General introduction
This thesis entitled ‘Beyond risk-reducing salpingo-oophorectomy: on breast cancer risk and bone health’ aims to increase knowledge of the effects of premenopausal risk-reducing salpingo-oophorectomy (RRSO) on breast cancer risk and bone health in women with a hereditary increased risk of breast and ovarian cancer. This general introduction summarises theories and background literature on RRSO in women at hereditary increased risk for breast and ovarian cancer, discusses existing knowledge on the consequences of RRSO on breast cancer risk and bone health, and identifies knowledge gaps. Furthermore, an overview of the aims and outline of this thesis are given.

**Risk-reducing salpingo-oophorectomy**

RRSO is the surgical removal of the fallopian tubes and ovaries, to reduce ovarian cancer risk. RRSO at premenopausal age is advised to women at hereditary increased risk of ovarian cancer, particularly women with hereditary breast and ovarian cancer (HBOC) syndrome caused by germline mutations in the BRCA1 or BRCA2 genes.

**Germline BRCA1 and BRCA2 mutations**

Women with a germline mutation in one of the BRCA1 or BRCA2 genes have substantially higher breast and ovarian cancer risks compared to women from the general population. Worldwide, breast cancer is the most common cancer in women, with a cumulative lifetime risk (CLTR) of 12% at age 85 (1,2). In contrast, CLTRs for breast cancer in BRCA1/2 mutation carriers are estimated to be 60 - 80% (3-7). CLTRs for contralateral breast cancer are elevated and related to age at diagnosis of the first breast cancer, the use of adjuvant systemic therapy, time since first breast cancer and family history (7-12).

Ovarian cancer is less common in the general population, being the 7th cancer in women worldwide (2). In developed countries, it is the most lethal gynaecological cancer (2). Of all ovarian cancers, 90% are from epithelial origin. Of these, 70% are high grade serous epithelial cancers (13). Ovarian, fallopian tube and peritoneal serous epithelial cancer were previously considered different disease entities. However, they have similar histology and clinical behaviour and it is now postulated that the majority has a common origin in the distal fallopian tubes (14,15). Other histological subtypes of epithelial ovarian cancer include clear-cell, endometrioid, and mucinous, which originate from endometrial, germ cell or transitional cell tissue (14,15). When ovarian cancer is mentioned in this thesis, this implies all types of non-uterine serous epithelial cancer and all other types of epithelial ovarian cancer.
General introduction

In the general Dutch population, CLTRs for ovarian cancer are a little over 1% at age 85, while for \( BRCA1 \) and \( BRCA2 \) mutation carriers CLTRs are estimated to be 30 - 60% and 5 - 35% respectively (1,4-7). Mean age at ovarian cancer diagnosis is about 50 years in \( BRCA1 \) mutation carriers and 55 - 60 years in \( BRCA2 \) mutation carriers (16,17), while diagnosis before age 40 is rare (4,7). Most ovarian cancers in \( BRCA1/2 \) mutation carriers are high grade serous cancers (18). In 10 - 20% of ovarian cancers, germline \( BRCA1/2 \) mutations are found (19-24).

Reducing ovarian cancer risk in \( BRCA1 \) and \( BRCA2 \) gene mutation carriers

Up until 2009, \( BRCA1/2 \) mutation carriers in the Netherlands and other developed countries were offered ovarian cancer screening, with the aim of improving survival by diagnosing ovarian cancer at an early, curable stage. Screening consisted of annual pelvic examination, transvaginal ultrasound and serum tumour marker CA-125 testing (25). This screening protocol was proven to be ineffective (26-30). Many screening visits were necessary to detect a low number of ovarian cancers and interval cancers were common. Most importantly, both interval and screen detected cancers were advanced stage disease and no survival benefit of screening was observed (26-30). In contrast, RRSO is highly effective in reducing ovarian cancer risk. Two meta-analyses reported ovarian cancer risk reductions of 80% after RRSO in \( BRCA1/2 \) mutation carriers, although a residual risk of peritoneal cancer remained (31,32). Individual studies showed risk reductions up to 96%, associated with a younger mean age at RRSO (33,34). RRSO in \( BRCA1/2 \) mutation carriers reduces overall mortality up to 68% (32). The laparoscopic procedure of RRSO in \( BRCA1/2 \) mutation carriers was shown to be safe with low conversion and complication rates (35).

