Long term somatic and psychosocial morbidity after risk-reducing salpingo-oophorectomy in women at risk for hereditary breast ovarian cancer

Trond Melbye Michelsen, MD

Department of Gynaecology, Sørlandet Hospital Arendal

Department of Gynaecological Oncology, The Norwegian Radium Hospital,

National Resource Centre for Women's Health, Rikshospitalet,

Department of Clinical Cancer Research, The Norwegian Radium Hospital,

Oslo University Hospital

Faculty Division The Norwegian Radium Hospital, Faculty of Medicine,

University of Oslo

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Acknowledgements

Last, but not least, I thank my family. To Merethe, my wife and dearest friend, thank you for always being there. To my dear children Fredrik, Christoffer and Nora, thank you for the happiness and joy that we share.

Abbreviations

BIQ - Body Image Questionnaire

BIS - Body Image Scale

BRCA1 - Breast Cancer Susceptibility Gene 1

BRCA2 - Breast Cancer Susceptibility Gene 2

CHD - coronary heart disease

CVD - cardiovascular disease

DNA - deoxyribonucleic acid

EORTC - European Organization for Research and Treatment in Cancer

FQ - Fatigue Questionnaire

GP - general practitioner

HABITS - Hormonal Therapy After Breast Cancer – Is It Safe?

HADS-A - Hospital Anxiety Depression Scale – Anxiety subscale

HADS-D - Hospital Anxiety Depression Scale – Depression subscale

HADS-T - Hospital Anxiety Depression Scale – Total scale

HBOC - hereditary breast ovarian cancer

HE4 - human epididymis protein 4

HERS - Heart and Estrogen/progestin Replacement Study

HRT - hormonal replacement therapy

HUNT - The Nord-Trøndelag Health Study

NORM - the norm sample collected by the Norwegian Radium Hospital

QoL - quality of life

RMI - risk of malignancy index

RRSO - risk-reducing salpingo-oophorectomy

UK - United Kingdom

USA - United States of America

WHI - Women's Health Initiative

List of papers

Paper I:

Michelsen TM, Pripp AH, Tonstad S, Tropé CG, Dørum A. Metabolic syndrome after risk-reducing salpingo-oophorectomy in women at high risk for hereditary breast ovarian cancer. A controlled observational study. Eur J Cancer 2009;45:82-89.

Paper II:

Michelsen TM, Tonstad S, Pripp AH, Tropé CG, Dørum A. Coronary heart disease risk profile in women who underwent salpingo-oophorectomy to prevent hereditary breast ovarian cancer. (Submitted 2009).

Paper III:

Michelsen TM, Dørum A, Tropé CG, Fosså SD, Dahl AA. Fatigue and quality of life after risk-reducing salpingo-oophorectomy in women at increased risk for hereditary breast-ovarian cancer. Int J Gynecol Cancer 2009;19:1029-36.

Paper IV:

Michelsen TM, Dørum A, Dahl AA. A controlled study of mental distress and somatic complaints after risk-reducing salpingo-oophorectomy in women at risk for hereditary breast ovarian cancer. Gynecologic Oncology 2009;**113**:128-33.

1. Introduction

1.1 Women's Health

It has long been established that women live longer than men, although they have higher morbidity rates (1). Men experience more life-threatening chronic diseases and die younger, whereas women live longer but have more nonfatal acute and chronic illnesses (1). Women outlive men in every region and almost every country in the world, and male mortality is higher at every age from infancy and throughout life (2). The main causes of death among Norwegian women are cardiovascular diseases (CVD) and cancer (Fig 1).

Fig 1. Deaths from cardiovascular disease (CVD), cancer and total mortality among Norwegian women, 2006. Data from the Causes of Death registry of Norway.
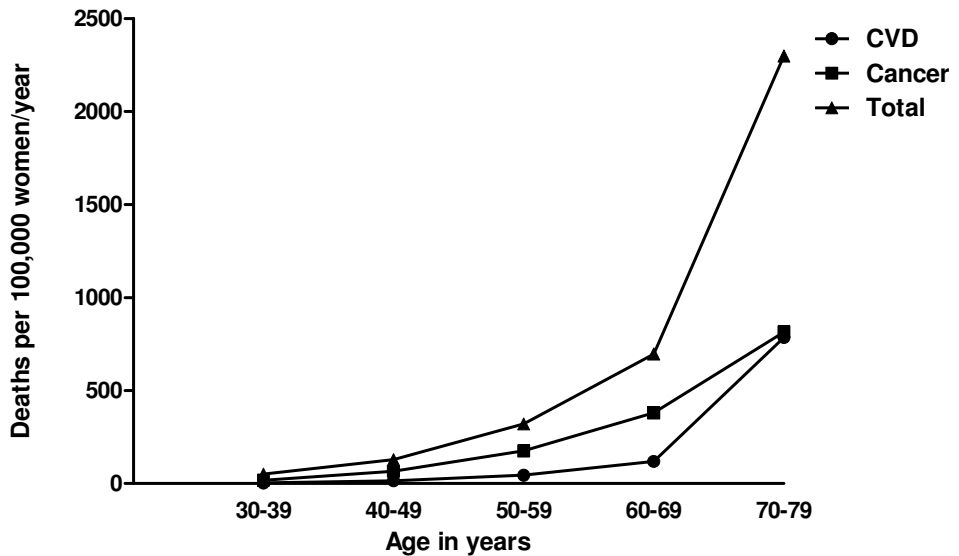
Life expectancy for Norwegian men and women at birth in 2007 was 78.2 years and 82,7 years, respectively (3). Up to the age of 80 years, cancer dominates as the leading cause of death. After the age of 70 years, death rates from CVD rise exponentially, although the mortality from CVD has decreased in Norway during the last 15 years (Fig 2).

Fig 2. Deaths from CVD, cancer and total mortality among Norwegian women, 1991-2006. Data from the Causes of Death Registry of Norway.
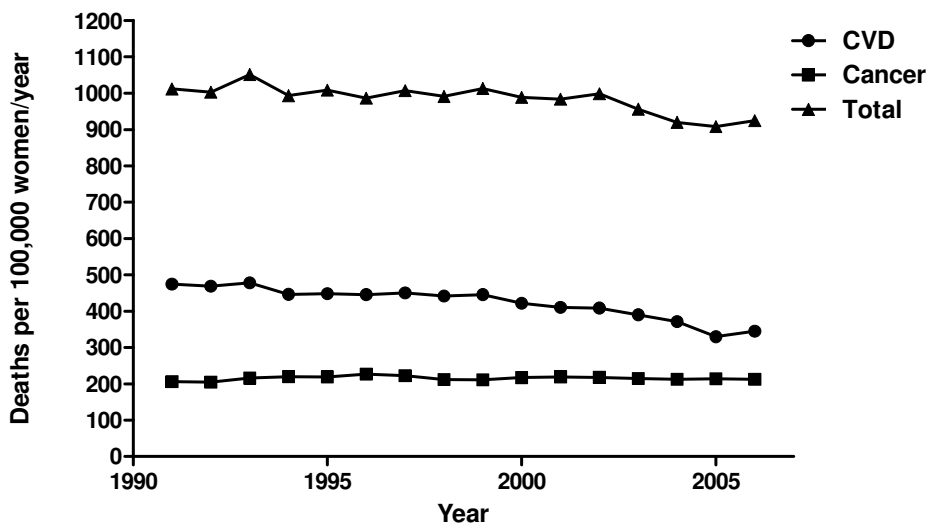
1.2 Cancer

In 2000, almost 11 million new cases of cancer were diagnosed worldwide, 7 million people died from cancer, and 25 million persons were alive with cancer (4). Cancer is a disease recognized by uncontrolled cell growth. Malignant tumours consist of neoplastic cells which do not perform useful functions in the host organism, have a tendency of continuous, indefinite growth, and are often irresponsive to the normal regulatory circuits

that control normal cell proliferation and homeostasis (5;6). Initiation is the first stage of carcinogenesis and is characterized by irreversible changes in the cellular DNA. Initiators can be chemical, physical or viral agents (7). Tumour promotion is the second stage, which involves stimuli from growth factors and hormones to produce expansion of malignant cells (7). Hanahan and Weinberg (5) described six essential alterations in cell physiology that are involved in malignant growth: self-sufficiency in growth signals, insensitivity to growth-inhibitory (anti-growth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. The authors proposed that the mentioned six capabilities are common by most and perhaps all types of human tumours (5).

1.3 Epithelial ovarian cancer

1.3.1 Epidemiology

Ovarian cancer is the sixth most common cancer and the seventh most common cause of cancer death among women worldwide, with 204,000 new cases and 125,000 deaths in 2002 (8). It is the ninth most common cancer in Norwegian women, and 449 new cases were diagnosed in Norway in 2007 (9). In Norway, the average incidence rate of ovarian cancer is 11.1 per 100,000 women per year, and the lifetime risk of ovarian cancer is estimated to be 1.3% (9). The incidence rates are highest in developed countries, and sporadic ovarian cancer mainly affects postmenopausal women (8).

Etiology
Epithelial cancer of the ovary derives from malignant transformation of the ovarian surface epithelium, although the cause is still unknown (10). Incessant ovulation is considered to be one of the primary risk factors for ovarian cancer, maybe because ovulation is followed by proliferation of the ovarian epithelium which may lead to mutations and carcinogenesis (11). The association between incessant ovulation and ovarian cancer was reported almost 40 years ago (12;13).

A strong family history of ovarian or breast cancer is the most important risk factor for ovarian cancer (10). Multiple parity, breast-feeding, and the use of oral contraceptives are factors associated with lower risk of ovarian cancer, as is a history of tubal ligation and hysterectomy (7). Early age at menarche, late natural menopause and older age are all associated with increased risk of ovarian cancer (7). Other potential aetiological factors include excessive gonadotropin secretion (14), effects of androgens and progestins (15) and stromal hyperactivity (16).

A new theory describes a subset of serous ovarian cystadenomas that evolve through serous borderline ovarian tumours to low-grade epithelial ovarian cancer (17). Epithelial ovarian cancer probably develops via either of two pathways (18). Type I tumours are slow growing, generally localized to the ovary at diagnosis and develop from borderline tumours. These tumours are genetically stable and carry mutations in KRAS, BRAF, PTEN and beta catenin oncogenes. Type I tumours include low-grade micropapillary serous carcinoma, mucinous, endometroid and clear cell carcinoma. Type II tumours are rapidly growing, highly aggressive cancers that lack well defined precursor lesions, and most cancers are at advanced stages at the time of diagnosis. These cancers include high grade serous carcinoma, malignant mixed mesodermal tumours and undifferentiated carcinomas. Type II tumours are characterized by mutations of the tumour-suppressor gene Tp53 and a high level of genetic instability (18).

1.3.2 Clinical presentation

Paulsen et al. (19) studied symptoms in all women who were diagnosed with invasive ovarian tumours in Norway in 2002 (N=486). They found that the majority of women with invasive disease experienced at least one symptom before diagnosis. Only 6% had no symptoms. The most common symptoms were abdominal pain or discomfort, distended or tense abdomen, bowel irregularities and persisting fatigue or weight loss.

Physical findings are diverse and typically include a palpable ovarian mass. Ovarian cancer should be considered in any premenopausal woman with an unexplained

enlargement of the ovary or any postmenopausal woman with a palpable ovary. Other findings may include ascites, pleural effusions, and an umbilical mass (10).

Diagnostic tools include transvaginal ultrasound examination and CT scans of thorax and abdomen. The serum CA-125 level is elevated in 80% of cases of ovarian cancer. The CA-125 test lacks sensitivity and specificity to be used as a diagnostic tool, but may be useful in the evaluation of treatment response (10). In women with an identified pelvic tumour, the risk of malignancy index (RMI) may be helpful in distinguishing between benign and malignant disease (20) so that the patient can be referred to a gynaecologic oncology unit for treatment if necessary. The RMI consists of a score based on the CA 125 level, ultrasound score (0,1 or 3) and menopausal status (1=premenopausal, 3=postmenopausal). A recent systematic review concluded that the RMI should be the prediction model of choice in the preoperative assessment of an adnexal mass (21).

1.3.3 Treatment

Classification and staging

The non-specific symptoms in many cases lead to a diagnostic delay, and the women are often referred to doctors with other speciality fields before they are examined by a gynaecologist (19). The diagnostic delay has major impact on ovarian cancer mortality, as two thirds of cases are diagnosed at advanced stages (22;23). Therefore, ovarian cancer is often called the silent killer and has the highest age-standardised mortality rates of genital cancers in Norwegian women (24).

Surgical staging, performed during exploratory laparotomy, is important to optimize post-operative treatment and to classify the disease. On entering the abdomen, aspiration of ascites or peritoneal lavage should be performed to obtain specimens for cytologic examination. Based on the staging system by the International Federation of Gynaecology and Obstetrics (FIGO), the disease is staged from stage I to IV with subcategories A, B and C (Table 1). Patients with early-stage disease (stage I or II) may have five-years survival rates as high as 90%, whereas patients with advanced disease

have lower survival rates (10%-30%) (9). Histopathologically, ovarian cancer is categorized in grade 1 to grade 3 (7). The pattern of spread includes direct spread, lymphatic spread and haematogenous spread (7).

Table 1. Carcinoma of the ovary: FIGO nomenclature (Rio de Janeiro 1988) (25)

Stage 1		Growth limited to the ovaries
	Ia	Growth limited to one ovary. No ascites present containing malignant cells. No tumour on the external surface; capsule intact.
	Ib	Growth limited both ovaries. No ascites present containing malignant cells. No tumour on the external surfaces; capsules intact
	Ic	Tumour either Stage Ia or Ib, but with tumour on surface of one or both ovaries, or with capsule ruptured, or with ascites present containing malignant cells, or with positive peritoneal washings.
Stage II		Growth involving one or both ovaries with pelvic extension
	IIa	Extension and/or metastases to the uterus and/or tubes
	IIb	Extension to other pelvic tissues
	IIc	Tumour either Stage IIa or IIb, but with tumour on surface of one or both ovaries; or with capsule(s) ruptured; or with ascites present containing malignant cells or with positive peritoneal washings.
Stage III		Tumour involving one or both ovaries with histologically confirmed peritoneal implants outside the pelvis and/or positive retroperitoneal or inguinal nodes. Superficial liver metastases equal Stage III. Tumour is limited to the true pelvis, but with histologically proven malignant extension to small bowel or omentum.
	IIIa	Tumour grossly limited to the true pelvis, with negative nodes, but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces, or histologically proven extension to small bowel or mesentery.
	IIIb	Tumour of one or both ovaries with histologically confirmed implants. Metastases of abdominal peritoneal surfaces.
	IIIc	Peritoneal metastasis beyond the pelvis >2cm in diameter and/or positive retroperitoneal or inguinal nodes.
Stage IV		Growth involving one or both ovaries with distant metastases. Malignant pleural effusion. Parenchymal liver metastases.

Surgery

The standard treatment for ovarian cancer is a laparotomy with a vertical midline incision (7;10). The surgery typically includes a hysterectomy with bilateral salpingo-oophorectomy, omentectomy, biopsies from para-aortal and pelvic lymph nodes and peritoneal washings. Optimal debulking to no residual disease after surgery improves prognosis (7;10). Ovarian cancer surgery requires well-trained and experienced surgeons. In Norway, centralization of ovarian cancer surgery improved short-term survival in a study including all women who were diagnosed with advanced ovarian, tubal or peritoneal cancer in 2002 (N=198) (26). Fertility-sparing surgery is an option for ovarian cancer patients, but requires careful selection (27).

Chemotherapy

All patients with advanced-stage and most patients with early-stage ovarian cancer are treated with post-operative chemotherapy. The standard primary regimen is intravenous taxane- and platinum based chemotherapy. The prior treatment of cisplatin and paclitaxel was replaced by carboplatin and paclitaxel because the latter regimen had fewer side-effects (28). In recurrent ovarian cancer, which is generally not curable, the treatment aim is symptom management and complication prevention. In patients who have recurrence within six months of first-line chemotherapy, the disease is considered to be platinum-resistant, and other chemotherapy regimens are chosen. Alternative regimens include docetaxel, topotecan, pegylated liposomal doxorubicin, gemcitabine and weekly paclitaxel (29). Intraperitoneal chemotherapy is an effective alternative, but it is highly toxic and therefore not implemented as standard adjuvant treatment (30;31).

At first relapse of ovarian cancer, a treatment option is secondary cytoreduction followed by chemotherapy. A recent Norwegian study showed that secondary cytoreduction followed by chemotherapy carried a survival benefit compared to chemotherapy alone when the tumour was localized (32).

Novel therapeutic interventions in ovarian cancer include the administration of targeted therapy. Angiogenesis-targeted therapy with bevacizumab is a promising

alternative aiming to inhibit vascular endothelial growth factor (VEGF), but at present there are only data available from phase II trials (33;34).

BRCA-related ovarian cancer may have a different biology than sporadic ovarian cancer, as described in chapter 1.6.2. The treatment of such cancers may require a different approach in the future. During the last years, promising studies describing the use of poly ADP-ribose polymerase-1 (PARP-1) inhibitors in BRCA-related cancers have been published (35;36).

Optimal debulking and improvement in chemotherapy over the last decades have increased the five-year ovarian cancer survival for all stages and histologies combined from 30% in the 1970ies to approximately 50% at present worldwide (25). The survival rate in Norway has improved from 38% in 1972-1976 to 47% in 1997-2003 (9). The five-year survival of the 2/3 of patients presenting with advanced disease is currently 28% in Norway (9).

1.3.4 Pathology

Epithelial ovarian cancer represents more than 90% of all malignant ovarian tumours. Although the disease is often considered to be a single entity, it consists of many tumour types, each with subtypes (7). Epithelial ovarian cancer is often categorized as serous (63%), endometroid (12%), mucinous (9%), clear cell (7%) or other types (9%) (10). Papillary serous carcinoma of the ovary is often associated with elevated CA-125 levels. Mucinous carcinoma is not typically associated with BRCA mutations. Clear-cell carcinoma is the most chemoresistant type of ovarian cancer (10).

1.4 Hereditary Cancer

The term hereditary cancer describes forms of cancer where the development is caused by a genetic predisposition. Detection of hereditary cancer is important as it gives opportunity to consider preventive measures in individuals at risk. During the last 15-20

years, rapid progress has been made in the understanding of genetic predisposition for common cancers, and genetic testing for familial colon cancer, endometrial cancer, breast cancer and ovarian cancer is available.

1.4.1 Main features of hereditary cancer

Main features of hereditary cancer are: 1) early age of cancer onset, often 10-20 years earlier than it occurs in the sporadic counterpart; 2) an excess of bilateral cancers in paired organs, such as breast or ovary in the HBOC syndrome; 3) multiple primary cancers; 4) autosomal dominant mode of inheritance (37).

The hereditary cancer features can be sees in BRCA mutation carriers. In *BRCA1* mutation carriers, the debut of ovarian cancer occurs at a younger age than in *BRCA2* mutation carriers and sporadic ovarian cancers (38-40). The risk of a contralateral breast cancer in BRCA mutation carriers with a breast cancer diagnosis was estimated by Metcalfe et al. (41) to be 40% in 10 years without tamoxifen treatment or risk-reducing salpingo-oophorectomy (RRSO). BRCA mutation carriers also have an increased risk of other cancers, for example stomach cancer, pancreatic cancer, colon cancer and prostate cancer (42;43).

1.5 Hereditary Ovarian Cancer

Ovarian cancer is the only common adult cancer which has a hereditary proportion that exceeds 10% (40;44).

1.5.1 History

In 1866 the French surgeon Paul Broca described his wife's family with four generations of breast cancer in 10 out of 24 relatives as hereditary cancer. Other tumours were also described, which provided the link between hereditary breast cancer and other tumours (Broca PP. Traité des tumours, vols. 1-2. Asselin, Paris, 1866-9) (45). This observation was published almost concurrently with Gregor Mendel's principles of genetics (46). More than 80 years later, Liber (47) reported the history of a family with increased incidence of ovarian cancer. One of the first descriptions of hereditary breast ovarian cancer (HBOC) based on pedigrees from families with increased risk for such cancers was made by Lynch et al. (48) in 1972. These observations documented autosomal dominant inheritance. By studying HBOC families, researchers were able to localize the genes involved in hereditary breast ovarian cancer (49;50). In 1994, the *BRCA1* gene was cloned by Miki et al. (51), and in 1995, Wooster et al. (52) were able to clone the *BRCA2* gene.

1.5.2 Clinical manifestations

Hereditary ovarian cancer presents as three distinct clinical syndromes: 1) Hereditary breast and ovarian cancer syndrome (HBOC) with mutations in the BRCA genes accounting for approximately 90% of all cases. 2) "Site-specific" ovarian cancer with mutations in the BRCA genes or unknown genes, and without the breast cancer phenotype. This syndrome accounts for approximately 5% of cases of hereditary ovarian cancer, although no certain disease-causing gene has been identified (53;54). High risk genes are not estimated to explain all familial ovarian cancers, and novel susceptibility genes are therefore sought. These include potential low-penetrant genes and the impact of single nucleotide polymorphisms (55). 3) Hereditary non-polyposis colorectal cancer syndrome (HNPCC) or Lynch syndrome accounts for 5-10% of hereditary ovarian cancer cases. This syndrome is associated with mutations in the DNA mismatch repair genes. The mutations are primarily found in MLH1 and MSH2, and these two genes account for

around 90% of all identified mutations associated with HNPCC (56). A smaller amount of mutations associated with HNPCC are found in MSH6 (57;58).

1.6 BRCA mutations and related cancers

Approximately 8-13% of epithelial ovarian cancer cases are considered to be attributable to heritable germ line mutations in the BRCA genes (40;59;60). *BRCA1* maps to chromosome 17q21. The *BRCA1* gene was the first breast and ovarian cancer susceptibility gene identified through studies that applied linkage analysis in families with multiple breast and ovarian cancers and early onset breast cancer (51). The gene includes 22 exons with a 7,2 kb long transcript that translates to an 1863 amino acid protein. The C-terminal end contains a region that functions in DNA damage repair and cell cycle control (61). *BRCA2* was identified through linkage analysis in families which did not show linkage to *BRCA1*, and is located to chromosome 13q12-23 (52). This large gene contains 26 coding exons and transcribes into an 11,2 kb mRNA and a 3418 amino acid protein (52;62). It takes part in homologous recombination, and the C-terminal end of the protein contains two nuclear localization signals for nuclear transport (63;64). Because the genes are large, genetic testing has been expensive and complicated until recently, as more than 4000 different BRCA mutations have been documented (65).

The frequency of *BRCA1/2* mutations in the general population is about 1 in 800 for *BRCA1* and slightly lower for *BRCA2*. The frequency can vary significantly between different geographic areas and ethnic groups, because of founder mutations (Fig 3).

