UiT Norges arktiske universitet

Faculty of Health Sciences

Department of Community Medicine

Female sex hormones and subtypes of breast cancer

The Norwegian Women and Cancer Study

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A dissertation for the degree of *Philosophiae Doctor*

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Summary

Background: Substantial evidence emphasizes a pivotal role of female sex hormones in the etiology of breast cancer. Intrinsic molecular subtypes of breast cancer represent distinct disease entities and have different etiological pathways, risk factors and prognosis. These molecular subtypes are commonly cross-classified as luminal A-like, luminal B-like, HER2-enriched, and triple-negative breast cancer (TNBC) based on surrogate clinicopathological criteria. The association between female sex hormones and subtypes of breast cancer is not fully identified.

Aim: The primary objectives of this thesis was to investigate the associations between endogenous (body fatness) and exogenous (oral contraceptives [OCs] and menopausal hormone therapy [MHT]) female sex hormones and subtypes of breast cancer in Norwegian women, and to determine whether these hormonal factors differentially affect the risk of various tumor subtypes.

Methods: This thesis used data from the Norwegian Women and Cancer Study (NOWAC) – a prospective national cohort of more than 170 000 women. Data on exogenous hormone use and body mass index (BMI) were collected from self-administered questionnaires. Incident breast cancer cases were obtained through linkage to the Cancer Registry of Norway, whereas the Cause of Death Registry provided updated information on causes of death. Cox proportional hazard models were employed to estimate associations between OC use (Paper I), body fatness (Paper II), and MHT use (Paper III) with subtypes of breast cancer incidence, mortality (Paper III), and survival (Paper III).

Results: While use of combined OCs (COCs) increased risk of hormone receptor-negative breast cancer, progestin-only OC (POC) use was associated with hormone receptor-positive breast cancer if used for five years or more. The risks of current COC use varied significantly between breast cancer subtypes. Overweight and obesity at baseline, increasing age at the onset of overweight and obesity, and increasing overweight duration were associated with increased risk of luminal A-like breast cancer compared to normal-weight women. Women belonging to trajectories with increasing BMI had a higher risk of luminal A-like cancer compared to those with a normal and stable trajectory, while those with decreasing weight

had nearly a 50% reduced risk. MHT use was associated with incident and fatal overall and luminal A-like breast cancer, and incident luminal B-like breast cancer. Among patients with breast cancer, duration of estrogen-progestin therapy (EPT) use was associated with worse survival from luminal A-like disease, whereas EPT use was associated with improved survival among patients with TNBC. Current MHT use was differentially associated with survival across intrinsic-like subtypes.

Conclusions: While COC use increased the risk of hormone receptor-negative breast cancer, body fatness and the use of POCs and MHT increased the risk of hormone receptor-positive subtypes. Moreover, differences in survival indicated heterogeneity in associations between MHT use and breast cancer progression across tumor subtypes. These findings underscore the complex interplay between estrogen and progestins and their role in breast cancer carcinogenesis. Limited statistical power in some of the subgroup analyses may have affected the robustness of the results.

Sammendrag

Bakgrunn: Det er gode holdepunkter for at kvinnelige kjønnshormoner har en avgjørende rolle i utviklingen av brystkreft. Subtyper av brystkreft utgjør distinkte sykdomsentiteter og har ulik etiologi, risikofaktorer og prognoser. Disse subtypene er ofte klassifisert som luminal A-lignende, luminal B-lignende, HER2-positiv og trippel-negativ brystkreft (TNBC) basert på immunhistokjemisk undersøkelse. Assosiasjonen mellom kvinnelige kjønnshormoner og subtyper av brystkreft er ikke fullstendig kartlagt.

Mål: Formålet med denne avhandlingen var å undersøke sammenhengen mellom endogene (kroppsmasseindeks [BMI]) og eksogene (p-piller [OC] og hormonbehandling i overgangsalderen [MHT]) kvinnelige kjønnshormoner og subtyper av brystkreft hos norske kvinner, og å undersøke om disse hormonelle faktorene påvirker risikoen for ulike subtyper forskjellig.

Metoder: Denne avhandlingen brukte data fra den norske Kvinner og Kreft-studien (NOWAC) – en prospektiv nasjonal kohort med mer enn 170 000 kvinner. Data om bruk av hormoner og BMI ble samlet inn fra selvadministrerte spørreskjemaer. Insidente tilfeller av brystkreft ble innhentet gjennom kobling til Kreftregisteret, mens Dødsårsaksregisteret supplerte med oppdatert informasjon om dødsårsaker. Cox proporsjonal hasard regresjon ble brukt til å estimere sammenhengen mellom bruk av OC (Paper I), BMI (Paper II) og MHT-bruk (Paper III) og insidens, mortalitet (Paper III) og overlevelse (Paper III) av subtyper av brystkreft.

Resultater: Bruk av kombinasjonspiller (COC) økte risikoen for hormonreseptor-negativ brystkreft, mens bruk av gestagenpiller (POC) var assosiert med hormonreseptor-positiv brystkreft hvis brukt i fem år eller mer. Risikoene ved nåværende bruk av COC varierte mellom brystkreftsubtypene. Overvekt og fedme ved baseline, økende alder ved overvekt og fedme, og økende varighet av overvekt var assosiert med økt risiko for luminal A-lignende brystkreft sammenlignet med normalvektige kvinner. Kvinner med økende BMI-trajektorier hadde høyere risiko for luminal A-lignende kreft sammenlignet med de med en normal og stabil BMI-trajektorie, mens de med avtagende vekt hadde nesten 50% redusert risiko. MHT-bruk var assosiert med økt forekomst av og dødelighet fra luminal A-lignende brystkreft, og

økt forekomst av luminal B-lignende brystkreft. Blant pasienter med brystkreft var varigheten av østrogen-gestagen terapi (EPT) assosiert med dårligere overlevelse fra luminal A-lignende sykdom, mens EPT-bruk var assosiert med økt overlevelse blant pasienter med TNBC. MHT-bruk påvirket overlevelsen av de ulike subtypene forskjellig.

Konklusjoner: Mens COC-bruk økte risikoen for hormonreseptor-negativ brystkreft, var overvekt og fedme og bruk av POC og MHT assosiert med økt risiko for hormonreseptor-positive subtyper. Forskjeller i overlevelse indikerte heterogenitet i sammenhengene mellom MHT-bruk og progresjon av brystkreft på tvers av subtyper. Disse funnene understreker det komplekse samspillet mellom østrogen og gestagen i brystkreftutvikling. Begrenset statistisk styrke i noen av subgruppe-analysene kan ha påvirket robustheten i resultatene.

List of papers

The thesis is based on the following papers, referred to in the text by their Roman numerals.

I. Busund M, Bugge NS, Braaten T, Waaseth M, Rylander C, Lund E. Progestin-only and combined oral contraceptives and receptor-defined premenopausal breast cancer risk: The Norwegian Women and Cancer Study. Int J Cancer. 2018;142(11):2293-302.

II. Busund M, Ursin G, Lund E, Wilsgaard T, Rylander C. Trajectories of body mass index in adulthood and risk of subtypes of postmenopausal breast cancer. Breast Cancer Res. 2023;25(1):130.

III. Busund M, Ursin G, Lund E, Chen SLF, Rylander C. Menopausal hormone therapy and incidence, mortality, and survival of breast cancer subtypes: a prospective cohort study. Breast Cancer Research. 2024;26(1):151.

Abbreviations

BMI	Body mass index
CI	Confidence interval
COC	Combined oral contraceptives
DAG	Directed acyclic graph
ER	Estrogen receptor
EPT	Estrogen-progestin therapy
ET	Estrogen therapy
FSH	Follicle-stimulating hormone
GBTM	Group-based trajectory modeling
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratio
IARC	International Agency for Research on Cancer
IHC	Immunohistochemistry
ISH	In situ hybridization
LH	Luteinizing hormone
MAR	Missing at random
MCAR	Missing completely at random
MHT	Menopausal hormone therapy
MICE	Multiple imputation by chained equations
MNAR	Missing not at random
NETA	Norethisterone acetate
NOWAC	Norwegian Women and Cancer Study
OBY	Weighted cumulative years of obesity
OC	Oral contraceptives
OWY	Weighted cumulative years of overweight
POC	Progestin-only contraceptives
PR	Progesterone receptor
SHBG	Sex hormone-binding globulin
TDLU	Terminal duct lobular unit
TNBC	Triple-negative breast cancer
WHO	World Health Organization

1 Introduction

Cancer is a group of diseases with a shared constellation of abnormal cell behaviors, including the ability of uncontrolled growth and spread of cell masses. Such malignant cells can invade adjacent tissues, and, at advanced stages, migrate to different sites of the body. The spread of malignant cells, either through blood or lymphatic systems, is a process termed metastasis. The Hallmarks of Cancer comprise a core set of alterations in cell physiology proposed to describe the transformation from normal to malignant cells (1-3). These traits are rapidly increasing as ongoing advances in cancer research continually enrich our understanding of carcinogenesis. Key characteristics include resistance to cell death, sustained proliferative signaling, induction of vasculature, and evasion of immune destruction. Universal to all attributes of cancer is the underlying damage in DNA. DNA can suffer alterations through mutations during cell division, but also via damage from agents inside (endogenous) or outside (exogenous) the body. Despite significant advancements in cancer research, principal aspects including etiology, biological mechanisms, and optimal treatment strategies continue to be inadequately understood. Underscoring its substantial health burden, cancer was the leading cause of death in Norway in 2023 (4).

Cancers are named after the site in which the primary tumor originates. They can arise in any organ, affect any gender, and manifest at any age. This thesis focuses on breast cancer among adult females. While it is widely acknowledged that tumorigenesis is a multistep process encompassing tumor initiation, promotion, and progression, the mechanisms of breast cancer carcinogenesis is heterogeneous and complex (5-8). Every phase of tumorigenesis can be influenced by risk factors, defined as determinants associated with increased cancer incidence (9, 10). Compelling evidence highlights the pivotal role of female reproductive hormones in the etiology of breast cancer. This thesis seeks to shed light on their intricate roles as risk factors for development and progression of the disease, with particular emphasis on various breast cancer subtypes.

1.1 Breast cancer

1.1.1 Epidemiology and burden

Breast cancer is the most commonly diagnosed cancer among women globally and in Norway (11, 12). It is the leading cause of cancer deaths among females worldwide, whereas in Norway, it ranks third after lung and colon cancer. In 2022, there were an estimated 2.3 million incident breast cancer cases and 666,000 breast cancer deaths globally (Figure 1) (11).

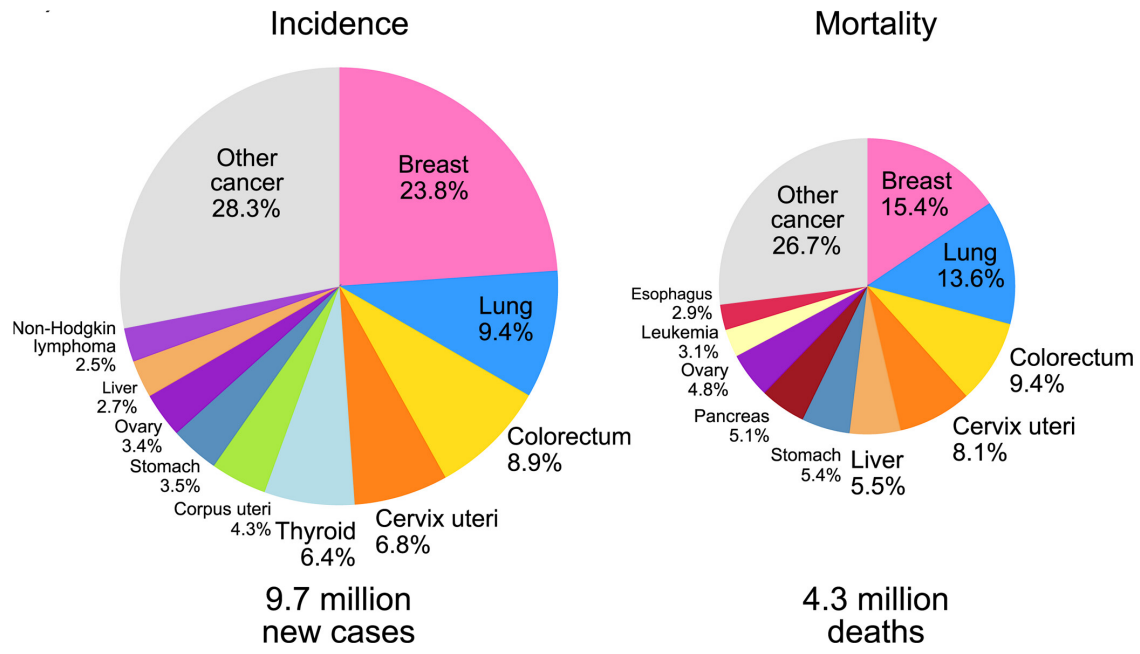

Figure 1. Pie charts of the distribution of incident cancer cases and deaths among females globally. Source: "Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries" (11). Permission to reuse obtained from John Wiley and Sons

Breast cancer incidence rates are higher in Australia/New Zealand, North-Western Europe, and North America than in South America, Central America, Africa, and Asia (11). The higher incidence rates in western countries are partly due to higher prevalence of reproductive, hormonal, and lifestyle risk factors, including early age at menarche, later age at menopause, older age at first birth, lower parity, reduced breastfeeding, use of menopausal hormone therapy (MHT) and oral contraceptives (OCs), alcohol consumption, excess body weight, and physical inactivity (13). Additionally, variability in cancer registration and detection through mammographic screening contribute to these differences (11). In

contrast, breast cancer mortality rates are generally higher in low- and middle-income non-western countries compared to western countries, reflecting the level of coverage of essential health services (14, 15). While increasing trends in both breast cancer incidence and mortality are seen in low- and middle-income countries in South America, Africa, and Asia, breast cancer mortality has declined in western countries over the past decades due to improved treatment and mammography screening (11).

In Norway, a steady increase in breast cancer incidence has been reported over recent decades. Figure 2 displays trends in breast cancer incidence, mortality, and survival in Norway over the last half-century. A steeper increase was observed in the mid 1990s, at which time the Norwegian Breast Cancer Screening Programme was initiated. Following a gradual implementation, the screening programme became nationwide by 2005, after which the curve declined slightly until 2009. The screening programme targets women aged 50-69 years for biennial mammography. Over the past decade, an increase in incidence rates has been observed in all age groups over 30 years, including women outside screening age (12). Despite increasing incidence, decreased mortality and increased survival have been reported since mid-1990s.

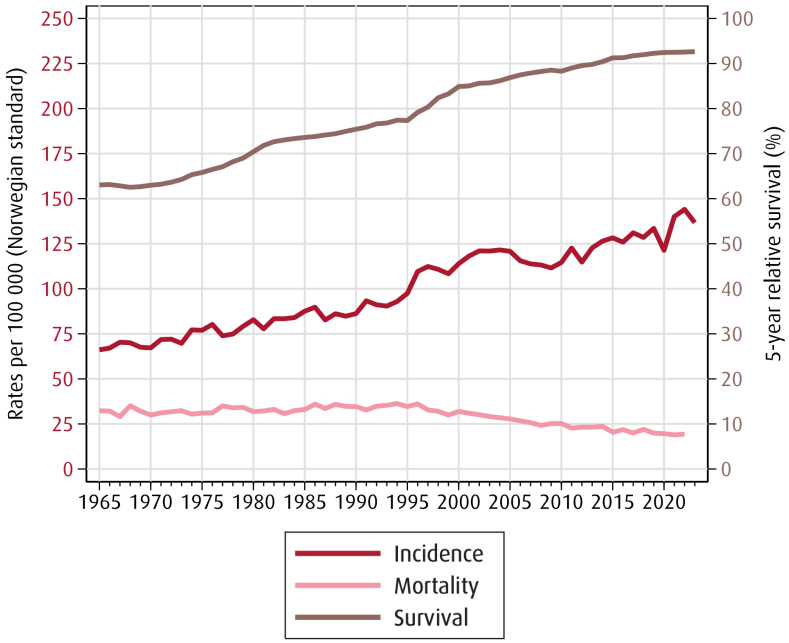

Figure 2. Trends in incidence and mortality rates and 5-year relative survival proportions among female breast cancers in Norway. Source: “Cancer incidence, mortality, survival and prevalence in Norway. Cancer in Norway 2023” (12)

These positive trends have been linked to improved diagnostics and treatment as well as earlier detection due to the implementation of the screening programme (12). Norwegian figures from 2023 show that 93% of females diagnosed with breast cancer survive the disease for 5 years or more (12). Findings from the United States as well as from Norway have found that increasing incidence is confined to hormone receptor-positive cancers, whilst the rates for hormone receptor-negative cancers have remained stable (16-18). This trend can be explained by hormonal factors such as increasing body mass index (BMI) and MHT use, along with the impact of mammographic surveillance.

