRSO ≥ 54 years of age)	1.00		
Age (per year increase)	1.09	0.93	1.28
No history of breast cancer	1.00		
History of breast cancer	1.04	0.49	2.25
0 children	1.00		
≥ 1 child	1.88	0.54	6.56
BMI	1.11	1.04	1.19
Premenopausal RRSO (RRSO ≤45 years of age)			
Early premenopausal RRSO (RRSO ≤40 years of age)	1.00		
Later premenopausal RRSO (RRSO at 41-45 years of age)	2.19	0.91	5.28
Age (per year increase)	0.99	0.89	1.09
No history of breast cancer	1.00		
History of breast cancer	1.39	0.59	3.27
BMI (per 1kg/m^2 increase)	1.09	1.02	1.17
0 children	1.00		
≥ 1 child	0.55	0.25	1.24
Current users MHT	3.26	0.78	13.57
Former users MHT	3.13	1.19	8.21
Never users MHT	1.00		

Supplementary references

1. Kramer HM, Kwee A, Bremer HA. [Once a C-section, always a C-section?]. Nederlands tijdschrift voor geneeskunde 2009;153(4):136-40. (In dut).
2. Gynaecologie NVvOe. Indicatiestelling sectio caesarea. 2011.
3. Utomo E, Korfage IJ, Wildhagen MF, Steensma AB, Bangma CH, Blok BF. Validation of the Urogenital Distress Inventory (UDI-6) and Incontinence Impact Questionnaire (IIQ-7) in a Dutch population. Neurourology and urodynamics 2015;34(1):24-31. (In eng). DOI: 10.1002/nau.22496.

Chapter 8

Summary, general discussion and future perspectives

Focus of this thesis

The research presented in this thesis aimed to evaluate the long-term health effects of risk-reducing salpingo-oophorectomy (RRSO) in women at high familial risk of breast and/or ovarian cancer. We investigated the long-term effects of a premenopausal RRSO on cardiovascular disease (CVD) risk, bone health, quality of life and urogenital functioning, including sexual functioning and urinary incontinence. All chapters in this thesis are based on results of the Health After Early Menopause Due to Oophorectomy (HARMOny) study. In this study we included 500 women at high familial risk of breast and/or ovarian cancer who underwent a premenopausal RRSO (≤45 years) and compared them with 240 women with comparable familial risk who underwent a postmenopausal RRSO (≥54 years). To participate in our study women had to be at least 55 years or older, leading to a follow-up time of at least 10 years after premenopausal RRSO. In this final chapter I will describe the main results of our study, compare our results with the current literature, discuss the methodology of the HARMOny study and provide clinical implications and future perspective for this research topic to be able to draw overall conclusions.

Main results and implications

In **Chapter 2,** we assessed the long-term effects of timing of RRSO on coronary artery calcium (CAC) as a marker of subclinical atherosclerosis and CVD risk. For this study we compared women who underwent a premenopausal RRSO with women who underwent a postmenopausal RRSO and with a reference group from the general population (ROBINSCA trial). In addition, we assessed if timing of premenopausal RRSO influenced CAC scores by comparing women who underwent an early premenopausal RRSO (<41 years) with women who underwent a late premenopausal RRSO (41-45 years. Twenty-one years after surgical menopause, we did not observe increased CAC scores in women with a premenopausal RRSO compared with women who underwent a postmenopausal RRSO (multivariably adjusted RR 0.93, 95% CI, 0.75-1.15 for any CAC; RR 0.71, 95% CI, 0.43-1.17 for at least moderate CAC; RR 0.81, 95% CI, 0.30-2.13 for severe CAC). CAC scores of the premenopausal RRSO group were also comparable to those of an external reference population of similar age (RR 1.05, 95% CI, 0.92-1.21 for any CAC; RR 1.11, 95% CI, 0.80-1.53 for at least moderate CAC; RR 1.05, 95% CI, 0.50-2.20 for severe CAC). Furthermore, an early premenopausal RRSO before the age of 41, compared to an RRSO between ages 41 and 45 years was not associated with increased CAC scores. We did not find a protective effect of MHT use on increased CAC scores, neither for ever use nor for the duration of use and

MHT use was not a confounder in our analyses. In conclusion, **Chapter 2** does not support a long-term adverse effect of surgical menopause on the development of cardiovascular disease.

In **Chapter 3,** we assessed the effects of timing of RRSO on arterial stiffness measured as pulse wave velocity (PWV), as a surrogate marker of arteriosclerosis and CVD risk. In addition, we studied the association between PWV and CAC in women with a premenopausal RRSO. Women who underwent a premenopausal RRSO did not have increased PWV levels compared with women who underwent a postmenopausal RRSO; remarkably, women who underwent a premenopausal RRSO even had reduced PWV levels (β -0.87, 95% CI, -1.45, -0.28 for continuous PWV in m/s; RR 0.80, 95% CI, 0.52, 1.25 for having a PWV level in the upper quintile). Among women who underwent a premenopausal RRSO, we observed an association between PWV level and presence of CAC (RR 1.07, 95% CI, 1.02-1.13 for PWV as continuous variable; RR 1.32, 95% CI, 1.04-1.68 for being in the upper PWV quintile). In conclusion, in **Chapter 3** we did not find long-term increased arterial stiffness in women who underwent a premenopausal RRSO compared with a postmenopausal RRSO.

In **Chapter 4,** we studied the association of timing of RRSO on bone mineral density (BMD). We showed that for both the lumbar spine (LS) and femoral neck (FN) the BMD Z-scores were lower in the premenopausal RRSO group compared with the postmenopausal RRSO group, suggesting a long-term effect of surgical menopause on BMD (β -0.88, 95% CI, -1.10,-0.66 for LS; β -0.51, 95% CI, -0.71,-0.31 for FN). In addition, women in the premenopausal RRSO group were at a significant higher risk of a lowered BMD Z-score (Z-score ≤ -1.0) of either the lumbar spine and/or femoral neck, compared with the postmenopausal RRSO group (absolute risk 18.2% vs 9.5%; RR 2.05, 95% CI, 1.30-3.25). However, in subgroup analyses in women aged 60-70 at study visit, we observed no difference in the prevalence of either osteopenia or osteoporosis (T-score ≤ -1.0) in the premenopausal RRSO group compared with the postmenopausal RRSO group (RR 1.04, 95% CI, 0.74-1.48 for LS; RR 1.09, 95% CI, 0.81-1.46 for FN). An early premenopausal RRSO before the age of 41, compared to an RRSO between ages 41 and 45 years was not associated with decreased BMD. **Chapter 4** shows that a premenopausal RRSO is associated with long-term lowered BMD; however, in subgroup analyses we did not observe a difference in the prevalence of osteopenia or osteoporosis.

In **Chapter 5,** we provided an overview of all the outcomes studied in the HARMOny study. In addition, we investigated the long-term effects of RRSO on quality of life (HRQOL) by using the 36-Item Short Form Health Survey (SF-36) and the Cancer Worry Scale (CWS). Twenty-one years after surgical menopause, women who underwent a premenopausal RRSO did not have a higher prevalence of impaired physical or mental quality of life compared with women who underwent a postmenopausal RRSO (RR 0.99, 95% CI, 0.72-1.37 for a lowered physical component score; RR 1.18, 95% CI, 0.38-3.62 for a lowered mental component score). Timing of RRSO did not influence fear of cancer (RR 0.81, 95% CI, 0.61-1.09). In conclusion, we did not find a long-term difference in quality of life between the premenopausal and postmenopausal RRSO groups.

In **Chapter 6,** we assessed the association between timing of RRSO and long-term sexual functioning using the Sexual Activity Questionnaire. Women who underwent a premenopausal RRSO reported similar sexual pleasure compared with women who underwent a postmenopausal RRSO (mean pleasure score 8.6, P=0.99). However, women who underwent a premenopausal RRSO did report more discomfort during sexual activity (OR 3.1, 95% CI, 1.04-9.4), mainly due to increased vaginal dryness (OR 2.6, 95% CI, 1.4-4.7). This seemingly contradictory finding might be explained by sexual pleasure not being limited to penetration, but also self-stimulation or non-penetrating sex. In conclusion, **Chapter 6** shows that more than 15 years after premenopausal RRSO, women experienced more severe complaints of vaginal dryness and more discomfort with sexual intercourse than women with a postmenopausal RRSO. However, this did not result in less pleasure during sexual activity.

In **Chapter 7,** we studied the effects of timing of RRSO on urinary incontinence. Women who underwent a premenopausal RRSO had no (significantly) increased risk of overall symptomatic incontinence compared with women who underwent a postmenopausal RRSO (OR 2.1, 95% CI 0.93-4.78). However, when we focused more on different types of incontinence, we observed increased risk of stress incontinence (absolute risk: 13% vs 8%; OR 3.5, 95% CI, 1.2-10.0). We observed no increased risk for urge incontinence in women who underwent a premenopausal RRSO compared with women who underwent a postmenopausal RRSO (OR 1.1, 95% CI 0.5-2.4). Remarkably, women who had a history of menopausal hormone therapy use experienced more symptomatic overall urinary incontinence (OR 1.9, 95% CI, 1.1-3.3). In conclusion, **Chapter 7** shows that women who underwent a premenopausal RRSO are at increased risk of stress urinary incontinence compared with women who underwent a postmenopausal RRSO.