Fig 3. The development of a founder effect

Original population | Population decreased. Proportion of mutation carriers higher. | Generations later

■ Mutation carrier   □ Non-mutation carrier
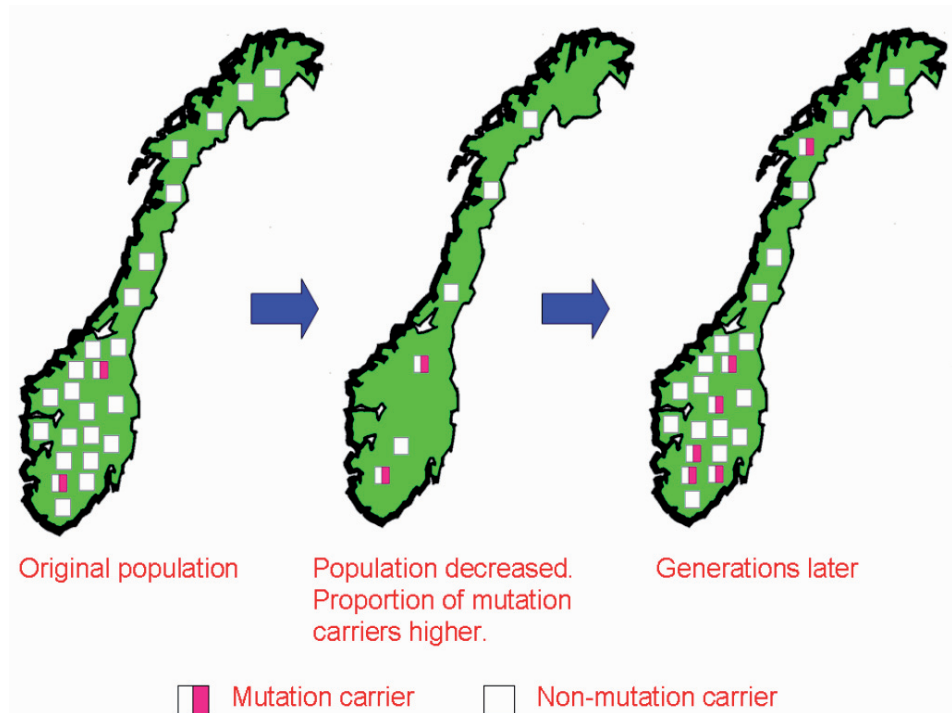
A founder mutation is a mutation in the DNA of one or more individuals who were founders of a distinct population. Founder mutations initiate with changes that occur in the DNA and are passed through generations, typically if a small subset of the population emigrates and establishes a new population or if the population is reduced due to war or epidemic diseases. The prevalence of the three *BRCA1/2* founder mutations among Ashkenazi Jews is approximately 1 in 50 (66-68). In Norway, four mutations account for about two thirds of the *BRCA1* mutation carriers. The reason for this aggregation is thought to be a reduced population size after the medieval Bubonic plagues in the 14th century, followed by a rapid expansion of the population (69). The 1675delA, 816delGT and 3347delAG families originated from the South-West coast of Norway with a few families in Northern Norway, while the traceable ancestors of the 1135insA families

clustered along the historical inland road from the South-East to mid-Norway. The carriers of each of the four mutations today are descendants of one or a few individuals surviving the plagues (69). More recently, altogether 10 Norwegian founder mutations have been described, including two *BRCA2* mutations (70).

Mutations in the *BRCA1* gene account for 60% of heritable ovarian cancer, *BRCA2* mutations for 30% and ovarian cancer as part of the Hereditary Non Polyposis Colorectal Cancer (HNPCC, Lynch syndrome) for about five percent (71).

The lifetime risk of ovarian cancer is about 40% for *BRCA1* and 18% for *BRCA2* mutation carriers (72), while the risk of sporadic ovarian cancer in the general Norwegian population is 1.7% (9). The lifetime risk of breast cancer is estimated to 57% for *BRCA1* and 49% for *BRCA2* mutation carriers (72).

In summary, BRCA mutations significantly increase the risk of both breast and ovarian cancer, and the risk is well documented in large, prospective multicentre studies. The risk of cancer is affected by environmental factors, reproductive factors and probably modifying genes.

1.6.1 Pathology

Serous adenocarcinoma predominates among BRCA-related ovarian cancers, but other histological types, like endometroid carcinomas are also found, and they tend to be poorly differentiated (73). Borderline and mucinous tumours seem to be uncommon (74).

1.6.2 Survival

BRCA-related ovarian cancer

The first important study on prognosis in BRCA mutation carriers with ovarian cancer was performed by Rubin et al. (75). They concluded that BRCA-related ovarian cancer had a more favourable clinical course than sporadic ovarian cancer. In contrast to this

finding, two other early studies on BRCA-related ovarian cancer concluded that the prognosis was similar or poorer than the prognosis of women with sporadic ovarian cancer (76;77). The validity of the latter studies has been questioned because the analyses were performed without stratifying for tumour stage. Recent studies of survival in BRCA mutation carriers with ovarian cancer have concluded that the prognosis is better than in sporadic ovarian cancer (54;78-81). Tan et al. (82) described a clinical syndrome of "BRCAness" in ovarian cancer including serous histology, high response rates to first and subsequent lines of platinum-based treatment, longer tumour free intervals between relapses, and improved overall survival.

The studies reporting a favourable outcome for BRCA mutation carriers with ovarian cancer can be criticized because of significant differences in the mean age at diagnosis between BRCA mutation carriers with ovarian cancer and women with sporadic ovarian cancers (54;78-81). BRCA-related ovarian cancers, and especially *BRCA1*-related cancers, have lower mean age at onset than sporadic ovarian cancers (79), and age is an important prognostic factor in advanced-stage ovarian cancer (83). In some of the studies the prognostic data are incomplete and there is lack of detailed information regarding the type of chemotherapy used (75;82;84). At last, there are possibilities of biases resulting from the length of time between the date of ovarian cancer diagnosis and positive BRCA mutation test. In some studies ovarian cancer cases were collected retrospectively, and the study populations were tested for mutations at inclusion (75;79). This approach could allow a selection of participants with a better prognosis if patients who have died from the disease before initiation of the study are not included in the analyses (85;86).

In summary, most studies tend to conclude that BRCA-related ovarian cancer has a better prognosis than sporadic ovarian cancer, but there is still some uncertainty regarding the role of age at diagnosis and tumour stage.

BRCA-related breast cancer

BRCA1 related breast cancers are more often of a higher grade and more often triple-negative (i.e. tumours that do not express estrogen and progesterone receptor genes or the tyrosine kinase HER-2/*neu*) than *BRCA2*-related and sporadic breast cancers (87). Triple-negative tumours are more often poorly differentiated and associated with poorer survival (88). Nonetheless, a recent large population-based study did not show differences in survival between BRCA-related and sporadic breast cancer (89). It is therefore possible that also BRCA-related breast cancers have a different biology and responsiveness to chemotherapy than sporadic cancers, leading to lower mortality rates than in sporadic breast cancers of the same stage and grade.

1.7 Ovarian cancer screening and prevention

1.7.1 Screening

Cancer screening aims at early detection of a malignancy common in a particular population. The rationale behind ovarian cancer screening studies is that screening could detect ovarian cancer at an earlier stage and that treatment at these stages would result in higher cure rates and improved survival (90). The new theory of low-grade (type I epithelial ovarian cancer) and high grade (type II epithelial ovarian cancer) pathways is interesting, as it may change the goal of screening tests. Present tests may detect low-grade type I tumours but miss the more aggressive type II tumours which account for most of the epithelial ovarian cancers (18;91). According to Kurman et al. (18), a more rational approach to early detection of epithelial ovarian cancer would be to focus on low volume rather than early stage of disease, because the type II tumours are unlikely to be identified in a preclinical state or, once invasive, confined to one organ of origin. Disease detection prior to clinical presentation could result in a selection of relatively healthier women affected by the disease, even if the disease is at an advanced stage. Detection of low volume disease can therefore presumably enhance the safety and completeness of

surgical resection, and optimize the tolerability and response to systemic or regional therapeutics, thus improving prognosis (91).

To the current date, no studies have been able to demonstrate a reduction in ovarian cancer mortality by ovarian cancer screening, neither in the general population nor in high-risk subpopulations (92-94).

Tumour markers

CA-125 is the most extensively studied tumour marker in ovarian cancer research. Studies on the use of CA-125 in ovarian cancer screening point out that isolated values of CA-125 lack adequate sensitivity or specificity to be an effective screening tool (95). The CA-125 is elevated in only 50% of cases of stage I ovarian cancer, and is therefore not suitable to detect early-stage cancers. Serial measurements of CA-125 or combinations between CA-125 and transvaginal ultrasound or other tumour markers may have a future role in ovarian cancer screening. Recently, measurements of human epididymis protein 4 (HE4) in addition to CA-125 have been documented to accurately triage patients with a pelvic mass into groups of high or low risk for malignancy (96).

Ultrasound

Previous studies have indicated that vaginal ultrasound screening in women from families with increased ovarian cancer risk may detect cancer at an early stage (97;98). Tailor et al. (98) presented a 10-year material containing 2.500 women who were self-referred to a familial ovarian cancer clinic. They concluded that transvaginal ultrasound could effectively detect ovarian cancer and borderline ovarian tumours related to the type of familial history. Van Nagell et al. (97) reported a 13-year material containing 14.000 women who were either in postmenopausal age at average risk for ovarian cancer or above 25 years of age and at increased risk for ovarian cancer. They found that transvaginal ultrasound screening, when performed annually, was associated with a lower cancer stage at detection and a decrease in case-specific ovarian cancer mortality. They also concluded that transvaginal ultrasound screening did not appear to be effective in detecting ovarian cancer in which ovarian volume was normal. In contrast to these studies, later studies in similar populations concluded that ovarian cancer screening was

ineffective (92;94). Woodward et al. (94) presented a material with 341 women divided into high risk, moderate risk and low risk of ovarian cancer. One ovarian cancer was detected at surveillance while three ovarian cancers were interval cancers. In the study group, 30 participants had surgery because of an abnormal scan, but only 2 of those had cancer. Oei et al. (92) reported from a prospective cohort study following 512 high-risk women who underwent annual transvaginal ultrasound and CA-125 measurements with a median follow-up of two years. They discovered one ovarian cancer (stage III) at surveillance and concluded that screening in women at high risk for ovarian cancer was inefficient considering the high number of surveillance visits and the advanced stage of ovarian cancer in the identified patient (92).

Currently, two large population-based ovarian cancer screening studies combining tumour markers and transvaginal ultrasound are conducted in the United Kingdom (UK) and in the United States. The published preliminary results from the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) trial are promising. According to the authors, the screening procedure had a high specificity (99.8% [95% CI, 99.7 to 99.9]) and an acceptable positive predictive value (19% [95% CI, 4.1 to 45.6]) for primary invasive ovarian cancer using the risk of ovarian cancer algorithm in postmenopausal women (90).

In general, screening is more likely to be effective in populations at high risk for the disease, indicating that screening could be more beneficial in an HBOC population than a population at average risk for ovarian cancer (99).

1.7.2 Preventive measures

Strategies for preventing breast and ovarian cancer include RRSO leading to estrogen deprivation, which in some cases may induce a premature (surgical) menopause. It is important to evaluate the acute and long term effects of inducing surgical menopause in young women at increased risk for breast and ovarian cancer

Among women with the predisposing BRCA mutations, risk of breast and ovarian cancer may be affected by environmental factors and modifying genes. Through cohort

studies of women who are BRCA mutation carriers, several risk factors have been identified.

Reproductive factors
Menarche
Early menarche, defined as at or before 11 years of age, was associated with increased risk of breast cancer in *BRCA1* carriers in a matched case-control study (100). The association was not found in *BRCA2* carriers.

Parity
McLauchlin et al. (101) conducted a case-control study where 799 BRCA mutation carriers with a history of invasive ovarian cancer were compared to 2,424 BRCA mutation carriers without ovarian cancer. Parity was associated with a reduced risk of ovarian cancer for carriers of *BRCA1* mutations (OR 0.67 [95% CI 0.46–0.96]; p=0.03), but with an increased risk of ovarian cancer for those with *BRCA2* mutations (OR 2.74 [95% CI 1.18–6.41]; p=0.02).

In the general population, pregnancy offers protection against breast cancer after the age of 40 years, but appears to increase the risk for very early-onset breast cancer (102). In a large matched case-control study, Cullinane et al. (103) reported that the risk of breast cancer in *BRCA1* mutation carriers did not decrease with pregnancy until four births were reached. Among *BRCA2* mutation carriers there was a statistically significant increase in breast cancer risk with each additional pregnancy. For *BRCA1* mutation carriers, parity is associated with a reduced risk of ovarian cancer, while the risk seems to increase by parity in *BRCA2* mutation carriers.

Contraceptives
Oral contraceptives reduced the risk of hereditary ovarian cancer by up to 50% in a large, multinational matched case-control study (104). However, in a population-based case-control study from Israel, oral contraceptives did not seem to have a protective effect (105). In the case-control study by McLaughlin et al. (101), use of oral contraceptives reduced the risk of ovarian cancer in carriers of *BRCA1* mutations (OR 0.56 [95% CI

0.45–0.71]; p<0.0001) and carriers of *BRCA2* mutations (OR 0.39 [95% CI 0.23–0.66]; p=0.0004). There was no association between tubal ligation and lower risk of ovarian cancer for BRCA mutations carriers (101). In a matched case-control study from Canada, the USA and the UK, tubal ligation was associated with a decreased risk of ovarian cancer in *BRCA1* mutation carriers, while no protective effect was seen among *BRCA2* carriers (106).

A large multinational study showed that in *BRCA1* mutation carriers, women who have started to use oral contraceptives before the age of 30, or who have used such medications for five or more years, may have an increased risk of early onset breast cancer compared to non-users. (107). In the same study, the use of oral contraceptives did not seem to increase the risk of breast cancer in *BRCA2* mutation carriers (107).

1.8 Risk-reducing salpingo-oophorectomy (RRSO)

The most effective way of reducing the risk of ovarian cancer is to surgically remove all tissue at risk. Women who have been identified as BRCA mutation carriers by testing, or are at risk for HBOC based on clinical criteria, may reduce the risk of ovarian cancer (and breast cancer if performed at an early age) by RRSO (108-113).

1.8.1 The RRSO procedure

When performing RRSO, it is important to be aware that the ovaries and fallopian tubes are both at increased risk of malignancies. Therefore, all tissue at risk should be removed, together with biopsies from peritoneum and cytology from peritoneal washings. The RRSO procedure is illustrated in Fig 4.

Fig 4. Division of the infundibulopelvic ligament (containing the ovarian vessels)
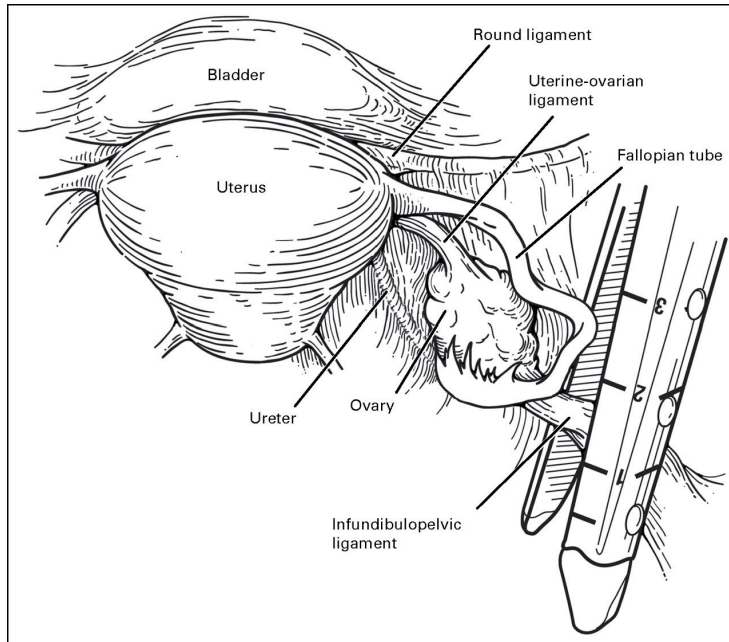
Kauff, N.D. et al. J Clin Oncol;25:2921-2927 2007

This procedure reduces the risk of leaving tissue remnants, because the approach is radical and removes the ovary with adequate margins. There are no documented reports of the development of ovarian cancer in an ovarian remnant in a BRCA mutation carrier, but as stated by Kauff et al. (114) in a review on RRSO, there are several reports in the literature of ovarian cancer occurring in an ovarian remnant after oophorectomy (115-117).

There is some controversy regarding the need to perform a hysterectomy at the same time as performing RRSO. The arguments for doing a concurrent hysterectomy are that the proximal intramural part of the fallopian tube will be removed, and that removal of the uterus will ease later administration of unopposed estrogen (i.e. without progesterone) or tamoxifen in case of breast cancer. If the uterus is removed, the patient can use estrogens without the addition of progesterone, because the risk of endometrial

cancer is eliminated. If the uterus is not removed, use of estrogens without progesterone will increase the risk of endometrial cancer. However, no studies or case-reports have reported on proximal tubal cancer in a BRCA mutation carrier after RRSO (114). Large studies on fallopian tube cancer underline that the majority of cancers arise in the distal parts of the fallopian tube (118;119). In 2007, Beiner et al. (120) published a case-control study demonstrating increased incidence of endometrial carcinoma among BRCA mutation carriers, and the increased incidence was linked to use of tamoxifen. There is still insufficient evidence to argue that easier administration of tamoxifen, if indicated, justifies a hysterectomy at the time of RRSO in women from HBOC families..

1.8.2 The impact of RRSO on subsequent BRCA-related cancer risk

The studies evaluating the impact of RRSO on subsequent BRCA-related breast or ovarian cancer risk, have estimated the reduction in ovarian cancer risk to be 79-96% (108;109;111-113;121) and the reduction in breast cancer risk to be 47-68% (108;111;112;121;122) (Table 2).

Previous studies have determined the risk reducing effect of RRSO in *BRCA1* and *BRCA2* mutation carriers together, which means that they did not distinguish between types of BRCA mutations. Recently, Kauff et al. (123) evaluated the risk of breast cancer after RRSO with separate analyses of *BRCA1* and *BRCA2* mutation carriers. They found less reduction of cancer risk in *BRCA1* related breast cancer than in *BRCA2* related breast cancer. This may be in accordance with the recent findings that *BRCA1* related breast cancers are more often triple-negative (60%) than *BRCA2* (23%) related breast cancers (87), as triple-negative breast cancer is more likely to develop early in life and has a poorer prognosis. It is likely that more studies with separate analyses of *BRCA1* and *BRCA2* associated cancers are to come.

Table 2. Studies evaluating the impact of RRSO on risk of gynaecologic cancer (including ovarian, tubal, and peritoneal cancer) and breast cancer in BRCA mutation carriers.

Study (ref no)	Year	Design	RRSO (N)	Gynaecologic Cancer HR 95% CI	Breast Cancer HR 95% CI
Kauff et al. (111)	2002	Prospective	98	0.15 (0.02, 1.31)	0.32 (0.08, 1.20)
Rebbeck et al. (112)	2002	Retrospective	259	0.04 (0.01, 0.16)	0.53 (0.33, 0.84)
Rutter et al. (113)	2003	Retrospective	251	0.29* (0.12, 0.73)	
Eisen et al. (122)	2005	Retrospective	1,439		0.46* (0.32, 0.65)
Domchek et al (108)	2006	Prospective	155	0.11 (0.03, 0.47)	0.36 (0.20, 0.67)
Finch et al. (109)	2006	Combined	1,045	0.20 (0.07, 0.58)	
Rebbeck et al. (121)	2009	Metaanalysis	2,871	0.21 (0.12, 0.39)	
Rebbeck et al. (121)	2009	Metaanalysis	5,503		0.49 (0.37, 0.65)

Abbreviations: RRSO, risk-reducing salpingo-oophorectomy; BRCA, breast cancer gene; Year, year of publication; RRSO (N), number of patients who underwent RRSO in each study; HR, hazard ratio; CI, confidence interval; ORs, odds ratios.

*values are ORs with 95% CIs

Adapted from Kauff, N.D. et al. J Clin Oncol; 25:2921-2927 2007

In *BRCA1* mutation carriers, the risk of ovarian cancer increases markedly from the age of 40 years (124) (Fig 5). Therefore, RRSO is recommended after ended childbearing or before the age of 40 in *BRCA1* carriers. Carriers of *BRCA2* mutations have a 2-3% risk of developing ovarian cancer by the age of 50 years (125;126), and therefore the RRSO procedure may be postponed to the age of 45 to 50 years. If the patient has undergone prophylactic mastectomy, the risk of breast cancer is markedly reduced, giving additional reason to postpone the RRSO procedure.

Fig 5. Cumulative risk of breast and ovarian cancer in BRCA1/2 mutation carriers.
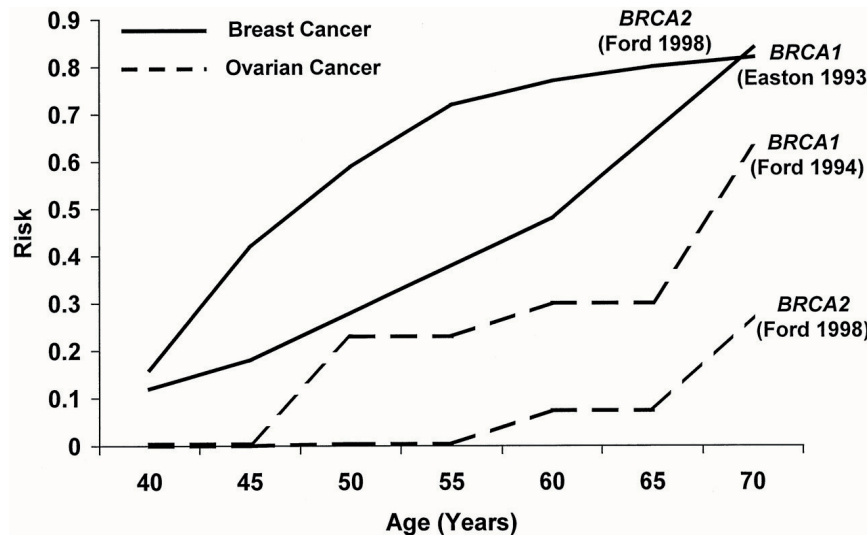
Rebbeck, T.R. J Clin Oncol;18:100s-103s 2000

Only 10% to 24% of *BRCA1*-associated breast cancers are estrogen-receptor (ER) positive, whereas 65% to 79% of *BRCA2*-associated breast cancers are positive for this receptor (127;128). This implies that there are possible biologic differences between tumours related to *BRCA1* and *BRCA2* mutations.