Therefore, since 2009, ovarian cancer screening has increasingly been discouraged. The most recent guideline recommends not to offer ovarian cancer screening to \( BRCA1/2 \) mutation carriers anymore (36). Currently, RRSO is advised to \( BRCA1 \) mutation carriers between age 35 and 40 and to \( BRCA2 \) mutation carriers between age 40 and 45, i.e. after childbearing is complete and before ovarian cancer incidence increases (4,36,37). Median age at natural menopause is 51 (38,39), thus RRSO in \( BRCA1/2 \) mutation carriers is advised between 6 and 16 years before the expected age of natural menopause.

Other indications for RRSO

In women from HBOC families without germline \( BRCA1/2 \) mutations, breast and ovarian cancer risks can be estimated by various models, and estimated risks depend on tumour characteristics and number and age of onset of affected
family members (40-42). The most recent Dutch guideline advises not to perform RRSO without previous genetic testing, and in families without a detectable BRCA1/2 mutation, to only perform RRSO in women whose CLTR of ovarian cancer estimated by a clinical geneticist exceeds 10%. This is the case in women with two or more relatives with ovarian cancer (including one first-degree relative) and women carrying a Lynch syndrome mutation (36). However, recommendations for RRSO in women with Lynch syndrome are under debate, because ovarian cancer associated with Lynch syndrome is often diagnosed in an early stage with frequently endometroid or clear cell histology, thus surveillance aiming at early detection seems a reasonable alternative (43).

Long-term health consequences of RRSO

RRSO at premenopausal age induces acute menopause at an early age, which results in short- and long-term health consequences. In order to understand possible consequences of RRSO, differences between natural and surgical menopause are explained below.

Natural menopause and ovarian steroid hormones

In premenopausal women, functional ovaries produce steroid hormones, including oestrogens (oestradiol, oestrone and oestrone sulphate), progesterone and androgens (dehydroepiandrosterone [DHEA], androstenedione and testosterone) (44,45). The maturing follicle produces oestrogens, progesterone and androgens, and stromal cells produce androgens (44,45). In the late reproductive years, typically after age 40, menstrual cycles are mostly ovulatory, but begin to shorten (46,47). Levels of oestrogens remain within normal ranges, while progesterone levels in the luteal phase of the menstrual cycle decrease (46,48). During menopausal transition, also called perimenopause, at a median age of 47, menstrual cycle length becomes irregular, levels of oestrogens start to fluctuate, with hyper- and hypo-oestrogenic cycles, and luteal phase progesterone levels decrease (39,46,49-51). After several years of irregular menses, women experience permanent cessation of menses and secretion of oestrogens and progesterone by the ovaries permanently stops (46,52). Natural menopause in women with an intact uterus is defined as the permanent cessation of menstrual periods resulting from the loss of ovarian follicular activity and can be established after 12 months of amenorrhea for which there is no obvious other pathological or physiological cause (53). Median age at natural menopause is 51 years (38,39). Levels of androgens in women decline with aging from the early reproductive
years (54). After natural menopause, ovarian stromal cells continue to produce androgens and the age-related decline of levels of androgens is not affected by natural menopause (45,54,55).

The adrenal glands also produce androgens (dehydroepiandrosterone sulphate, DHEA, androstenedione and testosterone) (44). Furthermore, 50% of testosterone production results from peripheral conversion of androstenedione by 17β-hydroxysteroid dehydrogenase in liver, adipose tissue and skin cells (44). The most potent androgen, dihydrotestosterone (DHT), is a product of peripheral conversion of testosterone by 5β-reductase in its target cells (44). Androgens can be converted to oestrogens in a reaction catalysed by the enzyme aromatase. In postmenopausal women, aromatase is primarily expressed in adipose tissue, skin fibroblasts, and in target tissues for oestrogens, such as breast, bone and brain cells (56). After natural menopause, levels of circulating oestrogens decrease, but target tissues locally produce oestrogens from circulating androgens (57).

Surgical menopause and ovarian steroid hormones
In contrast to gradual changes in ovarian steroid hormone levels during perimenopause and natural menopause, after surgical menopause, secretion of ovarian hormones is stopped acutely. As the indication for RRSO in BRCA1/2 mutation carriers is posed before the ovarian cancer incidence rises, this is mostly before the age of perimenopause or natural menopause. After surgical menopause, circulating levels of oestrogens and androgens are lower than after natural menopause (58).