General discussion and comparison with the literature

History of cardiovascular disease research in women and the effect of menopause

The study of differences in the pathogenesis of cardiovascular disease between women and men is a relatively young research area. Until the 1980s-1990s, most large epidemiological studies and drug trials were limited to men.[1] Therefore, evidence concerning CVD risk-assessment, prevention and treatment, including the traditional risk factors we use today, was initially based on studies performed predominantly on men. It was assumed that the results found in these studies were generalizable to the general population, irrespective of race and sex. Since that time we have come a long way to find out that this is certainly not true. Smoking and diabetes mellitus have been shown to be stronger CVD risk factors in women compared to men.[2] It stands to reason that female-specific risk factors of CVD, including the effects of (timing of) menopause were also scarcely studied before the 1980s. The first reports on the association between menopause and risk of CVD and ischemic heart disease (IHD) were published in 1976 and 1978 by the investigators of the landmark Framingham study.[3-4] These investigators found a twofold increased incidence of CVD in women aged 40-55 years who were postmenopausal compared with similarly aged women who were premenopausal. Furthermore, in the same cohort they found an odds-ratio of 2.7 for ischemic heart disease (IHD) in women who were postmenopausal compared with women who were premenopausal. These findings agreed with the observations made earlier that, before middle age, coronary artery disease was much less prevalent in women than in men, suggesting a possible protective effect of estrogen on IHD in premenopausal women.[5-6] Another important publication was a large prospective cohort study of 121,700 US women aged 30-55 years by Colditz et al. in 1987, showing that women who underwent bilateral oophorectomy were at increased risk of IHD compared to women who were premenopausal and of similar age (RR: 2.2, 95% CI 1.2-4.2).[7] In addition, they observed that this increased risk was canceled out by the use of estrogen-replacement therapy (RR: 0.9, 95% CI 0.6-1.6). These results, in combination with a number of more recent reports appear to imply that menopause is associated with increased CVD risk. A meta-analysis performed by Muka et al. reported an elevated risk (HR: 1.50, 95% CI, 1.28-1.76) for ischemic heart disease when comparing women with a natural menopause before age 45 with women aged 45 or older at menopause.[8] Another large meta-analysis by Roeters-van Lennep et al. showed significantly elevated CVD risk after natural menopause before 40 years (HR: 1.61, 95% CI, 1.22-2.12).[9] However, it remained unclear whether this association is indeed causal. Related to this, it remained unclear whether early surgical menopause is also associated with increased CVD risk.

Our results in the context of what is known about surgical menopause and CVD risk

The few studies investigating the direct association between surgical menopause and CVD risk provided inconsistent results, possibly because they were limited by study design or subject to bias due to confounding by indication. The latter bias could arise because the indication for surgical menopause may be associated with increased CVD risk. A large cohort study by Dam et al. showed an increased risk for IHD when comparing women with a surgical menopause (n=2206) with a natural menopause.[10] However, this increased risk could largely be explained by differences in established CVD risk factors such as high blood pressure and dyslipidemia. In addition, surgical menopause was defined by hysterectomy and/or any oophorectomy and a sensitivity analysis restricted to women who underwent bilateral oophorectomy showed no increased CVD risk. Another recent cohort study by Honigberg et al. found an increased risk of CVD after menopause by bilateral oophorectomy under age 40 (n=644), compared to women with either natural or surgical menopause after age 40.[11] Furthermore, in the Nurses' Health study, Parker et al. found an increased risk of IHD and stroke in women who had bilateral oophorectomy combined with hysterectomy before the age of 45 compared with women of similar age who had hysterectomy alone.[12] Finally, a large cohort study by Rivera et al. showed increased CVD-associated mortality following bilateral oophorectomy before the age of 45 (n=991) compared to a referent group matched by age.[13] However, in both the studies of Honigberg et al. and Parker et al., the indication for bilateral oophorectomy was not specified, rendering the results subject to potential confounding by indication. In the study of Rivera et al. the indications for bilateral oophorectomy were reported, with most frequent indications being RRSO, endometriosis and benign cysts. Endometriosis has been associated with an increased risk for CVD, irrespective of a history of surgical menopause, and whether CVD risk is increased in women with cysts remains unclear.[14-15] Yet, no subgroup analyses were performed by Rivera et al. among women with RRSO. Moreover, because of the relatively high proportion of *BRCA1/2* gPV carriers treated for (breast) cancer and the possible confounding effect of in particular radiotherapy and chemotherapy, comparisons of CVD risk between women who underwent RRSO and the general population may be biased.

Since the large majority of CVD events in women occur after the age of 65, the relatively young age of our study population (median 59.2 years at study in the premenopausal RRSO group) precluded analysis of the risk of CVD outcomes. We simply did not expect enough events to compare possible differences between the premenopausal and postmenopausal RRSO groups with sufficient power. Therefore, we used two different markers for CVD risk with excellent predictive value as

intermediary outcomes. In our study, we found no long-term differences in coronary artery calcification (CAC) scores in women who underwent a premenopausal RRSO compared with a postmenopausal RRSO after a median follow-up of 21 years. Our study is the first one to investigate the possible effect of surgical menopause on CVD risk by using CAC as a measure of subclinical atherosclerosis. Although surgical menopause does not appear to be associated with atherosclerosis, this does not entirely rule out the possibility of increased CVD risk after surgical menopause through other pathways. Another known pathway associated with CVD risk is vascular ageing caused by stiffening and thickening of the aortic wall, which can be measured by pulse wave velocity (PWV).[16-18] We did also not observe long-term increase of PWV levels in women in the premenopausal RRSO group compared with the postmenopausal RRSO group. Our findings in women who underwent a surgical menopause are consistent with a recent smaller study by van Bommel et al. who found no association between time since RRSO and several predictors of CVD events, including PWV and carotid intima-media thickness, another known marker of subclinical atherosclerosis, in a cohort of female *BRCA1/2* gPV carriers.[19]

While there are no studies investigating the association between CAC and *surgical* menopause, there are two studies that investigated development of CAC after early *natural* menopause. Freaney et al. showed no differences in having any CAC (CAC>0) or moderate CAC (CAC>100) in women with a menopause before the age of 40 compared with women of similar age with a menopause after 40 or who were still premenopausal.[20] In addition, Gunning et al. found no differences in CAC scores or MESA percentile in women with premature ovarian insufficiency (POI), compared with age and race-matched controls from the Multi-Ethnic Study of Atherosclerosis (MESA).[21] At first sight, the results of these studies might raise a question about CAC as a precursor of CVD risk after early menopause. However, a more plausible explanation might be the rather young median age of the study populations (median age 49.4 and 50 years, respectively, compared with 64.5 years in our study); therefore, participants were probably not yet old enough to expect significant differences in subclinical atherosclerosis using CAC, due to the lower prevalence of increased CAC at those ages and the lower sensitivity of CAC>0 for plaques at younger age, especially in women.[22-23]

Reverse causality hypothesis regarding menopause and CVD risk

The results described in this thesis suggest that *surgical* menopause is not associated with elevated future CVD risk. Interestingly, a study by Krul et al. did also not show an increase in CVD risk after another form of early artificial menopause, i.e. POI induced by chemotherapy in Hodgkin lymphoma survivors.[24] In contrast, two recent meta-analyses showed a clear association between early *natural* (non-artificial)

menopause and CVD risk.[8-9] These contradictory findings suggest that early natural menopause is associated with increased CVD risk, whereas an early surgical (or otherwise artificial) menopause is not. This discrepancy may be explained by the hypothesis that early natural menopause is the result of accelerated vascular ageing, leading to a statistical (non-causal) association between early natural menopause and increased CVD risk, which was hypothesized by Kok et al.[25] They showed that increased premenopausal weight, blood pressure and total cholesterol levels were associated with an earlier age at menopause in women in the Framingham Heart Study cohort. In addition, a decrease of serum total cholesterol during the premenopausal period was associated with a later age at menopause. This hypothesis offers an alternative explanation, next to the estrogen deficiency hypothesis, for the association found between early natural menopause and increased CVD risk. This may also explain our observations that surgical menopause was not associated with markers for increased CVD risk.