Eisen et al. (122) performed an international case-control study, and demonstrated a significant protective effect on breast cancer in *BRCA1* mutation carriers, while the effect on *BRCA2* related breast cancer was not significant. The results differ from the study by Kauff et al. (123). One explanation of the divergent results may be survival bias in the study by Eisen et al (122). Retrospective studies reporting risk-reduction after RRSO are subject to survival bias because the participants who are included may be healthier than the initial RRSO sample (129). There are also possibilities for confounding by indication, meaning that the women who chose RRSO had BRCA mutations with a higher penetrance than those who chose surveillance (129). Prospective studies may be influenced by detection biases, as cancers may be discovered more often in the group

opting for RRSO, for example as occult cancers at the time of RRSO (129). Such occult cancer will not be discovered in the control surveillance group. Kauff et al. (123) attempted to eliminate detection biases by excluding participants who had cancers discovered within the first six months after RRSO.

The study by Rebbeck et al. (121) is the most recent study estimating risk-reduction after RRSO. They performed a meta-analysis of studies reporting risk-reducing effect of RRSO on subsequent cancers and found that RRSO significantly reduced the risk of breast cancer in all BRCA mutation carriers analyzed as one group as well as in *BRCA1* and *BRCA2* mutation carriers analyzed separately. They also reported a significant risk-reduction of gynaecologic cancers in BRCA mutation carriers analyzed as one group (121).

Domcheck et al. (108) studied mortality after RRSO in a prospective cohort study. They compared BRCA mutation carriers who underwent RRSO to those who chose surveillance, and found that RRSO reduced both cancer-specific (breast and ovarian) and overall mortality compared to surveillance. In the secondary analyses, without matching for age at RRSO, the authors only found a reduction in overall mortality (108).

1.8.3 Risk of gynaecologic cancer in women who have undergone RRSO

Although RRSO has a considerable protective effect, there is a residual risk of gynaecologic cancer: ovarian cancer, fallopian tube cancer or peritoneal cancer after RRSO. The first retrospective studies estimated the residual risk of cancer to be as high as 10% (130-134). Later prospective studies have concluded that the risk is lower, probably because ovarian specimens after RRSO are studied more extensively to exclude occult cancer. Finch et al. (109) performed a multinational prospective study including 1,800 BRCA mutation carriers, and estimated a 4.2% cumulative incidence of peritoneal cancer in 20 years after the RRSO procedure.

1.8.4 Occult cancer at the time of RRSO

The risk of occult cancer in the ovaries or the fallopian tubes at the time of RRSO has to be considered. A study performed by Callahan et al. (119), demonstrated that seven out of 122 (6%) consecutive patients undergoing RRSO had an occult malignancy in the upper genital tract. All the occult cancers were fallopian tube malignancies. The authors concluded that the greatest proportion of serous cancer risk in BRCA mutation-positive women should be assigned to the fimbria rather than the ovary, and future clinical and research protocols should employ thorough examination of the fimbria, including multiple sections from each tissue block, to maximize detection of early malignancies in women going through RRSO. Detection of occult cancer at the time of RRSO may be important, as it would allow proper staging and proper treatment and follow-up, although the effect of such interventions is still under debate (119). Other studies have estimated the prevalence of occult genital cancer at the time of RRSO to be between 2 and 17% (111;112;131;132;135). Some of these materials are small, giving a possible explanation of the variable estimates. It is important that the pathologist who examines the surgical specimen is informed about the increased cancer risk, and that the entire specimen including the whole fallopian tube is sectioned and examined (Fig 6).

In summary, RRSO has well documented risk reducing effects on ovarian cancer and a probable effect on reducing risk of breast cancer. There are several large prospective studies documenting these effects, but the follow-up time in most of these studies is still short.

Fig 6. Recommended method for sectioning ovarian and fallopian tube specimens obtained at risk-reducing salpingo-oophorectomy in a *BRCA1* or *BRCA2* mutation carrier
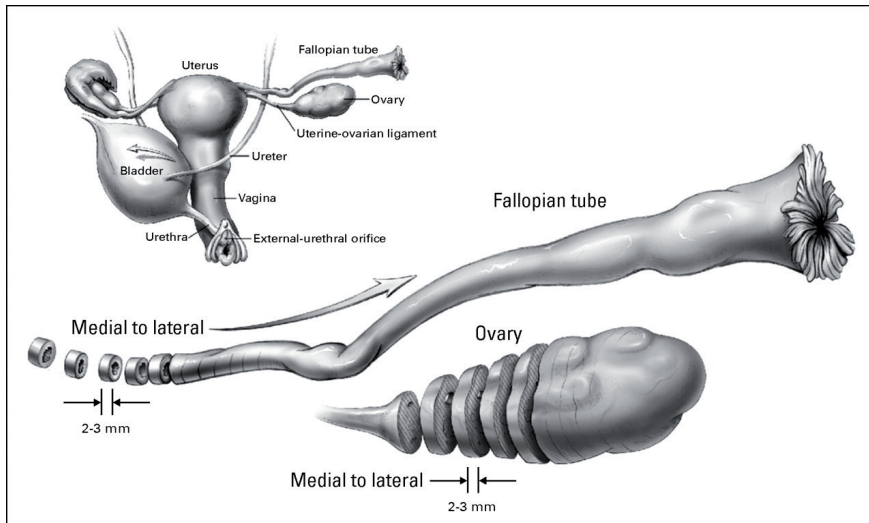
Kauff, N.D. et al. J Clin Oncol;25:2921-2927 2007

1.9 Somatic morbidity after RRSO

The mean age of natural menopause in Norway is 52.9 years (136). The mean levels of estradiol and especially testosterone seem to be lower in women with surgical versus natural menopause, since intact ovaries continue to produce steroid hormones also after the menopause (137-141). If a woman goes through RRSO before her natural menopause, immediate surgical menopause is induced.

1.9.1 Cardiovascular disease after RRSO

Studies in women who had a natural menopause show that the transition from pre- to post menopause is associated with several features of the metabolic syndrome, including 1) increased central (abdominal) body fat; 2) a shift towards a more atherogenic lipid profile, with increased low density lipoprotein (LDL) and triglyceride levels, and reduced high density lipoprotein (HDL); 3) increased glucose and insulin levels (142). Premature menopause is associated with increased cardiovascular mortality (143), and bilateral oophorectomy performed before natural menopause is associated with increased risk of CVD and increased cardiovascular mortality (144-146).

CVD is the main contributor to morbidity and mortality among women in the Western World (147;148). The mortality rates from CVD increase with age, and especially after the age of 50 years (Fig 7).

Figure 7. Deaths from cardiovascular disease among Norwegian women and men, 2006. Data from the Causes of Death Registry of Norway
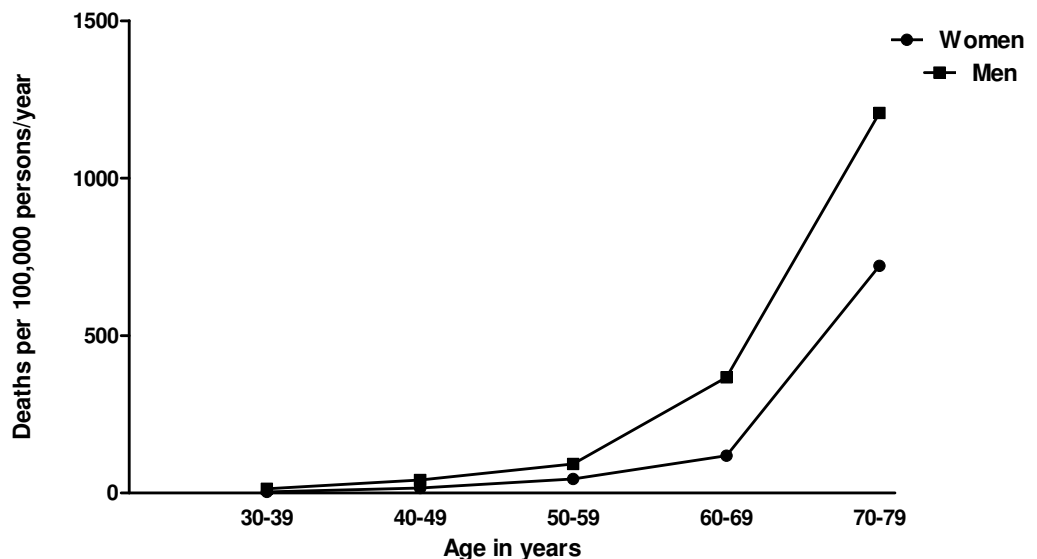
In Norway, CVD is the main cause of death among both men and women, although the mortality rates from CVD are decreasing (149). In 2005, the mortality rate among Norwegian women was 100/100,000 from CVD among which 50/100,000 died from coronary heart disease (CHD) (149).

Atsma et al. (145) summarized the results of 18 observational studies on the effect of menopause and menopausal age on CVD. Overall, the data pointed to an increased risk of CVD associated with postmenopausal status as opposed to premenopausal status and early menopause as opposed to those who reached menopause after the age of 50 years. The authors found a particularly higher risk of CVD among women who had undergone early bilateral oophorectomy compared to women who experienced natural menopause (145). Rocca et al. (150) prospectively followed a cohort of 2,390 American women who had undergone unilateral or bilateral oophorectomy for various reasons. They demonstrated an increased mortality risk in the subpopulation whose bilateral oophorectomy was performed before the age of 45 years compared to referent women without oophorectomy (150). Rivera et al. (144) published an increased cardiovascular mortality risk in women who had undergone bilateral oophorectomy before the age of 45 years compared to referent women with intact ovaries. It is unclear whether these increased risks were due to the bilateral oophorectomy or to an increased baseline risk. In a recent cohort study on Danish nurses, rates of ischemic heart disease were higher in women who had undergone bilateral oophorectomy before age 45 years compared to age after 45 years (146). The differences in rates of ischemic heart disease were non-significant after adjustment for use of estrogens (146).

Howard et al. (151) performed sub analyses of the Women's Health Initiative (WHI) material. The authors found that hysterectomy with or without oophorectomy was significantly associated with CVD, but that the association was non-significant after adjustments for CVD risk factors at baseline (smoking, hypertension, diabetes, high cholesterol, history of peripheral artery disease, and history of deep vein thrombosis).

The Nurses' Health Study prospectively followed 121,700 American women, and demonstrated an increased risk of CHD in the subgroup that had undergone bilateral

oophorectomy and reported no use of estrogens compared to referent women who had a natural menopause (152).

In summary, both premature and surgical menopause seems to be associated with increased risk of CHD and CVD compared to natural menopause. The menopausal transition is associated with metabolic disturbances, and women with metabolic syndrome are at increased risk for CVD. To our knowledge, no studies have examined Framingham risk score or metabolic syndrome in women from HBOC families who have undergone RRSO.

1.9.1.1 Metabolic syndrome

Metabolic syndrome is one of the most prevalent risk conditions for CVD and type 2 diabetes. Metabolic syndrome is a constellation of metabolic abnormalities including glucose intolerance, insulin resistance, central obesity, dyslipidemia and hypertension (153). Metabolic syndrome is associated with increased rates of CHD, CVD and all-cause mortality (154;155). In a recent study by Hildrum et al. (156) the association between metabolic syndrome and CVD was age-dependent in a Norwegian population-based sample. The authors found that the metabolic syndrome was associated with increased cardiovascular and total mortality in the middle-aged group (40-59 years of age), but not in older women.

Postmenopausal status is associated with a 60% increased risk of metabolic syndrome, after adjusting for age, body mass index (BMI), income and physical inactivity (157). Natural menopause affects body fat distribution and lipid metabolism leading to higher levels of total and LDL cholesterol, triglycerides and lipoprotein (a) (142), and these alterations may be related to the development of metabolic syndrome.

Several definitions of metabolic syndrome have been issued. The 2005 criteria of the International Diabetes Federation (IDF) (158) and the revised The National Cholesterol Education Program's Adult Treatment Panel III criteria (ATP) (159) are among the latest, both designed to facilitate clinical diagnosis of metabolic syndrome. The IDF definition differs from the ATP definition on the following features: 1) the cut-

off values for central obesity measured by waist circumference are lower, and the values are gender and ethnic-group specific and 2) central obesity is mandatory for the diagnosis of metabolic syndrome. The rationale for this requirement is that central obesity is highly correlated with insulin resistance and regarded as an important part of the metabolic syndrome (158) (Table 3).

The prevalence of metabolic syndrome according to IDF is expected to be higher than according to ATP, because the diagnostic criteria for central obesity has a markedly lower cut-off level. The prevalence of the components of the syndromes may vary between different geographic regions, as risk factors are influenced by differences in genetic background, diet, levels of physical activity, age and sex.

Table 3. Simplified criteria of metabolic syndrome according to the IDF and ATP definitions.

Diagnostic criteria	IDF definition	ATP definition
Central obesity *cm*	> 80	> 88
Triglycerides *mmol/l*	> 1.7	> 1.7
Blood pressure *mmHg*	> 130/85	>130/85
Glucose *mmol/l*	> 5.6	> 5.6
HDL-cholesterol *mmol/l*	< 1.29	< 1.29
Diagnostic criteria needed	Central obesity + 2/4	3/5

Abbreviations: IDF, International Diabetes Federation; ATP, The National Cholesterol Education Program's Adult Treatment Panel III; HDL, high density lipoprotein.

Details from Alberti et al. (158) and National Cholesterol Education Program Adult Treatment Panel III (159).

Previous relevant studies

Data is scarce regarding the association between surgical menopause and metabolic syndrome. Recently, an association between premenopausal oophorectomy and metabolic

syndrome was demonstrated by Dørum et al. (160) using data from the Nord-Trøndelag Health Study (HUNT-2). The authors found that patients with bilateral oophorectomy before 50 years of age had a higher prevalence of metabolic syndrome than age-matched controls without oophorectomy. This study included patients with both hysterectomy and/or oophorectomy and the indications for the procedures were benign uterine or ovarian diseases . To our knowledge, no published works have studied the prevalence of metabolic syndrome in women at risk for HBOC who have undergone RRSO.

1.9.1.2 Framingham risk score

The Framingham risk score estimates the ten-year risk of a CHD event (159). The Framingham risk score is based on age, level of total cholesterol, smoking status, level of HDL cholesterol and systolic blood pressure (159). A detailed description of the Framingham risk score is attached in Appendix 1. Point-based weights are assigned to the presence and/or level of each risk factor, and the points are summed. The total score can be converted to an estimated percentage risk of a CHD event occurring within the next ten years (161).

Some studies comparing metabolic syndrome with Framingham risk score have suggested that the latter may give a more valid prediction of CHD (162;163). However, in the Norwegian population-based study by Hildrum et al. (156), metabolic syndrome was associated with higher mortality risk than Framingham risk score.

Previous relevant studies
To the best of our knowledge, no studies have estimated Framingham risk score in a sample of women who have undergone RRSO because of increased risk of HBOC. Hsia et al. (164) calculated Framingham risk score in a subpopulation of the WHI study, and showed that women who had undergone hysterectomy with bilateral oophorectomy had increased risk of CHD whereas women with hysterectomy only had not. In multivariate analysis, hysterectomy with bilateral oophorectomy was an independent predictor of Framingham risk score (164). Hsia et al. (164) calculated Framingham risk score after

bilateral oophorectomy, but their sample had average ovarian cancer risk and the performed procedure was therefore not RRSO in a high-risk sample. Their results may therefore not be transferable to a sample that went through RRSO because of increased risk of HBOC.

1.9.2 Somatic complaints after RRSO

The symptoms associated with falling levels of estrogen include hot flashes, night sweats, insomnia, sexual dysfunction, depression and vaginal dryness (138;165;166). Compared to natural menopause which is a natural physiological process over time, the surgical menopause is abrupt and symptoms present immediately.

In addition to the loss of estrogen, the testosterone levels are lowered after oophorectomy. The Princeton consensus statement by Bachmann et al. (167) defines female androgen insufficiency (FAI). Among clinical symptoms of FAI are reduced self esteem, dysphoria, fatigue, reduced sexual functioning, and osteoporosis. FAI should only be considered in women with adequate levels of estrogen (167).

To our knowledge, no previous studies have examined osteoporosis, bowel function or musculoskeletal disease in a sample of women who have undergone RRSO. Increased incidence of osteoporosis is well documented after both early natural menopause and premature surgical menopause (defined as before the age of 51 years) (168). Some data suggest that the risk of osteoporosis is higher after surgical menopause than after natural menopause based on bone mass measurements (169;170). Estrogen deficiency is associated with loss of collagen (171), which also could increase the risk of other musculoskeletal diseases than osteoporosis.

Peri- and postmenopausal women have increased prevalence of bowel dysfunction and constipation compared to premenopausal women, although this finding may be explained by an age effect (172). The data regarding bowel function and relation to menopause seem to be scarce and difficult to interpret, as bowel function is affected by parity, sex and race in addition to age (173).

In summary, surgical menopause in general leads to increased risk of osteoporosis, but osteoporosis has not been studied in post-RRSO samples. Few previous studies have investigated the prevalence of somatic morbidity such as musculoskeletal disease and symptoms like palpitations and pain and stiffness after RRSO. More knowledge is needed as somatic morbidity may influence the well-being of women who have undergone RRSO.

1.10 Psychosocial Issues after RRSO

In this thesis, psychosocial issues comprise fatigue, quality of life (QoL) and mental distress.

Fatigue

Fatigue is a complex, subjective experience that has several definitions, and there are no objective measures or tests to define fatigue. Reports of fatigue are subjective and based on self-report of symptoms. Fatigue can be defined as: "a subjective inability to maintain effort of a markedly different quality and severity from ordinary fatigue, adversely impacting function and unrelieved by rest or sleep" (174). In some contexts, fatigue appears to be the most prevalent symptom associated with malignancy, as well as the most incapacitating symptom to the patients (175). Chemotherapy, radiation therapy, multimodal treatment, and biologic and hormonal therapies have all been found to increase the risk of fatigue (176). Fatigue is a common presenting symptom which negatively impacts work performance, family life, and social relationships. The differential diagnosis of fatigue includes lifestyle issues, physical conditions, depression, and treatment side effects. Fatigue can be classified as secondary to other medical conditions, physiologic, mental or chronic (177). In a normative study, 11.4% of Norwegian women from the general population reported chronic fatigue (CF) defined as substantial fatigue lasting for six months or more (178). Feeling tired or worn out, lack of physical strength and lack of stamina was commonly reported in a study of women going through the menopausal transition (179).

Fatigue is sometimes assessed by *multi-symptom scales* like the Short-Form 36 (SF-36) domain of Vitality (180) or fatigue symptoms of the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire C30 (EORTC-QLQ-C30) (181). Fatigue can also be assessed by *specific fatigue scales* like Fatigue Severity Scale (182) or Fatigue Questionnaire (FQ) (183). There is no consensus on which fatigue scale that is most valid for use in cancer patients, and a review identified 252 different ways to measure fatigue used in 2285 papers (184).

To the best of our knowledge, there are no published studies of fatigue in women who have undergone RRSO, and the earlier reports of associations between menopause and fatigue have not taken surgical menopause into consideration (179).

Quality of life (QoL)

QoL is also a subjective experience without objective measures. The World Health Organization has published a broad, but important QoL definition: "QoL is defined as an individual's perception of his position in life in the context of the culture and value system in which he lives and in relation to his goals, expectations, standards and concerns (185)." Health-related QoL is a narrower concept than QoL. Cella and Bonomi (186) defined the term as: "the extent to which one's usual or expected physical, emotional or social well-being are affected by a medical condition or its treatment." In medical studies, QoL is often used as a multidimensional concept into which both physical and mental dimensions are included. Instruments measuring QoL can be divided into generic and disease-specific ones. *Generic QoL instruments* like the SF-36 (180) can be applied to all somatic diseases and mental disorders, and the EORTC QLQ-C30 (181) can be applied to all types of malignancy. *Disease-specific QoL instruments* focus on side effects and late effects as well as specific symptoms of relevance for defined types of cancer. For ovarian cancer, for example, the following disease-specific QOL-instruments have been developed: the Functional Assessment of Cancer Therapy (FACT-O) (187) and the EORTC Ovarian Cancer 28 module (188). There are no specific QoL instruments for RRSO.

A paradox of QoL is that patients that obviously are in a bad somatic or mental state, still can report high levels of QoL. This may be related to personality features like coping styles/defence mechanisms or personality traits like optimism (189).

Mental morbidity

Mental morbidity covers both levels of mental symptoms (such as anxiety or depression) and occurrence of mental disorders (like social anxiety disorder). Mental or psychological distress are frequently used terms in the cancer literature, and such terms usually cover discomforting, emotional states experienced by an individual in response to the cancer illness and/or its treatment. In this thesis, mental distress covers self-reported levels of anxiety and depression. The Hospital Anxiety and Depression Scale (HADS) was designed to identify cases in need of further attention due to anxiety or depression among patients in somatic hospital clinics (190). The HADS has been used extensively in medical research and particularly in oncologic research (191).

Previous studies

During the last few years, eleven studies on psychosocial issues in women after RRSO have been published. Seven of the studies are quantitative (Table 4).

Table 4. Overview of quantitative studies reporting on psychosocial functioning after RRSO

Author (ref no)	Design	N	Controls (N)	QoL	Mental distress	Fatigue
Fry et al. (192)	Retrospective	29	Surveillance (28)	↓	↓	-
Elit et al. (193)	Cross-sectional	74	None	→	-	-
Tiller et al. (194)	Prospective	22	Surveillance (46)		↓*	-
Robson et al. (195)	Prospective	59	None	→	→*	-
Fang et al. (196)	Prospective		Surveillance	↓→	↓→	
Madalinska et al. (197)	Cross-sectional	369	Surveillance (477)	→	↓*	-
Van Oostrom et al. (198)	Prospective	14	Surveillance (37)		↓→	-

Abbreviations: RRSO, Risk-reducing salpingo-oophorectomy; N, number of women undergoing RRSO in each study; Controls (N), Types of control sample and number of controls in each study; QoL, Quality of life; Surveillance, Controls who opted for surveillance in stead of RRSO.

*Cancer related worries

→   Levels comparable to controls/the general population

↓   Lower levels than controls

Qualitative studies

Qualitative studies are suitable for getting information about the participants' experiences, thoughts and opinions (199). Qualitative studies may generate hypotheses, and may be used as pilot studies prior to quantitative studies. One can not generalize statistically from qualitative studies as the samples are small and the selection of cases are often biased (199).