Health consequences after natural and surgical menopause
Changes in ovarian steroid hormone levels during menopause are associated with several symptoms and health effects. Vasovagal symptoms, behavioural changes and decreased sexual desire may occur from perimenopause until long after menopause. Health effects that may become apparent after a longer time after menopause are decreased bone health, urogenital atrophy, increased risk of cardiovascular disease, changed risks of various cancers and possibly cognitive decline (45,46,59). Because surgical menopause occurs at a younger age, is more acute, and ovarian steroid hormone production is stopped completely, it is generally assumed that symptoms and health effects associated with menopause appear more rapidly and severely after surgical compared to natural menopause (60). This hypothesis is supported by the finding that surgical menopause before age 45 is associated with increased mortality in the general population (61).
Although premenopausal RRSO is currently the only proven effective measure to reduce ovarian cancer risk, a knowledge gap remains regarding its long-term health consequences (62-64). Knowledge on these long-term health consequences can be used to improve patient education, targeted prevention and treatment, and to avoid overdiagnosis and overtreatment. This thesis will focus on long-term consequences of RRSO on two health issues: breast cancer risk (Part I) and bone health (Part II).

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

Breast cancer and ovarian steroid hormones

Normal breast development as well as breast cancer risk are associated with circulating levels of ovarian steroid hormones. Actual mechanisms of the effects of ovarian steroid hormones on breast tissue are not fully understood.

Normal breast development

Human breast tissue contains multiple glandular structures formed by dichotomous branching of multiple ducts arising from the nipple. These ducts are lined with luminal epithelial cells, surrounded by a layer of myoepithelial cells and a basement membrane. The stromal tissue between the glandular structures consists of fat and fibrous tissue (65-67). Until puberty, breast growth is isometric in relation to the body under the influence of growth hormone (GH) and insulin-like growth factor 1 (IGF-1) (68). During puberty, the breast undergoes its first phase of allometric growth due to increasing volumes of ducts and stromal tissue (65). The ductal network grows and divides into bundles of primary and secondary ducts, ending with end buds. From the end buds and ductal side branches, terminal duct lobulo-alveolar units (TDLUs) form. In non-pregnant women, breast epithelium undergoes small cyclical changes in proliferative and apoptotic activity (66). During pregnancy, the breast undergoes its second phase of allometric growth. More TDLUs are produced and reach full differentiation to synthesize and secrete milk for lactation (66,69). After pregnancy and lactation, the mammary gland regresses to a pre-pregnancy state (69). After menopause, the breast undergoes involution by increasing atrophy of glandular tissue and fibrosis of the stromal tissues (70).

Normal breast development and ovarian steroid hormones

Ovarian steroids mediate their effect on breast tissue by binding to nuclear oestrogen (ER), progesterone (PR) and androgen receptors (AR), that subsequently
bind specific DNA sequences within gene promoter regions and regulate transcription (65,66,71). Two ER subtypes (ERα and ERβ), two PR isoforms (PRA and PRB) and one AR have been observed (71-73). The main receptor mediating the effects of oestrogens on breast tissue is ERα (74). ERα and PR are expressed in 15-30% of the luminal epithelial cells, where most proliferation occurs (65,66). PR expression is regulated by oestrogens (75,76). In normal breast tissue, cells expressing a single hormone receptor are more common than cells expressing both ERα and PR (77). Both oestrogens and progesterone mainly promote proliferation of ERα and PR negative cells through paracrine signals from ERα and PR positive cells (78-80). However, a small proportion (< 0.5 – 2%) of ERα and PR positive cells have proliferative capacity (81). AR is expressed in luminal cells, stromal fibroblasts and adipocytes (71). Androgens act directly on breast tissue by binding AR or indirectly after aromatisation into oestrogens (71). In normal breast tissue, androgens inhibit growth.

Women without functional ERα do not show breast development in puberty (82). Epithelial proliferation rates of breast tissue are maximal during the luteal phase of the menstrual cycle, when both oestrogens and progesterone are being secreted by the corpus luteum, and relatively low during the follicular phase, when progesterone levels are at their lowest (65,83,84). It is hypothesized that postmenopausal regression of breast tissue is a consequence of unopposed androgen action, as circulation oestrogen levels decrease more than androgen levels (71).