Bone mass and menopause

Osteoporosis is literally as old as the pyramids. Paleopathological studies show that osteoporotic fractures in women were already present in ancient Egypt, as early as 1990 BC. [26] The first to describe sex-specific differences in bone health was von Bruns in 1882.[27] He observed that, before the age of 50 years, men were at a 8 times higher risk for fractures compared to women. However, this changed dramatically after the age of 50; women were at an increased risk for fractures compared with men, especially for hip fractures. At that time, this difference was considered to be caused by women tripping over their long skirts.[28] In 1941, Albright et al performed the first study to describe the association between menopause and osteoporosis and the role of estrogen deficiency in bone loss.[29] In the years that followed numerous studies showed that menopause leads to accelerated bone loss, osteopenia/osteoporosis and subsequent risk of fractures. The generally assumed mechanism behind this phenomenon is that estrogen deficiency leads to an imbalance between bone resorption due to osteoclast activity and bone formation due to osteoblast activity.[30] However, the overall effect of menopause on bone loss can be challenging to measure because of the concomitant influence of ageing. Most men and women reach their peak bone mass in the third decade of life; after that they start to lose bone mass.[31] This age-related bone loss is progressive, especially after the age of 65, and studies suggest it to be independent of estrogen deficiency.[32] In addition to the overall effects on bone mass, the influence of menopause and age on bone mass differ between specific (type of) bones. Studies in women show that the lumbar spine (LS) is mainly influenced by estrogen deficiency, with a large portion of bone loss occurring during and after menopause, whereas the femoral neck (FN) is less influenced by menopause,

showing a more gradual decline throughout life directly after reaching the peak bone mass.[33] Although estrogen deficiency due to early menopause is associated with a short-term decrease of bone mass, it remains unclear whether these effects persist in later life or are attenuated by age-related bone loss. A recent longitudinal study from Australia with a median of follow-up of 23 years showed that women with an early menopause before the age of 45 had an increased risk of osteoporosis and fractures compared with women who underwent menopause after the age of 45 (OR: 1.37; 95% CI 1.07–1.77 for osteoporosis, OR: 1.45; 1.15–1.81 for fractures).[34] However, other studies investigating the long-term effects of early menopause on bone mass and fracture risk show inconsistent results.[35-36]

Our results in the context of what is known about surgical menopause and bone mass

Because surgical menopause leads to acute estrogen deficiency as compared to a more gradual decline of estrogen levels in women undergoing an early natural menopause, it has been hypothesized that surgical menopause has an even larger impact on bone mineral density (BMD). Our observation that women who underwent a premenopausal RRSO before the age of 46 had long-term reduced BMD compared with women who underwent the same surgery after the age of 54, is consistent with other recent studies in *BRCA1/2* GPV carriers.[37-39] However, these studies had a relatively short follow-up time (median 2-5 years) and their results could therefore be explained by the hypothesis of a rapid bone loss phase of 4-5 years after any type of menopause, followed by a less steep but stable decline later on.[40] Women who retained their ovaries might experience this rapid bone loss phase around their natural menopause at a later age, leading to an only temporary difference of BMD after premenopausal RRSO. Furthermore, on the long-term age-related bone loss might attenuate the effects of surgical menopause. To investigate the possible long-term differences in BMD between women who underwent a surgical menopause and a natural menopause, both groups would therefore have to be at least 5 years postmenopausal – if not more - at measurement of BMD, to be able to draw sound conclusions about the long-term outcomes. In the HARMOny study we observed that a reduction in BMD was still present 18 years after surgical menopause. Other studies investigating the long-term effects of RRSO on BMD are scarce and inconsistent.[41-42] Studies with a long-term follow-up not restricted to women at high familial risk of ovarian cancer reported short-term decreased BMD after early surgical menopause before the age of 45 compared with menopause after 45 years. However, the observed differences observed in these studies gradually disappeared after the age of 55, suggesting that the long-term effects of early menopause might be attenuated by factors related to ageing.[35-43]

Quality of life after premenopausal RRSO in the current literature

In our study, we found no clinically significant long-term effects of timing of RRSO on health-related quality of life (HRQOL). Other studies investigating the short-term effects of RRSO also observed no effect on HRQOL. However, most studies did not specify for timing of RRSO (pre- vs postmenopausal), and had relatively short follow-up periods (median up to 5 years).[44-45] Although RRSO is known to affect HRQOL-related outcomes including more vasomotor/menopausal complaints and a worsened sexual function, generic HRQOL appears to be unchanged.[46-47] Recently, new insights in the development of familial ovarian cancer have led to more research into potential novel strategies for risk-reducing surgery. As high-grade serous carcinoma appears to originate from the fallopian tubes it has been hypothesized that *BRCA1/2* GPV carriers may safely undergo risk-reducing salpingectomy without oophorectomy (RRS) in the same age period as recommended by the current guidelines, with a 5 year delay of bilateral oophorectomy and estrogen deficiency.[48-50] Two recent prospective trials suggest that, one year after surgery, women undergoing RRS experienced less sexual problems and menopausal symptoms compared with women who underwent RRSO according to current guidelines. However, in accordance with our study, HRQOL was unaffected.[51-52]

Urogenital functioning after surgical menopause in the current literature

Women in our study who underwent a premenopausal RRSO did not report long-term differences in sexual pleasure compared with women who underwent a postmenopausal RRSO or with the general population. However, we did find that women who underwent a premenopausal RRSO experienced significantly more vaginal dryness and discomfort during intercourse. This apparent contradiction could be explained by the influence of non-penetrating sex on sexual pleasure. Available studies investigating the influence of RRSO were consistent with our findings concerning vaginal dryness; however, they also reported more sexual problems and a reduced interest in sex.[53-54] These contradictory findings might be explained by differences in methodology; average time since RRSO in the current literature was up to 5 years, compared to 15 years in our study.

The HARMOny study is the first study to investigate urinary incontinence after surgical menopause. We found that women who underwent a premenopausal RRSO had significantly increased risk for stress incontinence compared with women who underwent a postmenopausal RRSO. This was an unexpected finding, as we know that urge urinary incontinence is more strongly associated with menopause than stress urinary incontinence.[55] In general, the peak prevalence of stress urinary incontinence occurs postpartum and around menopause. It is possible that the peak

prevalence of stress urinary incontinence is higher after surgical menopause than after natural menopause. Therefore, the prevalence of stress urinary incontinence may still be higher in women who had surgical menopause at an earlier age. The absence of other studies renders the interpretations of our results in the context of the current literature difficult. However, we did find that the mean urinary distress scores in our study population were higher compared with Dutch reference data, indicating more problems caused by urinary incontinence after a RRSO compared with the general population.[56]

Menopausal hormone therapy after early menopause

Menopausal hormone therapy (MHT), supplementation with estrogen alone or estrogen in combination with progestogen, is widely used by perimenopausal women to treat menopausal symptoms. In the general population, systemic MHT is mostly prescribed to treat vasomotor symptoms such as hot flashes and night sweats, whereas local estrogen is used to treat vulvovaginal symptoms. Because the risks and benefits of MHT are still not entirely clear, there is a continuing scientific debate about the indications and recommended dosage and duration of MHT treatment. There is limited epidemiological evidence that MHT could be used to prevent adverse effects of early menopause including CVD and osteoporosis.[57] However, a large study from the Women's Health Initiative in the early 2000's suggested an excess risk of ischemic heart disease (IHD), stroke and breast cancer in women using MHT. [58] Although these results turned out to be affected by selection bias, the study led to a drastic decline of women using MHT in the period 2001 to 2004 from 22% to 5% in the United States and from 5.6% to 2.4% in the Netherlands.[59-60] Based on more recent studies the current guidelines state that women aged 60 years or younger, within 10 years of menopause and without contraindications, can safely be treated with MHT for vasomotor symptoms.[61-62] However, an important contraindication for MHT is high (>10%) 10-year CVD risk, and women at moderate (5-10%) 10-year CVD risk are advised to only use transdermal MHT. In addition, these guidelines do not recommend MHT to prevent CVD or osteoporosis. Women with early menopause and without contra-indications are advised to use MHT until the age of 50, the mean age at menopause in the general population.

MHT in the HARMOny study

Due to the aforementioned scientific uncertainties and because a history of (specific types of) breast cancer is considered a contra-indication for MHT, a large proportion of *BRCA1/2* GPV carriers undergoing a premenopausal RRSO do not use MHT. In the HARMOny study, only 29.9% of the women who underwent a premenopausal RRSO had ever used any MHT (10.8% in the postmenopausal RRSO group). Nonetheless,

studies investigating long-term effects of early menopause should include MHT use as a confounder, as women with menopausal complaints or another indication for MHT use may have increased risks of the outcomes of interest. In the HARMOny study, we found that MHT had different effects on the outcomes and associations studied. MHT use turned out not to be a confounder in our CAC analyses and adding MHT use to our model did not influence the outcomes. In contrast, MHT was a confounder for the outcomes of BMD and sexual functioning. As expected, in women who underwent a premenopausal RRSO compared with women who underwent a postmenopausal RRSO, the ever use of MHT protected against having a low BMD (RR 0.59; 95% CI 0.36-0.96, for Z-score of the LS and/or FN ≤-1.0). Remarkably, when investigating the association of MHT use with PWV levels in the pre- and postmenopausal RRSO groups, we found that women who (ever) used MHT were at increased risk of having a PWV in the upper quintile (RR: 1.88, 95% CI, 1.09-3.22). If estrogen deficiency after early menopause would cause increased arterial stiffness, we would have expected lower PWV levels in (former) MHT users, instead of the higher PWV levels we found. An explanation of this finding might be that women who experience postmenopausal vasomotor symptoms, the most frequent reason for prescribing MHT, are at increased risk for (subclinical) CVD. Therefore, these findings could be caused by confounding by indication for MHT.[63-64] However, omitting MHT as a confounder in our analyses did not change our results regarding arterial stiffness, nor did sensitivity analyses in women without a history of MHT use. We observed the same phenomenon in our analyses concerning urinary incontinence. Women who ever used MHT were at increased risk of experiencing symptomatic urinary incontinence, possibly because they were prescribed MHT more often for this very reason. Since data on type of MHT use were largely missing, we were not able to investigate possible explanations regarding specific treatments further.