1.1.2 Clinicopathological characteristics

Breast tissue consists of adipose tissue, glandular tissue arranged in lobes, ducts, and connective tissue. These tissues develop predominantly during puberty, pregnancy and lactation in response to hormones such as estrogens, progesterone, insulin and growth factors. Breast cancer can derive from epithelial cells, mesenchymal cells, fibroepithelial cells, cells of the nipple or lymph node cells (19). The majority of breast neoplasms are ductal carcinomas originating from the epithelial cells that line the lactiferous ducts, which transport milk from the lobes to the nipple (Figure 3).

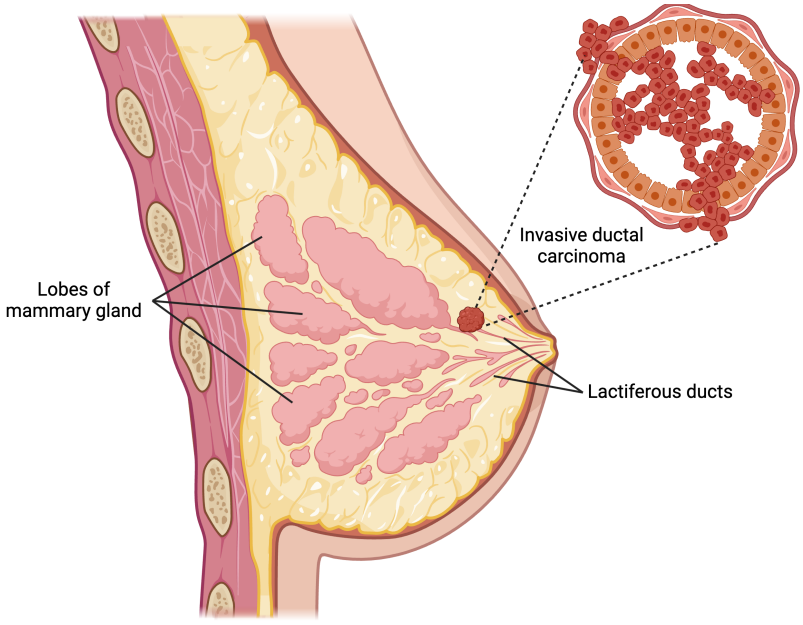

Figure 3. Invasive ductal carcinoma. Created by BioRender.com

The remaining tumors are mainly lobular carcinomas, deriving from the cells of the milk-producing sacs named lobes. Other less common types, such as tubular carcinoma,

mucinous carcinoma, medullary carcinoma, and adenoid cystic carcinoma, account for the rest. Almost 80% of invasive breast carcinomas are ductal type, and nearly 10% are lobular type (20). Carcinoma *in situ*, tumors which are not penetrating the basal membrane, and non-carcinoma cancers are not included in this thesis. In addition to histological classification, histological grade is a scoring system used on infiltrating carcinomas to indicate how closely the tumor resembles the glands of origin (21). This grade ranges from 1 to 3 and is based on the degree of differentiation, nuclear pleomorphism and proliferation assessed by the number of mitosis. Grade 3 is the most aggressive type of tumor.

Staging of breast cancer is a measure of the extent of disease involvement and is used to determine prognosis and treatment. Staging assessment is based on the tumor, lymph node, metastasis (TNM) classification system, which involves tumor size or expansion (T), involvement of lymph nodes (N) and metastasis (M). It is performed according to the 8th edition of the Cancer Staging Manual by the American Joint Committee on Cancer (22). Tumor stage ranges from stage I (least severe) to stage IV (most severe).

Comprising important prognostic and predictive factors, newly diagnosed breast cancers are tested for expression of the hormone receptors estrogen receptor (ER) and progesterone receptor (PR), and for overexpression of human epidermal growth factor receptor 2 (HER2). ER and PR expression predicts response to endocrine therapy and are related to improved breast cancer outcomes (23), whereas HER2 overexpression identifies patients who might benefit from HER2-directed therapy and contends an unfavorable prognosis in the absence of systemic therapy (24). Hormone receptor status is ascertained using immunohistochemistry (IHC), which identifies receptor proteins by their binding to specific antibodies. ER positivity is defined by a 1% cut-off, whilst the threshold-value for PR positivity is 10% (25, 26). HER2 overexpression is detected by IHC staining and supplemented by fluorescence *in situ* hybridization (ISH). IHC 0 or 1+ is accounted as negative values. IHC 2+ is uncertain and supplemented with ISH, and 3+ is positive (27).

1.1.2.1 Molecular intrinsic subtypes

Gene expression profile studies have identified intrinsic molecular subtypes of breast cancer that represent distinct biological entities and have differences in prognosis and response to treatment (28-32). These studies used gene expression array criteria to create a subtype

taxonomy. At least five subtypes have been identified; namely, the luminal subtypes luminal A and luminal B, HER2-enriched, basal-like, and normal-like subtype. The luminal subtypes resemble luminal epithelium of the breast and make up the majority of hormone receptor-positive tumors. Luminal A tumors are the most common breast cancer subtype, they have a high expression of ER-related genes, low expression of HER2-amplified genes, and has the most favorable prognosis of all subtypes in general (29-31, 33, 34). The normal-like subtype is rare and resembles luminal A. Luminal B have a relatively lower expression of ER-related genes, variable HER2 expression and relatively worse prognosis than luminal A (35). The HER2-enriched intrinsic subtype, although not synonymous with clinically HER2-positive breast cancer, typically has a high expression of HER2 genes and low hormone receptor gene expression and carries a less favorable prognosis compared with the luminal subtypes (35). Most basal-like subtypes fall under the category triple-negative breast cancers (TNBC) as they are typically negative of ER, PR and HER2 on clinical assays (28). This subtype carries the worst prognosis of all subtypes (35).

Gene expression analyses are costly and are not routinely performed on breast cancer tissues in clinical practice. The St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer approximated the definition of molecular subtypes using clinicopathological criteria (36, 37). Thus, the intrinsic molecular subtypes were cross-classified into a surrogate intrinsic-like subtype definition based on IHC measurements of ER, PR, and HER2 (with ISH confirmation if appropriate). Although the overlap is not complete, this makes a convenient approximation for research purposes (37, 38). This intrinsic-like subtype definition uses the proliferation marker Ki67 to differentiate between luminal A and luminal B subtypes. The current thesis will solely be focusing on intrinsic-like subtypes based on receptor expression (i.e. ER, PR and HER2), as mutually exclusive groups are created doing this, and because most studies do not include Ki67. Further, we merged luminal B (HER2 negative) and luminal B (HER2 positive) into one group. Table 1 displays intrinsic breast cancer subtypes and surrogate intrinsic-like cross-classifications as recommended by the St. Gallen guidelines and employed in this thesis.

Table 1. Surrogate definitions of intrinsic subtypes of breast cancer. Adapted from the St. Gallen guidelines (36)

<table>
<tr><th>Intrinsic subtype</th><th>Surrogate intrinsic-like subtype</th><th>Recommended surrogate markers</th><th>Surrogate markers used in thesis</th></tr>
<tr><td>Luminal A</td><td>Luminal A-like</td><td>ER positive and PR positive
HER2 negative
Ki-67 low</td><td>ER positive
PR positive
HER2 negative</td></tr>
<tr><td rowspan="2">Luminal B</td><td>Luminal B-like
(HER2 negative)</td><td>ER positive
HER2 negative
Ki-67 high or PR negative</td><td rowspan="2">ER positive
PR negative
HER2 negative
or
ER positive
PR negative or positive
HER2 positive</td></tr>
<tr><td>Luminal B-like
(HER2 positive)</td><td>ER positive
HER2 positive
Any Ki-67
Any PR</td></tr>
<tr><td>Erb-B/HER2 overexpression</td><td>HER2-enriched</td><td>ER negative
PR negative
HER2 positive</td><td>ER negative
PR negative
HER2 positive</td></tr>
<tr><td>Basal-like</td><td>Triple-negative</td><td>ER negative
PR negative
HER2 negative</td><td>ER negative
PR negative
HER2 negative</td></tr>
</table>

According to the Norwegian Breast Cancer Registry, incorporating Ki67 and tumor grade into the intrinsic-like subtype definition, 60% were luminal A-like, 28% were luminal B-like, 4% were HER2-enriched, and 8% were triple-negative in 2023 (39).

1.2 Female sex hormones

In order to unravel breast cancer risk factors and their relation to female sex hormones, a brief overview of these hormones is essential. Estrogen and progesterone, the primary endogenous ovarian hormones, are steroid hormones. Steroid hormones can be divided into three main groups: mineralocorticoids, glucocorticoids, and sex steroids. The production and secretion of these hormones are regulated by the hypothalamic-pituitary axis. In addition to the female reproductive hormones, sex steroids also encompass the male reproductive hormones collectively termed androgens (testosterone, androstenedione, dehydroepiandrosterone (DHEA), and androstenediol). Sex hormones are

derived from cholesterol, which undergoes several enzymatic transformations in the gonads to become progesterone, androgens, and estrogens (Figure 4) (40).

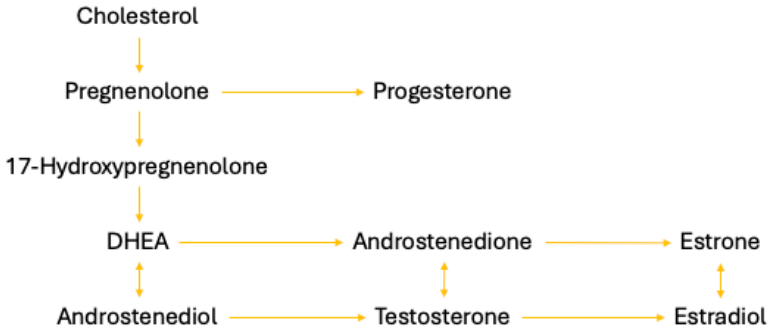

Figure 4. Biosynthesis of steroid hormones in the gonads. Adapted from Henderson (41)

Estrogen and progesterone are synthesized *de novo* in the ovaries and adrenal glands and are converted from precursors in peripheral tissues, including the placenta, adipose tissue, liver, muscle, and brain (41, 42). These hormones work by stimulating specific intracellular receptors, namely the ER and PR. There are two isoforms of the ER, namely the ERα and ERβ. ERα is the predominant isoform present in the mammary gland (43). Herein, unless otherwise specified, ER refers to ERα. As with the ER, there are also two isoforms of the PR termed A and B. While PR-A operates in the uterus and ovary and can suppress PR-B and ER expression, PR-B is predominantly a transcriptional activator and drives the proliferative progesterone-mediated effects in the mammary tissue (44). The hormone receptors are located in the cytosol of target cells and operate as transcription factors (Figure 5). Upon binding of the hormone to the ligand-binding domain, an allosteric change occurs, revealing DNA-binding sites on the receptor. This leads to migration to the nucleus and binding to specific hormone-responsive elements. Subsequently, genetic responses follow, involving metabolism, cell differentiation and proliferation and cell cycle regulation (45, 46). Ovarian hormones can regulate ER and PR expression (47).

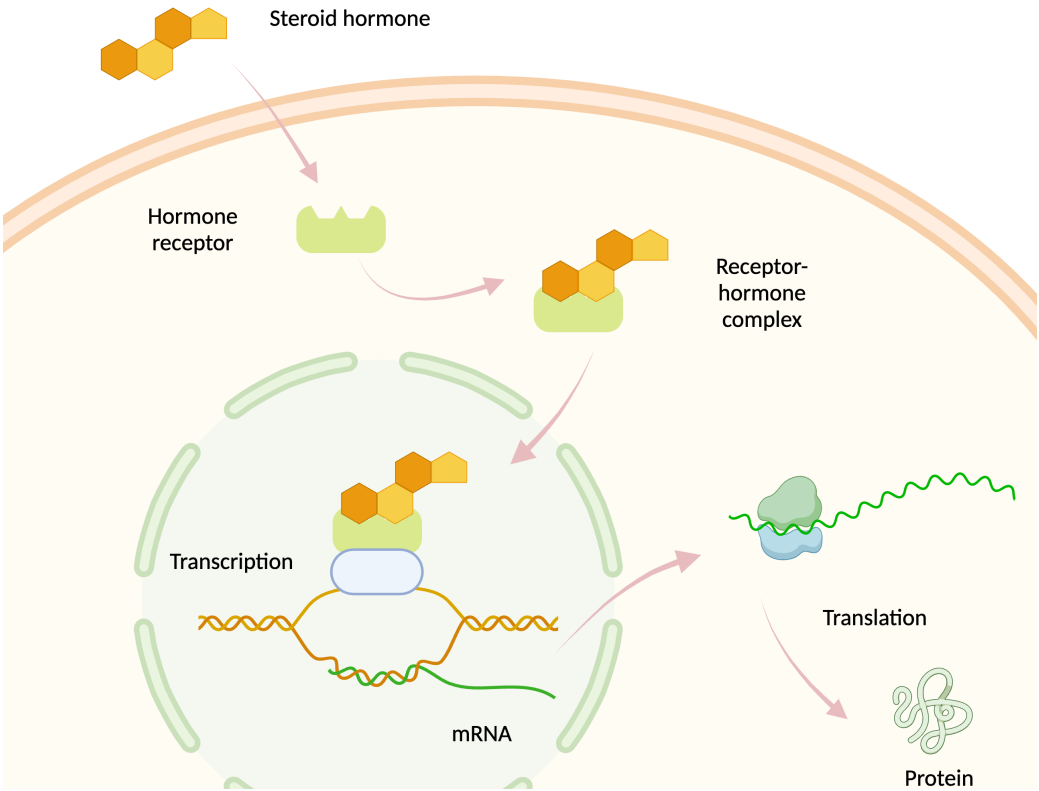

Figure 5. Simplified steroid hormone receptor signaling. Adapted from Saha (48) and created by BioRender.com

1.2.1 Estrogen

There are three major endogenous estrogens: estradiol (17β-estradiol; E2), which dominates in the reproductive years, is highly potent and is primarily produced in the ovaries; estrone (E1), which is the dominating estrogen after menopause and is mainly produced in adipose tissue; and estriol (E3), the pregnancy-estrogen, largely produced in the placenta and is considered the weakest estrogen (49-51). Estradiol is formed from an enzymatic alteration of testosterone called aromatization, a process requiring the enzyme aromatase. Estradiol can be further transformed to estriol in the placenta or liver. Androstenedione is secreted from the adrenal glands and ovaries and acts as a precursor to estrone through aromatization in extra-glandular tissues. Estrone may be further converted to estradiol or estriol. During pregnancy, the rate of estrogen production in the corpus luteum, and subsequently by the placenta after the first trimester, increases markedly. This leads to a gradual rise in circulating levels of estradiol, estrone, and estriol throughout pregnancy (52). After menopause, when the ovarian production of estradiol has ceased, the principal estrogen source is through aromatization of circulating androstenedione to estrone in peripheral tissues (53). Estrogen stimulates maturation and growth in multiple

organs. Aromatase activity and ER expression have been observed in multiple tissues in both males and females, including adipose tissue, brain, bone, prostate and testes (54). Estrogen binds to sex-hormone binding globulin (SHBG) as well as albumin in the circulation, and only a small portion remains free and bioavailable (2%) (45) .

1.2.2 Progesterone

Following ovulation, progesterone released from the corpus luteum serves biological functions, whereas adrenal-derived progesterone is predominantly converted into androgens or glucocorticoids (55). Progesterone stimulates glandular secretion in reproductive tissue, promote the maturation of certain estrogen-stimulated tissue, and induces secretory changes in the endometrium. It holds a vital role in promoting and supporting pregnancy. Progesterone circulates in the bloodstream bound to cortisol-binding globulin and albumin, and approximately 2% remains free (55).

Historically, estrogens and progesterone have been considered female reproductive hormones. Estrogens and progesterone work in concert to regulate numerous cellular processes in female reproductive organs such as the uterus, ovaries, and breasts. However, our comprehension of their physiological functions has expanded; estrogens and progesterone are now recognized as crucial in various non-reproductive physiological functions in both sexes. Estrogen is involved in the regulation of metabolism and the cardiovascular system and plays a critical role in maintaining bone and muscle homeostasis as well as prevention of osteoporosis (56, 57). Progesterone is important in the development and protection of both the central and peripheral nervous system (58).