**Breast cancer and ovarian steroid hormones**

Higher endogenous postmenopausal levels of oestrogens are associated with increased breast cancer risk (85,86). Several but not all studies find increased breast cancer risks for women with higher premenopausal levels of oestrogens (87-95). However, numbers of premenopausal breast cancers were low in most studies and associations might be different according to phase of the menstrual cycle and ERα/PR status of the breast cancer (87-95). It remains unclear if premenopausal levels of oestrogens are associated with postmenopausal breast cancer risk (88). Studies on endogenous progesterone levels describe either no association with breast cancer risk or a protective effect of higher levels (87,88,90,92,96). Higher endogenous levels of androgens were associated with increased breast cancer risk in both premenopausal and postmenopausal women in some, but not all studies (86,88-90,97).

Next to endogenous ovarian steroid hormones, exogenous hormones affect breast cancer risk. Several meta-analyses concluded that current and recent use
of combined oral contraceptives are associated with increased breast cancer risk. Ten years after cessation of oral contraceptive use, breast cancer risk is comparable to the breast cancer risk in never users (98,99). Studies on the effect of duration of use, different formulations of oral contraceptives and the risk of ERα positive and negative tumours were inconclusive (98-103).

Observational studies report an increased breast cancer risk after postmenopausal hormonal replacement therapy (HRT) with oestrogens only, but randomised controlled trials (RCT) report no significant relation with breast cancer risk or indicate a reduced risk (104-112). Postmenopausal HRT with oestrogen-progestogen is associated with increased breast cancer risk in both observational studies and RCTs (104-107,113-115). Breast cancer risk was not increased in women using exogenous androgens, but only few long-term studies are available (97).

Oestrogens may increase breast cancer risk by paracrine ERα dependent mechanisms through enhanced cell proliferation, which is associated with a high risk of errors in DNA replication and activation of proto-oncogenes and oncogenes (116). In addition, oestrogens might increase breast cancer risk by ERα independent mechanisms through DNA damage induced by metabolites of oestrogens (116). A more recent theory is the cancer stem cell theory, where only a small subset of cancer stem cells are believed to drive and sustain tumour growth. Breast cancer stem cells lack ERα and PR expression or express it at very low levels, but are hypothesized to be influenced via paracrine mechanisms (117). Progesterone stimulates proliferation of mammary stem cells in mice and there is growing evidence that it stimulates breast cancer stem cells and thus promotes breast cancer risk in humans (75). It is unclear if circulating androgens can increase breast cancer risk directly, or only indirectly after aromatisation to oestrogens (97).

Other breast cancer risk factors and ovarian steroid hormones

Reproductive factors associated with longer exposure to endogenous oestrogens, such as earlier age at menarche and older age at menopause, are associated with higher breast cancer risks (118,119). Obesity and adult weight gain are associated with an increased risk of postmenopausal breast cancer, which has mainly been attributed to high endogenous levels of oestrogens as a result of the aromatisation of androgens in peripheral fat tissue (120-126).

Women with a younger age at first pregnancy have a lower risk of ERα and PR positive breast cancer than women who are older at first pregnancy or nulliparous women (127,128). Several explanations for this finding were proposed, including
persistent alterations in systemic hormone levels, growth factors, stromal composition, hormone responsiveness, maintained differentiation of epithelial cells and decreased number of stem cells (129-131). Recent studies suggest that early pregnancy reduces the proportion of hormone receptor positive cells and induces decreased proliferation of stem and progenitor cells (129,132). Breastfeeding has been shown to reduce breast cancer risk independent from parity (133-137). Postulated mechanisms for this risk reduction include delayed reestablishment of ovulatory cycles, changes in levels of circulating oestrogens, prolactin and GH, and increased numbers of differentiated and decreased numbers of proliferative epithelial cells (118,135-137). There are indications that breast feeding particularly reduces ERα and PR negative breast cancer risk (136,137).

Women with higher breast density at mammography are at increased risk of breast cancer and higher breast density can mask breast cancer on mammograms (138-140). Breast density declines with age, and this decline is steeper at time of menopausal transition and after surgical menopause (141). However, studies on the relation between ovarian steroid hormone levels and breast density have been inconclusive, some reported a positive association between endogenous levels of oestrogens and breast density, but most concluded they were independent risk factors (142-146).