Methodological review of the HARMOny study

The HARMOny study has several strengths such as its nationwide character, relatively large sample size, good overall response of 61.8%, distinct difference in age at RRSO and a comparison group of women selected from the same cohort from which the exposed group was drawn. By directly comparing women at high familial risk of ovarian cancer with and without a premenopausal RRSO, potential selection bias and confounding by indication for surgical menopause could strongly be reduced. Such biases affected the results of most other studies in this research field because in these reports comparisons were made with the general population or with premenopausal women of similar age recruited from the same hospital or through advertisements.

Nevertheless, it is important to consider some important limitations of our study when appraising the results described in this thesis.

The HARMOny study was designed to investigate the long-term effects of a premenopausal RRSO. To avoid selection bias when comparing women at high familial risk for ovarian cancer who underwent an RRSO with the general population, we compared women who underwent a premenopausal RRSO with women with the same familial risk who underwent a postmenopausal RRSO. To be able to make a more distinct evaluation of potential long-term differences between the pre- and postmenopausal RRSO groups, we decided to consider women who underwent an RRSO before the age of 46 in the premenopausal group, and women who underwent RRSO after the age of 54 in the postmenopausal group. The rationale behind the chosen age at RRSO in the premenopausal group is that the guidelines for women at high familial risk recommend to undergo RRSO before the age of 46. In addition, most studies define early menopause using a cutoff of 45 years, based on the median age of menopause in high-income countries (50 to 52 years).[65] To be able to adequately distinguish between the pre- and postmenopausal RRSO groups, women in the postmenopausal group had to have a natural menopause after the age of 50, and an RRSO after the age of 54. Since the outcomes of interest in our study take time to develop and usually present themselves at a later age, women participating in our study had to be at least 55 years at study enrollment; therefore, by design women in the premenopausal RRSO group were at least 10 years after surgery at time of study.

Limitation 1: Difference in age at study between the pre- and postmenopausal RRSO groups

These distinct differences in age between the pre- and postmenopausal RRSO groups has led to a limitation of our study. During the inclusion period we observed a substantial difference in age at study (median 10.1 years) between the premenopausal and postmenopausal RRSO groups. This age difference was caused by a change in the 2007 guidelines for management of ovarian cancer risk in *BRCA1/2* GPV carriers, leading to a strongly decreased prevalence of postmenopausal RRSO.[66] In the past women were advised to undergo 3-monthly transvaginal ultrasound and serum cancer antigen 125 (CA-125) screening; however, studies have shown that screening for ovarian cancer is not effective, neither for hereditary nor for sporadic ovarian cancer.[67-68] This has led to an uptake of 81-99% of premenopausal RRSO in female *BRCA1/2* GPV carriers in the Netherlands.[69-72] Therefore, there are simply less relatively young women with a history of postmenopausal RRSO, leading to a median age of 69.8 years (67.0-73.2) at time of study in our postmenopausal RRSO group and median 59.2 years (57.5-62.1) in our premenopausal RRSO group. As most

outcomes investigated in the HARMOny study (subclinical CVD and BMD) were age-dependent, the distribution of age at study in the pre- and postmenopausal RRSO groups should overlap to a sufficient extent in order to adequately adjust for age in our analyses. However, since it turned out that there was not enough overlap in ages at study between both groups to adjust for this important variable we used several methods to address this problem. First, we restricted our analyses to women in the study population aged 60-70 years at study enrollment to investigate the outcomes of increased CAC score, risk of osteopenia/osteoporosis, PWV levels, quality of life, sexual pleasure and urine incontinence in **Chapters 2-6**. Furthermore, in **Chapter 4,** we were able to use the BMD Z-scores (already adjusted for age) of both the LS and FN to perform analyses in the entire study population. In addition, in all chapters of this thesis, we were able to use the entire premenopausal RRSO group to assess the influence of timing of premenopausal RRSO (before the age of 41 vs age 41-45). Finally, in **Chapter 2,** we had the unique opportunity to compare the CAC scores of our premenopausal RRSO group with CAC scores in similarly aged women in the ROBINSCA general population cohort.

Limitation 2: Cross-sectional study

Other possible limitations are related to the cross-sectional design of the HARMOny study. We decided to use this design because of the long follow-up required if we would have used a prospective longitudinal approach and the relatively limited resources available. Possible limitations consist of potential survival and selection bias. It is possible that women eligible for the HARMOny study did not participate in our study because of morbidity or mortality directly caused by the outcomes in our study, especially CVD-related outcomes. In addition, our cross-sectional study provides no information on short-term effects of an RRSO or on longitudinal changes in our outcomes of interest in the course of follow-up. It would have been interesting to know for especially BMD, cognition and sexual problems whether there were short-term effects of premenopausal RRSO measurable in our cohort, since other studies reported short-term effects for those outcomes which we did not observe in our long-term assessment. Some possible limitations were addressed by nesting our study in the Hereditary Breast and Ovarian cancer study Netherlands (HEBON) cohort. The HEBON cohort is a well-established nationwide cohort consisting of women at high familial risk of breast and/or ovarian cancer recruited from all eight Dutch University Medical Centers and the Netherlands Cancer Institute.[73] As our study was nested in the HEBON cohort, we had the unique opportunity to obtain the causes of death, from Statistics Netherlands, for all women who were otherwise eligible for the HARMOny study but died before the start of the study.[73] Only 1.9% of these women died because of a cardiovascular event. Unsurprisingly, the most

frequent cause of death was cancer (87.6%), with breast cancer as the main cause (55.2%). Selection bias might also have arisen due to differences in response rates between the premenopausal group (68.0%) and the postmenopausal group (50.8%). A likely explanation is that women in the postmenopausal RRSO group felt less inclined to participate as our research hypotheses focused on the consequences of premenopausal surgical menopause. However, we cannot completely rule out the possibility that the lower participation rate in the postmenopausal RRSO group is caused by CVD-related morbidity. We were able to address this potential bias using previously collected data from questionnaire surveys completed for the HEBON study.[73] In these questionnaires, current non-responders in our postmenopausal RRSO group did not report a lower or higher prevalence of CVD than our responders. Although it was not feasible to collect information about the prevalence of the other outcomes of our study in non-responders, we do not expect that potential morbidity related to these outcomes has led to lower participation. Another limitation of using a cross-sectional design was the inability to draw definitive conclusions on causality. Although very unlikely, it might be theoretically possible that the outcomes studied were already present at time of exposure and differed between the two groups at that time. In order to prove that the observed differences between the pre- and postmenopausal RRSO groups are definitely caused by timing of RRSO (longitudinal) prospective studies are needed. However, as mentioned before, it will be challenging in the future to select enough women who underwent a postmenopausal RRSO and are of similar age to provide sufficient power.

Clinical recommendations based on the HARMOny study

The aim of the HARMOny study was to provide insight into the long-term effects of premenopausal RRSO, for women who have undergone this impactful intervention and women considering to undergo RRSO in the future. The HARMOny study also aimed to generate information for health care professionals aiding and counseling these women to improve balancing health benefits and long-term adverse effects of a premenopausal RRSO. This thesis provides some reassuring evidence about these long-term effects.

Regarding (subclinical) CVD, women and their treating physicians can be informed that twenty-one years after a premenopausal RRSO, we did not find increased risk of two important markers of cardiovascular disease risk. Both the CAC score as a measure of subclinical atherosclerosis and PWV level as a measure of arterial stiffness were not increased in the premenopausal RRSO group compared with the

postmenopausal RRSO group. This is important information because of the existing concerns of women who underwent a premenopausal RRSO, due to the reported association between an early *natural* menopause and CVD risk. In addition, we found no evidence that timing of premenopausal RRSO (RRSO<41 years vs RRSO 41-45years) influences these outcomes. We therefore conclude that women who underwent a premenopausal RRSO do not need to undergo any extra screening for CVD, besides the conventional cardiovascular risk management screening which is current practice in the general population in the Netherlands.

Regarding bone health, women and their treating physicians can be informed that, eighteen years after a premenopausal RRSO bone mineral density is still decreased. Women in the premenopausal RRSO group are at an approximately 2 times higher risk of a lowered BMD Z-score of either the lumbar spine and/or femoral neck, compared with the postmenopausal RRSO group (absolute risk 18.2% vs 9.5%). Somewhat surprisingly, in analyses in a subgroup of 60-70 year olds, we did not find an increased risk for osteopenia/osteoporosis or fractures. We therefore advice to consider adding premenopausal RRSO to the risk factors for osteoporosis which are used when assessing the indication for a DXA scan. However, we do not advice that women with a history of a premenopausal RRSO should undergo periodical DXA scanning by default.

Regarding quality of life, when integrating our results with studies examining short-term effects of RRSO, women and their treating physicians can be informed that there does not appear to be a clinically significant impact of premenopausal RRSO on HRQOL, both in the short-term and 21 years after RRSO. In addition, we found no influence of timing of premenopausal RRSO on HRQOL many years later. This is a reassuring message for women undergoing a premenopausal RRSO. Among the women who underwent premenopausal RRSO in our study, 70.9% reported less fear of cancer after the decision to undergo RRSO. Although 75.9% of these women considered themselves to be well-informed when deciding to undergo premenopausal RRSO, 16.3% reported that they were not well-informed. These results show that there is still room for improvement for health care professionals counseling women undergoing a premenopausal RRSO.