Hallowell et al. (200) performed qualitative interviews of 23 English women who had undergone RRSO. The women complained about lacking information about menopausal symptoms and risks and benefits of hormonal replacement therapy (HRT). All the women were satisfied with the choice of RRSO (200). Hallowell et al. (201) also published a descriptive report concluding that the 23 English women all were positive to the choice of RRSO. Their conclusion was that there was a need to inform women about physical and emotional sequelae of RRSO prior to surgery (201). In 2000, Meiser et al.

(202) reported findings comparable to the studies by Hallowell in 14 Australian women with a mean follow-up of 20 months after RRSO. The women in that study were more satisfied with the preoperative information than the sample studied by Hallowell et al (200;201). Swisher et al. (203) conducted in-depth telephone interviews with 30 women who had undergone RRSO and 30 women with ovarian cancer surveillance. They concluded that few women undergoing RRSO had regrets about their decision, but half of the RRSO group would have liked more information prior to surgery. In the surveillance group, dissatisfaction with the choice of treatment was more frequent (203).

In summary, qualitative studies examining the experiences of women who have undergone RRSO conclude that the women are satisfied with their choice and that few have regrets. The women would have liked more information about the physical and emotional effects of the surgery before the surgery took place. These studies do not give information about levels of QoL, fatigue or mental distress.

Quantitative studies

Quantitative studies provide results that can be treated with descriptive or analytic statistics. The results from quantitative studies may therefore be easier to compare than qualitative studies, given that the same type of measurements are performed. Compared to qualitative studies, quantitative studies are less likely to present explanations, understandings or subjective opinions of the sample studied (204).

Fry et al. (192) compared 29 women who had undergone RRSO to 28 women who participated in a surveillance program. Both groups were at increased risk for ovarian cancer. The RRSO group reported lower levels of role-emotional and social functioning than the surveillance group, but the levels of general health were higher in the RRSO group. No significant group differences were observed concerning cancer-related worries or sexual interest and functioning (192). Elit et al. (193) contacted 74 women who had undergone RRSO because of family history of ovarian cancer, and 40 (54%) of the women participated in the study. The study had no control group. The women had the same levels of QoL as women in the general population. The Menopause-Specific Quality of Life scores were reduced compared to women of similar age on all parameters: vasomotor symptoms, psychosocial support, physical status and sexual

quality of life. Satisfaction with sexual functioning was moderately to extremely compromised in 42%-53% of the women (193). Tiller et al. (194) published a prospective study on the psychological impact of the RRSO procedure on a sample of Australian women with a family history of breast/ovarian cancer. The women were examined when they came for counselling about increased risk of HBOC and had a new examination three years later (N=68). Among the 68 women only 22 had undergone RRSO, and 12 of those had RRSO before baseline. The remaining 46 women followed a surveillance program. The authors found a greater reduction in cancer-related anxiety from baseline to follow-up in the RRSO group compared to the group who did not undergo RRSO. Most of the women were satisfied with their choice of procedure (194). In 2003, Robson et al. (195) reported a retrospective study where 59 of 101 American women who had undergone RRSO because of increased risk of ovarian cancer participated. There was no control group, and mean time since RRSO was 2 years. Vaginal dryness was reported by 35% and dyspareunia by 28% of the sample. The level of overall QoL measured by the SF-36 was comparable to the general female American population, while 21% continued to report ovarian cancer-specific worries despite surgery (195). Fang et al. (196) compared women at risk for ovarian cancer who underwent RRSO to women who chose surveillance, and found that women who underwent RRSO compared to surveillance reported poorer physical functioning, more physical role limitations and greater bodily pain, lower levels of vitality and social functioning, and greater discomfort and less satisfaction with sexual activities at 1-month assessment compared to baseline. In contrast, women undergoing surveillance experienced no significant reduction in levels of QoL or sexual functioning at 1-month assessment. Most reductions of the QoL scores observed in the surgical group were no longer significant by 6-month assessment (196).

The reviewed studies presented above can be criticized on sample selection and design. In short, the studies demonstrate few problems with QoL and a high degree of satisfaction after the RRSO procedure. Women who have undergone RRSO before their natural menopause report difficulties when it comes to sexuality and menopausal symptoms. As shown in table 3, most studies have small sample sizes and therefore may be subject to type II error, which is keeping the null hypothesis that there are no

differences between the groups when the null hypothesis is true. Low statistical power increases the risk of type II error.

The studies by Madalinska et al. (197;205) had larger sample sizes and the study by van Oostrom et al. (198) had better design than the studies presented so far. In 2005 Madalinska et al. (197) published a comparison of psychosocial issues in Dutch women at high risk for ovarian cancer. The participants had undergone either RRSO (N=369) or they participated in a surveillance program (N=477). Both groups had high levels of various QoL dimensions without significant differences, and the QoL mean scores in both groups were similar to those of the general population of the Netherlands. RRSO was associated with fewer breast and ovarian cancer worries but significantly more menopausal symptoms and poorer sexual functioning compared to the surveillance group. In the RRSO group, 86% would choose to undergo RRSO if they were to choose again (197). In 2007, Madalinska et al. (205) examined predictors of RRSO in 160 BRCA mutation carriers who had delivered baseline questionnaire data in a nationwide, longitudinal observational study of psychosocial consequences of RRSO versus periodic surveillance. They found that women with lower educational levels, poorer general health perception, who considered ovarian cancer an incurable disease and who believed more strongly in the benefits of surgery, were more likely to undergo RRSO than to choose surveillance (205).

The prospective study by van Oostrom et al. (198) followed 65 BRCA mutation carriers for five years. They found that the BRCA mutation testing itself was not associated with major mental health risks, and that RRSO was associated with lower levels of anxiety in the short term. However, at five years follow-up, there was a tendency towards increasing levels of anxiety, approaching the levels measured before surgery (198).

The comparisons in the previous studies examining psychosocial issues are mainly done between patients undergoing RRSO and patients with HBOC who chose ovarian cancer surveillance. None of the studies have compared the RRSO groups to age-matched normative controls from the general population, and five out of 11 studies do not have control samples. No studies have examined levels of fatigue after RRSO.

1.11 Hormonal Replacement Therapy After RRSO

Based on the risk of CVD and severe postmenopausal symptoms, many women going
through RRSO before natural menopause are advised to use HRT. The use of estrogen
has well-documented effects as to the relief of hot flashes and vaginal dryness in the
perimenopause (206). Concerning HRT after RRSO, Madalinska et al. (207) published
questionnaire-based data on endocrine symptoms and sexual functioning from 450
premenopausal, high-risk women who had participated in their nationwide, cross-
sectional, observational study. Among the 450 women, 162 (36%) had undergone RRSO
and 288 (64%) had opted for surveillance. In the RRSO group, 47% of the women were
current HRT users, and they reported significantly fewer vasomotor symptoms than
nonusers (p<0.05). However, compared with premenopausal women undergoing
surveillance, women who had undergone an oophorectomy and were HRT users were
more likely to report vasomotor symptoms (p < 0.01). Compared to the surveillance
group, women who had undergone an oophorectomy and were HRT users reported
significantly more sexual discomfort due to vaginal dryness and dyspareunia (p<0.01).
HRT users and nonusers after RRSO reported comparable levels of sexual functioning.
The authors therefore concluded that HRT may be less effective in women who have
undergone RRSO than previously assumed. Endocrine symptom levels remained well
above those of premenopausal women undergoing screening, and sexual discomfort was
not alleviated by HRT (207).

1.11.1 HRT and CVD

Some data suggest that estradiol has a cardio-protective effect before menopause, and
that reduction of the estradiol level increases the risk of CVD (208). The indications for
HRT in the general population have changed after the publication of the results from the
Heart and Estrogen/progestin Replacement Study (HERS) in 1998 (209) and the WHI
study in 2002 (210). Previous observational studies suggested that postmenopausal
hormone therapy was associated with a 40 to 50 percent reduction in the risk of CHD

(211;212). The HERS results (209) were published in 1998. The study aimed to evaluate the effect of HRT as secondary prevention against CHD in 2,763 postmenopausal women in a blinded, placebo-controlled design. During an average follow-up of 4.1 years, treatment with oral conjugated equine estrogen plus medroxyprogesterone acetate did not reduce the overall rate of coronary heart disease events in the treatment group compared to the placebo group. On the contrary, there was a pattern of early increase of CHD events (209). The WHI study results were published in 2002 (210). The randomized controlled primary prevention trial contained 16,608 postmenopausal women aged 50-79 years without previous CHD and an intact uterus at baseline. Participants received conjugated equine estrogens, 0.625 mg/d, plus medroxyprogesterone acetate, 2.5 mg/d, in 1 tablet (n = 8506) or placebo (n = 8102). Planned duration of the study was 8.5 years, but the study was stopped after a mean follow-up of 5.2 years because the breast cancer incidence in the treatment group exceeded the stopping boundaries and because the global index indicated that the risks exceeded the benefits. There were significantly more CHD, breast cancer, pulmonary embolism and stroke in the treatment group. At the same time, there were less hip fractures and colorectal cancer (210). A systematic review including earlier observational studies and the HERS and WHI studies support these findings, and concludes that benefits of HRT include prevention of osteoporosis-related fractures and colorectal cancer, while prevention of dementia is uncertain. Harms include CHD, stroke, thromboembolic events, breast cancer with five or more years of use, and cholecystitis (213).

Extrapolating the HERS and WHI results to women who have undergone RRSO represents difficulties. The HERS study concerns secondary prevention, and few women will have established CHD at the time of RRSO. The participants in the WHI study were included and treatment initiated at age between 50 and 79 years, and mean age was 63.3 years (210). Most RRSO procedures are performed before natural menopause, and other researchers have questioned whether the WHI data are relevant to HRT started in the perimenopause (214;215). In addition to this, there are data suggesting that HRT may have a protective effect against CHD in the period from premenopausal oophorectomy to the mean age of natural menopause (208). The HERS and WHI studies are large well-designed studies, but the sample selection may be questioned, especially because the

studies introduce HRT to a group of postmenopausal women that rarely use estrogen treatment in Norway. The estrogen used in the HERS and WHI studies, oral conjugated equine estrogen, is hardly in use in Europe.

1.11.2 HRT and breast cancer

A substantial proportion of the women undergoing RRSO because of HBOC already have a history of breast cancer. The results of the Hormonal Replacement Therapy After Breast Cancer – Is It Safe? (HABITS) trial indicated that HRT is contraindicated in women with a history of breast cancer (216). The HABITS trial was an open randomised clinical trial where women with a history of breast cancer were allocated to either HRT or best treatment without hormones. The main endpoint was any new breast cancer event. Until September 2003, 434 women were randomised and 345 had at least one follow-up report. After a median follow-up of 2.1 years, 26 women in the HRT group and seven in the non-HRT group had a new breast-cancer event. All women with an event in the HRT group and two of those in the non-HRT group were exposed to HRT and most women had their event while on treatment. The study group decided that these findings indicated an unacceptable risk for women exposed to HRT in the HABITS trial, and the trial was terminated in the end of 2003 (216).

1.11.3 HRT and hereditary breast cancer

BRCA mutation carriers without breast cancer may be reluctant to use HRT because studies have demonstrated increased risk of breast cancer for current users of estrogen (210;217;218). Rebbeck et al. (219) showed in a multi-centre study of BRCA mutation carriers with 3.6 years of prospective postoperative follow-up (N=426) that HRT of any type after RRSO did not significantly alter the reduction in breast cancer risk associated with RRSO. They thereby concluded that short-term HRT use was safe in BRCA carriers

after RRSO regarding the development of breast cancer (219). Mean age of RRSO participants in the study by Rebbeck et al. (219) was 42.7 years. Furthermore, Eisen et al. (220) demonstrated in a matched case-control analysis of 472 postmenopausal *BRCA1* mutation carriers that the use of HRT was not associated with an increased risk of breast cancer. In fact, being a HRT user versus being a HRT non-user was associated with a lower risk of breast cancer in their population (220). In Norway, women who have undergone RRSO are recommended to use HRT until the mean age of natural menopause.

1.11.4 HRT and ovarian cancer

Linkage between breast and ovarian cancer has lead to theories about hormonal involvement in the development of ovarian cancer. Two review articles from 2004 (221) and 2005 (222) both concluded that HRT given to women after bilateral oophorectomy for ovarian cancer did not increase the risk of recurrence of ovarian cancer.

The WHI writing group published data on the occurrence of gynaecological cancer after use of HRT in 2003, and demonstrated a non-significant tendency towards increased risk of ovarian cancer in the HRT group (223). All participating women in this randomized, double-blind, placebo-controlled trial (N=16,608) had an intact uterus at baseline and were postmenopausal (age 50-79 years). Mean follow-up was 5.6 years.

A meta-analysis of nine studies (1998) regarding use of HRT and occurrence of ovarian cancer, concluded that having ever used HRT was associated with an increased risk of developing invasive epithelial ovarian carcinoma (odds ratio [OR] 1.15; 95% confidence interval [CI] 1.05, 1.27) (224). Rodriguez et al. (225) (2001) reported from a large prospective study in the United States of America (USA) including more than 200,000 participants with 14 years of follow-up. They found that postmenopausal estrogen use for 10 or more years was associated with increased risk of ovarian cancer mortality that persisted up to 29 years after cessation of use (225). Such long-term, postmenopausal use of HRT is rare in Norway and not recommended for women who have undergone RRSO. Furthermore, Beral et al. (226) in the Million Women Study

(2007) concluded that the use of HRT in postmenopausal women was significantly associated with ovarian cancer by comparing almost half a million HRT users to half a million HRT non-users. None of the participants had a previous history of cancer or bilateral oophorectomy. The authors demonstrated that current HRT users were significantly more likely to develop and die from ovarian cancer than never users (relative risk 1.2 [95% CI 1.09-1.32]) (226;227).

The studies referred are conducted in other countries than Norway, and may not be transferable to Norwegian conditions. Studies from the United States, like the WHI study (210), often use equine estrogens. Such preparations are not in use in Norway. It is possible that the use of HRT in women who have undergone RRSO decreases risk of other diseases that may impact survival (colon cancer, osteoporosis, CHD and CVD).

1.11.5 HRT and hereditary ovarian cancer

There is only one study examining the risk of ovarian cancer in BRCA mutation carriers who use HRT. Kotsopoulos et al. (228) conducted a matched case-control study on 162 matched sets of women who carried a BRCA1 or BRCA2 mutation. Compared with those who had never used HRT, the odds ratio for ovarian cancer associated with ever use of HRT was 0.93 (95% CI = 0.56-1.56). There was no significant relationship with increasing duration of HRT use, and the authors concluded that HRT use did not appear to adversely influence the risk of ovarian cancer in BRCA mutation carriers (228).

In summary, the literature indicates that short-term use of HRT after RRSO in BRCA mutation carriers does not seem to negate the effect of the prophylactic surgery with regards to breast cancer risk. The only study performed on effects of HRT on ovarian cancer in BRCA mutation carriers showed no association.

1.12 Summary

Given the increased uptake of RRSO in women from HBOC families over the last decade, it is important to broaden the knowledge on long term morbidity in women who have undergone this procedure. RRSO provides significantly lower risk of ovarian cancer in women at high risk for HBOC, but RRSO may have a major impact on the physiology of premenopausal women. It is therefore important to study late effects. Few studies have addressed non-oncologic morbidity in post-RRSO women, and fewer yet have compared the findings to matched controls from the general populations. In this thesis, we sought to improve our understanding on both somatic and mental health of women after RRSO. No previous studies have investigated prevalence of metabolic syndrome, Framingham risk score or fatigue in women who have undergone RRSO, and no studies have examined mental morbidity, somatic complaints or QoL in Norwegian post-RRSO samples.

2. THIS THESIS

2.1 Aims of the thesis

This thesis is a clinical observational study with the aim to investigate the long-term non-oncologic morbidity in HBOC women after RRSO and also to compare their morbidity to controls from the general population. The four papers of this thesis examine the prevalence of metabolic syndrome, Framingham risk score and the prevalence of somatic complaints in women after RRSO as well as levels of fatigue, mental distress and quality of life and compare the findings to those of controls with intact ovaries from the general female population. In the following, we present the background, aims and hypotheses of the four papers included in this thesis:

Metabolic syndrome after risk-reducing salpingo-oophorectomy in women at high risk for hereditary breast ovarian cancer. A controlled observational study. (Paper I)

Background: No previous studies have investigated the prevalence of metabolic syndrome after RRSO in women from HBOC families. In general, surgical premature menopause seems to be associated with increased risk of CVD. Menopause is associated with metabolic syndrome, and metabolic syndrome increases the risk of CVD.

Aim: 1) to determine the prevalence of metabolic syndrome in women after RRSO and to compare the findings to controls from the general population. 2) To study associations between RRSO and metabolic syndrome and to examine variables associated with metabolic syndrome.

Hypotheses: 1) due to surgical menopause, women after RRSO have increased prevalence of metabolic syndrome. 2) RRSO is significantly associated with metabolic syndrome.

Framingham risk score after risk-reducing salpingo-oophorectomy in women belonging to hereditary breast ovarian cancer families. A controlled observational study. (Paper II)

Background: In general, surgical premature menopause seems to be associated with increased risk of CHD. No previous studies have examined the prevalence of CHD after RRSO in women from HBOC families, or examined future risk of CHD by using the Framingham risk score. The Framingham risk score is a risk assessment tool which indicates 10-year risk of a CHD event.

Aims: 1) to determine future risk of CHD in women after RRSO by using Framingham risk score and to compare the findings to age-matched controls from the general population. 2) To examine factors associated with Framingham risk score in the total sample.

Hypotheses: 1) women after RRSO would have increased CVD risk and therefore increased Framingham risk score compared to controls. 2) Having undergone RRSO would be associated with increasing Framingham risk score.

Fatigue and quality of life after risk-reducing salpingo-oophorectomy in women at increased risk of hereditary breast-ovarian cancer (Paper III)

Background: Belonging to a HBOC family, going through genetic counselling and testing, and going through RRSO might lead to lower levels of QoL and higher levels of fatigue. Previous studies of QoL after RRSO either had small sample sizes or lacked normative controls. At the time of the study, no studies had examined fatigue in post RRSO samples.

Aims: 1) To measure the levels of QoL and fatigue in women after RRSO and to compare the findings to age-matched normative controls from the general population. 2) To examine levels of QoL and fatigue in RRSO subgroups based on age at surgery and

presence of cancer. 3) To examine variables significantly associated with QoL and fatigue.

Hypotheses: 1) women after RRSO have lower levels of QoL and higher levels of fatigue compared to age-matched controls. 2) Women after RRSO who have a history of cancer have lower levels of QoL and higher levels of fatigue. Women with RRSO at premenopausal ages have lower levels of QoL and more fatigue. 3) RRSO is associated with lower levels of QoL and higher levels of fatigue.

A controlled study of mental distress and somatic complaints after risk-reducing salpingo-oophorectomy in women at risk for hereditary breast ovarian cancer (Paper IV)

Background: Due to estrogen deprivation after RRSO, the procedure with genetic counselling, BRCA mutation testing and confirmation of increased cancer risk, we expected women who had undergone RRSO to have increased levels of mental distress and more somatic complaints than women from the general population. Previous studies have shown that the level of mental distress is reduced after the RRSO procedure compared to controls who chose surveillance, but these studies have short follow-up time. The few long-term follow-up studies suggest that the levels of mental distress return to pre surgery levels. In general, surgical menopause leads to increased risk of osteoporosis, but osteoporosis has not been studied in post-RRSO samples. Little is known about the prevalence of musculoskeletal morbidity and impaired bowel function after RRSO.

Aims: 1) to determine levels of mental distress and prevalence of somatic complaints in women after RRSO and to compare the findings to age-matched controls from the general population. 2) To examine variables significantly associated with anxiety, depression and total mental distress in the RRSO group.

Hypotheses: 1) Women after RRSO have higher levels of mental distress and more somatic complaints than controls from the general population. 2) Having undergone RRSO is associated with higher levels of anxiety, depression and total mental distress.

2.2 Materials and methods

2.2.1 Design

This thesis consists of four clinical observational studies with cross-sectional design
(229). All studies used within-group comparisons of women who underwent RRSO, and
between group comparisons with two different control materials.

2.2.2 Patient selection

Through surgical records, we identified women who had undergone RRSO because of
increased risk of HBOC at The Norwegian Radium Hospital, Ullevål University Hospital
and Stavanger University Hospital after genetic counselling at The Norwegian Radium
Hospital. HBOC was defined according to European recommendations suggested by
eleven clinical genetic centres as described by Dørum et al. (230). The criteria for HBOC
families was either a female with ovarian cancer who: 1) had a first-degree relative, or a
second-degree relative through a male, with ovarian or breast cancer; and/or 2) had both
ovarian and breast cancer (breast cancer <= 60 years).

      The intention was to identify a sample of women who all had undergone surgery
after genetic counselling at the Norwegian Radium Hospital in the period from 1994 to
2006. The search was performed in the hospital archives based on diagnoses (ICD-10 Z
80.4: Family history of malignant neoplasm of genital organs) and procedures (NCSP –
the NOMESCO Classification of Surgical Procedures LAE20, Bilateral Oophorectomy;
LAE21, Bilateral Laparoscopic Oophorectomy; LAF10, Bilateral Salpingo-
oophorectomy; LAF11, Bilateral Laparoscopic Salpingo-Oophorectomy. For RRSO
performed before 1999, we performed manual searches in surgical protocols. Due to
administrative reasons we were not given access to the genetic counselling data at the
Department of Genetics at the Norwegian Radium Hospital.

      We found it correct to also include patients who had undergone bilateral
oophorectomy only, which is prophylactic surgery without removing the fallopian tubes,

as this procedure also was a risk-reducing procedure in the 1990's (231). Based on the present knowledge, remaining fallopian tubes increase the risk of gynaecological cancer after bilateral oophorectomy in women at risk for HBOC (119), and bilateral oophorectomy only is not considered to be sufficient risk-reducing surgery (114).

2.2.3 Organization and data collection

The material is a sample consisting of 503 women, where all have had RRSO because of HBOC risk or identified BRCA mutations. The women were sent an invitation and study information by ordinary mail. In the same delivery they received a written consent form, questionnaires concerning demographic data, anxiety, depression, fatigue, quality of life, body image and a referral for blood sampling to be presented to their regular general practitioner (GP). The non-respondents received one reminder after three weeks. Due to logistic reasons, the invitations were sent out in two waves; the first in 2006 and the second in 2007. In the first wave, 415 invitations were sent out, and 301 (73%) responded. In the second wave, 60/88 (68%) responded. Altogether, 361 (72%) of the 503 women responded and delivered written informed consent.