Breast cancer in BRCA1/2 mutation carriers

Most invasive breast cancers in both the general population and BRCA1/2 mutation carriers are invasive carcinoma of no special type (previously invasive ductal carcinoma) (147,148). BRCA1 associated breast cancers are often of the basal subtype (i.e. with a genetic expression profile similar to myoepithelial breast cells (149)), a higher mitotic index, higher grade and triple-negative (i.e. ER, PR and Human Epidermal growth factor Receptor 2 (HER2) negative) (148,150-152). BRCA2 associated breast cancers are often of the luminal type (i.e. with a genetic expression profile similar to luminal breast cells (153)) and ERα and PR positive (154,155). For BRCA1 associated breast cancer, the percentage of ERα and PR positive cancers increases with age, while for BRCA2, this percentage decreases (152,156). It is hypothesized that both in luminal and basal breast cancer, the cell of origin might be the same luminal progenitor cell that may either be ERα positive or negative (157). The eventual phenotype likely depends on genetic background and specific DNA mutations and germline BRCA1 mutations may pre-programme cancer cells to a basal-like phenotype (157,158).
Chapter 1

**Breast cancer screening in BRCA1/2 mutation carriers**

Breast cancer screening in BRCA1/2 mutation carriers consists of annual MRI and clinical breast examination from age 25, to which annual mammography is added from age 30 (159). MRI is important in this screening protocol, because sensitivity of mammography was lower in BRCA1/2 mutation carriers, in dense breasts, and in women younger than age 50 (160-162). Several large prospective studies reported that multimodality breast cancer screening with mammography and MRI, sometimes also adding ultrasound and clinical breast examination, is effective (160,163-179). The evidence on survival in BRCA1/2 mutation carriers screened with MRI and mammography is limited, but recent studies indicate improved metastasis free survival in BRCA1 and overall survival in BRCA2 mutation carriers (180,181). To prevent first or second breast cancer, women with BRCA1/2 mutations may opt for risk-reducing mastectomy (RRM). RRM reduces breast cancer risk with about 90% for both first and contra-lateral breast cancer (182,183) and is associated with limited improved overall survival (184). RRM may also reduce fear of developing breast cancer and prevent breast cancer treatment related morbidity.

**RRSO and breast cancer risk**

Because levels of oestrogens and breast density are decreased after surgical menopause, and higher levels of oestrogens and breast density are both associated with increased breast cancer risk, breast cancer risk is likely to be reduced after RRSO. The acute cessation of ovarian progesterone and androgen production might add to this risk reduction. A decline in breast density after RRSO might also increase sensitivity of breast cancer screening by mammography.

**Breast cancer incidence after RRSO**

A protective effect of surgical menopause on breast cancer risk in the general population was shown in several population based studies (185-187). For both BRCA1 and BRCA2 mutation carriers, a meta-analysis combining all studies on breast cancer risk after RRSO until 2009 reported a 50% risk reduction (31). Because most breast cancers in BRCA1 mutation carriers are ERα negative, while in BRCA2 mutation carriers ERα positive tumours are common (152), it has been hypothesized that RRSO has a different effect on breast cancer risk in BRCA1 and BRCA2 mutation carriers (188). Three studies included in the meta-analysis studied breast cancer risk after RRSO for BRCA1 and BRCA2 mutation carriers separately, but they report inconclusive results and had limited power to identify
a possible difference due to low numbers of BRCA2 mutation carriers (31,189-191). A more recent prospective study showed a risk reduction in both BRCA1 and BRCA2 mutation carriers, however the risk reduction in BRCA2 mutation carriers (HR 0.36; 95% CI 0.16 - 0.82) appeared stronger than in BRCA1 mutation carriers (HR 0.63; 95% CI 0.41 - 0.96) (192). It was not known if reduced breast cancer risks after RRSO alter the effectiveness of multimodality breast cancer screening in BRCA1/2 mutation carriers. More recently, it was postulated that previously reported RRSO-induced breast cancer risk reductions were mainly the result of bias (193). This theory was not actual at the time our studies were performed and will be discussed in more detail in the discussion of this thesis.

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

Bone health and ovarian steroid hormones

Normal bone development as well as osteoporosis risk are strongly associated with circulating levels of ovarian steroid hormones and the underlying mechanisms are increasingly well understood.

Normal bone development

During childhood, bone is modelled by formation of new longitudinal and radial bone to achieve the final shape and size of the skeleton (194). During adulthood, bone is remodelled by resorption of old bone and formation and mineralisation of new bone, to maintain structure and strength. This is called bone turnover (194). Osteoclasts are responsible for bone resorption and osteoblasts for bone formation (195). After completion of their function, all osteoclasts undergo apoptosis after 1 to 25 days, and most osteoblasts after 1 to 200 days. Some osteoblasts reside in the bone matrix and transit into osteocytes. Osteocytes are stellate cells that regulate bone formation and resorption in response to mechanical factors (195). Bone resorption and formation are linked in time and space (195). Bone resorption precedes bone formation and the process of resorption is faster, thus after a sudden increase in remodelling rate, there is a temporary imbalance (194).