Regarding sexual functioning, when integrating our results with studies examining short-term effects of RRSO, women and their treating physicians can be informed that a premenopausal RRSO does not influence long-term sexual pleasure. However, both in the short-term and on the long run, they may experience more vaginal dryness and discomfort during sexual intercourse. Health care professionals counseling

women undergoing RRSO should inform women about these effects and possible treatment options to minimize the possible influence on quality of life.

Regarding urinary incontinence, women and their treating physicians can be informed that after undergoing a premenopausal RRSO, they are at a 3.5 times higher risk of experiencing stress urinary incontinence compared with women who underwent a postmenopausal RRSO. However, this increased relative risk translates to quite a low absolute risk (13% vs 8%). In addition, this higher risk of stress urinary incontinence did not lead to a lower health-related quality of life. Long-term urge urinary incontinence does not appear to be affected by timing of RRSO. Regrettably, literature regarding the short-term effects of RRSO on urinary incontinence is lacking. Therefore, providing advice concerning this important topic is difficult.

Future perspectives

To our knowledge, our cohort of women who underwent a RRSO is internationally unique because of its size (N=740) and the long-term follow-up. To date there have been no other publications comparing the long-term effects of a premenopausal and a postmenopausal RRSO. As argued before, the cross-sectional design of our study has some limitations. However, changes in the guidelines for RRSO in 2007 have led to an increased uptake of premenopausal RRSO in the Netherlands in *BRCA1/2* GPV carriers, with estimated rates of 81% to 99%. Therefore, we think it will not be possible in the future to set up a prospective study comparing women with a premenopausal RRSO with women with a postmenopausal RRSO. Furthermore, because of the relatively high proportion of *BRCA1/2* GPV carriers with a history of treatment for (breast) cancer and the possible confounding effect of in particular radiotherapy and chemotherapy, we think it is not justified to compare women with a premenopausal RRSO with the general population alone for these outcomes. Consequently, it will be increasingly challenging to investigate the long-term effects of a premenopausal RRSO in the future. This renders the HARMOny study population even more valuable for future research on long-term effects of premenopausal RRSO. Therefore, we intend to perform a follow-up study of the HARMOny study, including the 500 participants in the premenopausal group and 240 participants in the postmenopausal group. At the time of the study visit, the median age of our premenopausal RRSO group was only 59.2 years. It would be very interesting to acquire more insight into especially, the development of cardiovascular disease, bone health and cognition after premenopausal RRSO at a later age, for example at the age of 65-70 years. This substantially higher age at study would also allow us to assess

outcomes for which we did not expect enough events in the current study to compare possible differences between the premenopausal and postmenopausal RRSO groups with sufficient power, such as CVD events and fractures. Prospective follow-up of our cohort using the same outcome measures as in the current study will also benefit from the acquisition of longitudinal data on coronary artery calcium scores, cognitive functioning and bone health. In addition, the aforementioned strategy of risk-reducing salpingectomy with a 5 year delay of bilateral oophorectomy might enable us to postpone estrogen deficiency by 5 years. If proven safe, this strategy might reduce potential adverse effects of menopause due to risk-reducing surgery in women at high familial risk of ovarian cancer in the future. It would be of great interest to assess long-term CVD risk, BMD, sexual functioning, urine incontinence and HRQOL in women who underwent this relatively new strategy compared with women who underwent a premenopausal RRSO.

Overall conclusions

This thesis describes the various outcomes of the HARMOny study on the long-term health effects of bilateral salpingo-oophorectomy before natural menopause on subclinical CVD, bone mineral density, sexual functioning, urine incontinence and quality of life. To summarize, we did not find any evidence for long-term increased risk of (subclinical) CVD in the premenopausal compared with the postmenopausal RRSO group. Although women who underwent a premenopausal RRSO had lower bone mineral density, they did not have increased risk of osteopenia or osteoporosis. In addition, women who underwent a premenopausal compared to a postmenopausal RRSO did not report a negative influence on sexual pleasure or urge urinary incontinence. We did observe, however, more vaginal dryness, sexual discomfort and more stress urinary incontinence in the premenopausal compared with the postmenopausal RRSO group. While these findings were statistically significant, they concerned relatively small differences and their clinical relevance is debatable. We did not observe differences in HRQOL or fear of cancer between the pre- and postmenopausal RRSO groups. Remarkably, in none of the studies presented in this thesis we observed differences in our outcomes of interest according to timing of premenopausal RRSO (RRSO<41 years vs RRSO 41-45 years). Overall, these are reassuring messages for women undergoing premenopausal RRSO and health care professionals involved in counseling these women.

References

1. Palca J. Women left out at NIH. Science. 1990;248(4963):1601-2.
2. Appelman Y, van Rijn BB, Ten Haaf ME, Boersma E, Peters SA. Sex differences in cardiovascular risk factors and disease prevention. Atherosclerosis. 2015;241(1):211-8.
3. Kannel WB, Hjortland MC, McNamara PM, Gordon T. Menopause and risk of cardiovascular disease: the Framingham study. Ann Intern Med. 1976;85(4):447-52.
4. Gordon T, Kannel WB, Hjortland MC, McNamara PM. Menopause and coronary heart disease. The Framingham Study. Ann Intern Med. 1978;89(2):157-61.
5. Oliver MF, Boyd GS. Coronary atherogenesis- an endocrine problem? Minn Med. 1955;38(11):794-9.
6. LEVY H, BOAS EP. CORONARY ARTERY DISEASE IN WOMEN. Journal of the American Medical Association. 1936;107(2):97-102.
7. Colditz GA, Willett WC, Stampfer MJ, Rosner B, Speizer FE, Hennekens CH. Menopause and the risk of coronary heart disease in women. N Engl J Med. 1987;316(18):1105-10.
8. Muka T, Oliver-Williams C, Kunutsor S, Laven JS, Fauser BC, Chowdhury R, et al. Association of Age at Onset of Menopause and Time Since Onset of Menopause With Cardiovascular Outcomes, Intermediate Vascular Traits, and All-Cause Mortality: A Systematic Review and Meta-analysis. JAMA Cardiol. 2016;1(7):767-76.
9. Roeters van Lennep JE, Heida KY, Bots ML, Hoek A, collaborators of the Dutch Multidisciplinary Guideline Development Group on Cardiovascular Risk Management after Reproductive D. Cardiovascular disease risk in women with premature ovarian insufficiency: A systematic review and meta-analysis. Eur J Prev Cardiol. 2016;23(2):178-86.
10. Dam V, van der Schouw YT, Onland-Moret NC, Groenwold RHH, Peters SAE, Burgess S, et al. Association of menopausal characteristics and risk of coronary heart disease: a pan-European case-cohort analysis. Int J Epidemiol. 2019;48(4):1275-85.
11. Honigberg MC, Zekavat SM, Aragam K, Finneran P, Klarin D, Bhatt DL, et al. Association of Premature Natural and Surgical Menopause With Incident Cardiovascular Disease. JAMA. 2019;322(24):2411-21.
12. Parker WH, Broder MS, Chang E, Feskanich D, Farquhar 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(5):1027-37.
13. Rivera CM, Grossardt BR, Rhodes DJ, Brown RD, Jr., Roger VL, Melton LJ, 3rd, et al. Increased cardiovascular mortality after early bilateral oophorectomy. Menopause. 2009;16(1):15-23.
14. Mu F, Rich-Edwards J, Rimm EB, Spiegelman D, Missmer SA. Endometriosis and Risk of Coronary Heart Disease. Circ Cardiovasc Qual Outcomes. 2016;9(3):257-64.
15. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2018;33(9):1602-18.
16. Cooper LL, Palmisano JN, Benjamin EJ, Larson MG, Vasan RS, Mitchell GF, et al. Microvascular Function Contributes to the Relation Between Aortic Stiffness and Cardiovascular Events: The Framingham Heart Study. Circ Cardiovasc Imaging. 2016;9(12).
17. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol. 2010;55(13):1318-27.

18. Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, et al. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. J Am Coll Cardiol. 2014;63(7):636-46.
19. van Bommel MHD, de Jong MA, Steenbeek MP, Bots ML, van Westerop LLM, Hopman MTE, et al. No signs of subclinical atherosclerosis after risk-reducing salpingo-oophorectomy in BRCA1/2 mutation carriers. J Cardiol. 2021;77(6):570-5.
20. Freaney PM, Petito L, Colangelo LA, Lewis CE, Schreiner PJ, Terry JG, et al. Association of Premature Menopause With Coronary Artery Calcium: The CARDIA Study. Circ Cardiovasc Imaging. 2021;14(11):e012959.
21. Gunning MN, Meun C, van Rijn BB, Maas A, Benschop L, Franx A, et al. Coronary artery calcification in middle-aged women with premature ovarian insufficiency. Clin Endocrinol (Oxf). 2019;91(2):314-22.
22. Mortensen MB, Gaur S, Frimmer A, Botker HE, Sorensen HT, Kragholm KH, et al. Association of Age With the Diagnostic Value of Coronary Artery Calcium Score for Ruling Out Coronary Stenosis in Symptomatic Patients. JAMA Cardiol. 2022;7(1):36-44.
23. Dzaye O, Razavi AC, Dardari ZA, Shaw LJ, Berman DS, Budoff MJ, et al. Modeling the Recommended Age for Initiating Coronary Artery Calcium Testing Among At-Risk Young Adults. J Am Coll Cardiol. 2021;78(16):1573-83.
24. Krul IM, Opstal-van Winden AWJ, Janus CPM, Daniels LA, Appelman Y, Maas A, et al. Cardiovascular Disease Risk After Treatment-Induced Premature Ovarian Insufficiency in Female Survivors of Hodgkin Lymphoma. J Am Coll Cardiol. 2018;72(25):3374-5.
25. Kok HS, van Asselt KM, van der Schouw YT, van der Tweel I, Peeters PH, Wilson PW, et al. Heart disease risk determines menopausal age rather than the reverse. J Am Coll Cardiol. 2006;47(10):1976-83.
26. Dequeker J, Ortner DJ, Stix AI, Cheng XG, Brys P, Boonen S. Hip fracture and osteoporosis in a XIIth Dynasty female skeleton from Lisht, upper Egypt. J Bone Miner Res. 1997;12(6):881-8.
27. von Bruns P. Die allgemeine Lehre von den Knochenbruchen1882. 630 p.
28. Gordan GS. Drug treatment of the osteoporoses. Annu Rev Pharmacol Toxicol. 1978;18:253-68.
29. ALBRIGHT F, SMITH PH, RICHARDSON AM. POSTMENOPAUSAL OSTEOPOROSIS: ITS CLINICAL FEATURES. Journal of the American Medical Association. 1941;116(22):2465-74.
30. Manolagas SC, Kousteni S, Jilka RL. Sex steroids and bone. Recent Prog Horm Res. 2002;57:385-409.
31. 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(5):468-89.
32. Manolagas SC. The Quest for Osteoporosis Mechanisms and Rational Therapies: How Far We've Come, How Much Further We Need to Go. J Bone Miner Res. 2018;33(3):371-85.
33. Hedlund LR, Gallagher JC. The effect of age and menopause on bone mineral density of the proximal femur. J Bone Miner Res. 1989;4(4):639-42.
34. Jones AR, Enticott J, Ebeling PR, Mishra GD, Teede HT, Vincent AJ. Bone health in women with premature ovarian insufficiency/early menopause: a 23-year longitudinal analysis. Hum Reprod. 2024;39(5):1013-22.
35. Hadjidakis DJ, Kokkinakis EP, Sfakianakis ME, Raptis SA. Bone density patterns after normal and premature menopause. Maturitas. 2003;44(4):279-86.
36. Anagnostis P, Siolos P, Gkekas NK, Kosmidou N, Artzouchaltzi AM, Christou K, et al. Association between age at menopause and fracture risk: a systematic review and meta-analysis. Endocrine. 2019;63(2):213-24.

37. Kotsopoulos J, Hall E, Finch A, Hu H, Murphy J, Rosen B, et al. Changes in Bone Mineral Density After Prophylactic Bilateral Salpingo-Oophorectomy in Carriers of a BRCA Mutation. JAMA Netw Open. 2019;2(8):e198420.

38. Chan LN, Chen LM, Goldman M, Mak JS, Bauer DC, Boscardin J, et al. Changes in Bone Density in Carriers of BRCA1 and BRCA2 Pathogenic Variants After Salpingo-Oophorectomy. Obstet Gynecol. 2023;142(1):160-9.

39. Fakkert IE, Abma EM, Westrik IG, Lefrandt JD, Wolffenbuttel BH, Oosterwijk JC, et al. Bone mineral density and fractures after risk-reducing salpingo-oophorectomy in women at increased risk for breast and ovarian cancer. Eur J Cancer. 2015;51(3):400-8.

40. Gallagher JC. Effect of early menopause on bone mineral density and fractures. Menopause. 2007;14(3 Pt 2):567-71.

41. 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(1):69-75.

42. Powell CB, Alabaster A, Stoller N, Armstrong MA, Salyer C, Hamilton I, et al. Bone loss in women with BRCA1 and BRCA2 mutations. Gynecol Oncol. 2018;148(3):535-9.

43. Francucci CM, Romagni P, Camilletti A, Fiscaletti P, Amoroso L, Cenci G, et al. Effect of natural early menopause on bone mineral density. Maturitas. 2008;59(4):323-8.

44. Vermeulen RFM, Beurden MV, Korse CM, Kenter GG. Impact of risk-reducing salpingo-oophorectomy in premenopausal women. Climacteric. 2017;20(3):212-21.

45. Wei X, Oxley S, Sideris M, Kalra A, Brentnall A, Sun L, et al. Quality of life after risk-reducing surgery for breast and ovarian cancer prevention: a systematic review and meta-analysis. Am J Obstet Gynecol. 2023;229(4):388-409 e4.

46. Madalinska JB, Hollenstein J, Bleiker E, van Beurden M, Valdimarsdottir HB, Massuger LF, et al. Quality-of-life effects of prophylactic salpingo-oophorectomy versus gynecologic screening among women at increased risk of hereditary ovarian cancer. J Clin Oncol. 2005;23(28):6890-8.

47. Hall E, Finch A, Jacobson M, Rosen B, Metcalfe K, Sun P, et al. Effects of bilateral salpingo-oophorectomy on menopausal symptoms and sexual functioning among women with a BRCA1 or BRCA2 mutation. Gynecol Oncol. 2019;152(1):145-50.

48. Labidi-Galy SI, Papp E, Hallberg D, Niknafs N, Adleff V, Noe M, et al. High grade serous ovarian carcinomas originate in the fallopian tube. Nat Commun. 2017;8(1):1093.

49. Boerner T, Long Roche K. Salpingectomy for the Risk Reduction of Ovarian Cancer: Is It Time for a Salpingectomy-alone Approach? J Minim Invasive Gynecol. 2021;28(3):403-8.

50. Steenbeek MP, van Bommel MHD, intHout J, Peterson CB, Simons M, Roes KCB, et al. TUBectomy with delayed oophorectomy as an alternative to risk-reducing salpingo-oophorectomy in high-risk women to assess the safety of prevention: the TUBA-WISP II study protocol. Int J Gynecol Cancer. 2023;33(6):982-7.

51. Steenbeek MP, Harmsen MG, Hoogerbrugge N, de Jong MA, Maas A, Prins JB, et al. Association of Salpingectomy With Delayed Oophorectomy Versus Salpingo-oophorectomy With Quality of Life in BRCA1/2 Pathogenic Variant Carriers: A Nonrandomized Controlled Trial. JAMA Oncol. 2021;7(8):1203-12.

52. Nebgen DR, Hurteau J, Holman LL, Bradford A, Munsell MF, Soletsky BR, et al. Bilateral salpingectomy with delayed oophorectomy for ovarian cancer risk reduction: A pilot study in women with BRCA1/2 mutations. Gynecol Oncol. 2018;150(1):79-84.

53. Kershaw V, Hickey I, Wyld L, Jha S. The impact of risk reducing bilateral salpingo-oophorectomy on sexual function in BRCA1/2 mutation carriers and women with Lynch syndrome: A systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2021;265:7-17.

54. Tucker PE, Bulsara MK, Salfinger SG, Tan JJ, Green H, Cohen PA. Prevalence of sexual dysfunction after risk-reducing salpingo-oophorectomy. Gynecol Oncol. 2016;140(1):95-100.
55. Quinn SD, Domoney C. The effects of hormones on urinary incontinence in postmenopausal women. Climacteric. 2009;12(2):106-13.
56. Uebersax JS, Wyman JF, Shumaker SA, McClish DK, Fantl JA. Short forms to assess life quality and symptom distress for urinary incontinence in women: the Incontinence Impact Questionnaire and the Urogenital Distress Inventory. Continence Program for Women Research Group. Neurourol Urodyn. 1995;14(2):131-9.
57. Maas A, Rosano G, Cifkova R, Chieffo A, van Dijken D, Hamoda H, et al. Cardiovascular health after menopause transition, pregnancy disorders, and other gynaecologic conditions: a consensus document from European cardiologists, gynaecologists, and endocrinologists. Eur Heart J. 2021;42(10):967-84.
58. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA. 2002;288(3):321-33.
59. de Jong-van den Berg LT, Faber A, van den Berg PB. HRT use in 2001 and 2004 in The Netherlands--a world of difference. Maturitas. 2006;54(2):193-7.
60. Faber A, Bouvy ML, Loskamp L, van de Berg PB, Egberts TC, de Jong-van den Berg LT. Dramatic change in prescribing of hormone replacement therapy in The Netherlands after publication of the Million Women Study: a follow-up study. Br J Clin Pharmacol. 2005;60(6):641-7.
61. Stuenkel CA, Davis SR, Gompel A, Lumsden MA, Murad MH, Pinkerton JV, et al. Treatment of Symptoms of the Menopause: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2015;100(11):3975-4011.
62. The NHTPSAP. The 2017 hormone therapy position statement of The North American Menopause Society. Menopause. 2017;24(7):728-53.
63. Thurston RC, Aslanidou Vlachos HE, Derby CA, Jackson EA, Brooks MM, Matthews KA, et al. Menopausal Vasomotor Symptoms and Risk of Incident Cardiovascular Disease Events in SWAN. J Am Heart Assoc. 2021;10(3):e017416.
64. Thurston RC, El Khoudary SR, Tepper PG, Jackson EA, Joffe H, Chen HY, et al. Trajectories of Vasomotor Symptoms and Carotid Intima Media Thickness in the Study of Women's Health Across the Nation. Stroke. 2016;47(1):12-7.
65. Mishra GD, Pandeya N, Dobson AJ, Chung HF, Anderson D, Kuh D, et al. Early menarche, nulliparity and the risk for premature and early natural menopause. Hum Reprod. 2017;32(3):679-86.
66. Daly MB, Pal T, Berry MP, Buys SS, Dickson P, Domchek SM, et al. Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2021;19(1):77-102.
67. Henderson JT, Webber EM, Sawaya GF. Screening for Ovarian Cancer: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA. 2018;319(6):595-606.
68. 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(4):919-23.
69. Domchek SM, Friebel TM, Singer CF, Evans DG, Lynch HT, Isaacs C, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA. 2010;304(9):967-75.
70. 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(2):80-7.