1.2.3 The menstrual cycle

The gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH) are secreted from the anterior pituitary gland in response to the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus (45). FSH and LH stimulate the ovaries to produce and secrete estradiol and progesterone, which in turn inhibits FSH and LH secretion through a negative feedback mechanism. The menstrual cycle comprises a follicular phase and a luteal phase (Figure 6). The follicular phase initiates with menstruation, at which time hormone levels are low. As the ovarian follicles are growing under influence of FSH in the

follicular phase, increasing amounts of estradiol is secreted. When estradiol values reach a threshold, inhibition of the anterior pituitary gland switches to stimulation, resulting in secretion of LH and FSH. A high LH-surge facilitates ovulation on day 14 after onset of menstruation, and an egg is released from the follicle. The emptied follicle transforms to corpus luteum, which has LH-receptors and produces high amounts of progesterone but also estradiol. As such, the follicular phase is characterized by dominating levels of estradiol with increasing levels towards the end and a negligible amount of progesterone, whereas the luteal phase has high levels of progesterone and lower levels of estrogen, both peaking mid-phase (45).

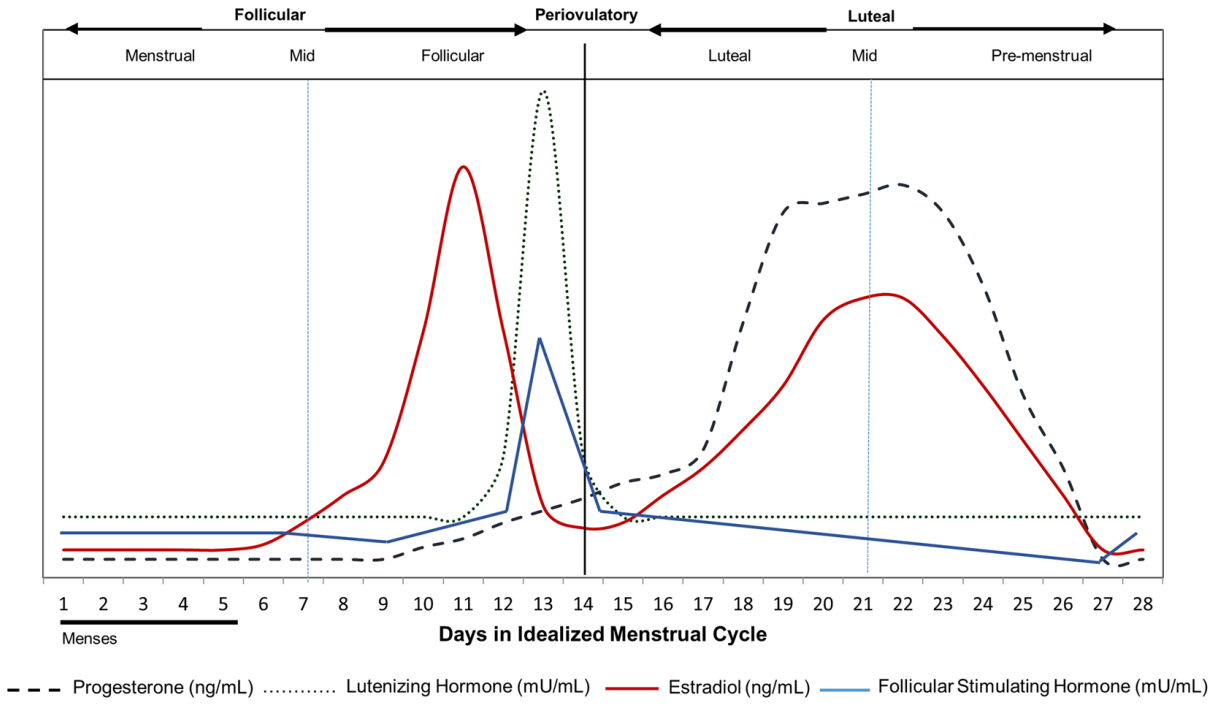

Figure 6. Hormone levels during normal menstrual cycle. Source: "Menstrual Cycle rhythmicity: metabolic patterns in healthy women" (59)

1.2.4 Hormonal effects on the breast

Estrogen and progesterone mediate development and growth of breasts in puberty and their maturation during pregnancy (60). Estrogen facilitates the development of ducts and stromal tissue, as well as fat deposition. In order for the breast to develop into a milk-producing organ, progesterone and prolactin are needed. Progesterone cause development of the lobules and alveoli of the breast, making the alveoli secretory. Milk secretion is further stimulated by prolactin (61).

1.2.5 Hormonal carcinogenesis

In the beginning of the 18th century, dr. Bernadino Ramazzini remarked on the notably high occurrence of the so-called “accursed pest” – breast cancer – among nuns (62). Although he made no direct connection between the hazards of nulliparity in nuns and breast cancer, his observations are considered as the initial clues of such association (63). The notion of the hormone dependency of breast cancer was first proposed in the 19th century by the surgeon dr. Thomas Beatson, who oophorectomized a patient with metastatic breast cancer and achieved temporary regression (64). Almost one century later, it became apparent, after originally suggested by Furth (65), that hormones act as carcinogens by increasing cellular proliferation and hence the chance of random genetic errors which in turn can lead to malignant transformation (41).

Underpinning this, the proliferation rate of breast epithelial cells varies across the menstrual cycle and is low in the follicular phase and higher in the luteal phase (66). As noted above, the luteal phase is characterized by increasing concentrations of progesterone and, to a lesser extent, estrogen. Thus, the increased proliferation rate in breast cells in the luteal phase reflects high levels of both female sex hormones. The accumulation of genetic errors caused by increased proliferation can occur in tumor suppressor genes, oncogenes, and also genes involved in DNA repair and hormone metabolism and transport (8). Evidence from experimental studies suggest estrogen indirectly and directly cause DNA damage, genetic instability and mutations in mammary tissue (67). The role of estrogen in breast carcinogenesis is further supported by the efficacy of chemopreventive agents such as aromatase inhibitors and selective estrogen receptor modulators on breast cancer risk reduction or treatment.

1.3 Breast cancer risk factors

Breast cancer etiology is multifactorial, with risk factors including age, history of benign breast disease, dense breast tissue, family history, exposure to ionizing radiation, hormonal influences, lifestyle choices, and reproductive history (68). Hormonal risk factors include exposure to endogenous and exogenous hormones, the latter involving use of MHT and OCs (69-72). Lifestyle factors, such as low premenopausal BMI, high postmenopausal BMI,

sedentary behavior, alcohol consumption, and smoking, increase the risk of breast cancer (73, 74). Knowledge on differences in risk profiles according to the underlying molecular subtypes of breast cancer has recently begun to emerge. In the following subchapters, hormone-related risk factors will be elaborated further.

1.3.1 Reproductive and menstrual history

Reproductive and menstrual factors associated with increased risk of breast cancer include high age at first birth, nulliparity or low parity, low age at menarche and high age at menopause (68). Compared to nulliparous women, parous women experience nearly half the risk of breast cancer, and the risk is further reduced with increasing parity (75, 76). Although a transient increase in breast cancer risk is seen within the first few years following delivery, the net long-term effect of parity is protective. Low age at first birth is also associated with decreased risk of breast cancer (75). The inverse association between parity and young age at first birth and breast cancer risk has constantly been reported with hormone receptor-positive or luminal cancers, and less conclusive effects have been reported for other subtypes (77-80). Breastfeeding is inversely associated with breast cancer, and the magnitude of the effect is dependent on duration of breastfeeding and on confounding by parity (81). The inverse association between breastfeeding and breast cancer is demonstrated to be stronger for ER-negative and TNBC, whereas weaker and less consistent effects have been observed for ER-positive subtypes (82, 83). Early onset menarche and late onset menopause is associated with hormone receptor-positive or luminal A-like breast cancer (78, 79, 84-86).

Although the role of reproductive and menstrual factors in breast cancer etiology is well recognized, the underlying biological mechanisms remain poorly defined. Although further insight is required, changes in endogenous hormonal profiles are assumed to be involved. An increased number of ovulatory cycles, with corresponding hormonal fluctuations and prolonged exposure to elevated levels of estrogen and progesterone, are proposed to contribute to the elevated breast cancer risk associated with early onset of menarche and/or late onset of menopause (87). The opposite occurs with prolonged lactation and increasing parity, which reduce the number of ovulatory cycles. Further, the postpartum hormonal milieu, especially among lactating women who have lower estradiol levels, may

also play a significant role (76). The proliferation and differentiation of lobular units in the breast during and after pregnancy are hypothesized to be key mechanisms that link parity and younger age at first birth to reduced risk of breast cancer, potentially enhancing the breast's resilience against cellular damage (88, 89). Emerging focus is raised on the role of involution of terminal ductal lobular units (TDLUs), which are the functional structures of the breast and the primary origin sites for breast cancers. Post-pregnancy and -lactation, the breast undergoes involution which is a wound healing-like process with regression of milk-producing structures (90). Women with less TDLU involution are more likely to develop breast cancer. Studies have shown a greater involution following lactation and among parous women compared to nulliparous women (91, 92).

1.3.2 Endogenous female sex hormones

Assessing the relationship between endogenous hormone levels and breast cancer risk is challenging due to measuring difficulties, variability in methods, menstrual cycle variations in premenopausal women, the complexity of interrelated markers, and large inter-individual metabolic differences (68). High endogenous levels of estrogen (estradiol and estrone) have consistently been linked to increased breast cancer risk in postmenopausal women (68, 93-96), and also in premenopausal women according to some studies (97, 98). This effect is mostly reported in hormone receptor-positive (94-97), but also hormone receptor-negative subtypes (95). Testosterone is also associated with increased risk of breast cancer in postmenopausal (93-96, 99) and premenopausal women (97, 98), predominantly in hormone receptor-positive subtypes (94-97). Few studies have examined the relationship between endogenous progesterone levels and breast cancer risk, and the findings from these studies have generally been non-significant (94, 97, 98). The absence of an association between progesterone levels and breast cancer risk in epidemiological studies could be due to methodological limitations or a true null relationship. Mechanistic studies have proposed a role of progesterone in the development of breast cancer (100), although the majority of in vivo studies have pointed towards no association (101). Arguing against progesterone having a cancer-promoting effect on breast tissue are findings that natural micronized progesterone added to estrogens in cyclic combined regimens does not increase breast cancer risk (102). However, subsequent publications have not supported this finding (72). In

sum, the etiological role of endogenous progesterone in breast cancer development remains uncertain.

1.3.3 Exogenous female sex hormones

Millions of females worldwide use exogenous hormones in the form of OCs or MHT. OCs prevent pregnancy by inhibiting ovulation and preventing sperm from penetrating through the cervix and upper genital tract (103). OCs were first registered in Norway in the 1960s. Since 2002, the implementation of the reimbursement scheme has secured Norwegian teenagers with contraceptives free of charge (104). In the last two decades, the use has been increasing, and combined OCs (containing estrogen and progestin; COCs) accounts for the most commonly used hormonal contraceptive method in all age groups (104).

MHT is the most effective treatment for alleviating menopausal symptoms, with estrogen effectively managing vasomotor symptoms such as hot flashes and night sweats (105). In Norway and in western countries, the use of MHT increased during the 1990s (72, 106). In the early 2000s, following clinical trial results such as the widely debated Women's Health Initiative randomized trial, which demonstrated adverse cardiovascular effects and excess breast cancer risk with MHT use (107), usage declined drastically, and there was a shift from high-dose to low-dose formulations (108). In recent years, the use of oral MHT has stabilized in Norway and in western countries, while the use of transdermal and vaginal regimens is increasing (109).

Universal to OCs and MHT is the content of estrogen and/or progestin. While numerous non-oral hormonal contraceptives and MHT regimens are available, this thesis concentrates on those administered orally. OCs consist either of a combination of estrogen and progestin or solely of progestin (progestin-only contraceptives [POC]). MHT is either taken as unopposed estrogen therapy (ET) or combined estrogen-progestin therapy (EPT). Due to the increased risk of endometrial hyperplasia and cancer associated with prolonged use of unopposed estrogen, non-hysterectomized women are recommended using EPT (110). Both OCs and MHT can be administered in either a cyclic or continuous regimen, with the cyclic treatment inducing withdrawal bleeding.

In MHT, the oral estrogen component is most commonly a micronized 17-beta estradiol which is bioidentical to the estradiol produced in the premenopausal ovary. Other estrogen formulations involve conjugated equine estrogens, esterified estrogens and ethinyl estradiol, the latter in which is almost exclusively used in OCs (111). Progestin refers to a synthetic compound emulating the action of progesterone (112). There are numerous types of progestins used in OCs and MHT, and they can be classified according to generation or structural properties. The structural classification includes three major groups: 1) pregnanes (medroxyprogesterone acetate, nomegestrol acetate), 2) estranes (norethindrone/norethisterone, norethindrone/norethisterone acetate (NETA), ethynodiol diacetate, norethynodrel), and 3) gonanes (levonorgestrel, desogestrel, norgestimate, gestodene) (113). Pregnanes are derived from progesterone, while estranes and gonanes are derived from testosterone. In Norway, NETA is the progestin predominantly used in MHT, whereas in other countries, including the US, the most commonly used progestin is medroxyprogesterone acetate (114).

COC and EPT are classified as carcinogenic to humans (group 1) by the International Agency for Research on Cancer (IARC) Monographs (115). The current evidence will be elaborated in the subsequent paragraphs.

1.3.3.1 Oral contraceptives

The use of OCs slightly increases risk of premenopausal breast cancer in current or recent users (69, 70, 116-118). The elevated risk is temporary and subsides within 5-10 years since last use. Emerging evidence demonstrate similar risk patterns associated with contemporary OCs. A Danish study of almost two million women aged 15-49 years showed a relative risk of breast cancer of 1.19 (95% CI 1.13-1.26) among current and recent users of COCs that were available on the market during 1995-2014, compared to never users (69). These figures resemble those of The Collaborative Group on Hormonal Factors in Breast Cancer from 1996 including 53,297 women with breast cancer, indicating a relative risk of 1.25 (95% CI 1.15-1.33) among current and recent users of COCs.

POCs have been classified as possibly carcinogenic to humans (group 2B) by the IARC (119). The mentioned Danish study found a small increased risk of breast cancer associated with levonorgestrel-containing POC use administered orally and intrauterine (69). This has later

been supported by a recent meta-analysis, where current/recent POC use yielded a 29% (95% CI 1.21-1.37) increase in breast cancer risk (118).

The association between OC use and breast cancer subtypes is not well understood, as prospective studies investigating these risk associations are sparse (116, 120, 121). Among them, associations with hormone receptor-negative (116, 121), hormone receptor-positive (116), and no association with either subtypes (120), were reported. The latest update from the Nurses' Health Study reported increased risks of HER2-enriched and TNBC subtypes associated with current OC use, however associations did not differ significantly by tumor subtype (116). Moreover, a recent meta-analysis of case-control studies reported increased risk of TNBC among ever users of OCs (122).

1.3.3.2 Menopausal hormone therapy

MHT has been identified as an important risk factor for postmenopausal breast cancer over the last three decades (71, 72, 109, 123-129). A recent meta-analysis of 143,887 women with breast cancer by the Collaborative Group on Hormonal Factors in Breast Cancer, demonstrated an increased risk of breast cancer associated with all types of MHT, except for vaginal estrogens (72). Risk increased with duration of use, and, depending on duration of use, remained for more than 10 years after cessation of use. Further, EPT use was associated with greater breast cancer risk (RR 1.60; 95% CI 1.52-1.69) than ET use (RR 1.17; 95% CI 1.10-1.26) with 1-4 years duration of use, and were twice as great during years 5-14 (RR 2.08; 95% CI 2.02-2.15) compared with never use (72). Age at start of use did not affect risk estimates, and the risk for breast cancer was greater with continuous than cyclic progestin use.

It is well established that MHT use is associated with hormone receptor-positive subtypes, predominantly luminal A-like (80, 109, 130-132) but also luminal B-like subtypes (80, 109, 132). Less is known regarding the association between MHT use and hormone receptor-negative subtypes. Some findings on increased risk of hormone receptor-negative (133) and TNBC (109) have been reported.

Whether MHT use affects developmental pathways of carcinogenesis that in turn influence tumor aggressiveness is inadequately understood. Publications on MHT use and breast

cancer lethality, measured as breast cancer-specific mortality or survival, has begun to accrue (134-146). While breast cancer mortality refers to the incidence of breast cancer deaths among healthy women at baseline, breast cancer survival measures the case-fatality among those diagnosed. As such, mortality reflects the effects of both incidence and lethality, whereas survival specifically measures lethality. The evidence on the association between MHT use and breast cancer-specific mortality and survival is conflicting. In general, positive associations between MHT use and breast-cancer specific mortality have been reported (134, 137), whereas studies of patients with breast cancer have indicated improved survival among pre-diagnostic MHT users (135, 139-146). The relationship between MHT and mortality and survival from breast cancer subtypes is unclear. A pooled analysis from the Breast Cancer Association Consortium with 121,435 breast cancer cases and 8,554 breast cancer-specific deaths demonstrated improved survival among MHT users in all breast cancer subtypes (139).