Normal bone development and ovarian steroid hormones

Prior to menarche, longitudinal and radial growth of the skeleton is influenced by GH, IGF-1, thyroid hormones and glucocorticoids (196). After menarche, oestrogens stimulate rapid mineralisation of the skeleton and additional longitudinal and radial growth (197). Mechanisms of bone mass acquisition during puberty and relative roles of oestrogens and androgens are not fully understood (198). Levels of
oestrogens and GH are tightly related during puberty and oestrogens stimulate growth through increased pituitary GH secretion after stimulation of ERα and ERβ in the hypothalamus and anterior pituitary (199). Testosterone stimulates GH secretion after conversion to oestrogens, but DHT can increase growth velocity independent of the GH – IGF-1 axis (199). In late puberty, oestrogens induce epiphyseal closure, by accelerating growth plate senescence (194,196,199). During puberty bone mineral density (BMD) increases until peak bone mass is reached at the age of about 25 to 35 years (194,196,197). Timing of peak bone mass within individuals varies between skeletal sites and bone compartment, and between individuals depending on genetic and epigenetic factors, sex, timing of adolescent growth spurt and lifestyle factors (198). After acquiring peak bone mass, age-related bone loss occurs. Rate of bone loss increases in perimenopause. Postmenopausal women experience rapid bone loss for 8 to 10 years, because bone resorption increases more than bone formation after cessation of ovarian production of oestrogens (196,197). The lifetime changes in BMD are shown in Figure 1.1.

**Figure 1.1: Hypothetical influence of RRSO at age 40 on femoral neck BMD**

Curves for childhood BMD up to age 16 (200) and adult curves (201) are not from the same population and peak bone mass is achieved at a relatively young age in this curve. Abbreviations: BMD is bone mineral density; NM is natural menopause; RRSO is risk-reducing salpingo-oophorectomy.

Postmenopausal bone loss is partially caused by deficiency of oestrogens (197). Oestrogens act directly on osteoblasts, osteoclasts, their precursors, and cells that modulate bone cell activity such as B-lymphocytes, by binding to ERα in the nucleus, on the cell membrane and in the cytosol, to regulate gene transcription.
Oestrogens promote osteoclast apoptosis and inhibit osteoblast and osteocyte apoptosis (196,197). In addition, oestrogens suppress receptor activation of nuclear factor kappa B ligand (RANKL) production in osteoblasts and B-lymphocytes. RANKL is required for osteoclast activation and survival (196,197,202). Also, oestrogens increase osteoprotegerin (OPG) production by osteoblasts, which neutralises RANKL (196,197). Deficiency of oestrogens leads to an increased RANKL/OPG ratio which favours bone resorption (197). Oestrogens modulate production of cytokines by bone marrow stromal mononuclear cells and osteoblasts that control osteoclast activity by paracrine action (197). Because bone loss starts in perimenopausal women, with often normal or high oestrogen levels and decreasing luteal phase progesterone levels, it is hypothesized that progesterone affects BMD as well (203). Progesterone might stimulate bone formation by osteoblasts, because progesterone stimulated osteoblast differentiation after oestrogen induction of PR in cell line studies (203). Markers of bone formation increased in perimenopausal women with ovulatory cycles compared to an-ovulatory cycles (203). Postmenopausal hormone therapy with oestrogen-progestin increased BMD more than oestrogen only preparations (204). Androgens affect bone cells directly by binding AR and indirectly after aromatisation to oestrogens (195). In vitro evidence suggests that testosterone and DHT act directly on osteoclast progenitors and mature osteoclasts to inhibit osteoclastogenesis and promote apoptosis. However, in mice with osteoclast-specific AR deletions, there is no change in osteoclast number or bone mass (195).