71. Harmsen MG, Arts-de Jong M, Horstik K, Manders P, Massuger L, Hermens R, et al. Very high uptake of risk-reducing salpingo-oophorectomy in BRCA1/2 mutation carriers: A single-center experience. Gynecol Oncol. 2016;143(1):113-9.

72. van der Aa JE, Hoogendam JP, Butter ES, Ausems MG, Verheijen RH, Zweemer RP. The effect of personal medical history and family history of cancer on the uptake of risk-reducing salpingo-oophorectomy. Fam Cancer. 2015;14(4):539-44.

73. Brohet RM, Velthuizen ME, Hogervorst FB, Meijers-Heijboer HE, Seynaeve C, Collee MJ, et al. Breast and ovarian cancer risks in a large series of clinically ascertained families with a high proportion of BRCA1 and BRCA2 Dutch founder mutations. J Med Genet. 2014;51(2):98-107.

Appendices

Nederlandse samenvatting

Het onderzoek gepresenteerd in dit proefschrift had als doel de lange-termijn gezondheidseffecten te evalueren van het preventief verwijderen van beide eierstokken en eileiders (een profylactische bilaterale salpingo-oöforectomie ofwel PBSO) bij vrouwen met een verhoogd familiair risico op borst en/of eierstokkanker. Wij onderzochten de lange-termijn effecten van premenopauzale PBSO op hart- en vaatziekten, botdichtheid, kwaliteit van leven, seksueel functioneren en urine incontinentie. Alle hoofdstukken in dit proefschrift zijn beschrijvingen van de resultaten van de "Health After Early Menopause Due to Oophorectomy" (HARMOny) studie. In deze studie hebben we een vergelijking gemaakt tussen 500 vrouwen met een verhoogd familiair risico op borst en/of eierstokkanker die een PBSO hebben ondergaan vóór de menopauze (premenopauzale PBSO ≤ 45 jaar) en 240 vrouwen met een vergelijkbaar familiair risico die een PBSO ná de menopauze (postmenopauzale PBSO ≥54 jaar) hebben ondergaan. Om mee te kunnen doen moesten vrouwen minstens 55 jaar oud zijn, waardoor de metingen in dit onderzoek minstens 10 jaar na de premenopauzale PBSO gedaan zijn. Hieronder volgt een samenvatting van de resultaten van dit proefschrift.

Vrouwelijke *BRCA1* en *BRCA2* mutatie draagsters hebben een sterk verhoogd risico op eierstokkanker; de kans om in de loop van het leven eierstokkanker te krijgen bedraagt respectievelijk 44% en 17%. Uit wetenschappelijke studies blijkt dat het bij deze vrouwen niet goed mogelijk is om eierstokkanker te voorkomen door periodieke controles in het ziekenhuis. Daarom worden vrouwen met een verhoogd familiair risico op eierstokkanker geadviseerd om een PBSO te ondergaan na het krijgen van kinderen (bij een kinderwens) op een leeftijd van 35-40 jaar voor *BRCA1* mutatie draagsters en op een leeftijd van 40-45 jaar voor *BRCA2* mutatie draagsters. Alhoewel deze operatie zeer effectief is in het voorkómen van eierstokkanker, leidt het wel tot een acute menopauze, wat een grote impact kan hebben op deze vrouwen. De gemiddelde leeftijd waarop Nederlandse vrouwen in de menopauze komen is 50 tot 52 jaar, maar na een premenopauzale PBSO komen vrouwen vele jaren eerder en plotseling in een vroege operatieve menopauze doordat de eierstokken die oestrogeen produceren zijn verwijderd.

Eerdere studies suggereren dat vrouwen met een *natuurlijke* vroege menopauze (<45 jaar) een verhoogd risico hebben op hart- en vaatziekten, verminderde botdichtheid, seksuele klachten, urine incontinentie en verminderde kwaliteit van leven. Echter, de uitkomsten van deze studies zijn niet consistent en het is niet bekend of deze gezondheidsproblemen ook spelen na een *operatieve* menopauze. Het mechanisme van ontstaan is immers totaal anders: natuurlijke menopauze is een langzaam proces

van afnemende productie van oestrogeen, terwijl deze oestrogeen productie door de eierstokken bij een operatieve menopauze acuut stopt. Daarnaast hebben de beperkte studies die gedaan zijn bij vrouwen met een operatieve menopauze met name gekeken naar de korte termijn gevolgen, en in veel mindere mate naar de lange-termijn effecten. Het doel van dit proefschrift is om duidelijkheid te verschaffen over eventuele lange-termijn effecten van een premenopauzale PBSO voor zowel vrouwen die deze operatie (hebben of overwegen te) ondergaan, als zorgverleners die hen begeleiden in dit traject.

In **Hoofdstuk 2** hebben we de lange-termijn effecten van de timing van PBSO onderzocht op aderverkalking van de kransslagaders. De hoeveelheid kalkafzetting (aderverkalking) van de kransslagaders (Coronary Artery Calcium) is een goede voorspeller voor het risico op toekomstige hart- en vaatziekten en wordt gemeten met een CT-scan van het hart. We vergeleken vrouwen met een premenopauzale PBSO zowel met vrouwen met een postmenopauzale PBSO (allen uit de HARMOny studie) als met een externe referentiegroep van vrouwen uit de Nederlandse algemene bevolking met dezelfde leeftijd (ROBINSCA studie). Daarnaast hebben we gekeken wat de invloed is van een vroege premenopauzale PBSO (<41 jaar) ten opzichte van een iets latere premenopauzale PBSO (41-45 jaar) op de mate van aderverkalking van de kransslagaders. Eenentwintig jaar na de PBSO vonden we geen verschil in de aderverkalking van de kransslagaders vrouwen met een premenopauzale PBSO vergeleken met vrouwen met een postmenopauzale PBSO of vrouwen. Er was ook geen verschil met vrouwen in de algemene bevolking. Daarnaast vonden we ook geen invloed van de timing van premenopauzale PBSO (vroeg vergeleken met laat) op het ontstaan van aderverkalking van de kransslagaders. Concluderend, in **Hoofdstuk 2** vonden we geen nadelig effect van een vroege menopauze ontstaan door operatie aan de eierstokken en eileiders op het ontstaan van aderverkalking van de kransslagaders. We verwachten daarom dat er geen nadelig effect is op de ontwikkeling van hart- en vaatziekten in de toekomst.

In **Hoofdstuk 3** onderzochten we de lange-termijn effecten van een premenopauzale PBSO op de vaatstijfheid, een andere voorspeller van de kans op toekomstige hart- en vaatziekten. We hebben deze bij de studiedeelneemsters gemeten door de Pulse Wave Velocity (PWV) van de grote lichaamsslagader te meten met een arteriograaf. Daarnaast hebben we gekeken of er een verband bestaat tussen aderverkalking van de kransslagaders en vaatstijfheid van de grote lichaamsslagader bij vrouwen met een premenopauzale PBSO. We vonden geen verschil in vaatstijfheid tussen vrouwen met een premenopauzale PBSO en vrouwen met een postmenopauzale PBSO. Onder de vrouwen met een premenopauzale PBSO vonden we dat met het stijver worden van de vaatwand van de grote lichaamsslagader, ook de kans op aderverkalking van de kransslagaders toenam.

In **Hoofdstuk 4** hebben we gekeken naar het lange-termijn effect van een premenopauzale PBSO op de botdichtheid. Hiervoor werd bij alle de studiedeelneemsters een botdichtheidsscan gemaakt. Vrouwen die een premenopauzale PBSO hadden ondergaan bleken 18 jaar later een, voor hun leeftijd, lagere botdichtheid te hebben dan vrouwen met een postmenopauzale PBSO (2.35 keer zo vaak een significant lagere botdichtheid dan vrouwen van hun leeftijd). We vonden echter geen verschil tussen de premenopauzale en postmenopauzale PBSO groep in het voorkomen osteopenie en osteoporose op de lange-termijn (respectievelijk 70.4% en 67.3%). Dit is belangrijk omdat het bestaan van osteopenie en osteoporose een reden is om te starten met eventuele medicamenteuze behandelingen om de botdichtheid te verbeteren. In deze studie vonden we geen invloed van de timing van de premenopauzale PBSO (<41 jaar versus 41-45 jaar) op de botdichtheid.