1.3.4 Body fatness in postmenopausal women

Overweight and obesity, herein collectively referred to as body fatness, are defined by respective BMIs of ≥ 25 kg/m^2 and ≥ 30 kg/m^2 by the World Health Organization (WHO) (147). In 2022, more than 1 billion people were living with obesity worldwide (148). Corroborating an ongoing epidemic, the prevalence of adult obesity has more than doubled in most countries since 1990 (148). The IARC Working Group estimated 4.5 million deaths worldwide caused by body fatness in 2013, and identified 13 cancer sites for which there were sufficient evidence that body fatness increases cancer risk (149). One of these cancer sites is postmenopausal breast cancer. Postmenopausal body fatness is considered a hormone-related risk factor for breast cancer due to the correlation between amount of adipose tissue and estrogen levels, owing to the endogenous estrogen synthesis which occurs mainly in adipose tissue in postmenopausal women (150, 151).

Body weight is associated with breast cancer in different ways through the life cycle; while birth weight and postmenopausal BMI is positively associated with breast cancer risk, childhood, adolescent, and premenopausal BMI are inversely related to risk in both pre- and postmenopausal women (73, 149, 150, 152-161). Among postmenopausal women, high BMI and/or adult weight gain is associated with hormone receptor-positive or luminal A-like

tumors (78, 133, 149, 158, 159, 162-168). A meta-analysis of over 1000 epidemiologic studies reported a relative risk of 1.1 (95% CI 1.1-1.2) per 5-unit increase in BMI, particularly in ER-positive postmenopausal breast cancer (149). While the majority of studies have reported no association with hormone receptor-negative subtypes (78, 158, 159, 162-167), two studies have found increased risks of TNBC with increasing BMI (133, 169). The association between BMI and postmenopausal breast cancer seems to be confined to non-users of MHT (149, 158, 160, 168). Correspondingly, postmenopausal weight loss reduces the risk of breast cancer among women not using MHT (168, 170, 171).

In contrast to postmenopausal breast cancer, higher BMI is associated with lower risk of breast cancer in premenopausal women (156, 157). A strong and linear association has been reported, and apparent for both ER-positive and ER-negative disease (157). A commonly hypothesized mechanism is obesity-associated anovulation, whereby increased estradiol synthesis in women with obesity leads to negative feedback in the hypothalamic-pituitary-axis, resulting in decreased ovarian function, fewer ovulatory cycles, and reduced exposure to ovarian hormones (172, 173). However, this suggestion has not been supported by studies adjusting for menstrual cycle patterns (174, 175). Estradiol levels have been found to be associated with premenopausal breast cancer risk in numerous studies (97, 98, 176-178). As premenopausal women with higher BMI are reported to have lower estradiol levels (179), differences in sex hormone levels related to BMI may contribute to the inverse association between BMI and breast cancer risk. The underlying mechanistic action could also be dependent on the timing of overweight/obesity, as stronger inverse associations have been reported with increased BMI at younger ages (157). The inverse association between childhood and adolescent body fatness and breast cancer risk could be mediated by mammographic density, as lower breast density has been observed in women who were overweight at a young age (161, 180).

Recent efforts have been focusing on dynamic aspects of body fatness in relation to breast cancer. A clear dose-response association between intensity and duration of body fatness and risk of postmenopausal breast cancer has been reported (181, 182). Moreover, studies assessing lifetime trajectories, i.e. time-varying fluctuations, of body fatness and risk of breast cancer are emerging (183-186). Such dynamic aspects could be relevant for disease

development. However, the association between BMI trajectories and subtypes of breast cancer is unclear.

1.4 Research rationale

While some causal associations between hormonal risk factors and breast cancer subtypes are established, significant gaps remain in understanding how specific exposures influence subtype development. For instance, further investigation is needed to understand the relationships between hormonal exposures and hormone receptor-negative subtypes. Similarly, the effects of progestins and the dynamic aspects of body fatness on breast cancer subtypes, and the hormonal impact on breast cancer survival each warrant further study. Further, determining whether hormonal factors differentially affect risk of tumor subtypes is central for deepening our understanding of subtype-specific carcinogenesis. Investigating these relationships is purposeful within the large Norwegian Women and Cancer (NOWAC) cohort, which provides detailed data on hormone exposure and a continuously increasing number of invasive cancer cases among its participants. As such, we wanted to elucidate the impact of female hormonal exposures, including the use of OCs, adult body fatness, and MHT use, on breast cancer subtypes within this extensive cohort of Norwegian women.

2 Aims

The aim of this thesis was to study the association between female hormonal factors and breast cancer subtypes in middle-aged Norwegian women, and to test whether these associations varied across subtypes.

Specific aims:

- Estimate the association between combined and progestin-only oral contraceptives and subtypes of premenopausal breast cancer (Paper I)
- Estimate the association between dynamic aspects of body fatness, i.e. duration, intensity, timing, and trajectories, through adulthood and subtypes of postmenopausal breast cancer (Paper II)
- Estimate the association between use of menopausal hormone therapy and incidence, mortality, and survival of subtypes of postmenopausal breast cancer (Paper III)

3 Materials and methods

3.1 The Norwegian Women and Cancer study

The NOWAC study is a national, population-based prospective cohort. Initiated in 1991, the study was created to investigate the etiology of cancer in a representative selection of the Norwegian female population (187). Women born in 1927-1965 and between the ages of 30 to 70 were invited to participate based on a random sampling from the Norwegian Population Register. Recruitment was conducted in several calendar periods, primarily across three enrollment waves: 1991-92, 1995-97, and 2003-07. Invited women received an information letter and a questionnaire (Appendix). Of the 327,476 women invited, 172,472 responded and returned a completed questionnaire covering anthropometry, lifestyle habits, reproductive factors, self-reported diseases, medication use, diet, and other variables. Repeated questionnaires were collected from the participating women; participants were invited to return a second follow-up questionnaire in 1998-2014, a third in 2003-10, and a fourth in 2017 (Appendix). The unique national identification number assigned to every resident in Norway allows for complete follow-up through linkages to national registries (188).

3.2 Study samples and design

All three papers in the thesis had study samples extracted from the NOWAC study and were of prospective cohort design (Figure 7). In Paper I, subjects were excluded from the total cohort of 172,472 women if they were postmenopausal or 53 years of age or older at enrollment (n = 88,258), if they were MHT users at enrollment (n = 6,786), if they had prevalent cancers other than non-melanoma skin cancer (n = 1,018), emigrated or died before the completed questionnaire was returned (n = 16). Moreover, subjects with extreme values for age at first birth ($\leq$ 10 years; n = 2) and those with missing values of OC use at enrollment (n = 1,540) were also excluded. This left a total of 74,862 subjects who were eligible for analysis, out of whom follow-up information from a second questionnaire was available for 51,850 subjects.

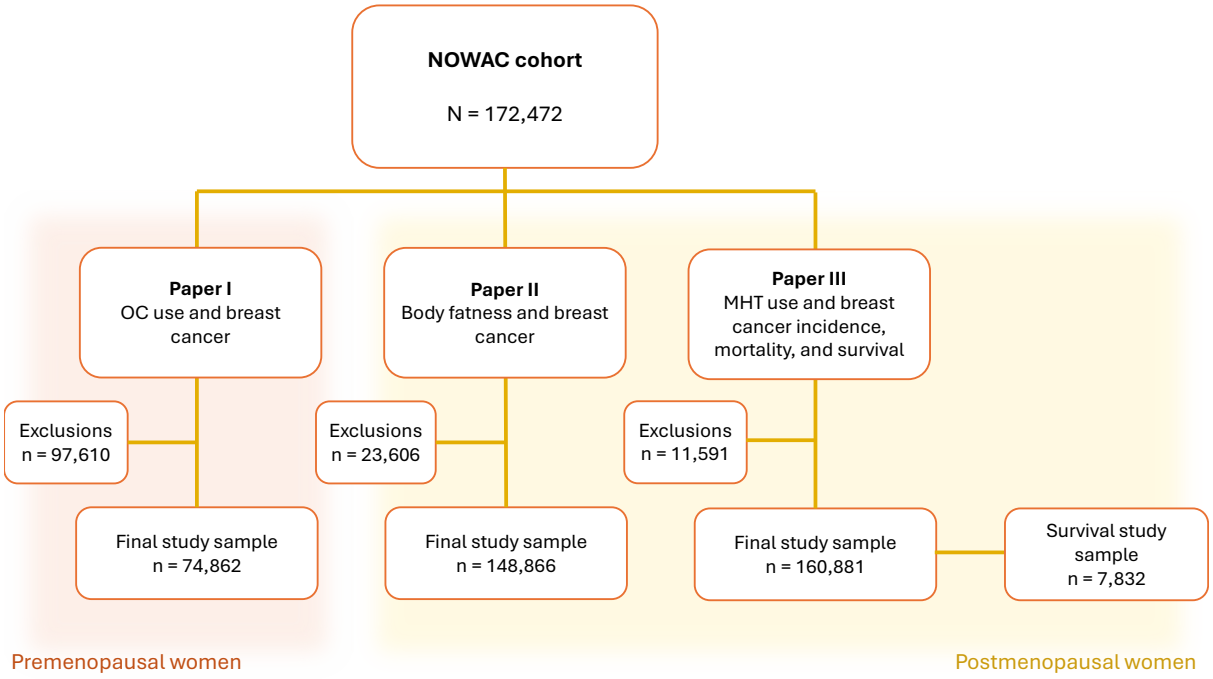

Figure 7. Study samples at a glance

In Paper II, subjects with less than two BMI measurements (n = 8,156) and no information on physical activity and tobacco smoking (n = 6,697) were excluded. Further exclusions included subjects with prevalent cancers (other than non-melanoma skin cancer) and cancers diagnosed within 1 year of first BMI measurement (n = 8,150), those who had died or emigrated prior to start of follow-up (n = 457), and women with extreme reported values for age at menarche (< 8 or > 20; *n* = 30), age at menopause (< 25 or > 60; *n* = 111), or age at first birth (< 12 or > 50; *n* = 5). The final study sample consisted of 148,866 women.

In Paper III, we excluded those with missing MHT status at the start of follow-up (n = 2,063), prevalent cancers (other than non-melanoma skin cancer; n = 8,866), participants who had died or emigrated before follow-up (n = 501), and those with extreme values for age at menarche (< 8 or > 20 years; n = 30), age at menopause (< 25 or > 60 years; n = 125), and age at first birth (< 12 or > 50 years; n = 6). The final study sample comprised 160,881 participants. For breast cancer survival analyses, 7,832 women diagnosed with incident postmenopausal breast cancer between 1991 and 2020 were included. In this subsample, women without breast cancer and those who were diagnosed post-mortem or after emigration (n = 12) were excluded.

3.3 Exposures

3.3.1 Exogenous hormone use

Information on exposure to OCs and MHT was obtained from self-administered questionnaires. The questionnaires included general inquiries about hormone use, covering ever use, age at first use, duration of use, and current use (Figure 8). Additionally, the women were asked to denote specific periods of use, defined as continuous use of a particular hormone brand for at least one month. To facilitate recall, the questionnaires included a photo booklet displaying images and names of various OC and MHT brands available in Norway up to the date of mailing. Follow-up questionnaires provided updated information on exogenous hormone exposure. Women who reported ever use at the baseline questionnaire and had missing status or reported never use at second or third questionnaires were categorized as ever users at follow-up.

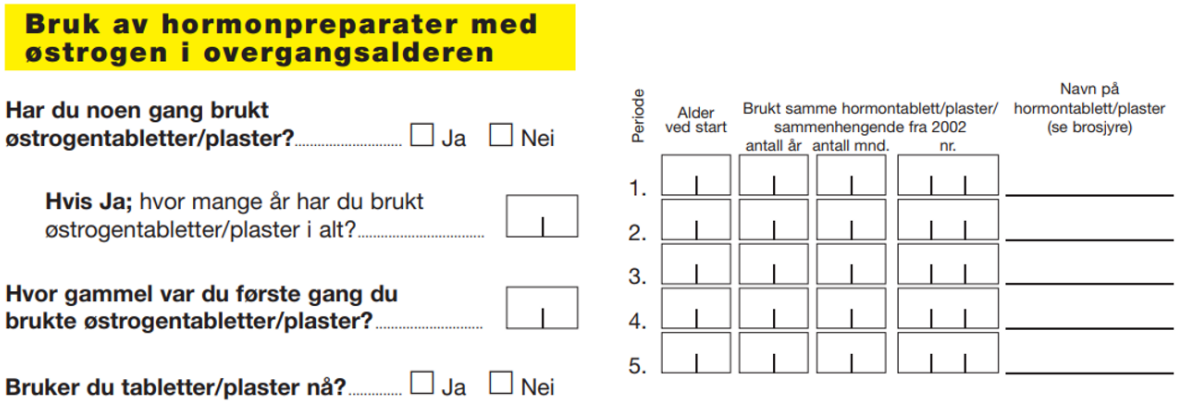

Figure 8. Excerpts from NOWAC questionnaire on MHT use

3.3.2 Body fatness

Self-reported weights at age 18 years and from the first, second, and third questionnaires, along with height from the first questionnaire, were used to calculate BMI at up to four time points. BMI was calculated as weight in kilograms divided by the square of height in meters and categorized according to the WHO's definition, with overweight defined as BMI ≥ 25 and < 30 kg/m^{2,} and obesity as a BMI of ≥ 30 kg/m^2 (189). Based on self-reported BMI at up to four points in time, we modeled duration (A), intensity (B), and timing (C) of body fatness

using linear mixed effects models, and BMI trajectories (D) using group-based trajectory modeling, explained in detail in section 3.6.1.1 and 3.6.1.2, respectively (Figure 9).

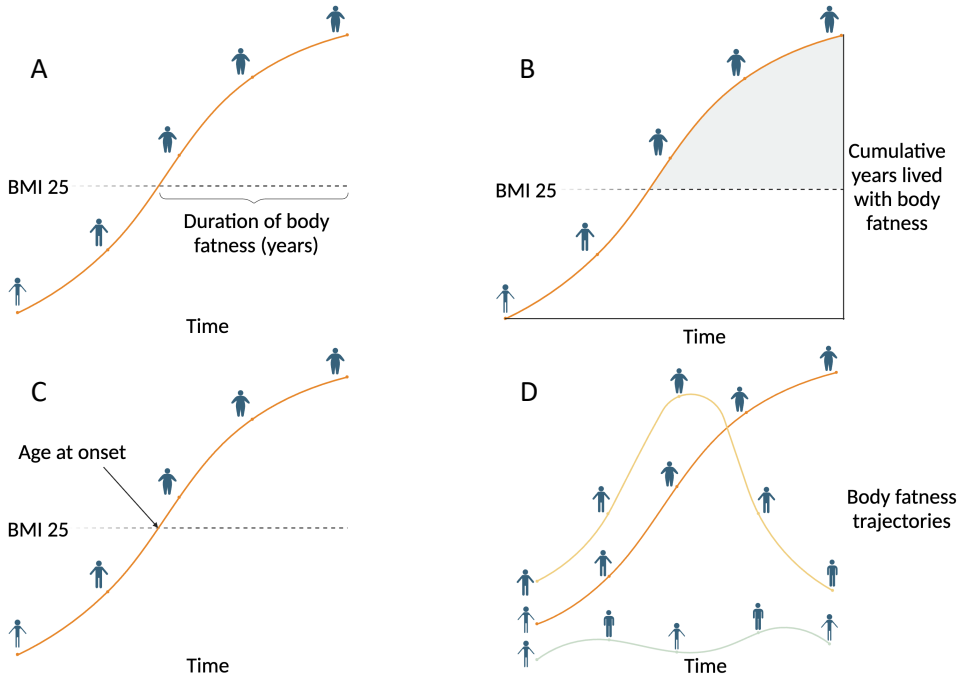

Figure 9. Schematic illustration of the different quantitative measures of changes in body fatness through adulthood: A) Duration of body fatness; B) Cumulative years (intensity) lived with body fatness; C) Age at onset of body fatness; D) Examples of body fatness trajectories. Created by BioRender.com

3.4 Outcomes

Incident invasive breast cancer cases were identified through linkage to the Cancer Registry of Norway and were classified according to the International Classification of Diseases 10th Revision (ICD-10, C50). Information on death and emigration were acquired through linkage to the Cause of Death Registry and the Central Population Register, respectively. These registries provide annual endpoint information, including the date of cancer diagnosis, death, emigration, and cause of death.