**Osteoporosis and ovarian steroid hormones**

Osteoporosis is a condition of increased fracture risk due to enhanced bone fragility, characterised by low bone mass and micro-architectural deterioration (202). The association between the decline of levels of oestrogens due to menopause and an increased risk of osteoporosis is long known (205). In the Netherlands, 43.1 per 1000 women are estimated to have osteoporosis, compared to 7.5 per 1000 men (206). From age 50, the year prevalence of osteoporosis is approximately five times as high for women as for men (206). Before age 50, fracture incidence is greater in men than in women, while after age 50, fracture incidence is about 50% higher in women than in men (207,208). Higher levels of endogenous circulating oestrogens and testosterone are associated with higher BMD in postmenopausal women (209-213). BMD is lower in adolescent women using combined oral contraceptives, while BMD in adult premenopausal women is similar in women who do and do not use oral contraceptives (214,215). In peri-
and postmenopausal women oral contraceptive use maintains BMD (214,215). No overall associations were described for oral contraceptive use and fracture risk, however, fracture risk might be increased in several subgroups, including long-term users (216). Postmenopausal HRT with oestrogens only or oestrogen-progestogen increased BMD and decreased fracture incidence (106,108,113,217-219).

**Other osteoporosis risk factors and ovarian steroid hormones**

Although many factors related to exposure to endogenous ovarian steroid hormones have been proposed to affect osteoporosis risk, few studies provide consistent results. A later age at menarche does not seem to be an important risk factor for low BMD (220,221). In women that never used HRT, lower age at natural menopause is associated with lower BMD and higher fracture risk after controlling for other risk factors for osteoporosis (222). Low body mass index (BMI) is a risk factor for most fractures, while higher BMI is associated with lower fracture risk, although these associations are not linear and not applicable to all fracture sites (223). Parity is associated with decreased hip fracture risk, which might be explained by higher exposure to oestrogens during pregnancy (224). However, other explanations were proposed including pregnancy associated weight gain, increased bone formation or altered loading of the hip joint (224,225).

**Fracture risk assessment related to T- and Z-scores**

Osteoporosis is diagnosed by measurement of BMD by dual energy X-ray absorptiometry (DXA), as advised by the world health organisation (WHO) in 1994 (226,227). Osteoporosis diagnosis is based on a T-score for BMD, representing the number of SDs an individual’s BMD differs from young adult mean BMD (peak bone mass). A T-score ≤ -1 and > 2.5 is considered osteopenia and a T-score of ≤ 2.5 is considered osteoporosis (226). However, these cut-offs are arbitrary and fracture risk increases gradually with decreasing BMD. Each SD decrease in BMD is associated with a two to three fold increase in fracture risk (228-230). Most fractures occur in persons with osteopenia, indicating an important role for other risk factors for fractures, next to BMD. Because BMD is strongly age-related, international guidelines advise to use Z-scores when evaluating fracture risk in premenopausal women (231). Z-scores represent the number of SDs an individual’s BMD differs from the mean BMD for their age. Figure 1.2 shows the relations between BMD in g/cm², T-scores and Z-scores.
Although BMD measurement by DXA is advised to diagnose osteoporosis, several other methods to evaluate bone health and estimate fracture risk are available. Bone turnover markers (BTMs) in blood and urine have been suggested to predict fracture risk independently of BMD (232). These BTMs are collagen breakdown products and other molecules released from osteoclasts and osteoblasts during the process of bone remodelling. Markers specific for bone formation include bone-specific alkaline phosphatase (BALP), osteocalcin (OC) and N-terminal propeptide of type 1 procollagen (PINP). Markers specific for bone resorption include N-telopeptide of type 1 collagen (NTX), C-terminal telopeptide of type 1 collagen (CTX) and pyridinoline cross-links (232). The use of BTMs in individual patients in clinical practice is yet limited, due to a large random within-patient variability, biologic variability, and poor standardization of assays (232). Serum PINP and CTX have been identified as most promising BTMs for clinical use by the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (233).

**Guidelines on fracture risk assessment and RRSO**

Current Dutch guidelines advise to measure BMD and perform vertebral fracture assessment in patients with a fracture at age ≥ 50 or in patients > 60 with a fracture risk score of more than four points. Within this risk score, bilateral oophorectomy
without HRT is considered a secondary cause of osteoporosis and is valued one point. Thus, systematic BMD measurement for women with surgical menopause is not advised, unless they have other risk factors for fractures (227). In addition, according to the Dutch guideline on hereditary ovarian cancer, women with RRSO and without a history of breast cancer can be prescribed HRT until the assumed age of natural menopause. BMD measurement can be performed in those women with RRSO before age 45 who do not use HRT (36).