In **Hoofdstuk 5** hebben we een overzicht gegeven van alle uitkomsten van de HARMOny studie. Daarnaast hebben we onderzocht of er een lange-termijn effect is van een premenopauzale PBSO op kwaliteit van leven en zorgen over kanker. Hiervoor hebben we gebruik gemaakt van de 36-Item Short Form Health Survey en de Cancer Worry Scale. Vrouwen met een premenopauzale PBSO rapporteerden even goede fysieke en mentale kwaliteit van leven als vrouwen die een postmenopauzale PBSO hadden ondergaan. We vonden ook geen verschil tussen de pre- en postmenopauzale PBSO groep in de zorgen over het krijgen van kanker (51.8% versus 50.0%).

In **Hoofdstuk 6** onderzochten we de lange-termijn effecten van een premenopauzale PBSO op seksueel functioneren. Hiervoor hebben we gebruik gemaakt van de Sexual Activity Questionnaire. Vrouwen in de premenopauzale PBSO groep waren even vaak seksueel actief als vrouwen van dezelfde leeftijd in de postmenopauzale PBSO groep. Dit percentage van 48% is ook vergelijkbaar met de algehele bevolking. Vijftien jaar na de PBSO vonden we geen verschil in het gerapporteerde plezier in seks tussen de premenopauzale en postmenopauzale PBSO groep. Daarentegen gaven vrouwen in de premenopauzale PBSO groep wel meer klachten aan van seksueel ongemak, voornamelijk als gevolg van meer vaginale droogheid. Deze schijnbare tegenstilling zou verklaard kunnen worden door het feit dat seksueel plezier ook beïnvloedt kan worden door niet penetrerende seks.

In **Hoofdstuk 7** onderzochten we de effecten van premenopauzale PBSO op het voorkomen van urine incontinentie. Vrouwen met een premenopauzale PBSO hadden een mild en niet statistisch significant verhoogd risico op het voorkomen van incontinentie voor urine. We vonden dat vrouwen met een premenopauzale PBSO een 3.5 keer groter risico hadden op stress incontinentie (urine verlies bij

druk verhogende momenten zoals hoesten of lachen) vergeleken met vrouwen met een postmenopauzale PBSO. We vonden daarentegen geen verschil in risico op aandrang incontinentie, urine verlies bij een zeer volle blaas, tussen de pre- en postmenopauzale PBSO groep. Dit was een onverwachte bevinding aangezien we op basis van eerder onderzoek weten dat de natuurlijke menopauze meer invloed heeft op aandrang incontinentie dan op stress incontinentie.

Dit proefschrift geeft inzicht in diverse lange-termijn gevolgen van een premenopauzale PBSO. Eerdere studies waren voornamelijk gefocust op de korte termijn, of gebruikten alleen controle groepen met vrouwen uit de algehele populatie. Samenvattend vonden we geen aanwijzingen voor een mogelijk verhoogd risico op hart- en vaatziekten in de premenopauzale vergeleken met de postmenopauzale PBSO groep. Hoewel vrouwen die een premenopauzale PBSO hadden ondergaan een lagere botdichtheid hadden, vonden we geen verhoogd risico op osteopenie en osteoporose vergeleken met de postmenopauzale PBSO groep. Ondanks dat vrouwen in de premenopauzale PBSO groep vaker vaginale droogheid en seksueel ongemak ervoeren vergeleken met de postmenopauzale PBSO groep, was er geen verschil in plezier in seks. Vrouwen met een premenopauzale PBSO rapporteerden meer klachten van stress urine incontinentie vergeleken met vrouwen met een postmenopauzale PBSO, deze verschillen waren daarentegen relatief klein en of deze in de klinisch praktijk veel invloed hebben is de vraag. We vonden geen verschil in aandrang incontinentie tussen de pre- en postmenopauzale PBSO groep. Daarnaast vonden we ook geen verschil in gezondheid gerelateerde kwaliteit van leven of angst om kanker te krijgen tussen beide groepen. In geen van de studies beschreven in dit proefschrift vonden we bewijs dat vrouwen met een vroege premenopauzale PBSO (<41 jaar) andere uitkomsten hadden dan vrouwen met een iets latere premenopauzale PBSO (41-45 jaar). Concluderend zijn de uitkomsten van ons onderzoek grotendeels geruststellend, zowel voor vrouwen die deze ingrijpende operatie op jonge leeftijd (nog moeten of al hebben) ondergaan, als voor de zorgverleners die deze vrouwen informeren in het traject voor en na het preventief verwijderen van de eierstokken en eileiders.

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Research Data Management paragraph

Ethics and privacy

All chapters in this thesis are based on the HARMOny study which involved human participants. The HARMOny study was conducted according to the standards of Good Clinical Practice, in agreement with the principles of the Declaration of Helsinki and with the Dutch law as stated in the Medical Research Involving Human Subjects Act (WMO). The study was approved in writing by the Institutional Review Board of the AVL/NKI to be conducted in all nine University Medical Centers and the Antoni van Leeuwenhoek. Privacy of the participants was warranted by the use of pseudonymization. The pseudonymization key was stored on a secured network drive that was only accessible to members of the project who needed access to it because of their role within the project. The pseudonymization key was stored separately from the research data. All participants included in the HARMOny study signed informed consent. Participants were also asked for consent for reuse of their data outside the HARMOny study.

Data collection and storage

Data for all chapters was obtained using Exploratio and ALEA for secured online questionnaires and from the Electronic Health Records of the participants in the participating hospitals. Raw data was stored and analyzed on the department server only accessible by project members working at the NKI. Processed data and documentation concerning research protocol and readme file are stored on the department server only accessible by project members working at the NKI. These secure storage options safeguard the availability, integrity and confidentiality of the data.

Data sharing according to FAIR principles

The NKI repository was used for long-term archiving and publishing (https://repository.nki.nl/, dataset not yet uploaded) with restricted access. With publication, de-identified data collected for the study, including participant data, will be made available upon signing a Data Use Agreement, only of participants that agreed to have their data shared with other (non-commercial) parties on the signed informed consent. Data were made reusable by adding sufficient documentation (research protocol and read me file).

Dankwoord

Graag wil ik alle studiedeelneemsters danken voor het vrijwillig deelnemen aan de HARMOny studie. Tijdens het onderzoek bleken velen van u bereid om een dag vrij te nemen en (midden in een pandemie) naar het ziekenhuis te komen voor de verschillende metingen, en daarnaast openhartig te vertellen over uw privéleven. Zonder u was het niet mogelijk geweest om vrouwen die in de toekomst met dezelfde moeilijke keuzes geconfronteerd worden, beter te kunnen informeren over de eventuele consequenties van hun beslissing. Daarnaast wil ik graag Hart voor Vrouwen, de Maarten van der Weijden Foundation en het KWF bedanken voor het mogelijk maken van dit proefschrift.

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PhD portfolio

Department: Cardiology
PhD period: 1-4-2020 t/m 1-6-2024
PhD Supervisor(s): Prof. A.H.E.M. Maas, Prof. Ir. F.E. van Leeuwen
PhD Co-supervisor(s): Dr. M.J. Hooning

Training activities	Hours
Courses	
• Radboudumc - Introduction day (2020)	6.00
• RIHS - Introduction course for PhD candidates (2020)	15.00
• RIHS introductory course (2020)	21.00
• Basic oncology course (2020)	84.00
• Radboudumc - Scientific integrity (2021)	20.00
• Klinimetrie (2021)	84.00
• OOA ethics and integrity course	56.00
• Logistic Regression (2022)	40.00
• Attending patient consultations (2020-2023)	56.00
• English writing course (2023)	84.00
Seminars	
• Scientific Meeting of the Medical Oncology Department Erasmus MC, oral presentation (2023)	7.00
• Staff meeting NKI-AvL, oral presentation (2024)	7.00
Conferences	
• EMAS virtual meeting (2020)	12.00
• EMAS congress, oral presentation (2023)	56.00
• Hebon congress 2023, oral presentation (2023)	56.00
Other	
• EBROK (2021)	12.00
• OOA retreat (2022)	56.00
• RIHS retreat (2022)	56.00
• OOA retreat (2023)	56.00
• PSOE department meeting NKI (2024)	112.00
• Epidemiology journal club (2024)	112.00
Teaching activities N/A	
Total	**998.00**

About the author

Maarten was born on November 8, 1991, in Amstelveen. He graduated from Comenius College in Hilversum in 2009 and began studying Econometrics and Operational Research at Vrije Universiteit Amsterdam. In 2011, he switched to Medicine at the University of Amsterdam. After obtaining his Master's degree in Medicine in 2018, Maarten started working as a Resident Internal Medicine (not formally in training) at Flevoziekenhuis in Almere. In 2020, he began his PhD at the Department of Psychosocial Research and Epidemiology (PSOE) at the Netherlands Cancer Institute In 2024, he resumed working as a Resident Internal Medicine (not formally in training) at Spaarne Gasthuis. In October 2025, Maarten will start as a Resident Internal Medicine (in training) at Rode Kruis Ziekenhuis and Amsterdam University Medical Center.

Radboudumc university medical center

IN DEI NOMINE FELICITER

Radboud University