3.4.1 Breast cancer subtypes

Information on ER, PR and HER2 status was obtained from the Cancer Registry of Norway and assessed by IHC techniques by pathological departments nationwide. Prior to January 2012, ER negativity was defined as less than 10% reactivity. From February 2012, the threshold for ER-negative tumors was revised to less than 1% reactivity, following changes in treatment protocols in Norwegian clinics. We employed these official thresholds. PR

negativity was defined as < 10% reactivity. HER2 expression status was determined by IHC and/or ISH. Tumors with no or weak immunostaining were defined as HER2 negative, while moderate or strong immunostaining were considered HER2 positive. ISH was generally used to confirm moderate staining. In Paper I, breast cancer subtypes were classified according to ER and ER/PR defined status. We did not incorporate HER2 status into the subtype classification due to a significant number of missing values in this subset of premenopausal women. In Papers II and III, breast cancer subtypes were defined by IHC surrogates for molecular subtypes according to the St. Gallen 2013 criteria without using Ki67 in the subtype definition: luminal A-like (ER+ PR+ HER2-), luminal B-like (ER+ PR- HER2- or ER+ PR- HER2+ or ER+ PR+ HER2+), HER2 positive (ER- PR- HER2+) and triple-negative (ER- PR- HER2-) (36).

3.5 Covariates

Self-reported information on sociodemographics, anthropometry, lifestyle factors, reproductive and menstrual history were extracted from the comprehensive NOWAC questionnaires to assemble potential confounding factors. Assessment of covariates that could lead to confounding was carried out with different approaches in Paper I and Papers II and III. In Paper I, a backward elimination procedure was performed, where potential confounders were excluded from the model if their inclusion altered the regression coefficient by less than 10% (except for age at menarche). In Papers II and III, potential confounding factors were identified based on *a priori* knowledge using directed acyclic graphs (DAGs) (190). DAGs provide a transparent way to identify and demonstrate causal relationships between variables, thereby depicting potential confounders and their assumed association with exposure and outcome.

In Paper I which involved premenopausal women only, multivariable models were adjusted for age at menarche (continuous), a combined variable with parity and age at first birth, history of breast cancer in mother, BMI, and alcohol consumption. For the analysis addressing COC exposure, the model was adjusted for POC use (ever, never), and vice versa.

In Paper II including postmenopausal women only, multivariable analyses were adjusted for age at menarche, a combined variable with parity and age at first birth, breast cancer in

mother, MHT use, physical activity and smoking status. Due to delayed entries and thus varying possible time spent with overweight or obesity according to age at enrollment, we constructed a variable based on age at enrollment (10-year groups) that were included in the cox regressions of age at overweight/obesity onset and overweight/obesity duration as stratum variables. This way, we allowed the baseline hazard to vary across stratum, but the coefficients were equal across groups.

In Paper III, also on postmenopausal women, multivariable analyses for incidence, mortality, and survival outcomes were adjusted for age at menarche, a combined variable with parity and age at first birth, family history of breast cancer, BMI, physical activity, smoking status and education level.

3.5.1 Sociodemographic and family health covariates

All analyses in all papers were controlled for age as the underlying time metric. Age at baseline questionnaire, cancer diagnosis, death and emigration were calculated using year of birth and dates for baseline and follow-up questionnaires, cancer diagnosis, death and emigration. Education level, measured as the number of years of schooling, served as a proxy for socioeconomic status and was grouped into an ordinal category variable in four groups ($\leq$ 9 years, 10-12 years, 13-16 years, $\geq$ 17 years). Hereditary breast cancer was accounted for using reported family history with the disease. In Papers I and II, breast cancer in mother (yes/no) was used as surrogate for genetic susceptibility. In Paper III, first-degree relatives (mother and sister, only mother, only sister, none) was used in multivariable analyses.

3.5.2 Lifestyle covariates

Physical activity level was reported using an ordinal scale from 1 to 10 corresponding to very low to very high activity level, and subsequently grouped into three categories (low, moderate, high). Smoking status was reported and categorized as never, former or current. Alcohol consumption was reported as number of units of beer, wine or liquor per day/week/month. This was converted to daily intake of alcohol in grams and further grouped into ordinal categories (0.1-4.9 g/day, 5-9.9 g/day, $\geq$ 10 g/day).

3.5.3 Menstrual history and reproductive covariates

Menopausal status among study participants was based on reported menstrual history. A woman was defined postmenopausal if her menstrual period had stopped naturally or by bilateral oophorectomy. Age at menopause was defined as the reported age at which the woman´s menstruation stopped. Women with unknown menopausal status or irregular menses were considered postmenopausal at age 53 or older. Since menopausal status can be masked by a hysterectomy or by use of MHT before natural menopause, these women were also considered postmenopausal at age 53 or older. This cutoff has been used previously in NOWAC and is based on the Million Women Study convention (123, 191). For women who were current smokers, the age of 53 was substituted with 51 since current smoking can reduce age at menopause by approximately two years (192). Other menstrual and reproductive factors included age at menarche (continuous), parity and age at first full-term pregnancy. Parity (0, 1, 2, ≥ 3) and age at first full-term pregnancy (< 25, 25-29, ≥ 30 years) were combined into a single variable.

3.6 Statistical analysis

All statistical analyses were performed using STATA versions 14 and 17 (193, 194). We used Cox proportional hazard regression to model the time-to-event of interest and estimate hazard ratios (HR) with 95% confidence intervals. The proportional hazards assumption was evaluated by testing Schoenfeld residuals and by graphically inspecting log–log survival plots. All *p*-values were two-sided, allowing a type I error rate of 5%.

In Paper I, follow-up began at the time of return of the baseline questionnaire (Figure 10). Women were followed until date of cancer diagnosis, death, emigration, or the end of the study (December 31, 2015), whichever occurred first. Time-varying OC exposure was applied in the regression model, using updated information from a second questionnaire during the follow-up period.

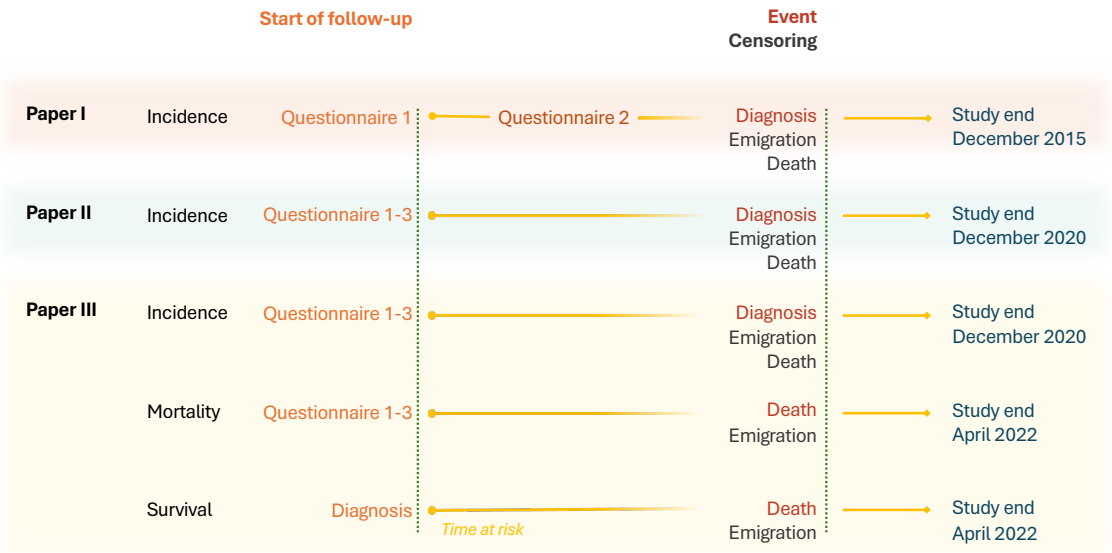

Figure 10. Follow-up in Papers I-III

In Paper II, the start of follow-up was defined as the date of the last questionnaire used for BMI modeling if the woman was postmenopausal or at the date of menopause if it occurred later than the last returned questionnaire. Women were followed until date of cancer diagnosis, death, emigration, or the end of the study (December 31, 2020), whichever occurred first.

In Paper III, start of follow-up for incidence and mortality analyses began at the date of the baseline questionnaire for postmenopausal participants. If menopause occurred later, start of follow-up was set to age at menopause or age at MHT initiation. Follow-up ended at the first occurrence among cancer diagnosis, death, emigration, or end of study (December 31, 2020 for incidence outcomes; April 30, 2022 for mortality outcomes). For analyses on breast cancer survival, follow-up was defined from date of breast cancer diagnosis until date of death, emigration, study end (April 30, 2022), or 10 years post-diagnosis, whichever occurred first.

Separate regression models were constructed for ER, ER/PR and ER/PR/HER2 (intrinsic-like) defined subtype outcomes, censoring subjects who developed a subtype other than the one defined as failure at the time of diagnosis (195). In all papers, one of our objectives were to investigate whether the exposure of interest was differentially associated with breast cancer subtypes. To assess heterogeneity between risk estimates across subtype outcomes, heterogeneity between HRs of breast cancer subtypes were tested by the Wald test in Paper

I. In Papers II and III, heterogeneity was tested by competing risks analyses using the data duplication method and likelihood ratio tests, as described by Lunn and McNeil (196, 197).

3.6.1 BMI variable constructions

In Paper II, linear mixed-effects models and group-based trajectory modeling (GBTM) were employed to construct the BMI variables of interest.

3.6.1.1 Linear mixed-effects models

Linear mixed-effects models were used to model duration, intensity, and age at first onset of overweight and obesity (198). Changes in BMI for each study participant was modeled as a function of age, physical activity (time-varying) and tobacco smoking (time-varying) (182). A cubic effect of age and random intercepts and slopes were employed. As the number of samples exceeded the number of measurement occasions, we did not assume any specific covariance pattern for the random effect; instead, an unstructured covariance matrix was fitted (199). BMI values were interpolated annually for each participant from age 18 up to the last valid BMI measurement. Using these interpolated values, years spent with BMI ≥ 25 (overweight duration) and BMI ≥ 30 (obesity duration) were calculated. The duration variables did not necessarily represent successive years of overweight or obesity, as intervals with normal weight could occur between periods of overweight or obesity. Further, the age at first onset of overweight or obesity from age 18 years were calculated. Lastly, we computed the weighted cumulative years of overweight (OWY) and obesity (OBY) as measures of intensity. This was done by multiplying the duration of overweight or obesity in years by the excess BMI units above the normal range (≥ 25 kg/m^2 for overweight and ≥ 30 kg/m^2 for obesity) for each increment of age. The durations of overweight and obesity were evaluated in 10-year increments, and intensity was assessed per 100 units, consistent with methods previously described (181, 182).

3.6.1.2 Group-based trajectory modeling

We characterized fluctuations in BMI from age 18 years to the age at the last valid BMI measurement using Nagin´s approach to group-based trajectory modeling (GBTM) (200, 201). GBTM, a semiparametric finite mixture model, identifies the evolution of BMI over age per individual and group individuals with relatively homogeneous BMI trajectories into

clusters. Trajectories were constructed by censored normal model using the Traj package in STATA, whereby the optimal number of groups and trajectory shapes were evaluated by the Bayesian information criterion using a two-stage approach (202). Consequently, the BMI fluctuations among the participants were best described through five-group trajectories based on a cubic function of age and adjusted for time-varying physical activity and tobacco smoking. Ultimately, the average posterior probability and odds of correct classification were calculated, yielding satisfactory results with high assignment accuracy according to Nagin´s criteria (202).

3.6.2 Multiple imputation

In Papers I and III, we performed multiple imputation with chained equations (MICE) to handle missing values which were assumed missing at random (MAR), as detailed in section 5.3.4. As per recommendations, 20 duplicate datasets with 10 iterations were made in order to reduce sampling variability from the imputation simulations in both papers (203). In Paper I, multiple imputation was applied to handle missing values among covariate variables (alcohol consumption, age at menarche, and BMI) and some exposure variables (duration of and time since last OC use) at baseline and missing exposure variables at follow-up. Among missing variables at baseline, the degree of missingness ranged from 1.6-2.2%. The fraction of missing exposure variables at follow-up due to non-response of a second questionnaire were approximately 31%. To avoid possible inconsistencies in status of use at baseline and follow-up, possible changes in OC status and duration of use was imputed and used to assign status and duration at follow-up. The imputation model included all covariates used in the multivariable analyses, age at baseline and follow-up, and the Nelson-Aalen cumulative hazard estimator as predictors. In Paper III, multiple imputation was performed to handle missing data on covariate variables only. Covariates with missing values were BMI, age at menarche, smoking status, physical activity, education and age at first birth. The fraction of missing information ranged from 0.03%-7.4%. Family history and parity were treated as auxiliary variables due to no missing values. A MICE model was constructed for each subtype outcome within incidence, mortality and survival study samples. The model included all covariates used in the multivariable analyses, age at study entry, a MHT variable, a binary outcome variable and the Nelson Aalen cumulative hazard estimator. In both papers, the parameter estimates and standard errors in the imputed datasets were

averaged according to Rubin´s rule to account for within- and between-imputation variances (204). After the multiple imputation process, we performed imputation diagnostics to evaluate the imputation model. As recommended (205), we checked for convergence and compared frequency and means between observed and imputed values. No abnormality was observed.

3.7 Ethical considerations

The NOWAC study has been approved by the Regional Committee for Medical Research Ethics in Northern Norway (REK NORD 141/2008) and the Norwegian Data Inspectorate. The participants provided informed consent for the collection and storage of their information, as well as linkage to the Cancer Registry of Norway, Mammography Registry of Norway, and the Norwegian Cause of Death Registry.

4 Results

4.1 Paper I

The aim of Paper I was to estimate the association between the use of COCs and POCs and subtypes of breast cancer in premenopausal women. Among the entire study sample of 74,862 premenopausal women, 1,245 incident invasive breast cancer cases occurred during 580,017 person-years of follow-up. Among them, 679 were ER+ (78.0%), 191 ER- (22.0%), 540 ER+ and PR+ (78.1%), and 151 ER- and PR- (21.9%). 475 cases had missing ER status (38.2%), whereas 554 cases had missing ER/PR status (44.5%). Mean follow-up time was 7.8 years. Women who developed premenopausal breast cancer were more likely to report family history of the disease, be younger, have a lower BMI, fewer children, and higher age at first birth compared to the total study sample.

Ever use of OCs (COCs and POCs combined) was associated with ER-negative (HR 1.48; 95% CI 1.06-2.06) and ER/PR-negative (HR 1.61; 95% CI 1.10-2.35) breast cancer, and marginally associated with overall breast cancer (HR 1.12; 95% CI 0.99-1.26). Current OC use increased risk of overall breast cancer with 36% (95% CI 1.09-1.71), and was positively associated with ER-positive, ER-negative, and ER/PR-negative breast cancer. Duration of use was associated with risk of all subtypes, whereby a significant trend by duration of use was observed for all subtypes. Considering time since last use, the association with overall breast cancer was limited to less than 10 years since last use (p_{trend} 0.02).

Stratified analyses on COC and POC use indicated that COC use (ever, current, and former use) were consistently associated with increased risks of ER-negative and ER/PR-negative breast cancer compared to non-use. Current use of COCs more than doubled risk of ER/PR-negative breast cancer (HR 2.39; 95% CI 1.14-5.04) compared to never OC use, and significant heterogeneity was observed across subtypes ($p_{heterogeneity}$ 0.04). POC use for 5 years or more was associated with ER-positive (HR 1.59; 95% CI 1.09–2.32; p_{trend} 0.03) and ER/PR-positive (HR 1.63; 95% CI 1.07–2.48; p_{trend} 0.05) subtypes, compared to those who had never used OCs. However, we observed no significant heterogeneity in risk estimates between subtypes with POC use. We observed similar effect estimates when considering POC users who had never used COCs, and in COC users who had never used POCs.