**RRSO and bone health**

Because surgical menopause occurs at a younger age than natural menopause and ovarian steroid hormone production is stopped acutely and completely, it is likely that bone loss would occur at an earlier age and a faster rate than after natural menopause. This might lead to lower BMD and increased fracture risk in women after RRSO when compared to women of the same age without surgical menopause. In Figure 1.1, a dotted line represents the hypothetical course of BMD after surgical menopause.

**Bone health after RRSO**

Several retrospective observational studies reported deterioration of bone health after RRSO (64,234-236). However, most of these studies were small with few events, did not measure BMD systematically in a consecutive sample and often lacked a reference population without RRSO. Michelsen et al. reported that 8% of women with RRSO were ‘told by their physician that they had osteoporosis’, compared to 3% of age-matched controls ($p = 0.02$) (234). Challberg et al. described that in 119 women with RRSO before the age of 48, women who had ≥ 24 months of deprivation of oestrogens had significantly more osteopenia and osteoporosis (16%) than women without deprivation of oestrogens (46%; $p = 0.03$) (235). Cohen et al. reviewed the medical files of 226 women with RRSO, of whom 152 had DXA results available (236). Osteoporosis prevalence did not differ significantly between women with RRSO < age 50 (9%) or ≥ 50 (20%), while mean age in the RRSO ≥ 50 women was higher. Pezaro et al. described 61 women with premenopausal RRSO, of whom 35 reported to have a DXA scan of whom 13 reported to be diagnosed with osteoporosis (64). To date, no studies on BTMs after RRSO in *BRCA1/2* mutation carriers have been published. However, studies on BTMs after surgical menopause in the general population indicate that BTMs increase immediately after surgical menopause (237-239). Bone resorption markers increase faster than bone formation markers, but with time their ratios
normalise (237,238,240). Studies comparing BTMs in women with surgical and natural menopause provide conflicting results as some find increased BTMs after surgical menopause compared to natural menopause (241), while others find no differences (242,243).

Thus, although current literature supports the hypotheses of decreased BMD and increased fracture risk after RRSO, the published studies are liable to bias and do not provide solid comparisons to women without RRSO.

Outline of this thesis

This thesis aims to gain more knowledge on two specific consequences of RRSO: a possible positive effect on breast cancer incidence and a possible negative effect on bone health.

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

After RRSO, breast cancer risk is reported to be reduced by 50% compared to high risk women without RRSO, and breast density is assumed to decrease. These factors are likely to influence the effectiveness of the individual breast cancer screening modalities, with probably a higher sensitivity for mammography and less need for MRI-screening. Therefore, in Chapter 2, the effectiveness of breast cancer screening in BRCA1 and BRCA2 mutation carriers after RRSO was studied in a retrospective cohort study. Sensitivity, specificity, positive and negative predictive value of MRI, mammography and clinical breast examination after RRSO were evaluated and compared to the effectiveness of breast cancer screening before RRSO described in literature.

In Chapter 2, breast cancer incidence was higher than expected after RRSO based on earlier findings and theories. Therefore, in Chapter 3, the observed breast cancer incidence after RRSO was compared to expected incidence, using previous breast cancer risk estimates in BRCA1 and BRCA2 mutation carriers in the Northern Netherlands and based on a hypothetical risk reduction of 50%.

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

We hypothesized that after surgical menopause, BMD would decrease faster and fracture risk would increase more than after natural menopause. Therefore, in Chapter 4, current knowledge on bone health after surgical menopause was summarised in a systematic review and meta-analysis of BMD and fracture incidence, using studies that compared women after surgical menopause with age-matched women without surgical menopause.
Chapter 1

Because a deteriorating effect of RRSO was described in several studies, a consecutive cohort of women that had undergone RRSO because of familial or hereditary elevated risks of ovarian cancer, were invited for measurement of BMD by DXA, blood sampling and a questionnaire on fracture incidence and risk factors for osteoporosis. Chapter 5 describes the findings of this study with respect to BMD and fracture incidence. As BMD changes slowly, and fractures are long-term complications of low BMD, it would be practical to have early markers that can predict osteoporosis and future fracture risk. Bone turnover markers have been shown to predict changes in BMD and fracture risk in the general population, so they might be potential predictive markers of osteoporosis and fractures in women with RRSO. In Chapter 6 the results of the analysis of bone turnover markers in the cohort of women 5 years after RRSO were described.

In Chapter 7 the findings of the previous chapters are summarised and discussed in the context of current literature and knowledge. Suggestions for clinical implications of our results and for future research are provided.
PART I
Risk-reducing salpingo-oophorectomy and breast cancer risk