The papers had different data sources. In paper I, we used demographic data and blood samples to determine the prevalence of metabolic syndrome, and compared the data to 679 non-age matched controls from HUNT-2. In paper II, we used demographic data and blood samples to determine Framingham risk score, compared to 1,630 age-matched controls from HUNT-2. Among the respondents, 326/361 (90%) delivered all necessary data for paper I and II. Paper III was written after the first wave of sampling, and therefore comprised a lower sample size of women with RRSO than the other papers (N=301). Demographic data, and data on QoL and fatigue were used in paper, and the data were compared to controls from NORM. In paper IV we used demographic data, data on anxiety and depression, and self-reported medical data. Out of the 361 respondents, 338 (94%) delivered all relevant data, and the cases were compared to 1,690 age-matched controls from HUNT-2.

2.2.4 Control samples

The HUNT Sample

The second HUNT study (HUNT-2) was carried out from 1995-97 in Nord-Trøndelag County, which is a both rural and urban part of Norway. The study is considered representative for the Norwegian population and is described in detail elsewhere (232). Between 1995 and 1997 all inhabitants aged ≥20 years were invited to a health examination. The invitation letter included a questionnaire and date and time for an appointment with physical measurements and blood tests. At that appointment, the participants received a second questionnaire (Form 2), that should be filled in at home and returned in a prepaid envelope, which lead to a 10% lower response rate to Form 2. The questionnaires covered demographic characteristics, somatic diseases, somatic and mental symptoms, physical impairments, and use of medications, lifestyle, and health-related behaviour. The self-reported data were not confirmed by medical records.

We used data from the HUNT-2 study in the papers I, II and IV. In paper I, we used 679 controls, which represented all eligible participants with blood samples drawn in the fasting state (defined as fasting nine hours or more before the HUNT-2 appointment). In paper II and IV, we were able to randomly allocate five age-matched controls per case, and therefore the number of controls was 1,630 in paper II and 1,690 in paper IV.

The NORM sample

In order to obtain normative population samples, the Cancer Clinic at The Norwegian Radium Hospital performed two similar studies one year a part (233). Using public address lists, an anonymous age-representative sample of the Norwegian female population (3,500 females in 2004 and 2005) aged 20 to 79 years received a questionnaire containing the QoL questionnaire EORTC QLQ-C30 in 2004 and the fatigue measure Fatigue Questionnaire (FQ) in 2005 as well as questions about demography and use of medication. Among the women invited in 2004, 41% responded, and 988 were 30-71 years old, cancer-free and with completed EORTC QLQ-C30

(NORM-2004). In the 2005 survey 38% responded, and of them 957 delivered completed FQ (NORM-2005). We used controls from the NORM 2004 and NORM 2005 samples in paper III. Because of the low participation rate, we were only able to age-match two controls per case from NORM 2004 and one control per case from NORM 2005. The control group therefore consisted of 602 and 301 controls, respectively. These controls were used in paper III.

2.2.5 Questionnaire data

The questionnaires were returned to Service Office of Clinical Research, The Norwegian Radium Hospital, where the data were scanned and converted into SPSS data files. The following questionnaires were used:

The Follow-up questionnaire of the Norwegian Radium Hospital has been used during the last year in follow-up studies of different groups of cancer patients. In this study, a modified version with 100 questions for women who had undergone RRSO was used. The questionnaire collected information on marital status, level of education, working status, smoking, sleep disturbances, medical history, and use of medications, physical activity and family history of disease.

The Hospital Anxiety and Depression Scale (HADS) consists of the depression (HADS-D) and the anxiety (HADS-A) subscale each with 7 items that are rated from 0 (not present) to 3 (maximally present) (191). The subscale scores may be added to calculate HADS-total (HADS-T) (234). The HADS-A and the HADS-D scores both range from 0 to 21, and the HADS-T scores range from 0 to 42. Higher scores represent higher symptom loads. The psychometric properties of the HADS have been well documented in HUNT-2 (235). We studied HADS-T as cancer researchers have claimed that the HADS is a unidimensional scale, and that the HADS-T forms a higher-order single factor structure (234;236). In our samples the internal consistency of the HADS-A was $\alpha=0.84$, the HADS-D $\alpha=0.78$ and the HADS-T $\alpha=0.81$ in the RRSO group, and $\alpha=0.84$ of the HADS-A, $\alpha=0.79$ of the HADS-D and $\alpha=0.83$ of the HADS-T in the control group in paper IV.

The European Organization for Research and Treatment of Cancer (EORTC-C30) (181) comprises 30 questions and is an international standard instrument for assessing QoL in cancer patients. The schedule contains five functional dimensions; an overall QoL dimension, three symptom scales, and six single symptom items (181). The scores are transformed onto a 0–100 scale; and on these scales increasing scores represent better QoL. On the symptom items higher scores mean more symptoms. The instrument has been validated in Norwegian patient samples, and the psychometric properties are considered to be good (237;238). The internal consistencies were α=0.93 for role functioning, α=0.82 for emotional functioning, α=0.60 for cognitive functioning, α=0.83 for social functioning, α=0.91 for physical functioning and α=0.91 for overall QoL in our RRSO sample. In the NORM sample the internal consistencies were α=0.92 for role functioning, α=87 for emotional functioning, α=0.62 for cognitive functioning, α=0.88 for social functioning, α=0.76 for physical functioning and α=0.91 for overall QoL in paper III.

The Fatigue Questionnaire (FQ) (178) consists of 13 items. Seven items assess physical fatigue and four items assess mental fatigue. Each item is rated from 0 ("less than usual") to 3 ("much more than usual"). The scorings are summed up as the total fatigue score. Cases of chronic fatigue (CF) are defined by an algorithm based on the 11 fatigue item scores and duration $\geq$6 months (183). The FQ is well validated and widely used in fatigue research (239). Internal consistency of physical fatigue was α=0.89, mental fatigue α=0.75 and total fatigue α=0.89 in the RRSO sample, while in the NORM sample the internal consistency of physical fatigue was α=0.90, mental fatigue α=0.74 and total fatigue α=0.90 in paper III.

The Body Image Scale (BIS) contains 10 questions about body image of cancer patients. Five of the questions are general and five are connected to disease and treatment. The BIS focuses on the patient's feelings about her appearance and changes due to cancer and/or treatment during the past week. Each item is scored on a four point Likert scale scored: from 'not at all' (0) to 'very much very' (3), and a higher BIS score represents poorer body image. The internal consistency was α=0.89 for BIS in the RRSO sample in paper III.

2.2.6 Physical measures

The RRSO group
The women who had undergone RRSO and agreed to participate in the study were asked to contact their regular GP and make an appointment for anthropomorphic measurements and blood tests. Instructions concerning these issues were given in an enclosed formal letter sent with the questionnaire. The GP measured systolic and diastolic blood pressures guided by the following procedure: After at least five minutes rest, the mean of the second and third measurements was used. Cuff size was adjusted after measuring the arm circumference. The GP also measured waist circumferences in a standardized manner above the iliac crest. Height and weight were self-reported by the participants.

The control group (HUNT)
Systolic and diastolic blood pressures were measured in a standardized manner by trained nurses using a Dinamap 845XT (Criticon) based on oscillometry. The measurements were started after the participant had been seated for two minutes with the cuff on the arm, and blood pressure was measured three times at one-minute intervals. The mean of the second and third reading was used in this study. Waist circumference was measured above the iliac crest.

2.2.7 Laboratory data

The RRSO group
According to the instruction, the blood samples were drawn in the fasting state at the GP's laboratory. The blood samples were sent by mail to the laboratory at Sørlandet Hospital, Arendal. The blood samples were analyzed on a Hitachi 911 auto-analyser. Glucose was measured with an enzymatic hexokinase method, triglycerides with an enzymatic colorimetric method and total and HDL cholesterol with an enzymatic colorimetric cholesterol esterase method.

The control group (HUNT)

Fasting blood samples were analysed at Levanger Hospital, Norway on a Hitachi 911 auto-analyser. Glucose was measured with an enzymatic hexokinase method, triglycerides with an enzymatic colorimetric method and total and HDL cholesterol with an enzymatic colorimetric cholesterol esterase method.

2.2.8 Statistical methods

In papers I-IV, continuous measures were analyzed with independent sample t-tests, or with non-parametric tests when applicable due to skewed distributions. Categorical measures were analyzed by chi-square tests or by Fisher's exact test (when expected values were less than five in 2x2-tables). Significant differences on continuous variables and 2x2 contingency tables were calculated as effect sizes (ESs) and values >0.40 were considered as clinically significant (240;241). The internal consistency of scales was tested with Cronbach's alpha coefficient. For papers I-IV, the level of significance was set at $p<0.05$ and all tests were two-tailed. The statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS, version 14.0-16.0, SPSS Inc, Chicago, IL, USA).

In paper I, the RRSO group and control groups were not systematically matched for age or other demographic and lifestyle factors. Therefore, the tables covering demography and risk factors presented both the crude data and the age-adjusted data. Age-adjusted mean differences or odds ratios between the RRSO and control groups were calculated with linear regression or binary logistic regression, respectively. The age-adjusted mean differences expressed age-adjusted differences between mean scores in the RRSO and control groups. The age-adjusted odds ratios expressed the risk of each outcome given RRSO. Associations with metabolic syndrome as defined by IDF and ATP were modelled by multivariate logistic regressions with age, education, civil status, smoking, BMI, RRSO, physical activity, BRCA mutation status, history of cancer, level of total cholesterol and HRT as independent variables. The strength of association with

each independent variable was expressed as odds ratio (OR) with 95% confidence intervals (CI).

In paper II, The Framingham risk score was modelled by multivariate logistic regression analyses with RRSO, history of cancer, level of education, paid work, cohabitation, use of HRT, level of physical activity, history of stroke and waist circumference as independent variables. We used Framingham risks of ≥5% and >10% risk as dependent variables (159). We examined possible presence of multicollinearity in the multivariate regression models. The contribution made by each covariate was expressed as odds ratio (OR) with 95% confidence intervals (CI).

In paper III, we performed univariate and multivariate linear regression analyses with overall QoL and total fatigue as dependent variables both in the total sample (RRSO + NORM-2004 and RRSO + NORM-2005) and in the RRSO group only. Only variables that were significant in the univariate analyses were entered into the multivariate analyses. The strength of associations in the linear regression analyses was expressed as standardized beta (β) coefficients.

In paper IV, differences between the RRSO and control groups concerning HADS-A, HADS-D and HADS-T were calculated with Mann-Whitney U test, due to skewed distributions. When comparing the RRSO and control groups on mental distress, we adjusted for having had cancer and use of HRT. When comparing the RRSO subgroups based on surgery before or after 50 years of age and presence of cancer, we adjusted for use of HRT, follow-up time and age at survey. We performed univariate and multivariate linear regression analyses with RRSO, use of HRT, history of cancer, paired relations, paid work, level of education, HADS-depression, smoking, palpitations, constipation, musculoskeletal disease, osteoporosis and pain and stiffness as covariates. In the regression analyses, HADS-A, HADS-D and HADS-T as dependent variables. e did not use HADS-D as a covariate when analyzing HADS-A and vice versa, as HADS-D and HADS-A were highly correlated (r=0.60). The strength of associations in the regression analyses was expressed as standardized beta coefficients.

3. MAIN RESULTS

3.1 Attrition analysis

Altogether, 503 women who had undergone genetic counselling and RRSO were identified and invited, and 361 (72%) delivered written informed consent. The 361 respondents had mean age 54.7 (SD 9.3) years at survey while the non-respondents had mean age 54.2 (SD 10.0) years at survey (p=0.58). The respondents had mean age 48.4 (SD 8.3) years at RRSO, whereas the non-respondents had mean age 48.3 (SD 8.8) years at RRSO (p=0.91). We did not have medical information about the non-respondents, so a full attrition analysis could not be performed. Although no significant differences were observed concerning age at survey and age at RRSO, we had too little data on the non-respondents to conclude that the findings of the respondents could be generalized to the whole sample.

3.2 Papers I-IV

Paper I

Mean age of the RRSO group at survey was 54.4 (SD 8.9) years while mean age in the control group was 48.5 (SD 13.1) years. Mean time since RRSO was 6.5 years (SD 4.4). The prevalence of metabolic syndrome was 31% in the RRSO group and 27% in the control group according to the IDF criteria, while the prevalence was 26% in the RRSO group and 24% in the control group according to the ATP criteria. These differences did not reach statistical significance. Our first hypothesis of a significantly higher prevalence of metabolic syndrome in women with RRSO compared to controls, was therefore not confirmed.

In a multivariate logistic regression analysis with metabolic syndrome as dependent variable in the total samples (RRSO+controls), RRSO was significantly associated with metabolic syndrome according to the ATP (odds ratio [OR] 2.46 [95% CI

1.63, 3.73]) and according to the IDF (OR 2.49 [CI 1.60, 3.88]), as were increasing age and BMI. Our hypothesis that RRSO was significantly associated with metabolic syndrome was therefore confirmed.

The RRSO group had a more favourable cardiovascular risk profile than the control group, even after age-adjustment, with lower levels of total cholesterol, lower BMI, lower levels of triglycerides, higher levels of HDL cholesterol, lower levels of systolic and diastolic blood pressures, lower levels of fasting glucose and a lower proportion of daily smokers. In contrast to these findings, the RRSO group had higher mean waist circumference than the control group.

Paper II

Going further from the metabolic syndrome study (paper I), we wanted to examine the CHD risk profile and determine future risk of CHD by using the Framingham risk score and examine factors associated with this risk score. The mean age of RRSO cases and controls were 54.4 years at survey. Mean time since RRSO was 6.5 years (SD 4.4), and mean age at RRSO was 48.5 (SD 13.1) years.

The RRSO group had lower Framingham total score than the control group (12.9 [SD 5.1] versus 14.5 [SD 5.2]), p=0.02) and the RRSO group contained a significantly lower proportion with Framingham risk ≥5% (22% vs. 38%, p=0.006). There was no significant differences between the RRSO and control groups regarding Framingham risk >10% 9% vs. 16%, p=0.43).

In addition, the RRSO group had a more favourable CHD risk profile (higher education, more physical activity, less smokers, lower total cholesterol, higher HDL cholesterol, lower systolic blood pressure and lower BMI) compared to controls. The hypothesis of a higher CHD risk in the RRSO group was not confirmed.

The second aim was to examine risk factors associated with Framingham risk score in the total sample (RRSO+controls). In multivariate regression analyses RRSO showed a negative association with Framingham 10-year risk ≥5% (Odds ratio 0.49 [0.34, 0.71], p<0.001), while history of cancer, lower levels of education, not having paid

work, lower level of physical activity, history of stroke and increasing waist circumference were significantly associated with increasing Framingham risk score. The hypothesis of an association between RRSO and increasing Framingham risk score was therefore not confirmed.

Paper III

Comparisons between the RRSO and control groups
For RRSO women mean age at survey was 53.7 (SD 9.2) years, at RRSO 48.4 (SD 8.4) years, and median time since RRSO was 5.0 years (range 1-15).

Compared to controls, the RRSO group had higher scores on physical functioning (87.5 versus 86.0, p=0.01), role functioning (85.0 versus 81.9, p=0.01) and overall QoL (74.7 versus 72.1, p=0.01). None of these differences reached clinical significance based on effect size calculations. There were no significant differences in levels of mental fatigue, physical fatigue or total fatigue between the groups. The hypotheses of lower levels of QoL and higher levels of fatigue in the RRSO group were not confirmed.

RRSO subgroups
In subgroup analyses of the RRSO group no clinically significant differences in QoL and fatigue were observed between those who had surgery before or after 50 years, or between BRCA1/2 carriers and women with unknown mutation status.

Women who had cancer (32%) however, showed clinically significantly lower levels of QoL and more fatigue than women without cancer. The RRSO subgroup with cancer compared to the RRSO subgroup without cancer had lower levels of physical functioning (82.3 vs. 90.0, p=0.003), lower levels of cognitive functioning (80.6 vs. 86.0, p=0.02) and lower levels of social functioning (79.2 vs. 86.1, p=0.01). The RRSO subgroup with cancer compared to the RRSO subgroup without cancer had higher levels of physical fatigue (9.1 vs. 7.9, p=0.001), mental fatigue (4.8 vs. 4.4, p=0.007) and total fatigue (14.0 vs. 12.3, p=0.001). Our hypothesis of lower levels of QoL and more fatigue among women who had a history of cancer was confirmed, while the hypothesis of lower

levels of QoL and more fatigue in women who had RRSO at an early age (premenopausally), was not confirmed.

Variables associated with QoL and fatigue

The third aim was to examine variables associated with QoL and fatigue. In univariate analyses of the total sample (RRSO + NORM-04), having lower levels of education, not being in a paired relationship and using antihypertensive or psychotropic medication were all significantly associated with lower levels of QoL. In the multivariate analysis, not being in a paired relationship and using antihypertensive or psychotropic medication were significantly associated with lower overall QoL.

In the multivariate analyses in the RRSO sample, only higher level of depression, poorer physical condition, and more pain and stiffness were significantly associated with lower levels of QoL, while use of analgesics showed a borderline significance (p=0.05). The strongest associations were shown by depression and physical condition in the RRSO group.

In the total sample (RRSO + NORM-05), having had cancer, not being in a paired relationship, not having paid work and using psychotropic medication were all significantly associated with higher levels of total fatigue in the univariate analyses and the same variables except non-paired relationship showed significant association in the multivariate analyses of the total group.

In the multivariate analyses in the RRSO sample, higher levels of depression, poorer physical condition, and more pain and stiffness were significantly associated with more fatigue. The strongest associations with total fatigue in the RRSO group were shown by depression and physical condition. RRSO was not significantly associated with QoL or fatigue in these analyses, thereby disconfirming our third hypothesis.

Paper IV

Comparisons between the RRSO and control groups

The RRSO and control groups had mean age 54.6 (SD 9.3) years and 54.6 (SD 9.4) years respectively at survey. Mean age at RRSO was 48.5 (SD 8.3) and mean time since surgery was 5.3 (SD 3.3) years.

After adjustments for having had cancer and current use of HRT, the RRSO group showed similar levels of anxiety but significantly lower levels of depression ($p<0.001$) and total mental distress (HADS-T) ($p=0.002$) compared to the control group. The RRSO group had significantly more palpitations ($p=0.02$), constipation ($p=0.01$), pain and stiffness ($p=0.02$), more musculoskeletal disease ($p=0.01$) and osteoporosis ($p=0.02$) than the control group. The hypothesis of more mental distress in the RRSO group was not confirmed while the hypothesis of more somatic complaints in the RRSO group was confirmed.

After adjustment for the presence of osteoporosis, the association between belonging to the RRSO group and pain and stiffness was not statistically significant ($p=0.09$). After adjustment for levels of anxiety, the association between belonging to the RRSO group and palpitations was still significant ($p=0.005$).

Variables associated with anxiety, depression and total mental distress

In multivariate analyses of the total sample (RRSO+controls), not having paid work, history of cancer, daily cigarette smoking, palpitations, constipation and pain and stiffness, were significantly associated with higher levels of HADS-A, while being a BRCA mutation carrier versus not having a positive BRCA mutation test almost reached statistical significance.

In multivariate analyses of the total sample (RRSO+controls), belonging to the control group, not having paid work, history of cancer, palpitations, musculoskeletal disease and pain and stiffness were significantly associated with higher levels of HADS-D.

In multivariate analyses of the total sample (RRSO+controls), belonging to the control group, not having paid work, history of cancer, daily smoking, palpitations,

constipation, musculoskeletal disease and pain and stiffness were significantly associated with higher levels of HADS-T. The hypothesis that RRSO would be associated with increasing levels of mental distress was not confirmed.

4. General discussion

4.1. Design and attrition analysis

4.1.1 Cross-sectional design

All papers in the present thesis had a cross-sectional observational design. A cross-sectional study is less time-consuming and more cost-effective to perform than a longitudinal study. Cross-sectional studies may give important information on the prevalence of a disease or a condition, and they may also be helpful in assessing the health care needs of various samples of individuals. Since this thesis investigated several issues that have not been examined in previous studies of RRSO samples like fatigue, metabolic syndrome and the risk of future CHD estimated by the Framingham risk score, a cross-sectional design was chosen for a first assessment of these issues.

The classic cross-sectional study measures exposure and effect at the same time. The main limitation of cross-sectional studies is therefore that causation cannot easily be assumed. The time frame represents a factor of major importance. If the exposure took place before any effect occurred, the data from a cross-sectional study can be treated like data from a cohort study (242). Without baseline measurements, however, the timing of exposure and effect is difficult to establish. In this thesis, we have identified a sample of RRSO cases, and the measurements are performed at a median of six years after the surgery took place. The effects are therefore measured after the exposure. However, both somatic and mental variables may be influenced by exposures before the RRSO procedure and these exposures cannot be corrected for. Metabolic syndrome or CHD develop over time, with increasing metabolic disturbances and accelerating atherosclerosis. As we do not have measurements at baseline before the RRSO procedure, causation cannot be assumed in our studies. This is a major limitation of the cross-sectional design.

4.1.2 Representativeness

We only had data on age at survey and age at RRSO for the non-respondents. Attrition analysis showed no statistical differences between the respondents and the non-respondents. As we had no medical information about the non-respondents, we can not generalize our findings beyond our RRSO sample, but we can describe findings that are of interest for future studies.

This thesis is limited to women who underwent the RRSO procedure after genetic counselling at the Norwegian Radium Hospital. The sample is based on searches in surgical records, and therefore not based on all women who underwent genetic counselling and were referred for RRSO. We cannot be certain that the sample studied in this thesis is representative for the initial cohort who went to genetic counselling. This is a major weakness. When the findings from our sample deviate from those of other studies of women who have undergone RRSO because of increased risk of HBOC, the problem of the representativeness could be part of the explanation. We do not find it likely that our RRSO sample differs significantly from the total sample of women who had genetic counselling at other Norwegian hospitals, although we do not have data to explore this issue.

4.2. Validity

4.2.1. Internal and external validity

Validity expresses if a test is able to measure what it is intended to measure and is often divided into internal and external validity.

Internal validity
Internal validity describes to which degree the results of an observation are correct for the particular group of people being studied (242;243), and can be threatened by all sources of systematic error, such as confounding, selection bias and information bias. Internal

validity can be improved by good design in order to secure better representativeness (242).

In a study of exposure and outcome, confounding can occur when another exposure is present in the study population and is associated with both the exposure and outcome studied. Especially, this is so if the confounding variable is unevenly distributed in the study's subgroups. Confounding is present when the effect of two exposures have not been separated and the analysis concludes that the effect is due to one variable rather than the other (242).

In this study, there are several potential confounding variables. In paper I, potential confounding variables associated with both the exposure (RRSO) and the outcome (metabolic syndrome) are age, level of education, work status, level of physical activity, smoking status, and BMI. We have corrected for these variables in the multivariate logistic analyses. However, we cannot exclude that there may be confounding variables that are unidentified or not measured.