4.2 Paper II

Paper II aimed to examine the associations between duration, intensity, timing, and fluctuations of body fatness in adulthood and subtypes of breast cancer among postmenopausal women. Among the study sample of 164,316 postmenopausal women, 7,223 incident cases of invasive breast cancer occurred during 2,221,544 person-years of follow-up. The average follow-up time was 14.9 years. Changes in BMI were modeled over a range of 3 to 58 years, with mean modeling duration of 36 years. Among the breast cancer cases, 5,674 were ER+ (86.8%), 866 ER- (13.2%), 3,549 luminal A-like (62.8%), 1,387 luminal B-like (24.4%), 248 HER2-enriched (4.4%), and 466 triple-negative (8.2%). 1,573 cases had missing intrinsic-like subtype (21.8%). Five distinct BMI trajectories were identified (Figure 11): 43.5% of women had a consistent normal BMI (T1 “Normal-stable”) through adulthood; 40.3% started with normal weight and developed overweight in late adult life (T2 “Normal-overweight”); 12.8% evolved from normal to overweight in early adult life and had obesity in late adulthood (T3 “Normal-obesity”); 2.5% progressed from overweight to obesity (T4 “Overweight-obesity”); and 0.8% had a descending curve from obesity to overweight (T5 “Obesity-decrease”).

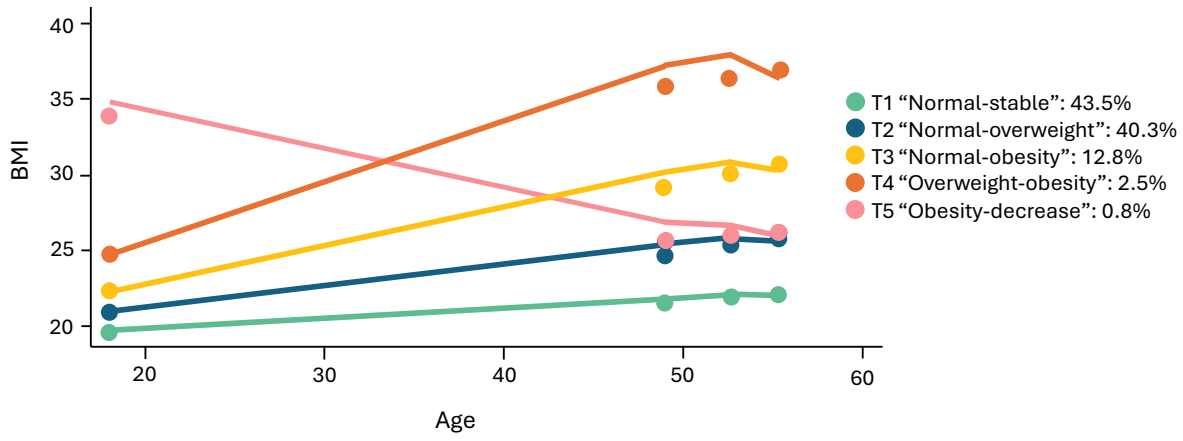

Figure 11. BMI trajectories in Paper II

Higher age at overweight was associated with an increased risk of overall postmenopausal breast cancer ($p_{trend} < 0.01$) compared to women who never became overweight. Generally, body fatness was associated with the luminal A-like subtype. Specifically, increased risks were observed among women with overweight (HR 1.11; 95% CI 1.02-1.20) or obesity (HR 1.13; 95% CI 1.00-1.28) at baseline, older age at the onset of overweight and obesity, and

increasing overweight duration (HR per 10-year increment 1.04; 95% CI 1.00-1.07). Several body fatness-related exposures were inversely associated with luminal B-like cancer: i.e. overweight duration (HR per 10-year increment 0.93; 95% CI 0.88-0.99), and weighted cumulative years of overweight and obesity (HR 0.85; 95% CI 0.74-0.99 and HR 0.61; 95% CI 0.38-0.99, respectively). We observed significant heterogeneity of effect estimates of overweight duration ($p_{heterogeneity}$ 0.03) and age at overweight ($p_{heterogeneity}$ 0.03) across breast cancer subtypes. Women in the “Obesity-decrease” trajectory group experienced decreased risk of overall breast cancer (HR 0.71; 95% CI 0.52-0.96) compared with those who remained in normal weight through adulthood. Women with a consistently increasing BMI throughout adult life had a higher risk of luminal A-like cancer compared to those with a "Normal-stable" trajectory (HR 1.09; 95% CI 1.01–1.17 for “Normal-overweight”; HR 1.20; 95% CI 1.07–1.33 for “Normal-obesity”), while those with decreasing weight had nearly a 50% reduced risk (HR 0.54; 95% CI 0.33–0.90 for “Obesity-decrease”). The “Overweight-obesity” trajectory was associated with a borderline-significant decreased risk of luminal B-like breast cancer (HR 0.64; 95% CI 0.41–1.00). No significant associations were found for HER2-enriched or TNBC subtypes.

Stratified analyses on MHT use revealed significant associations between body fatness and ER/PR-positive breast cancer in never MHT users and not in ever MHT users, thus suggesting effect modification by MHT use. In detail, in women who never used MHT, older age at overweight and obesity onset, overweight duration and weighted cumulative years with overweight increased the risk of ER/PR-positive breast cancer. Moreover, ascending trajectories from normal BMI were associated with ER/PR-positive breast cancer, where the “Normal-obesity” trajectory increased risk by 34% (95% CI 1.18–1.52). Women belonging to the descending trajectory appeared to be at 59% decreased risk (HR 0.41, 95% CI 0.20–0.87). Age at overweight onset ($p_{heterogeneity}$ 0.04), overweight duration ($p_{heterogeneity}$ 0.04) and the “Normal-overweight” ($p_{heterogeneity}$ 0.01) and “Normal-obesity” ($p_{heterogeneity}$ 0.01) trajectory were differentially associated with ER/PR-positive and ER/PR-negative breast cancer among never MHT users.

4.3 Paper III

In Paper III, we aimed to estimate the associations between MHT use and incidence, mortality, and survival of breast cancer subtypes in postmenopausal women. Among the study sample of 160,881 participants, 7,844 invasive breast cancer cases and 721 breast cancer-specific deaths occurred over median follow-up periods of 15.8 and 18.0, respectively. For analyses on 10-year survival outcomes, breast cancer patients were followed for a median of 8.5 years. Among incident breast cancer cases, the distribution of intrinsic-like subtypes was as follows: 3,784 luminal A-like (62.8%), 1,480 luminal B-like (24.6%), 264 HER2-enriched (4.4%), and 500 triple-negative (8.3%). 1,816 cases had missing intrinsic-like subtype (23.2%). Among the incident breast cancer cases, deaths from 163 luminal A-like (41.8%), 113 luminal B-like (29.0%), 33 HER2-enriched (8.5%), and 81 TNBC (20.8%) occurred. 331 breast cancer-specific deaths had missing intrinsic-like subtype (45.9%).

MHT use at study entry was associated with increased risks of overall, luminal A-like, and luminal B-like breast cancer compared with never use. Current EPT use increased risk of overall, luminal A-like, and luminal B-like breast cancer with 44% (95% CI 1.36-1.52), 41% (95% CI 1.31-1.52), and 23% (95% CI 1.09-1.40), respectively, and the associations varied by subtype ($p_{heterogeneity} = 0.04$). Among these outcomes, a significant trend for duration was observed, with respective increase in HRs by 4%, 4%, and 2% per year of EPT use. Former use of ET (HR 0.68; 95% CI: 0.49–0.94) and EPT (HR 0.86; 95% CI: 0.75–0.99) were associated with decreased risk of overall and luminal A-like breast cancer compared with never use, respectively. Increasing associations with the overall, luminal A-like, and luminal B-like subtypes were observed with increasing cumulative estrogen and progestin doses. > 2 g NETA equivalence was associated with HER2-enriched breast cancer (HR 1.79; 95% CI: 1.08–2.98), whereas high estrogen dose (≥ 5 g) combined with low progestin dose (< 1 g) was associated with a 2-fold increased risk of TNBC (HR 2.23; 95% CI: 1.22–4.09).

Among the entire study sample, ever and current use of EPT at study entry were associated with an increased risk of mortality from luminal A-like breast cancer (HR 1.74; 95% CI: 1.24–2.44 and HR 2.15; 95% CI: 1.51–3.05, respectively). The association with breast cancer mortality increased by 2% per year of EPT use, and ≥ 5 years of EPT use was associated with

a 2-fold risk of dying from luminal A-like breast cancer (HR 2.16; 95% CI: 1.42–3.29). No association was observed between MHT use and mortality from luminal B-like, HER2-enriched, or triple-negative disease. Relationships between current MHT use and breast cancer mortality varied across intrinsic-like subtypes ($p_{heterogeneity}$ = 0.03).

Among patients with breast cancer, EPT use was inversely, although non-significantly, associated with overall breast cancer survival, thus higher 10-year survival compared with non-users. Positive associations, i.e. worse survival, were observed with luminal A-like subtype, although non-significant effect estimates were dominant also for these results, except marginal significance for duration of EPT use (HR death 1.04; 95% CI: 1.00-1.09 per year increment). Ever and current use of EPT at study entry were associated with improved survival from TNBC compared with never users (HR death 0.57; 95% CI: 0.34–0.96 and HR death 0.48; 95% CI: 0.26–0.87, respectively). Moreover, current MHT use was differentially associated with survival by intrinsic-like subtypes ($p_{heterogeneity}$ = 0.02).

1

5 Discussion

5.1 Summary of the results

This thesis identified dissimilar associations between hormonal exposures and risk of breast cancer across tumor subtypes. In OC users, those who used COC regimens were at increased risk of hormone receptor-negative breast cancer, whereas POC users experienced increased risk of hormone-receptor positive breast cancer. Body-fatness related exposures, including duration, intensity, timing, and trajectories with increasing body weight were associated with hormone receptor-positive and luminal A-like subtype and predominantly among never users of MHT. MHT use increased risk of incident and fatal luminal A-like disease, whereas pre-diagnostic MHT use was associated with increased survival among TNBC patients. Hence, our results indicate that hormone receptor-negative breast cancer is not completely hormone-insensitive.

5.2 Discussion of the main results

5.2.1 Overall breast cancer risk

5.2.1.1 Exogenous hormones

The slight increase in overall premenopausal breast cancer risk by 36% among current OC users observed in our study was similar to risk estimates reported in previous large studies (69, 70, 116). The risk of overall breast cancer increased with increasing duration of use and ceased after 10 years since last use, the latter underpinning the established temporary association which attenuates with time since last use (70, 116). The finding that POC use was associated with breast cancer if used for five years or more is in line with the results from Morch et al., who discovered increased breast cancer risk with current or recent use of POC, confined to levonorgestrel-containing products (69). Due to the relatively small fraction of POC users in our cohort, we were not able to study the effects of specific progestins.

Our findings of a 24% increased risk of overall breast cancer among current MHT users aligns with risk estimate from large, prospective studies (109, 125, 127). Consistent with a recent meta-analysis, risk increased with duration of use, and, as the increased risk was confined to current users, was dependent on recency of use (72). In contrast to this study, we did not

observe increased breast cancer risk among ET users. This could be due to the small proportion of women who were ET users in our study sample, resulting in insufficient statistical power to detect the modest association reported. Underscoring the hormone dependency of breast cancer development, we observed increased risks of breast cancer with increasing cumulative dose of estrogen and progestin in a dose-response relationship. Together, the findings on exogenous hormones and overall breast cancer add to the empirically supported knowledge that OC and MHT use is causally related to a modest increase in breast cancer risk.

5.2.1.2 Body fatness

In our study, we observed 2% increased risk of overall breast cancer per 10-year increment in overweight duration. Arnold et al., who assessed duration and intensity of body fatness in relation to breast cancer risk, reported a corresponding 5% increase in risk (181). Moreover, they discovered 8% risk increase per 100 units OWY, which we did not capture in our results. Of note, our study sample were leaner and experienced less weight gain compared to theirs. Arnold et al. found more pronounced results among women who never used MHT, and, in another multicenter study by the same author, that significant results were confined to never MHT users (182). Consistent with these findings, our stratified analyses by MHT use also revealed results that were more pronounced and, for some exposures like overweight intensity, only became significant among never users of MHT. Another potentially relevant effect modifier on the association between body fatness and breast cancer is breast density (206-208). Unfortunately, we were not able to assess these relationships as we did not have access to such data.

A previous study on body shape trajectories discovered increased breast cancer risk with trajectories representing weight gain from lean body shape, with more pronounced risks among never users of MHT (186). This study started the trajectory modeling from childhood until mid-adulthood, and did not capture a distinct trajectory with decreasing body fatness. Moreover, they applied perceived body silhouettes as a measure of body fatness. These differences could explain some of the disagreeing results, as we did not capture these associations with overall breast cancer. Other similar studies modelled trajectories from childhood until early adulthood and would not be comparable to our study (183-185).

5.2.2 Hormone receptor-positive breast cancer risk

5.2.2.1 Exogenous hormones

The novel association of POC use with hormone receptor-positive breast cancer remains unconfirmed, as, to our knowledge, no other studies have yet examined POC use and breast cancer subtypes. However, POC use have been linked to overall breast cancer risk, as previously mentioned (69). We did not demonstrate differential associations of POC use across subtypes, which could be due to a true null difference, or because of lack of statistical power. The association between POC use and hormone receptor-positive breast cancer is based on a small number of cases. Thus, cautious interpretation is necessary.

Our results indicated that MHT use increases risk of luminal A-like breast cancer, agreeing to this well-established association (80, 109, 130-132). The results on luminal A-like subtype mirrored those of overall breast cancer due to the large proportion of this subtype. Surprisingly, we observed an inverse association between luminal A-like subtype and former use of MHT. This has not been supported by previous publications. While follow-up started at the baseline questionnaire for those who were postmenopausal at the time completing the questionnaire, nearly half of the study sample entered the study at menopausal onset that occurred later. As MHT status was updated at start of follow-up whereby a large portion had recently entered menopause, a fairly small proportion were former users. The interpretation of this finding is challenging because we did not consider time since cessation of use. Moreover, we detected increased risk of the luminal B-like subtype with ever and current use of MHT, which has also previously been reported in large studies (80, 109, 132).

5.2.2.2 Body fatness

Our study aligns with the emerging empirical consensus that adult body fatness increases risk of hormone receptor-positive or luminal A-like disease in postmenopausal women (78, 149, 158, 159, 162-167). Contrarily, previous reports do not support an inverse association between body fatness and luminal B-like cancer, as we observed for overweight duration and overweight/obesity intensity. Most studies have demonstrated no association between body fatness and luminal B-like disease (84, 165, 209, 210), while one study reported a positive association with this subtype (78). Differences in study design, participant age, exposure measurement, sample size, and subtype definitions could account for these inconsistencies. Importantly, our results concerning luminal B-like breast cancer should be

interpreted cautiously due to the low statistical power with broad CIs, raising the possibility that the findings could be due to chance as we did not adjust for multiple testing.

We demonstrated an inverse association between weight loss from obesity and breast cancer, which was confined to the luminal A-like subtype. The results on overall breast cancer are in line with previous publications (168, 170, 171, 211, 212), whereby some of them found inverse associations with hormone receptor-positive tumors (168, 170, 171).

5.2.3 Hormone receptor-negative breast cancer risk

5.2.3.1 Exogenous hormones

The association between COC use and hormone receptor-negative subtypes, where ever, current, and former use increased risk, is in agreement with some pre-existing prospective and retrospective studies (121, 213, 214), as well as a recent prospective study (116) and meta-analysis (122). We detected significant heterogeneity with current COC use by hormone receptor-subtype, suggesting true differences in risks of these subtypes. In this sample of premenopausal women, we were unable to apply HER2 status into the subtype definition due to substantial missing data. In the Nurses' Health Study, heterogeneity by OC use was only observed across subtypes when considering tumor subtype defined by ER, PR and HER2 (116). It is possible that the more detailed subtype categorization would provide a more nuanced picture of the associations between COC use, POC use and subtypes of breast cancer.

Consistent with several studies, we did not observe associations between general MHT use and HER2-enriched or triple-negative subtypes (80, 130, 131). The observed associations between increasing cumulative progestin dose and HER2-enriched disease, and high cumulative estrogen dose combined with low cumulative progestin dose and TNBC are based on small sample sizes and should be interpreted without inferring causality. However, association between MHT use and estrogen receptor-negative (72) and triple-negative (109) breast cancer has been reported which provides support to our finding on cumulative hormonal dose and TNBC. Further research is required to confirm or disprove the association between MHT use and hormone receptor-negative subtypes.

5.2.3.2 Body fatness

Our findings of a null association between body fatness and hormone receptor-negative subtypes are in agreement with most prospective studies (78, 158, 159, 162-167). However, other studies have indicated positive associations between increasing BMI and TNBC. One study using data from the Women's Health Initiative found a 1.35-fold increase, though non-significantly, among women in the highest versus lowest BMI quartile (169). The other study, using the European Prospective Investigation into Cancer cohort, found a 1.47-fold increase in third versus first BMI tertile among never MHT users (133). Positive associations between body fatness and TNBC have also been observed in premenopausal women (215). We might have been restricted by low statistical power to detect such potential associations.

5.2.4 Breast cancer mortality and survival

While MHT use was associated with increased population mortality from overall and luminal A-like breast cancer, pre-diagnostic MHT use among breast cancer patients was not associated with worse survival from overall breast cancer. In fact, pre-diagnostic MHT use was associated with increased survival among TNBC patients. Current use of MHT showed significant differences in its association with mortality and survival across tumor subtypes.

Our findings are coherent to those in previous studies, suggesting that MHT use increases risk of overall breast cancer-specific mortality (134, 136, 137), while improves survival among patients who develop breast cancer (135, 139-146, 216), albeit non-significantly in our results. While studies on breast cancer survival begin follow-up at breast cancer diagnosis and tend to adjust for stage, tumor characteristics, and/or treatment, mortality studies begin follow-up at study entry and typically adjust for traditional breast cancer risk factors. Despite similar adjustments, we observed divergent effect estimates for overall breast cancer. The inability to detect significant inverse associations with overall breast cancer survival in our results could be due to differences in recruitment periods. In our study, start of follow-up was in median year 2004, and consequently we expect a mixture of user patterns seen prior to and following the millennium shift. As most prior studies on MHT use and breast cancer mortality and survival were from earlier recruitment periods, it is plausible that the presumably higher amount of low-dose formulations used in our cohort

could dilute associations. Moreover, differences in age at initiation of MHT and time from menopausal onset to initiation could contribute to differential results.

The finding where MHT use was associated with increased survival among patients with TNBC has previously been reported (139). However, unlike in our study, the authors reported similar effect estimates with improved survival for all subtypes and did not detect heterogeneity by intrinsic-like subtypes.

5.2.5 Possible mechanisms

5.2.5.1 Exogenous hormones and breast cancer development

According to the multistep view, carcinogenesis can be divided into tumor initiation, promotion, and progression. After tumor initiation, which is the result of genetic alterations, a reversible stage of tumor promotion follows, which is mediated through promoter-receptor interactions. If the stage of promotion is not reversed, it culminates into tumor progression, which is an irreversible stage characterized by malignant growth (217). It is proposed that hormones influence hormone-sensitive cancers, including breast cancer, through alternative pathways that do not require a specific tumor initiator (Figure 12) (8).

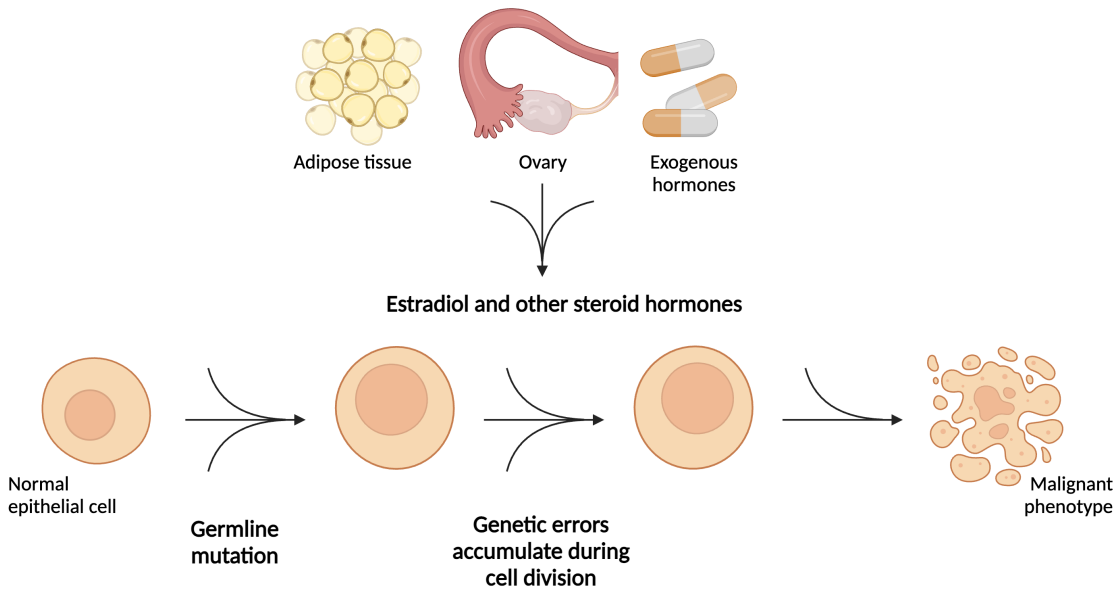

Figure 12. Hormonal cell proliferation model. Adapted from Henderson (8) and created by BioRender.com

Estrogen, both endogenous and exogenous, stimulates cell division in breast epithelial cells, thereby increasing the chance of random genetic error and cancer development. Our results

indicated increased breast cancer risk associated with EPT use, whereas ET use did not increase risk. The findings where EPT use confers a greater breast cancer risk than unopposed estrogen has been consistent in previous literature, as well as in our study. This has been hypothesized to be due to the mitotic effects of progestins on breast tissue, which, similarly to estrogen, leads to the accumulation of DNA errors that ultimately result in breast cancer, or increased proliferation of malignant cells (218). Supporting this is the observation that women treated with EPT exhibit a greater increase in mammographic density and higher cell proliferation in benign breast biopsies compared to those taking ET (219). As such, it is plausible that estrogen and progestins play a crucial role at all stages in the development of breast cancer (66).

The carcinogenic effect of progestins may depend on the regimen and type of progestin used. Continuous use of progestins, in contrast to cyclic regimens, has been associated with higher risk (109, 128, 129). A proposed mechanism is the inhibition of sloughing (cell shedding) of lobular duct epithelium with continuous use (101). The type of progestin used in EPT may also influence breast cancer risk, as synthetic progestins have been associated with excess risk in contrast to MHT with natural progesterone (220). Several studies have reported a relatively higher breast cancer risk associated with NETA compared to other progestin constituents (72, 109, 220, 221). Moreover, second-generation progestins, including levonorgestrel, used in OCs have predominantly been associated with breast cancer risk (69, 116, 222). Levonorgestrel has proved higher potency than other commonly used progestins in animal assays (223). In addition to the PR, progestins interact with other hormone receptors including ER, androgen receptor, glucocorticoid receptor and mineralocorticoid receptor and exert various functions other than progesterone-like effects (224). Levonorgestrel exhibits a high affinity for androgen receptors and induces more potent androgenic effects compared to other progestins (224, 225). Given the high proportion of androgen receptor expression found in breast cancer tumors (226), the stimulation of these cells by progestins is a plausible mechanism.

5.2.5.2 Exogenous hormones and breast cancer progression

The understanding of the underlying mechanisms by which MHT affects breast cancer aggressiveness and prognosis is incomplete. The opposing results on MHT use and breast cancer-specific mortality among the entire study population and survival among breast

cancer patients observed in our results, as well as in previous literature, could be due to several circumstances. First, the improved survival observed in pre-diagnostic MHT users could be attributed to early detection due to mammography screening. The consequences of early detection include lead-time bias, owing to the receding time of diagnosis, and length bias, which involves the identification of relatively slow-growing tumors with good prognosis (227). These circumstances inflate the survival time while the course of the disease remains unaltered. Given that MHT users are more likely to attend mammographic screening (228, 229) and consistent reports that MHT users have more favorable tumor characteristics than never users (135, 141-143, 145, 146, 230), the presence of these biases seems plausible. Second, differences in socioeconomic status, health seeking behavior and lack of exchangeability in MHT users and non-users could also afflict survival time. Third, the presence of collider stratification bias is another possible explanation and will be elaborated further in section 5.3.2.3. Together, these circumstances comprise implications of biased inverse associations between MHT use and breast cancer survival.

The favorable impact of MHT on breast cancer survival could also stem from underlying biological actions. Arguing against bias due to early detection are evidence that MHT use increases risk of interval cancers compared to screen-detected cancers (109, 230, 231), presumably due to increased mammographic density among MHT users (232, 233). Patients with breast cancer mostly die of systemic metastatic disease (234). According to the multistep view, progression into metastatic disease is a result of the sequential accumulation of mutations and, consequently, phenotypic alterations in a single cell followed by clonal expansion (235, 236). From this point of view, it is plausible that MHT acts as a growth promoter to occult tumors, where ceased use after a cancer diagnosis could be beneficial in breast cancer cases that occurred due to MHT. The discontinuation of MHT could potentially make the cancer less aggressive compared to cancers that develop in individuals who did not use MHT. Alternatively, it is possible that MHT induces tumors of distinct phenotypes with favorable prognosis. When exposed to estrogens and progestins, the expression of both ER and PR can result in crosstalk between the receptors and, consequently, yield better disease outcome (237). Receptor conversion, i.e. discordance of ER, PR and HER2 status between the primary breast tumor and paired metastases, occurs in 10-30% of receptor expressions in the primary tumor (238), with a majority of change from

positive to negative receptor status (238, 239). However, whether MHT can influence these alterations is not known. In summary, there is currently insufficient knowledge about the impact of MHT on breast cancer progression.

5.2.5.3 Exogenous hormones and hormone receptor-negative breast cancer

We identified positive associations between COC use and cumulative estrogen and progestin doses in MHT and hormone receptor-negative breast cancer, and inverse associations between MHT use and TNBC survival. While the association between female sex hormones and hormone receptor-positive breast cancer has a plausible underlying mechanistic action of tumor promotion through ER and PR stimulation, the link between estrogens, progestins and hormone receptor-negative breast cancer is not well understood. Non-hormonal mechanisms have been proposed, whereby estrogen can promote growth of ER-negative cancers by acting on cells distinct from the cancer cells to stimulate angiogenesis (240).

Several mechanisms could explain the potential association between exogenous hormones and TNBC. First, emerging focus has been raised on the influence of the androgen receptor in TNBCs. The presence of androgen receptor expression has been reported in a substantial proportion of triple-negative tumors and represents a distinct subtype of TNBC (241, 242). In estrogen receptor-negative tumors, the presence of androgen receptors is associated with postmenopausal status and improved survival (243). Synthetic progestins used in OCs and MHT have affinity to androgen receptors (244, 245), which in turn can promote epithelial-mesenchymal transition, migration and invasiveness in androgen receptor-positive TNBC (246). Second, progestins can also indirectly stimulate proliferation through paracrine signaling on neighboring cells (247). Third, despite TNBC tumors being estrogen receptor-negative (i.e. ERα-negative), they can express alternative estrogen-binding receptors, including ERβ or G-protein-coupled estrogen receptor (247). Thus, estrogen may still exert direct growth-stimulating effects in TNBC. Although hormonal mechanisms in TNBC continue to accrue, the direction of these effects remains unclear. In sum, the alternative pathways in which estrogen and progestin could influence hormone receptor-negative tumor initiation and progression is poorly understood and need to be further investigated.

5.2.5.4 Body fatness and breast cancer

We demonstrated associations between body fatness and postmenopausal breast cancer, which was confined to luminal A-like disease. A biological mechanism describing the association between body fatness and breast cancer is the altered circulating levels of sex hormones in women with overweight and obesity (150, 248). In the postmenopausal state, in which ovarian hormone levels are low, the primary source of estrogen is through aromatization of estrogen precursors in peripheral tissues such as adipose tissue. Consequently, increased body fatness in postmenopausal women leads to higher estrogen levels. Epidemiological studies have confirmed that the association between body fatness and postmenopausal breast cancer is largely explained by the increase in estradiol levels with higher BMI (151, 249). Indeed, even among women with normal BMI, a higher percentage of body fatness increased breast cancer risk (159). In addition to estrogen, a correlation between BMI and androgen levels, i.e. testosterone and androstenedione, and an inverse correlation between BMI and SHBG, have also been demonstrated and linked to breast cancer risk (248, 249). Given that high insulin levels are associated with both breast cancer and high BMI, hyperinsulinemia may, directly or indirectly via increased insulin-like growth factor 1, contribute to the association between body fatness-breast cancer relationship (150, 250). Moreover, elevated concentrations of adipokines and chronic inflammation are also proposed mechanisms (150).

The effect modification by MHT use on the association between BMI and breast cancer risk is proposed to be due to a dilution of the elevated endogenous sex hormones caused by adiposity, whereby the relatively elevated doses of exogenous hormones diminish the BMI – breast cancer risk association (150).

Despite ambiguous results regarding body fatness and hormone receptor-negative subtypes, indications exist that there might be an association. Thus, hormone receptor-positive and -negative subtypes may share common adiposity-driven mechanistic pathways.

5.3 Methodological considerations

The strengths and limitations of this thesis will be considered in light of methodological considerations outlined in the following paragraphs.

5.3.1 Study design

The papers in this thesis employed a prospective cohort study design to address the aims. A cohort study is an observational study in which a predefined population is followed over time from study inclusion until the occurrence of the outcomes of interest (251, 252). Commonly, incidences in groups with different exposure levels are compared. In contrast to cross-sectional and case-control studies, prospective cohort studies have the ability to explore the natural history of disease and the temporality between exposure and outcome (253). Temporality is, among others, key criteria of causality (254). While randomized controlled trials are considered gold standard for studying causal inference, they are costly and carries considerable ethical considerations. It could be considered unethical, in our scenario, to assign study participants to exogenous hormones or weight gain to study the effects on breast cancer. Thus, cohort studies are well-suited for examining the effects of risk factors on disease development. Another advantage of cohort studies is their capacity to study multiple outcomes, which we took advantage of by studying incidence and mortality. This is in contrast to case-control studies where subjects are recruited to a study if they have an outcome of interest. Disadvantages of cohort studies applicable to our study involve participant loss to follow-up and inefficiency with rare outcomes (in our scenario rare subtypes) (255).

5.3.2 Internal validity

Internal validity refers to whether the observed effect of an exposure on an outcome is genuinely attributable to the exposure rather than to chance or bias (252). Bias represents systematic errors that skew the results away from the truth, leading to inaccurate estimations of the association. In the uttermost consequence, these errors can result in false positive estimations (type I errors), where a statistically significant result is obtained when the null hypothesis (no difference between groups) is true. Conversely, false negative estimations occur when a true association fails to be detected in the results (type II errors) (251). In this work, internal validity would refer to the degree of confidence that the observed associations between hormonal risk factors and breast cancer risk represents true causality. Observational studies are encumbered by three major types of systematic errors: confounding, selection bias, and information bias, as described below (251).

5.3.2.1 Confounding

Confounding refers to a situation in which the association between an exposure and an outcome is distorted due to the presence of common causes of the exposure and the outcome (252, 256). The common causes, also referred to as confounding variables or confounders (C), are not on the causal pathway between exposure (X) and outcome (Y) (252). Contrary to a confounder, a variable that occurs on the causal pathway from exposure to outcome is called an intermediate variable, or a mediator (M; Figure 13). While adjustment for a confounding variable is necessary to ascertain the true causal effect of an exposure on an outcome, conditioning on an intermediate variable can cause overadjustment bias (251). Distinguishing between confounders and mediators can sometimes be challenging. In our scenario in Paper II, we considered physical activity as a confounder on the association between BMI and breast cancer. However, BMI might also subsequently affect physical activity, and thus, place physical activity as a mediator on the causal pathway between BMI and breast cancer. As such, the temporality between these variables are crucial.

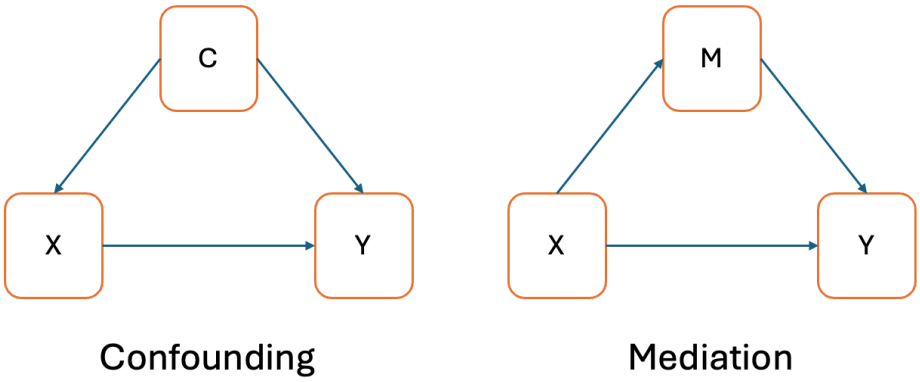

Figure 13. The concept of confounding (C) and mediation (M). Adapted from Lash (251)

Confounding is of particular concern in epidemiological research aimed at investigating causality. Ways to minimize confounding in statistical analysis involve stratification and adjustment in regression analysis. To achieve this, confounding factors must be identified. There are multiple procedures to select variables for model inclusion. As described in the Methods section, two distinct approaches were used to select covariates in Paper I and Papers II and III, respectively. A statistical confounder selection method was applied in Paper I. In detail, we performed a backward selection procedure with adjustment for all potential

confounding factors and iteratively removing each covariate unassociated with the outcome conditional on the exposure and other covariates. Although widely used in research, this method carries significant disadvantages, such as the reliance on a single *p*-value to determine covariate association with the outcome, which does not distinguishing between confounding factors and mediators (251). In Papers II and III, covariates were selected based on *a priori* knowledge and their assumed causal relationship with exposure and outcome was depicted in DAGs (190). We adjusted for a minimal sufficient adjustment set identified from the DAG. Employing the same method for covariate selection in Paper I as in Papers II and III could have resulted in different adjustments.