In paper II, there may be important confounders that have not been measured. As discussed later (4.3.2 and 4.3.3), the results of paper II are in contrast to previous studies and expected findings. The results of lower future CHD risk in the RRSO group compared to the control group may be due to selection bias among cases, but may also be explained by confounders that we have not been able to correct for.

In paper III, the potential confounders may be related to the RRSO group seemingly belonging to a higher socioeconomic group than the controls. We attempted to correct for these confounders in the multivariate analyses.

In paper IV, important confounders may be related to socioeconomic differences, which are known to affect the prevalence of mental distress. We have attempted to correct for these differences by including level of education, having paid work and cohabitation status in the multivariate analyses.

Bias

All papers in this thesis are observational studies. Observational studies have built-in biases, which may undermine the internal validity of the studies (244). Bias is systematic error and occurs when results differ systematically from the true values. A study with

small systematic error has high accuracy. Accuracy is defined as the difference between a measured value and the true value, and is not affected by sample size (242).

Selection bias occurs when there is a systematic difference between the characteristics of the people selected for a study and the population from which the sample is drawn. Thus, a selection bias is operating if we select patients and controls that systematically differ from their respective populations.

In this study, one of the important limitations may be a selection bias. This thesis includes some of the first women to undergo RRSO in Norway, the so called "early adopters" of RRSO (245). Women who undertake genetic testing and RRSO may be self-selected in regard to higher education, more paid work and a healthier lifestyle. Lerman et al. (246) demonstrated that rates of BRCA test use may be higher in families with higher socioeconomic status, although their study sample probably differ from the sample of the present study, as genetic counselling and RRSO are essentially free of cost in Norway. If there are such baseline differences in socioeconomic status and general health, these differences may affect the risk of CVD and CHD, and probably also levels of fatigue, levels of QoL and levels of mental distress.

This is an observational study, meaning that the researchers do not assign the participants to the different interventions. Observational studies are therefore vulnerable to selection biases. In randomized experimental studies the participants are randomly allocated to an intervention. Therefore, selection biases in the randomization process can be avoided, although exclusions prior to the randomization can decrease the external validity.

Given the solid documentation of the risk-reducing effects of RRSO, it will not be possible, or ethically justifiable, to conduct a study where participants are randomized to RRSO or surveillance. Therefore, all follow-up studies of women who have undergone RRSO will be subject to selection bias. The cases are retrospectively collected from medical records based on diagnoses and surgical procedure codes. The data collection in this study may introduce selection bias because coding may vary between different hospitals and different surgeons.

An additional selection bias may be present if the respondents differ systematically from the non-respondents. It is well documented that potential participants

with lower income and lower levels of education are less likely to respond to mailed questionnaire surveys (247;248). We do not have enough data on the non-respondents to perform a full attrition analysis, and although the respondents did not differ from the non-respondents regarding age at survey and age at RRSO, there may be unidentified differences. It is possible that the 72% who responded belonged to a higher socioeconomic group with higher levels of education. If such a selection bias was present, it may have affected CHD risk factors, levels of mental distress, levels of QoL and levels of fatigue.

Information bias occurs when the individual measurements of disease or exposure are inaccurate. Paper I and paper II could have been vulnerable to bias because the blood samples from the RRSO group and controls were analyzed in different laboratories. The blood samples from both the RRSO and control groups were analyzed on a Hitachi 911 auto analyzer using the same analyzing kits. However, the samples from the RRSO group were analyzed at Sørlandet Hospital Arendal, while the samples from the controls were analyzed at Levanger Hospital. Analyses from different laboratories may give rise to systematic errors resulting in information bias. It is not possible for us to determine if an information bias was present or what influence such error might have had.

The cancer diagnoses in the NORM material are self-reported. It has been documented by Nord et al. (249) that cancer patients are not exact concerning their self-report of a cancer diagnosis. Precise information can be collected from the Norwegian Cancer Registry, but we did not have the opportunity to do so in these anonymous questionnaire studies. The cancer diagnoses in the HUNT-2 material and the RRSO sample are also self-reported, and we do not have reason to believe that the self-report of cancer introduced systematic errors between the RRSO and control groups.

Recall bias is operating if the patients by will, or without realizing it, systematically distort their responses. Recall bias occurs when there is a differential recall of information by cases and controls (242). Recall bias is a possibility when relying on subject memory, because the cases and controls by definition differ with respect to their exposure or disease experience at the time of their recall (243). Participants in the RRSO group knew that they were recruited because they had gone through the RRSO procedure. The RRSO group contains participants with a history of cancer in the family, loss of

family members, genetic testing and surgery. A dramatic medical history could influence the ability to recall earlier events. Hypothetically, the RRSO group could have been more likely to recall exposure because they may have had more focus on their medical history. In some instances they could also be more likely to deny exposure, because they might have suppressed part of their medical history. Therefore, recall bias can either exaggerate the degree of effect associated with the exposure – or underestimate it – if cases are more likely than controls to deny past exposure.

Another bias possibly present is survivor bias. Survivor bias can be thought of as a special case of selection bias. If survivorship is selective, the sample examined can differ from the original sample (250). As we included post-RRSO patients who were alive at a median of six years after surgery, the data set may be influenced by a survival bias. In the initial material, 12 of the 515 (2.3%) identified cases were deceased, and we may have invited an RRSO group who was healthier than the cohort who initially went through surgery. For example, if there was an association between RRSO and CHD, women who had fatal CHD after RRSO before the study took place will not contribute to the estimates in this thesis. The proportion of 2% is low, and we do not find it likely that our estimates would be systematically affected by a survivor bias.

In order to achieve a large sample size, the inclusion period had to be long (the surgery took place from 1994-2005). This makes the patient sample vulnerable to cohort effects as the level of general health increases with time (251). Especially the mortality from CVD has decreased during the last 15-20 years in Norway (Table 2). Therefore, the patient sample may be heterogeneous in relation to risk factors and may differ from the control samples which were collected during a shorter time span. However, most cases underwent surgery during the last ten years before the survey, with the median time since surgery being six years. One can also argue that the mortality rates from CVD have decreased from the gathering of data for HUNT-2 (1995-7) to the gathering of data for the present survey (2006) (Table 2). Therefore, the participants from the HUNT-2 study may have a higher prevalence of CVD and metabolic syndrome and a higher risk of future CHD than if the control sample data had been collected at the same time as the RRSO data. Such error would tend to underestimate the associations between RRSO and

the outcomes measured because the control sample data were gathered in a time period with higher CHD and CVD mortality.

External validity

External validity describes to which degree the results of an observation are correct for the population outside the sample being studied (242;243). External validity is not only based on whether the sample studied is representative for the population it has been obtained from. It is also dependent on the internal validity of the study, which is a necessary, but not a sufficient factor for external validity. The external validity of a study is improved by study designs that examine clearly-stated hypotheses in well defined samples.

In this study, we had limited information about the non-respondents. Although we did not find any differences between respondents and non-respondents regarding age at invitation and age at RRSO, the information is not extensive enough to conclude that our study sample is representative for the entire RRSO sample. Furthermore, the recruitment strategy represents a selection of the women who have chosen RRSO. The sample may be affected by selection bias, information bias, recall bias and survivor bias as discussed above. These biases decrease the internal validity, which again will affect the external validity. The external validity was additionally weakened because we did not have data to assess whether the RRSO sample in this thesis was representative for the cohort who were referred to genetic counselling.

4.2.3. Issues of the questionnaires

A major part of this study was based on questionnaires sent to the invited participants by mail. The intent was to get information from the relatively large sample in an efficient way and at relatively low costs. An alternative would be to do an interview study, or interviewing a representative subsample of women who have undergone RRSO. The interview design was found to be too expensive and time-consuming within the frames of this study. In a mailed questionnaire design, one can not be certain whether the invited

participants get the questionnaire, and we do not know to what degree the non-respondents have been reached by the initial invitation and the reminder after three weeks. The addresses were confirmed by the National Population Registry, which is considered to be updated and valid.

Paper I and paper II were based on questionnaires for information on demographic issues and medical history (*The follow-up questionnaire of The Norwegian Radium Hospital*).

Paper III and paper IV were entirely based on questionnaires: *the follow-up questionnaire of The Norwegian Radium Hospital, the Hospital Anxiety and Depression Scale (HADS), the European Organization for Research and Treatment of Cancer (EORTC-C30), the Fatigue Questionnaire (FQ), and the Body Image Scale (BIS).* We tried to use established and validated questionnaires in order to get systematic information about the study subjects. We did not test the validity of the questionnaires used in this thesis, but relied on data collected by other researchers, such as Mykletun et al. (235) regarding the HADS, Loge et al. (178) regarding the FQ and Ringdal et al. (237;238) regarding the EORTC-C30.

The internal consistency of scales was tested by using the Cronbach's alpha in paper III and paper IV as described in the methods section. The Cronbach's alpha is an indicator of the internal consistency of the scale that is used, that is, a measure indicating that the items of the scale are measuring the same underlying construct. If the inter-item correlation increases, the Cronbach's alpha increases. Therefore, the Cronbach's alpha is a measure of the reliability of the scale being used. In paper III and IV, all scales being used showed Cronbach's alpha values well over the threshold of 0.7. We therefore concluded that the internal consistency was adequate.

4.2.4 Laboratory measures

The blood samples from the RRSO group were all analyzed in the same laboratory. The preferred method would have been to draw all participants' blood at Sørlandet Hospital Arendal to avoid differences in centrifuging, transportation, storage temperatures and

time from sampling to analyses. However, the project resources did not allow us to bring the more than 300 participants to the same laboratory.

It is considered a weakness that the blood samples from the control groups were analyzed in two different laboratories. Analyses from different laboratories may give rise to systematic errors because the machinery might not be equally calibrated.

Accordingly, the measurements of waist circumferences and blood pressures in the HUNT sample (controls in papers I and II) were carried out by trained nurses using the same routine on all participants. Measurements of waist circumferences and blood pressures in the RRSO group were performed by the GPs. Although GPs generally have considerable experience performing such measurements, the introduction of many measurers gives rise to errors. That is, although each GP performs reliable and reproducible measures, GPs may classify blood pressures differently. Such inter-observer differences may have affected our classification of blood pressures and waist circumferences. We are not able to determine if such errors were present.

4.2.5 Control sample selection

The NORM Sample
The NORM 2004 and 2005 samples allowed us to compare the findings of the RRSO group to an age-matched normative sample that ideally was representative for the general Norwegian female population. The controls used in paper III were drawn from the NORM sample, and the main limitation of the paper is the low participation rate in the control sample. In addition to this, no attrition analysis could be made in the NORM sample and only a minor attrition analysis was performed among cases. We do not know to what degree the scores of the participants are representative for the non-participants. The NORM sample may therefore not be representative for the general Norwegian population, and the external validity is therefore limited.

The HUNT Sample

The HUNT-2 sample represents 76% of a total population within a geographical area and thereby has a high participation rate. Nevertheless, 24% did not participate, and the participation rates were lower in the youngest and oldest age groups (232), indicating that a selection bias might be operating.

The main strength of this control material is that it is derived from a large, population-based sample with an acceptable participation rate, and that the HUNT-2 material is considered to be representative for the Norwegian population. The data were collected by questionnaires, and the self-report of illnesses without cross-checking with medical records is considered a weakness of the HUNT data. As both the cases and controls self-reported their medical history, we considered it less likely that there were systematic differences between the groups. An exception would be if the exposure recall of the RRSO group systematically differed from the controls as discussed under recall bias.

In paper I, a selection of 679 out of 21,650 controls increased the risk of a selection bias, and we therefore made a comparison between our controls and the total HUNT-2 sample. The controls used in paper I (N=679) were significantly younger than the whole HUNT-2 sample (N=20,911). After age-adjustment, the controls from this study had lower levels of education, more paid work, more diabetes, higher total cholesterol, higher waist circumference, higher systolic blood pressure, higher diastolic blood pressure, higher Framingham total point score and more smokers compared to the whole HUNT-2 sample. These examinations showed that the control sample was a biased subset compared to the HUNT-2 sample. The selection bias limits our ability to generalize the association between RRSO and metabolic syndrome. Overall, the controls in paper I seem to have a worse cardiovascular risk profile than the total HUNT-2 material. Presumably, this worse risk profile would tend to lessen the strength of the association between belonging to the RRSO group versus the control group and metabolic syndrome.

Controls for paper II and IV were drawn from the HUNT-2 sample. Apart from the possibilities of selection bias in the total HUNT-2 sample mentioned above, we find that the way of selecting controls for the papers II and IV is appropriate. The random selection of age-matched controls should not introduce further selection bias, and five

age-matched controls per case should ensure proper statistical power. However, no matching is perfect. There is always a possibility of selection bias when a subset of a sample is used, because a large part of the total sample is not included in the analyses. An alternative would be to use the whole HUNT-2 sample as a control sample. Such a procedure would minimize the risk of selection biases, but introduce the need for additional corrections in the analyses.

4.2.6. Statistical issues

Statistical power

Prior to designing the study, we performed power analyses. With p value <0.05 and statistical power of 0.80, balanced groups with N=100 will reliably show clinically significant differences with an effect size of 0.40. An effect size of 0.40 is considered to be the lower threshold for clinically relevant findings in most circumstances (240;241). We therefore concluded that our RRSO sample was large enough for us to reveal clinically important findings.

Data analysis: regression analyses

In paper III and IV, we used multivariate linear regression analysis to estimate associations between independent variables and QoL (paper III) and HADS-A and HADS-T (paper IV) as dependent variables. Multivariate linear regression analysis is most often used when one attempts to predict a single continuous variable using two or more independent or predictor variables (252). The independent variables must be nominal, but can be both categorical and continuous. Performing linear regression analysis requires a continuous dependent variable (252). In both the EORTC-QLQ-C30 and the HADS, the data are originally ordinal and not continuous. Performing linear regression requires a transformation to continuous data by summing up the scores from

each item. Statistically this procedure can be questioned, but in medical research this way of treating data from the HADS is well established (253).

In paper I and II, we used multivariate logistic regression analysis to estimate associations with metabolic syndrome (paper I) and Framingham risk score (paper II). In logistic regression analysis, the dependent variable is dichotomous rather than continuous. That is, the variable has only two values instead of several values, while the predictors can be continuous or non-continuous. The outcomes must be statistically independent.

In both studies I and II the dependent variables are dichotomous and the outcomes are statistically independent. Paper I revealed some interesting findings. In the crude and age-adjusted data, there were no differences between RRSO and controls regarding prevalence of metabolic syndrome. In the multivariate logistic regression analysis, however, we demonstrated a significant association between RRSO and metabolic syndrome. This finding was probably the result of us being able to adjust for confounders in the multivariate logistic regressions.

4.3. Discussion of specific results

4.3.1 Paper I

To our knowledge, this is the first study to examine metabolic syndrome in women at risk for HBOC who have undergone RRSO. The RRSO and control groups had the same prevalence of metabolic syndrome. However, in multivariate regression analyses, RRSO was associated with metabolic syndrome, both according to the IDF and ATP definitions, with odds ratios approaching 2.5.

We did not find differences in the prevalence of metabolic syndrome between the RRSO and control groups. A possible explanation is that there is no difference between the groups. Another explanation is that there are confounding variables not taken into account. A possible association between RRSO and metabolic syndrome may be masked by the fact that the RRSO group had higher levels of education, more paid work and were

more often physically active compared to the control group, as these factors are known to be inversely associated with CVD (254). In addition to this, the median time from RRSO to survey may be too short to uncover metabolic disturbances with such impact that the prevalence of metabolic syndrome is increased.

Our research group recently demonstrated an increased prevalence of metabolic syndrome in women from the general population who had bilateral oophorectomy before the age of 50 years compared to age-matched controls with intact ovaries (160). Our finding of the same prevalence of metabolic syndrome in the RRSO and control groups is not in accordance with the findings in this article. The groups in the two studies cannot be directly compared, as the previous study examined women from the general population at average risk for ovarian cancer who underwent bilateral oophorectomy due to several benign indications (160).

There are so far no other studies of associations between bilateral oophorectomy and metabolic syndrome, but there are some studies on bilateral oophorectomy and CVD. These studies are of interest as metabolic syndrome is associated with CVD (156;255). Rivera et al. (144) recently showed that in an American cohort, bilateral oophorectomy for several indications before the age of 45 years was associated with increased cardiovascular mortality. However, it is still unclear whether this association is due to a causal effect or to a risk profile in the population who underwent early oophorectomy. Our prevalence findings are not in accordance with these studies and earlier studies linking menopause to CVD (145;146), as one would expect that increased risk of CVD could be expressed as increased prevalence of metabolic syndrome. Atsma et al. (145) conducted a metaanalysis of 18 studies regarding the relation between postmenopausal status and age at menopause and CVD. They demonstrated that bilateral oophorectomy before age 50 substantially increased the risk of CVD. Lokkegaard et al. (146) performed a prospective cohort study among Danish female nurses and found that early surgical menopause was associated with increased risk of CHD, while the use of HRT seemed to outweigh this risk.

Although the RRSO participants were older, had a healthier lifestyle and belonged to a higher socioeconomic group than controls, RRSO was associated with metabolic syndrome in the multivariate logistic regression analyses. The RRSO group had lower

BMI, higher HDL-cholesterol and lower systolic blood pressure. In addition to this, they were more physically active and included a lower proportion of smokers. Some of these findings may have been caused by a change of lifestyle. The RRSO group might have been more concerned about their general health because of their high HBOC risk and the RRSO status, and adapt to a healthier lifestyle, such as their increased physical activity and reduced smoking habits, which are changes known to decrease the risk of CVD (256). The RRSO group had higher levels of education, well known to be associated with lower smoking rates (257;258). Also, the RRSO women may be aware of studies suggesting that lifestyle variables and weight control through restricted dietary energy intake may reduce the risk of breast cancer (259). Such lifestyle changes seem to affect breast cancer risk in BRCA individuals as well (260). Additionally, the rates of genetic testing seem to be higher in groups of higher socioeconomic status (246). Even after adjustments for these demographic and lifestyle characteristics, RRSO was still significantly associated with metabolic syndrome.

This study demonstrates a significantly higher waist circumference in the post-RRSO group, even after age-adjustment. The association between increased waist circumference and cardiovascular risk is well-known. Central obesity, either caused by visceral obesity or subcutaneous fat accumulation, is agreed as essential in the IDF definition of metabolic syndrome because of the strength of the evidence linking waist circumference with cardiovascular disease and other metabolic syndrome components (158). The most probable explanation of our finding is that the loss of estrogen caused by RRSO leads to alterations in body fat distribution with increased waist circumference and central obesity. The differences between the RRSO and control group regarding socioeconomic factors and other cardiovascular risk factors may outweigh the possible increased CHD risk imposed by the RRSO procedure and therefore the association between RRSO and metabolic syndrome is not revealed unless analyzed with multivariate models.

The results of paper I must be interpreted with caution because of possible selection biases both in the RRSO and control groups. The RRSO group is self-selected and seems to belong to a higher socioeconomic group than the control group. The control group is a non-age-matched subgroup from the HUNT-2 study. Comparisons with the

whole HUNT-2 sample indicate that the control group used in this study is a biased subset, as the controls seem to have a worse cardiovascular risk profile than the total HUNT-2 material. The results therefore need to be reproduced in longitudinal studies.

4.3.2 Paper II

The RRSO group had a lower Framingham total score than the control group, and the RRSO group contained a significantly lower proportion with Framingham risk ≥5%. In addition to this, the RRSO group had a more favourable CHD risk profile (higher education, more physical activity, less smokers, lower total cholesterol, higher HDL cholesterol, lower systolic blood pressure and lower BMI) compared to controls. We found that belonging to the RRSO group was associated with significantly lower prevalence of Framingham risk score ≥5%. Lower levels of education, not having paid work, lower levels of physical activity, history of stroke and increasing waist circumference were associated with increased risk, as shown in other studies (261;262).

It has been demonstrated that bilateral oophorectomy before the age of 45 years is associated with increased overall mortality (150) and increased cardiovascular mortality (144). These studies are in contrast to our finding of a more favourable CHD risk profile in the RRSO group, as one would expect the RRSO group to be at increased risk for CHD and therefore have an adverse risk profile. However, it is still unclear whether the described associations between bilateral oophorectomy and increased overall and cardiovascular mortality are causal in the sample that underwent early oophorectomy. In addition, these studies are performed in samples at average risk for ovarian cancer and with several benign indications for oophorectomy. The results are therefore not directly transferable to our sample of high-risk women who have undergone RRSO.

In contrast to this novel finding of lower CHD risk in a population who have undergone bilateral oophorectomy, we have previously demonstrated increased levels of Framingham risk score in women <50 who had their ovaries removed because of benign diseases (160). However, this sample may have had an unfavourable CHD risk status at baseline, as the indication for pelvic surgery may be associated with CHD. This is

especially important in studies looking at bilateral oophorectomy for several indications, because co morbid conditions such as obesity, diabetes and hyperinsulinemia put women at risk for having CHD as well as increased risk of undergoing a hysterectomy with bilateral oophorectomy. Previous studies, like the sub analysis from WHI data by Howard et al. (151) indicated that women who went through a hysterectomy, regardless of oophorectomy status, had a poorer socioeconomic profile than controls without such surgery. Howard et al. (151) reported that the observed association between hysterectomy and CVD was non-significant after adjustment for baseline CVD risk factors. These findings indicate that increased risk of CVD after hysterectomy and/or oophorectomy may be confounded by increased baseline risk.

Our findings do not agree with the previous studies that have linked bilateral oophorectomy to increased risk of CHD (145;146;152;164). One reason may be that the Framingham risk score has not been validated as a predictor of CHD in Nordic women, and the instrument may not be transferable from American women (263). In addition to this, Hildrum et al. (156) found that metabolic syndrome was a better predictor of mortality than Framingham risk score in participants of the HUNT-2 study. Studies performed in European populations point out that socioeconomic differences, as revealed in paper II, may affect risk prediction (264). On the other hand the Framingham risk score is based on the presence of classical CHD risk factors that confer risk in all populations.

Another possible explanation of the finding is that RRSO leads to alterations in body fat distribution with increased waist circumference and central obesity. Central obesity, either caused by visceral obesity or subcutaneous fat accumulation, is strongly correlated with metabolic syndrome and CHD risk (158), but central obesity is not included in the Framingham risk score. The omission of central obesity may explain the contrast to our former finding of an association between RRSO and metabolic syndrome (265).

It is reasonable that it takes time before estrogen deficiency leads to metabolic disturbances with such impact that the Framingham risk score is altered, and the median time since surgery of six years in this study may not be sufficient to detect effects.

The RRSO group had a higher level of physical activity. These findings may have been caused by a response shift (266), meaning that the RRSO group has adapted a healthier lifestyle. Also, women who undertake genetic testing and RRSO may be self-selected in regard to higher education, more paid work and a healthier lifestyle. This form of selection bias may explain differences in physical activity and also in CHD risk profile.

Notably the use of HRT did not seem to be associated with CHD risk in this patient sample. Indeed, the WHI study demonstrated that primary prophylaxis against CHD is no indication for use of estrogens (210), but later publications have questioned the timing of HRT use in the original study (267). As far as we know, this is the first study to report an a association between lower Framingham risk score after RRSO in women at risk of HBOC compared to controls from the general population, as previous studies have documented increased future CHD risk after bilateral oophorectomy for several indications.

4.3.3 Incoherence regarding results in paper I and paper II

Although we found an association between RRSO and metabolic syndrome in multivariate analyses (paper I), we found a negative association between RRSO and Framingham risk score (paper II). This should imply that RRSO is associated with increased risk for CVD but decreased risk for CHD. These findings are conflicting, and they are in need of further explanation.

One reason for the discrepancy may be the selection of control samples. We investigated the same 326 cases in paper I and II, but the controls were not the same, although all controls were drawn from the HUNT-2 sample. In paper I, we used all eligible controls that had their blood samples drawn in the fasting state. These 679 HUNT-2 participants may have had reasons for showing up without having breakfast that made them differ systematically from the rest of the HUNT-2 sample. Comparisons with the whole HUNT-2 sample may indicate that the control group used in this study might be a biased subset. The possible selection biases in paper I limit the external validity, while the risk of selection biases seems smaller in paper II.

Another explanation of the conflicting findings may be that metabolic syndrome and Framingham risk score predict different outcomes. Studies comparing metabolic syndrome with Framingham risk score suggest that the latter may be a better predictor of CHD, while metabolic syndrome is better at predicting type 2 diabetes and CVD (162;163). Hence, it is possible that RRSO is linked to increased risk of CVD through metabolic syndrome but not to increased risk of CHD, and therefore the Framingham risk score would not be increased in the RRSO group compared to the control group.

Both paper I and II show a significantly higher waist circumference in the RRSO group, even after age-adjustment. The association between increased waist circumference and CVD risk is well-known. Central obesity, either caused by visceral obesity or subcutaneous fat accumulation, is agreed as essential in the IDF definition of metabolic syndrome. The rationale for this requirement was that central obesity was more strongly correlated with metabolic syndrome than other parameters and was highly correlated with insulin resistance (158;268). A probable explanation of our finding is that the loss of estrogen caused by RRSO leads to alterations in body fat distribution with increased waist circumference and central obesity. Central obesity is not included in the Framingham risk score. The omission of central obesity may explain the contrast to our former finding of an association between RRSO and metabolic syndrome (paper I).

The results in papers I and II may be confounded by a cohort effect. The case data were collected in 2006, and the control data (papers I and II) in HUNT-2 were collected in 1995-97. The mortality rates from CVD among Norwegian women have decreased during the decade from HUNT-2 to this survey (Table 2). It is possible that the average Norwegian woman would have had a higher Framingham risk score in 1995-97 than in 2006, and that the result in paper II partly is caused by this fact.

An important limitation of this thesis is the possible presence of selection bias (as described in 4.2.2). If a selection bias is present, it could jeopardize the internal and even the external validity of the study. However, as the same 326 cases are studied in both paper I and II, it is not likely that such bias would explain the conflicting findings.

Finally, this is the first study to examine CVD and CHD risk in a post-RRSO population at risk for HBOC. All comparable studies have a different selection of cases making direct comparisons difficult to interpret.

4.3.4 Paper III

Fatigue

Fatigue is associated with menopause and reduced QoL (179), but to the best of our knowledge, there are no published studies of fatigue in women after RRSO. In this setting our finding of similar levels of fatigue in the RRSO and control group is new. The finding is somewhat surprising, as we had thought that surgical menopause in women who underwent RRSO would be associated with increased levels of fatigue. We had expected that the RRSO group, and particularly those who underwent surgery at young ages, would report higher levels of total fatigue than NORM. One explanation of this finding is that surgical menopause does not affect levels of fatigue. The fact that levels of fatigue were similar in the RRSO subgroup that went through surgery before the age of 50 years compared to those that had RRSO at the age of 50 years or above, supports this explanation. Another explanation is that response shift could be operating (266). Response shift implicates that women who have undergone RRSO adapt to their reduced cancer risk and changed hormonal state, self-image and life expectancy without increasing levels of fatigue. We are not able to draw solid conclusions on this issue, as we do not have data obtained before the surgery took place. We suggest that the reduced risk of future cancer or relapse of cancer with reduction of cancer related worries may cancel out negative effects of RRSO.

More women in the RRSO than the control group had paid work, despite the fact that the control group had more cancer. This finding is in accordance with the finding of similar levels of fatigue in the RRSO and control groups, as we believe that higher levels of fatigue have impact on work ability.

We observed that women in the RRSO subgroup with cancer had significantly higher levels of physical and total fatigue than women without cancer. Cancer was also significantly associated with fatigue in the univariate and multivariate regression analyses. This subgroup finding is new in the RRSO setting, but well-known from previous fatigue research where particularly breast cancer patients have higher levels of fatigue compared to normative controls (269;270).

RRSO was not associated with total fatigue in the univariate or the multivariate regression analyses. The main finding is therefore that RRSO was not associated with fatigue, but that women who had a history of cancer and RRSO had more fatigue. Fatigue in women who have undergone RRSO therefore seems to be associated with a history of cancer rather than the RRSO procedure in our study.

QoL

The symptoms caused by sudden surgical menopause may affect QoL in women (271;272). In this thesis the RRSO group reported higher levels of QoL as to physical and role functioning than NORM, reaching statistical significance, but these differences did not reach clinical significance (ES<0.40). In the RRSO subgroup analyses no significant differences were observed concerning QoL between women who had RRSO before or after 50 years of age, or between the *BRCA1/2* carriers or those without identified mutations. However, we observed that RRSO women who had a history of cancer reported significantly poorer physical functioning than RRSO women without cancer. In the present material, about one third had a history of cancer, and more than 80% of those had a history of breast cancer. In accordance with our findings, a link between a history of cancer and reduced levels of QoL has been reported in breast cancer patients (273-275).

In accordance with our study, Madalinska et al. (197) found no significant differences concerning QoL between women who had RRSO (N=369) and normative data from the general population. Madalinska et al. (197) used the Short Form 36 for QoL measurements and only reported normative data on four of its eight dimensions, while we used the EORTC QLQ-C30 QoL instrument with six dimensions controlled by an age-matched normative sample. Considering these differences in design, both studies concluded that levels of QoL in the RRSO group were similar to those of controls. Based on these findings, it seems possible that RRSO does not affect levels of QoL. The fact that levels of QoL were similar in the RRSO subgroup that went through surgery before the age of 50 years compared to the RRSO subgroup that had RRSO at the age of 50

years or above, supports the view that surgical menopause in this group does not affect QoL.

The measurements of QoL in the RRSO sample may be affected by response shift (266). There is substantial research documenting that people change their internal standards, values or conceptualization of QoL when they experience changes in their health. These changes may affect QoL measurements (276).

This negative finding may be reassuring for women who chose RRSO for cancer prevention. This result has now been reached both by Madalinska et al. (197) and our research group in two different countries and with two different, but well-established, QoL instruments. Other studies of QoL in women with HBOC risk have small sample sizes or lack normative controls (193;195). We are not able to draw conclusions on causal relationships between RRSO and levels of QoL, as we do not have pre-surgery data. However, we suggest that the reduced risk of future cancer or relapse of cancer with reduction of cancer related worries may cancel out possible negative effects of RRSO, and probably contribute to higher levels of QoL.

4.3.5 Paper IV

The RRSO group had more somatic symptoms than controls. The RRSO subgroup with surgery before the age of 50 years had significantly more palpitations, and the subgroup with cancer had significantly more nausea than the rest of the RRSO group. The RRSO group had significantly less depression (HADS-D) and total mental distress (HADS-T) compared to controls. In multivariate analyses, RRSO versus controls was significantly associated with lower levels of depression and total mental distress.

Mental distress

Anxiety

Our findings of the same level of anxiety in the RRSO and control groups were in contrast to a prospective study by Van Oostrom et al. (198), which found that RRSO was associated with a reduced level of anxiety measured by the HADS. However, although van Oostrom et al. (198) documented lower levels of anxiety in post-RRSO women at short term, they found increasing levels of anxiety approaching pre-surgery levels after five years. Our sample filled in the HADS questionnaire at a median of six years after their RRSO procedure, and our findings seem to be in accordance with the measurements five years after the RRSO procedure in the van Oostrom study. The results in the van Oostrom study suggested that genetic predisposition testing and prophylactic surgery alter the levels of anxiety temporarily, but that other characteristics determine the intensity of psychological distress at long-term. We cannot determine whether this was so in our study, as we did not obtain measurements at baseline or short term. The van Oostrom study found that women who lost a family member to breast/ovarian cancer tended to be more worried about developing cancer. Furthermore, women with young children at baseline reported more distress 5 years later. The authors concluded that this finding may be related to the participants' fear of leaving young children behind and to difficulties with informing children about their cancer risks (198). Our results also suggested that other factors than the procedure itself was associated with anxiety. In the multivariate regression analyses, not having paid work, history of cancer, daily cigarette smoking, palpitations, constipation and pain and stiffness were significantly associated with increasing levels of anxiety, whereas positive BRCA mutation status reached borderline significance.

Some somatic complaints may be expressions of anxiety. Palpitations are part of the anxiety syndrome (277). Smoking is well known to be associated with anxiety (278). It is reasonable that a history of cancer is linked to anxiety, and this fact may be explained by cancer-related distress and fear of recurrence. The borderline significant association between positive BRCA mutation status and anxiety is interesting. This link may be an expression of increased anxiety levels in women who are at higher risk for

developing hereditary breast and ovarian cancer, but the finding is in need of further investigation.

Regarding anxiety after bilateral oophorectomy in samples at average risk for ovarian cancer, Rocca et al. (279) recently found that women who underwent premenopausal bilateral oophorectomy had an increased risk of developing de novo symptoms of anxiety compared to referent women. Their finding of an increased risk of anxiety is in contrast to our finding, but the two studies can not be compared directly, as we have examined RRSO patients at risk for HBOC while Rocca et al. (279) included all women with bilateral oophorectomy for non-cancer indications.

There are several studies linking bilateral oophorectomy to increased levels of anxiety. There is also evidence suggesting that although RRSO may decrease levels of anxiety at short term, the levels of anxiety seem to increase again at long term. More longitudinal studies in samples of women who have undergone RRSO because of increased risk of HBOC are needed to explore and confirm these findings.

Depression

The RRSO group had significantly lower levels of depression than controls, and in multivariate regression analyses RRSO was associated with lower levels of depression and total mental distress. As far as we know, no studies have published levels of depression in women who have undergone RRSO compared to controls from the general population. Several studies have examined levels of depression after oophorectomy in women from the general population.

In contrast to our result, a longitudinal study of 2,500 middle-aged American women at average risk for ovarian cancer, concluded that women with surgical menopause had significantly higher depression scores than women with natural menopause (280). This American study is supported by several observational studies from the 1980's linking oophorectomy to depression (137;281;282). Rocca et al. (279) recently found that women who underwent premenopausal bilateral oophorectomy had an increased risk of developing depressive symptoms compared to referent women (279). These findings are not in accordance with our findings of lower levels of depression in

the RRSO group compared to the control group. The mentioned studies cannot be compared directly to other studies, as we have examined RRSO patients at risk for HBOC while all the other studies have included women at average or unknown risk for ovarian cancer.

In the multivariate regression analyses, belonging to the RRSO group rather than the control group was negatively associated with increasing levels of depression. One explanation of this finding is the possible presence of selection bias in the RRSO group. The self-selection of a sample belonging to a higher socioeconomic group may implicate that the RRSO group is less prone to develop depressive symptoms. The association between belonging to a lower socioeconomic group and common mental disorders is well documented in the general population (283).

Regarding depression after bilateral oophorectomy in samples at average risk for ovarian cancer, In general, anxiety is a reaction to danger in the future, while depression is a reaction to loss (284). With this interpretation, one might assume that the participating women have dealt with the initial loss constituted of the HBOC diagnosis, loss of affected family members, and loss of their ovaries. The finding is in accordance with Liavaag et al. (253), who studied long term survivors after ovarian cancer and found lower levels of depression in the cancer group compared to normative controls.

Somatic complaints

The RRSO group reported more osteoporosis than the control group. This is in accordance with Melton et al. (285;286), who reported from longitudinal American studies that bilateral oophorectomy both pre- and postmenopausally was associated with increased risk of fractures. However, no studies have examined the association between RRSO in women at risk for HBOC and osteoporosis. The most probable explanation of this finding is that loss of endogenous estrogen has decreased the bone mass and induced osteoporosis. The use of HRT after menopause is reported to reduce osteoporosis-related fractures by 50% (287). However, in our material the difference in the prevalence of osteoporosis between the RRSO and control groups remained significant after adjustment

for use of HRT. Women who have undergone RRSO may be at high risk for developing osteoporosis and medical follow-up should evaluate risk factors.

The women in the RRSO group reported more pain and stiffness than did controls, and this finding may be associated with osteoporosis. These symptoms may also be explained by estrogen deficiency which is associated with loss of collagen (171), and thus could increase the risk of other musculoskeletal diseases. This is in accordance with our finding that RRSO was associated with long-standing musculoskeletal diseases, such as fibromyalgia and osteoarthritis. After adjustments for the presence of osteoporosis, the association between belonging to the RRSO group and presence of pain and stiffness was statistically insignificant. This finding supports that the pain and stiffness finding was related to osteoporosis.

Hot flashes and vasomotor symptoms are well known associates of estrogen deficiency in menopausal women (288), and studies of post-RRSO samples have demonstrated more vasomotor symptoms and poorer sexual functioning compared to controls that chose ovarian cancer surveillance programs rather than RRSO (197).

As reported by Madalinska et al. (197), the RRSO group in their study had more palpitations than controls, which may be related to vasomotor symptoms after surgical menopause. However, palpitations are also associated with increased anxiety levels, as symptoms of autonomic arousal (palpitations, sweating, trembling or dry mouth) are common parts of the anxiety syndrome (277). In this study, we found no association between RRSO and increasing levels of anxiety. Adjustment for levels of anxiety did not affect the association between belonging to the RRSO group and presence of palpitations. This finding supports that the reported palpitations cannot be explained by the level of anxiety.

Peri- and postmenopausal women have been reported to have increased prevalence of bowel dysfunction and constipation, although an aging effect cannot be ruled out (172). The data regarding bowel function and relation to menopause seem to be scarce and somewhat contradictory, and bowel function is also affected by parity, sex and race in addition to age (173). The effect of sudden onset of menopause on bowel function is not well-known. Concerning women who have undergone RRSO, our finding of more constipation compared to controls is new. Alterations of bowel frequency due to

menstrual cycling have been reported, leading to hypotheses that constipation may be related to hormonal change. An American questionnaire study found no association between menopause and constipation when asking healthy women who accompanied patients who attended a clinic for colorectal surgery. Further research on the effect of sudden menopause on bowel function is needed.

Our hypothesis of more somatic complaints in the RRSO than the control group was confirmed for palpitations, constipation, pain and stiffness, osteoporosis and musculoskeletal diseases. As discussed above, all of these complaints may be linked to surgical menopause and lack of estrogen. One would imagine that at least some of these complaints could be relieved by the use of HRT, as this ideally is a replacement of the lost endogenous estrogen. All results were adjusted for the use of HRT, but the differences were still significant, indicating that the use of HRT did not have an impact on the somatic complaints. It has to be kept in mind that only 40 per cent of the RRSO group used HRT at the time of the study, and this material is not suitable to evaluate the effects of HRT use on somatic complaints.

In the analyses of the RRSO subgroup that underwent RRSO before the age of 50 years compared to those that had surgery at or beyond the age of 50 years, only palpitations were significantly more prevalent in the younger age group. This finding may support the assumption of a multifactorial explanation of the somatic complaints apart from loss of estrogen.

In the comparison of the subgroups with and without a history of cancer, the differences in somatic complaints were surprisingly few, and only the finding of more nausea in the cancer group reached significance. The presence of nausea, especially associated with adjuvant chemotherapy, has been documented in both breast and ovarian cancer patients (289). As the RRSO group had more cancer than the control group, this finding was not surprising. We do not know how many patients who currently received chemotherapy at the time of the study or the time since the last chemotherapy treatment. To our knowledge, no studies have examined the prevalence of somatic complaints in patients who have undergone RRSO with and without a history of cancer, and these findings are in need of further investigation.

5. General conclusions

The main findings in this study of women who have undergone RRSO because of risk of HBOC are:

1) Paper I
The RRSO and control groups had the same prevalence of metabolic syndrome. In multivariate analyses of the total sample, RRSO was significantly associated with metabolic syndrome, as was increasing age and BMI.

2) Paper II
Women who had undergone RRSO had a more favourable CHD risk profile than population-based controls. The RRSO group had a lower Framingham total score than controls, and in multivariate analyses of the total sample RRSO was associated with lower Framingham risk score.

3) Paper III
Women who had undergone RRSO had similar levels of QoL and fatigue as controls from the general population. Women who had RRSO and a history of cancer, had lower levels of QoL and more fatigue compared to the RRSO group without cancer.

4) Paper IV
Women who had undergone RRSO had the same levels of anxiety, but significantly lower levels of depression and total mental distress compared to controls from the general population. In multivariate analyses of the total sample, RRSO was negatively associated with depression and total mental distress. The RRSO group had more palpitations, constipation, pain and stiffness, osteoporosis and musculoskeletal diseases than the control group.

6. Implications for clinical practice

The CVD and CHD findings in paper I and paper II merit further research. Because possible presence of selection bias in the current thesis, these issues should be investigated in new, longitudinal studies. Previous studies performed of samples at average risk for ovarian cancer with bilateral oophorectomy from several benign indications, suggest increased risk of CVD. Therefore, medical follow-up should aim to identify risk factors for CVD and CHD in women who have undergone RRSO. Looking for the contributors of risk included in the metabolic syndrome algorithms may be an efficient way of identifying risk in women who have undergone RRSO.

Our results indicate that women who have undergone RRSO have levels of fatigue and QoL that are comparable to the general population. In addition to this, they had lower levels of mental distress. We do not have baseline measurements, and we can therefore not assume that the levels of fatigue, QoL and mental distress have been changed after RRSO. The RRSO subgroup with a history of cancer had higher levels of fatigue and lower levels of overall QoL. Medical follow-up after RRSO should therefore pay attention to the subgroup that have a history of cancer.

The RRSO group had more somatic morbidity than the control group. It is important to be aware of the risk of osteoporosis and musculoskeletal disease in post-RRSO women. Also, doctors should be aware of symptoms like palpitations, pain and stiffness and constipation in women who have undergone RRSO, and carefully evaluate possible somatic or mental causes, as well as need for HRT.

7. Future studies

A prospective study of patients belonging to HBOC families would represent a more robust design, for example by including all women who were referred to RRSO after genetic testing or genetic counselling. This approach would give opportunity to obtain baseline measures, and the identification of an HBOC cohort. An HBOC cohort allows evaluation of external validity by attrition analyses of those who are lost to follow-up. In addition to this, the cohort could be followed through the RRSO procedure and collection of post-surgery data. A cohort design requires considerable resources and is probably best performed as a multicentre study. Several studies compare BRCA mutation carriers or women at risk for HBOC who have undergone RRSO to controls who chose surveillance. This approach is vulnerable to selection biases because the selection of treatments is not performed randomly. The women who choose RRSO may differ from the ones who choose surveillance, both regarding general health and penetrance of their mutation. It would not be ethically or scientifically justifiable to perform randomized studies in HBOC samples. Therefore, all future follow-up studies will face the problem of representativeness, although some of these confounders can be adjusted for.

Some questions have been answered by the studies presented in this thesis, but more research is needed regarding BRCA mutation carriers, RRSO and long-term non-oncologic morbidity. Important themes are:

- Long-term cardiovascular morbidity and mortality measured with hard end-points after bilateral salpingo-oophorectomy and RRSO
- Cardiovascular morbidity and mortality in subgroups that have undergone RRSO before and after natural menopause
- The relation between CHD risk and novel risk markers as high-sensitive CRP, apolipoprotein A, apolipoprotein B, lipoprotein (a) and homocysteine after RRSO
- Menopausal symptoms in relation to hormonal status after RRSO
- Sexual functioning in relation to hormonal status and body image after RRSO
- The relation between metabolic syndrome and levels of testosterone, estrogen and SHBG after RRSO

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9. Appendix

Table B2. Estimate of 10-Year Risk for **Women** (Framingham Point Scores)

Age, y	Points
20-34	-7
35-39	-3
40-44	0
45-49	3
50-54	6
55-59	8
60-64	10
65-69	12
70-74	14
75-79	16

Total Cholesterol, mg/dL	Points				
	Age 20-39 y	Age 40-49 y	Age 50-59 y	Age 60-69 y	Age 70-79 y
<160	0	0	0	0	0
160-199	4	3	2	1	1
200-239	8	6	4	2	1
240-279	11	8	5	3	2
≥280	13	10	7	4	2

	Points				
	Age 20-39 y	Age 40-49 y	Age 50-59 y	Age 60-69 y	Age 70-79 y
Nonsmoker	0	0	0	0	0
Smoker	9	7	4	2	1

HDL, mg/dL	Points
≥60	-1
50-59	0
40-49	1
<40	2

Systolic BP, mm Hg	If Untreated	If Treated
<120	0	0
120-129	1	3
130-139	2	4
140-159	3	5
≥160	4	6

Point Total	10-Year Risk, %
<9	<1
9	1
10	1
11	1
12	1
13	2
14	2
15	3
16	4
17	5
18	6
19	8
20	11
21	14
22	17
23	22
24	27
≥25	≥30

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II

CORONARY HEART DISEASE RISK PROFILE IN WOMEN WHO UNDERWENT SALPINGO-OOPHORECTOMY TO PREVENT HEREDITARY BREAST OVARIAN CANCER

Trond M. Michelsen, MD[1,2], Serena Tonstad, MD, PhD[3]

Are H Pripp, MSc, PhD[4], Claes G Tropé, MD, PhD[5], Anne Dørum, MD, PhD[5]

[1] Department of Gynecology, Sørlandet Hospital, 4809 Arendal

[2] National Resource Centre for Women's Health, Oslo University Hospital, 0027 Oslo, Norway

[3] Department of Internal Medicine, Ullevål, Oslo University Hospital, University of Oslo, Division Ullevål University Hospital, 0407 Oslo, Norway

[4] Department of Biostatistics, Rikshospitalet, Oslo University Hospital, 0027 Oslo, Norway

[5] Department of Gynecologic Oncology, The Norwegian Radium Hospital, Oslo University Hospital, 0310 Oslo, Norway

Corresponding author:

Trond Melbye Michelsen, MD

Department of Gynecology, Sørlandet Hospital Arendal,

Service box 605, N-4809 Arendal, Norway

Phone 47-37014000, Fax 47-37014041

Mail: trond.melbye.michelsen@sshf.no

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Disclosure of interests

The authors declare that there are no conflicts of interest.

Abstract

Introduction

We examined coronary heart disease risk profile in women from hereditary breast ovarian cancer families who had undergone risk-reducing salpingo-oophorectomy, and compared the results to controls from the general population.

Methods

A sample of 326 (65% of invited) women with previous risk-reducing salpingo-oophorectomy after genetic counseling provided data and blood samples (cases). Controls were 1,630 age-matched women from a Norwegian population-based health study. We examined coronary heart disease risk profile and Framingham risk score in both groups.

Results

The cases had a lower mean Framingham total score compared to controls (12.9 [SD 5.1] versus 14.5 [SD 5.2]; p=0.02). Except for higher waist circumference, the cases had a more favorable coronary heart disease risk profile including more physical activity, lower levels of total cholesterol, higher levels of HDL-cholesterol, lower systolic blood pressure, and lower body mass index compared to controls. In multivariate logistic regression analyses, belonging to the risk-reducing salpingo-oophorectomy group was inversely associated with Framingham 10-year risk score ≥5% (odds ratio 0.49, 95% CI [0.34, 0.71]; p<0.001). Lower levels of education, not having paid work, a history of stroke and greater waist circumference were significantly associated with Framingham risk score >10% in the total sample.

Conclusion

Self-selection of women seeking risk-reducing salpingo-oophorectomy, changes in lifestyle after surgery, and survival bias may explain that women who underwent risk-reducing salpingo-oophorectomy had a more favorable coronary heart disease risk profile compared to

controls. Longitudinal studies are needed to further clarify the associations observed in this cross-sectional study.

Introduction

Risk-reducing salpingo-oophorectomy (RRSO) is the most effective method for preventing ovarian cancer in women at risk for hereditary breast ovarian cancer. RRSO leads to an 80% reduction in risk of ovarian and fallopian tube cancer (1). RRSO before menopause induces immediate surgical menopause, which may be associated with cardiovascular diseases (CVD).

Atsma et al. (2) demonstrated in a meta-analysis of 18 studies that bilateral oophorectomy before the age of 50 years substantially increased the risk of CVD. In two prospective studies, American women who had undergone bilateral oophorectomy before the age of 45 years had increased total and cardiovascular mortality compared to control groups without such surgery (3;4). In sub-analyses from the Women's Health Initiative (WHI) study, hysterectomy with bilateral oophorectomy was an independent predictor of higher Framingham risk score (5). The American Nurses' Health Study (6) demonstrated an increased risk of coronary heart disease (CHD) in the subgroup that had undergone bilateral oophorectomy with no subsequent use of estrogens. These studies examined CVD or CHD after bilateral oophorectomy for several different indications.

The reduction in estrogen levels after surgical menopause may lead to a rise of atherogenic lipoproteins and enhanced low-density lipoprotein (LDL) oxidation, which both are factors that may accelerate the development of CHD (7).

However, many factors other than lipids contribute to the CHD risk. The Framingham risk score estimates the 10-year risk for a CHD event based on an algorithm including age, level of total cholesterol, smoking status, and level of HDL–cholesterol, level of systolic blood pressure and use of antihypertensive medication (8). An increased Framingham risk score identifies individuals at future risk for CHD and allows targeted preventive efforts.

In a Norwegian population-based sample, our group reported that bilateral oophorectomy due to benign diseases before the age of 50 years was associated with a higher

Framingham risk score (9). We also reported a significant association between RRSO and metabolic syndrome in women at risk for hereditary breast ovarian cancer (10). These findings triggered further examination of CHD risk after RRSO, which is an important issue for carriers of BRCA mutations facing the option of prophylactic surgery. In contrast to previous studies, the present study allowed us to examine CHD risk profiles in a sample of women who all belonged to hereditary breast ovarian cancer families and had undergone RRSO.

Our primary goal was to examine CHD risk profile and Framingham risk score in women at risk for hereditary breast ovarian cancer at a follow-up examination after RRSO. We also compared the CHD risk profile of the RRSO women to age-matched women from the general population with intact ovaries. Finally, we examined variables associated with increased Framingham risk score in the total sample (cases and controls).

Materials and Methods

Study population

Cases

Through surgical records from three university hospitals in Norway we identified a sample of 515 women from families with hereditary breast ovarian cancer who had undergone RRSO from 1980 to 2005 after genetic counseling at The Norwegian Radium Hospital, Oslo, Norway. Of the 515, 12 women (2%) were not alive at 1st of January 2006 according to the National Death Registry. The 503 women alive were invited to the study and sent a mailed questionnaire. Non-respondents were sent one reminder three weeks later. Among the women invited, 361 (72%) responded, and 326 (65%) delivered completed questionnaires, physical measures, and blood samples.

Controls

The Health Study of Nord-Trøndelag County of Norway (HUNT-2) was carried out in a mixed rural and urban area in1995-97. All inhabitants aged 20 years and above were invited to a general health study, described in detail elsewhere (11). Of the 46,709 women invited, 34,518 (74%) between 20 and 98 years participated, and 28,025 were between 30 and 79 years. Among these, 25,529 had completed the questionnaires relevant for this study. We excluded women with cancer (N=1,192), women who had answered either "yes" or "do not know" to the questions: "have you had one ovary removed?" or "have you had both your ovaries removed?" (N=1,710) or "have you had your uterus removed" (N=975), and women who had incomplete data for calculating Framingham risk score (N=40). Of the 21,612 potential controls left, we randomly allocated five age-matched controls per case (N=1,630).

Measurements

Physical measurements and blood sampling

Both the RRSO and control group received a mailed questionnaire. The control group got an appointed date for physical measurements and blood tests, while the RRSO group made an appointment at their regular general practitioner's office.

In the RRSO group, blood samples were analyzed at Sørlandet Hospital, Arendal. The general practitioners were asked to measure blood pressures using a described procedure; Systolic and diastolic blood pressures were measured based on. Cuff size was adjusted after measuring the arm circumference. The cuff was placed on the upper arm and the blood pressures measured three times after five minutes rest. The mean of the second and third measurements was used.

In the control group, blood samples were analyzed at Levanger Hospital. Systolic and diastolic blood pressures were measured by specially trained nurses using a Dinamap 845XT (Criticon) based on oscillometry. Cuff size was adjusted after measuring the arm circumference. The measurements were started after the participant had been seated for two minutes with the cuff on the arm, and blood pressure was measured three times at one-minute intervals. The mean of the second and third reading was used in this study.

In both groups, total and HDL cholesterol was measured with an enzymatic colorimetric cholesterol esterase method on a Hitachi 911 auto-analyzer, and the waist circumference was measured above the iliac crest.

Questionnaire variables

The questionnaire administered to both groups covered demographic characteristics, somatic and mental morbidity, types of impairment, use of medication as well as life-style and health-related behavior.

Educational level was dichotomized into low (≤12 years) and high (>12 years) based on the number of completed school years. *Paid work* was defined as having income from employment or independent business, and employment was dichotomized into paid work and not paid work. The latter group consisted of housewives, participants on sick leave, students and pensioned participants. Women who were married or lived in a paired relationship were defined as *cohabiting*.

Current use of HRT consisted of those who answered "yes" to the question "Do you use estrogen pills or patches?" *Physical activity* was categorized as having minimal or moderate or more physical activity (12). *Smoking* concerned those with daily smoking of any number of cigarettes. The diseases *angina*, *myocardial infarction*, *stroke* and *diabetes* were

reported as present if ever diagnosed by a medical doctor. *Having had cancer* was defined by report of a positive history of any kind of cancer.

BRCA mutation status was defined by the responses to the question: "Did you have surgery because of a positive genetic test?" We checked the medical records of those not responding to this question. Our data do not discriminate between those who had a negative mutation test of unknown significance and those who did not have a test due to unknown mutations. We did not have data on BRCA mutation status in the control group.

Framingham risk score

We used the definition of Framingham risk score published by the National Cholesterol Education Program's Adult Treatment Panel III (8). The Framingham total score was calculated from weighted scores of age, level of total cholesterol, smoking status, and level of HDL–cholesterol, level of systolic blood pressure and use of antihypertensive medication. The total score was then converted into the Framingham risk score estimating a percentage risk of a CHD event within the next 10 years (8).

Statistics

Data were described by mean and standard deviations (SD) for continuous and by proportions for categorical variables. Differences between groups were assessed with t-test and chi-square-test, and adjustments were performed by linear and logistic regression analyses. The Framingham risk score ≥5% and >10% was analyzed by multivariate logistic regression with RRSO, history of cancer, level of education, paid work, cohabitation status, use of HRT, level of physical activity, history of stroke, waist circumference and BRCA mutation status as independent variables. These variables were chosen because they were known to be

associated with CHD risk (13), and because they differed between the RRSO and control groups. Age, smoking, blood pressure, total cholesterol and HDL cholesterol were not included as independent variables since they were part of the Framingham risk score algorithm.

We compared the RRSO participants who had RRSO before the age of 45 years (N=114) and before the age of 52 years (N=221) to separately allocated age-matched controls to evaluate whether these groups were worse off regarding Framingham risk score.

We examined possible presence of multi-collinearity in the multiple logistic regression models by using SPSS tools for collinearity statistics in linear models. The strength of association of each independent variable was expressed as odds ratio (OR) with 95% confidence intervals (CI). The level of significance was set at $p<0.05$ and all tests were two-tailed. SPSS version 15.0 (SPSS Inc., Chicago, Il, USA) was used for the statistical analyses.

Ethics and consent issues

The RRSO Study was approved by the Regional Ethics Committee of the Southern Norway Health Region and the Norwegian Data Inspectorate. The HUNT-2 study was approved by the Regional Ethics Committee of the Mid-Norway Health Region. All participants in both studies delivered written informed consent.

Results

Demographic variables

Women in the RRSO group were similar in age (mean [SD], 54.4 [8.9] years) compared to non-participants (mean [SD], 54.7 [10.4]; p=0.79). At the time of RRSO, participants had a mean age of 48.0 (SD 7.8) years compared to 48.7 (SD 9.3) years for non-participants (p=0.47). Mean time since RRSO was 6.5 years (SD 4.4). In the RRSO group, 93/326 (29%) had a history of any kind of cancer, and 75/326 (23%) had breast cancer, while 60/1,630 (4%) had a history of any kind of cancer in the control group. In the RRSO group, 64/321 (20%) had a positive BRCA mutation test, while the BRCA mutation status could not be documented in five cases. A significantly larger proportion of the RRSO group had paid work and more than 12 years of education compared to controls. Significantly more RRSO patients than controls were cohabiting (Table 1). Of the participating women, 221/326 (68%) underwent RRSO before the age of 52 years, which was the average age of natural menopause in Norway.

Life style variables and medication
The RRSO group used more HRT and reported more physical activity than controls (Table 1).

Physical measures and blood tests
After adjustments for having had cancer, level of education, employment status, cohabitation status, current use of HRT, and level of physical activity the RRSO group had significantly lower levels of mean total cholesterol, higher levels of mean HDL-cholesterol, lower mean systolic blood pressure, lower mean BMI, but higher mean waist circumference compared to controls (Table 2).

Framingham risk score and associated covariates

After adjustments for having had cancer, level of education, employment status, cohabitation status, current use of HRT, and level of physical activity, the mean Framingham total point score was significantly lower in the RRSO group than the control group (Table 2). In the subgroups that underwent RRSO before the age of 45 years and before the age of 52 years, Framingham scores were significantly lower than among age-matched controls ($p<0.03$ and $p<0.01$, respectively, data not shown). Significantly fewer women in the RRSO group had Framingham risk score $\geq 5\%$ (Table 2).

In the multivariate logistic regression analyses belonging to the RRSO group was negatively associated with Framingham risk score $\geq 5\%$ ($p<0.001$) (Table 3). A history of cancer, lower levels of education, not having paid work, lower levels of physical activity, a history of stroke, and greater waist circumference were significantly associated with Framingham risk score $\geq 5\%$ in multivariate analyses (Table 3). Lower levels of education, not having paid work, a history of stroke and greater waist circumference were significantly associated with Framingham risk score $>10\%$ (Table 3).

Discussion

Except for significantly higher waist circumference, the RRSO group had a healthier CHD risk profile with more physical activity, lower levels of total cholesterol, higher levels of HDL-cholesterol, lower systolic blood pressure, and lower body mass index compared to controls. In multivariate logistic regression analyses, Framingham 10-year risk $\geq 5\%$ was inversely associated with being a RRSO case, and the RRSO group had a lower Framingham score than controls. To our knowledge, this is the first study to examine Framingham risk score in women with RRSO in hereditary breast ovarian cancer families, as previous studies have studied CHD risk after bilateral oophorectomy for many different indications.

Our findings are in contrast to the previous studies that have linked bilateral oophorectomy to increased risk of CHD (2;5;6;14). Studies performed in European populations indicate that socioeconomic differences, as found in the present study, may affect CHD risk prediction (15). The Framingham risk score has not been validated as a predictor of CHD in Nordic women, and the value of this instrument may differ in different populations (16). On the other hand the Framingham risk score is based on the presence of classical CHD risk factors that confer risk in all populations.

Another possible explanation of the finding is that the surgical menopause induced by RRSO may lead to alterations in body fat distribution with increased waist circumference and central obesity. Central obesity, either caused by visceral obesity or subcutaneous fat accumulation, is strongly correlated with metabolic syndrome and CHD risk (17), but central obesity is not included in the Framingham risk score. The omission of central obesity may explain the contrast to our former finding of an association between RRSO in hereditary breast ovarian cancer families and metabolic syndrome (10).

It is reasonable that it takes time before estrogen deficiency leads to metabolic disturbances with such impact that the Framingham risk score is altered, and the median time since surgery of six years in this study may not be sufficient to detect such effects.

The RRSO group had a higher level of physical activity and a lower proportion of smokers than controls. These findings may be due to changed lifestyle behavior, implying that women diagnosed to be at risk for hereditary breast ovarian cancer and performing RRSO have established a healthier lifestyle with less smoking and more physical activity. The RRSO group may also have had a healthier lifestyle before the RRSO procedure, but we do not have data to investigate that.

Notably the use of estrogen therapy was not significantly associated with either higher or lower CHD risk in the RRSO sample. Indeed, the WHI study demonstrated that primary

prophylaxis against CHD is no indication for use of estrogens (18), but later publications have questioned the timing of HRT use in the original study (19). The RRSO group had significantly more HRT users, and according to the WHI study such use should increase the risk of CHD. However, the WHI group initiated HRT in postmenopausal women while the majority of RRSO procedures in this study were performed before natural menopause.

Women who have undergone RRSO may theoretically have an increased risk of CHD because bilateral oophorectomy before natural menopause removes endogenous estrogen. Loss of estrogen can accelerate the atherosclerotic process (7). In our study, about two thirds of the women went through RRSO before the average age of menopause, and the excess risk of CHD should be limited to these two thirds. However, we found in separate analyses that the Framingham total score was significantly lower in the RRSO subgroups with surgery before the age of 45 years and before the age of 52 years compared to their age-matched controls. This finding is in contrast to previous studies (4;14), who reported increased risk of CVD in women who underwent bilateral oophorectomy before the age of 45 years. None of these two publications studied high-risk women, however.

Strengths and limitations

A strength of the study is that the HUNT sample covers a total population within a geographical area and has a high participation rate (11).

The responder rate in the RRSO group of 72% at a median of five years follow-up is considered to be acceptable. Use of established instruments for evaluating cardiovascular risk is a strength of the study. All participants in the RRSO group went through surgery after genetic counseling at the same hospital. The RRSO group delivered blood samples that all were analyzed in the same laboratory, and samples from cases and controls were analyzed using the same type of machinery.

The interpretation of our findings should take the study's limitations into consideration. Self-reported pelvic surgery in the control group is considered a weakness. Earlier trials, like the Nurses´ Health Study (6), found that the reliability of self-reported medical history and pelvic surgery was good.

This study estimates future risk of a CHD event, and it was not designed to assess the true incidence of CHD. We were unable to assess risk factors in non-respondents, as we did not have access to their medical records. The cross-sectional design of the study means that causality in the relation between RRSO and low risk scores cannot be assumed.

There is no reason to believe that premenopausal oophorectomy has a positive impact on CHD risk profile. Rather, it is probable that women who seek RRSO have a different lifestyle, belong to a different socioeconomic group and have a different CHD risk prior to RRSO than the average woman.

It is possible that women with an increased risk of CHD tended to choose alternative options to RRSO. In that case, CHD risk could predict the RRSO decision, thus affecting the risk profile in the RRSO sample chosen, and RRSO could still be associated with CHD despite the findings in this study.

As we included post-RRSO patients who were alive at a median of six years after surgery, the data set may be influenced by a survival bias. In the initial material, 12 of the 515 identified cases were deceased. We may have invited an RRSO group that was healthier than the cohort who initially went through surgery, although it is not likely that such a minor proportion would have major impact on the analyses.

The most important source of selection bias in this study is the self-selection of cases. Given the solid documentation of the risk-reducing effects of RRSO, it will not be possible, or ethically justifiable, to conduct a study where participants are randomized to RRSO or surveillance. Follow-up studies of women who have undergone RRSO will therefore be

subject to systematic errors. As we included cases based on surgical records, we do not know to which degree our sample is representative of the cohort of women seeking genetic counseling.

This study includes some of the first women to undergo RRSO in Norway. These women may belong to a higher socioeconomic group, have higher levels of education, and be more proactive according to healthcare and their own health. A previous study (20) demonstrated that rates of BRCA test use were higher in families with higher socioeconomic status. The mentioned biases may all lower the risk of CHD, and therefore lower the Framingham risk score estimates in the RRSO group.

Conclusion

Uptake of genetic counseling and self-selection of women seeking RRSO, changes in lifestyle behavior after surgery, and survival bias may explain that women at risk for hereditary breast ovarian cancer with RRSO had a more favorable coronary heart disease risk profile compared to controls. The finding deserves further investigation in longitudinal studies.

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Table 1. Demographic and lifestyle variables in the RRSO control groups

	RRSO (N=326)	Controls (N=1,630)	P
	Mean (SD)	Mean (SD)	
Age at survey	54.4 (8.9)	54.5 (9.0)	0.93
Age at RRSO	48.0 (7.8)		
Years since RRSO	6.5 (4.4)		
	N/total (%)	N/total (%)	
Having cancer	93/326 (29)	60/1,630 (4)	<0.001
Having breast cancer	75/326 (23)		
BRCA mutation positive	64/321 (20)		
Higher level of education	152/312 (49)	317/1,566 (20)	<0.001
Paid work	205/313 (66)	956/1,630 (59)	0.02
Cohabiting	262/315 (83)	1230/1,630 (76)	0.003
Current use of HRT	127/326 (39)	213/1,630 (13)	<0.001
Moderate or more physical activity	283/326 (87)	1168/1,630 (72)	<0.001

Abbreviations**:** : RRSO. risk-reducing salpingo-oophorectomy; SD. standard deviation; BRCA, breast cancer susceptibility gene; HRT, hormonal replacement therapy.

Table 2. Characteristics of the RRSO and control groups in relation to risk factors for CHD and Framingham risk score

	RRSO (N=326)	Controls (N= 1,630)	P*
	N (%)	N (%)	
Smoking	70/326 (22)	489/1,630 (30)	0.18
History of angina	7/326 (2)	44/1,627 (3)	0.48
History of myocardial infarction	2/326 (1)	21/1,628 (1)	0.77
History of stroke	5/326 (2)	23/1,622 (1)	0.44
Diabetes	6/326 (2)	37/1,626 (2)	0.95
	Mean (SD)	Mean (SD)	
Total cholesterol, mmol/L	5.8 (1.2)	6.3 (1.2)	0.001
HDL – cholesterol, mmol/L	1.7 (0.4)	1.5 (0.4)	<0.001
Systolic blood pressure, mmHg	128 (17)	139 (23)	<0.001
Diastolic blood pressure, mm Hg	79 (10)	81 (12)	0.18
BMI, kg/m^2	25 (4.0)	27 (4.5)	0.001
Waist circumference, cm	87 (12)	83 (11)	<0.001
Framingham total point score	12.9 (5.1)	14.5 (5.2)	0.02
	N (%)	N (%)	
Framingham risk score ≥5%	70/326 (22)	611/1,630 (38)	0.006
Framingham risk score >10%	30/326 (9)	262/1,630 (16)	0.43

Abbreviations: CHD, coronary heart disease; RRSO. risk-reducing salpingo oophorectomy; SD. standard deviation; HDL, high density lipoprotein; BMI, body mass index.

* p-values adjusted for having had cancer, level of education, employment status, cohabitation status, current use of HRT, and level of physical activity.

Table 3 Multivariate binary logistic regression of Framingham risk score ≥5% and Framingham risk score >10% in the total sample (RRSO+controls)

Characteristics	Framingham risk score ≥5%			Framingham risk score >10%		
	OR	95%CI	p	OR	95%CI	p
RRSO (ref: control)	0.49 (0.33, 0.72) <0.001			0.61 (0.35, 1.05) 0.07		
History of cancer (ref: no cancer)	1.72 (1.13, 2.64) 0.01			1.33 (0.78, 2.29) 0.30		
Lower level of education (ref: higher level)	2.82 (2.08, 3.83) <0.001			3.44 (2.05, 5.78) <0.001		
No paid work (ref: paid work)	2.14 (1.73, 2.65) <0.001			2.72 (2.03, 3.63) <0.001		
Not cohabiting (ref: not cohabiting)	1.06 (0.83, 1.35) 0.66			0.90 (0.65, 1.25) 0.53		
Current use of HRT (ref: no use of HRT)	0.88 (0.65, 1.19) 0.40			1.01 (0.67, 1.51) 0.98		
Lower level of activity (ref: moderate or more)	1.47 (1.16, 1.87) 0.002			1.25 (0.92, 1.68) 0.16		
History of stroke (ref: no history of stroke)	5.19 (1.90, 14.2) 0.001			3.51 (1.50, 8.18) 0.004		
Waist circumference	1.03 (1.02, 1.04) <0.001			1.03 (1.02, 1.04) <0.001		
BRCA positive (ref: not BRCA postitive)	1.05 (0.50, 2.21) 0.89			1.47 (0.58, 3.78) 0.42		

Abbreviations: OR, odds ratio; CI, confidence interval; RRSO, risk-reducing salpingo oophorectomy; ref, reference value; HRT, hormonal replacement therapy; BRCA, breast cancer susceptibility gene.

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