In all papers, all analyses were adjusted for age, a crucial confounding factor in the association between exogenous hormone use (Paper I/III), body fatness (Paper II) and breast cancer. Age was included as time metric in all papers, which is a recommended approach to eliminate confounding by age when the hazards of the outcome are expected to change with age rather than time-on-study (257). Further, we adjusted for reproductive factors including age at menarche, parity and age at first birth in the analyses of all three papers. These factors were considered important confounding factors between exogenous hormone use, body fatness and breast cancer. Family history of breast cancer is a strong risk factor for breast cancer and is also assumed to influence exogenous hormone use. Although not directly associated with adult BMI, it was included in the minimal sufficient adjustment set and thus adjusted for in analyses on postmenopausal BMI and breast cancer. Additional variables adjusted for in the multivariable models were smoking status (Paper II/III), BMI (Paper I/III), physical activity (Paper II/III), alcohol consumption (Paper I), MHT use (Paper II), and education level (Paper III). To exclude confounding by menopausal status, we restricted study samples to premenopausal women (Paper I) and postmenopausal women (Papers II/III).

Controlling for variables that can cause confounding may remove bias. However, the existence of residual confounding caused by measurement error or unmeasured confounding can leave biased effect estimates (258). The comprehensive NOWAC questionnaires embodies numerous potential confounders for the effect of exogenous hormone intake and body fatness on breast cancer. However, there is always a possibility that not all potential confounding factors are taken into account and thus that unmeasured

confounding exists. We did not have access to a few established risk factors for breast cancer which could also impact exogenous hormone use and BMI. For instance, benign breast disease and exposure to ionizing radiation could serve as confounding factors on the association between exogenous hormone use and breast cancer (259).

Given the self-reported nature of covariate variables and the potential lack of detail in these covariates, there is also a potential of residual confounding in our results. For example, we know that genetic susceptibility to breast cancer is an important confounding factor for the association between exogenous hormone use and breast cancer. As we did not have access to such data, we used family history of breast cancer as a surrogate covariate. However, even though family history is an important risk factor irrespective of genetic susceptibility (260), it is possible that this variable is too vague to cover the confounding caused by hereditary breast cancer. Moreover, the impact of socioeconomic status is particularly relevant for breast cancer mortality and survival, as it also affects MHT use and health seeking behavior in addition to morbidity and mortality. Education level, used as a surrogate for socioeconomic status in this thesis, does not fully capture the complexities of socioeconomic status. Income, occupation, and subjective indicators are also relevant in the prediction of health inequalities. Education is less prone to reverse causation (i.e. less likely to be affected by diseases in adult life) and non-response error than income and occupation (261). Also, education level has been found to be the main driver of subjective socioeconomic status in Norwegian population studies (262). Despite these benefits, residual confounding cannot be definitively ruled out.

5.3.2.2 Selection bias

Selection bias occurs when the disease occurrence or the estimated association between an exposure and an outcome is distorted due to systematic errors in recruitment and follow-up of the study population (251, 252). In studies examining disease occurrence, selection bias arise if the outcome, or factors related to the outcome, influence participation (251). The temporality of the prospective cohort design used in Papers I-III, where participants were recruited prior to disease occurrence, should substantially minimize the differential selection of participants based on outcome status. However, other forms of selection bias are relevant in prospective cohort studies.

Non-response bias occurs when non-responders, i.e. those who are invited but do not participate in the study, differ from responders (253). In general, responders in epidemiological studies are more likely to be female, have higher socioeconomic status and completed more years of schooling, and be married (263). In the NOWAC study, validation by linkage to national registers demonstrated no material differences between responders and the source population, except for a somewhat higher educational level among responders (264). Moreover, comparing responders to non-responders, no differences were observed for parity, oral contraceptive use or years of schooling, suggesting no major differences in important exposures for breast cancer research. A higher response rate reduces the risk of non-response bias. In the NOWAC study, the response-rates were approximately 60% from age groups 30-34 to 55-59 years, and 45% for ages 65-70 years (264). Indicative of minimal selection bias in the NOWAC cohort, the cumulative incidence rates of breast cancer were close to identical to those of national figures from the Cancer Registry of Norway (187).

Bias due to differential loss to follow-up is also a form of selection bias that can afflict prospective cohort studies. This form of bias, also referred to as informative censoring bias, occurs if the study participants who are lost to follow-up have different risks of experiencing the disease compared to those that remain until the study end (265). In all papers, participants were censored when they emigrated from Norway or when they died. As there were no reason to expect that emigrated participants had different risk of breast cancer than those who did not, simple censoring was an adequate way of handling these participants. Conversely, given the shared risk factors for cancer and death, censoring due to death may not have been independent of the event of interest. Hence, participants that died from other causes than breast cancer could have had a higher risk of the disease if they had not died. Such informative censoring of competing risks is also applicable to our regression analysis on breast cancer subtype outcomes, whereby participants who experienced another subtype than the one defined as failure were censored. Moreover, analyses on breast cancer-specific mortality and survival also carried this restriction due to competing risks by deaths from other causes. An alternative approach to the cause-specific hazards provided by standard Cox models in dealing with competing risks is the use of subdistribution hazard models (266). Several authors have encouraged this method as appropriate when used in

prediction settings, whereas cause-specific hazards have been considered applicable for explanatory/etiological/causal research questions (267-269). Given the explanatory nature of our research questions, we considered cause-specific hazards more fit. In fact, competing risks by deaths from other causes than breast cancer could also be unrelated to risk factors for breast cancer and breast cancer-specific mortality. This has been demonstrated previously, whereby the Kaplan-Meier and competing risk approach provided similar cumulative incidence of breast cancer-specific mortality (270).

In Papers I-III, exclusions were made from the total cohort due to extreme or missing values, thereby creating the analytic study samples. The excluded participants could be different from the participants who remained in the study sample. For instance, in order to model BMI in Paper II, we excluded women who had less than two BMI measurements and women who had missing values of physical activity and smoking status at all time points. The excluded women were slightly older, had higher BMI and lower education than the women included in the analytical study sample. Hence, the possibility of selection bias is evident, as women with high BMI may have been more likely not to report their weight at several occasions. As such, the study sample may be leaner than the general population. Also applicative to Paper II, participants with missing covariate values were excluded in complete-case analyses. The proportion of missing information was equally distributed across BMI trajectory groups, except for a higher percentage of missing values in the trajectory group with women with obesity who lost weight. We compared characteristics of women in the complete-case analyses study sample with characteristics of women in the full study sample, and differences were negligible.

5.3.2.3 Collider stratification bias

A form of selection bias or overadjustment bias relevant to Paper III is collider stratification bias. A collider is a variable that is the common effect of an exposure and an outcome. When conditioning on a collider, i.e. controlling for the collider through stratification, restriction or adjustment, so-called M-bias or collider stratification bias occurs (252). Collider stratification bias, also known as index event bias, arises from conditioning on an intermediate variable (M) situated between the exposure (E) and outcome (O), coupled with the existence of unmeasured confounding (U) that affects the mediator's impact on the outcome (271-275).

In this way, the intermediate variable is a collider between the exposure variable and the unmeasured variable. A well-known example of collider stratification bias is the “birth weight paradox”, where researchers demonstrated a protective effect of maternal smoking on infant death, conditional on the mediator birth weight (Figure 14). The explanation was bias due to conditioning on the intermediate variable (276). Due to the presence of unmeasured common causes (U) of low birth weight (M) and infant mortality (O), such as birth defects, the observed association between maternal smoking (E) and infant mortality among low-birth-weight infants could be contrary to what would be observed if adjustment for U were possible.

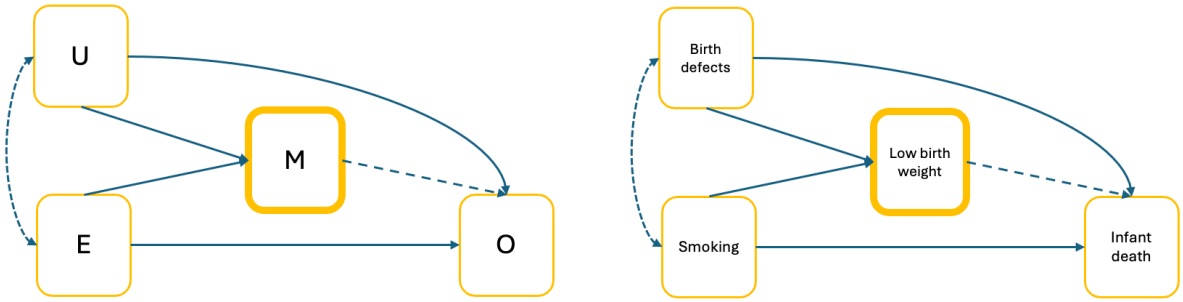

Figure 14. Collider stratification bias. Adapted from Greenland (273) and Hernández-Diáz (276)

Paper III focused, in part, on the effect of MHT use on survival of breast cancer subtypes. We applied baseline information on MHT use to a restricted subset of study participants (those with distinct breast cancer subtypes) and follow them until death from breast cancer. Here, a breast cancer subtype diagnosis is an intermediate variable (M) between MHT use (E) and death from breast cancer (O; Figure 15). Moreover, genetic susceptibility represents unmeasured confounding (U) for the effect of a breast cancer subtype on death from breast cancer, as susceptible genes are causally linked to both subtype diagnosis and mortality (277, 278). It is difficult, if not impossible, to overcome this barrier when studying risk factors in relation to cancer survival. We attempted to mitigate this by adjusting for family history of breast cancer, a surrogate for hereditary breast cancer, but our results may still be subject to collider bias due to imperfect adjustment.

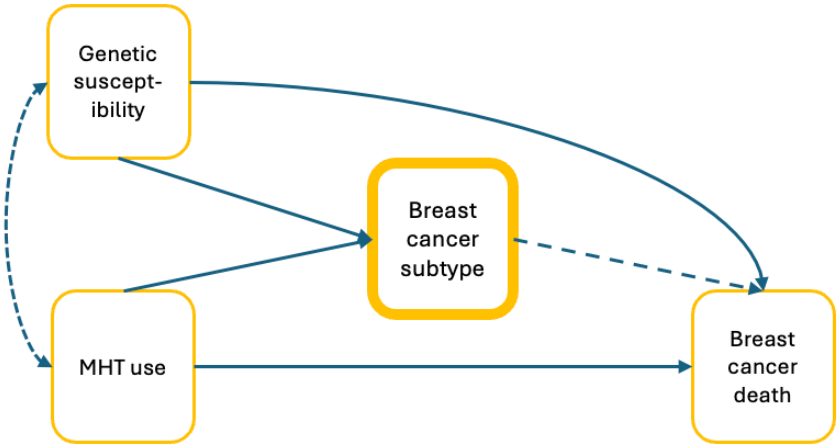

Figure 15. Collider stratification bias in Paper III

5.3.2.4 Information bias

Information bias arises from systematic errors in the measurement, recording, or classification of exposure and/or outcome data (251, 265). These errors can lead to variable misclassification. Misclassification of exposures can be non-differential or differential. Differential misclassification, where the likelihood of misclassification varies by exposure or outcome status, may skew risk estimates toward or away from the null. Non-differential misclassification refers to equal probability of misclassification between groups in the study, and, depending on number of categories and presence of confounding factors, can also over- and underestimate results (251, 279).

5.3.2.4.1 Misclassification of exposure variables

In this thesis, the exposure variables were derived from self-administered questionnaires. As such, the potential of inaccurate recollection and misclassification is feasible. Universal to all variables used, differential misclassification due to recall bias was likely absent due to the collection of exposure and covariate variables before cancer diagnosis. The use of OCs and MHT was denoted in questionnaires containing a photo booklet of specific brands available on the Norwegian market to facilitate recall. Although general use variables such as “ever use” and “current use” are likely not affected by misclassification, duration of use, specific periods of use, and specific brands used could be prone to such error. Both OC use and MHT use has been validated in the NOWAC study with satisfactory agreement. A test-retest study was undertaken among 2000 invited women and demonstrated a kappa estimator of agreement of 0.95 for OC use (187). In Paper I, we reduced the potential of misclassification

of OC use by applying time-varying OC use, where updated information on OC use was applied from a follow-up questionnaire. As such, baseline information was applied until follow-up information became available. The validity of MHT use in NOWAC has previously been assessed by comparison to plasma E2 levels. Current MHT users showed 100% specificity and 88% of MHT users had plasma E2 levels above the 95% CI of non-users (280).

Weight and BMI are commonly underestimated in self-report instruments, a tendency that intensifies with increasing age and BMI (281, 282). In a NOWAC validation study where self-reported weight was compared to values measured by medical staff, weight was underestimated in all BMI categories, but to a larger degree in overweight and obese categories (283). However, a substantial agreement was observed between self-reported and measured values (weighted kappa 0.73).

In Papers II and III, we applied the last non-missing value of exposures and covariates from follow-up questionnaires prior to study inclusion to ensure the use of the most updated information. The potential misclassification in our study samples were most likely non-differential, i.e. unrelated to exposures or outcome. Yet, due to several exposure categories and covariates in our multivariable models the potential misclassification can bias the estimates both toward and away from the null (279).

5.3.2.4.2 Misclassification of outcomes

Misclassification of cancer diagnoses was unlikely due to the high classification accuracy in the Cancer Registry of Norway, estimated at 98.8% completeness (284). The potential of misclassification of breast cancer subtypes is more likely. Tumor receptor status is routinely assessed by nationwide pathological departments. Varieties in laboratory techniques, scoring methods and interpretation of data can result in subjective influence and misclassification. Further, the overlap between intrinsic molecular subtype and intrinsic-like subtypes based on IHC is far from complete (37, 38). Limitations of the St Gallen surrogate classification has been demonstrated, where the main limitation laid in differentiating luminal A and B from each other (285).

We employed a mixture of cutoff values for ER positivity depending on diagnosis date. Before January 2012, ER positivity was defined by a threshold of > 10% reactivity. From February 2012 and onward, this threshold was replaced by > 1% reactivity due to alterations

in the national treatment guidelines owing to updated recommendations (25). This blend could have displaced true ER positives into the ER negative category prior to 2012, as the true threshold of ER positivity is recognized as 1% in clinical practice (286). If the threshold of 10% is more correct, which has been advocated based on response to endocrine therapy (287), then true ER negatives are misplaced as ER positives after 2012. A consequence for our results is a dilution of associations.

In Paper III, breast cancer-specific deaths were among the main outcomes. The Norwegian Cause of Death Registry holds a 98% degree of coverage (288). Several international quality evaluations of death registries have been conducted, focusing on the degree of coverage and completeness, the use of updated coding systems, and the amount of unspecific diagnostic codes used. The Norwegian Causes of Death Registry have been ranked “medium” (289), “medium-high” (290) and in the “best” group (291). The classification of cause of death can be challenging, and the frequent use of unspecific codes for underlying cause of death affects the data quality (288). In Paper III, we only had information on first incident breast cancer subtype. It is possible that some deaths could have resulted from recurrent subtypes that differed from the one identified at initial diagnosis.

The misclassification of outcomes was assumingly unrelated to our exposure variables of interest. Consequently, we anticipate the misclassification of our binary outcome variables to have biased effect estimates toward the null (251).

5.3.3 External validity

External validity assesses the extent to which study results can be extrapolated to other populations (292). The women invited to participate in the NOWAC study were predominantly white Caucasians. Thus, results can only be generalized to the ethnic Norwegian female population in the corresponding age groups. The generalizability of our results to non-Caucasians is limited due to varying prevalence of breast cancer subtypes across different ethnicities (293). Nonetheless, the external validity in NOWAC is considered high. As stated in section 5.3.2.2, the distribution of exposures were generally independent of the response rate and incidence rates for all cancer sites in NOWAC were almost identical to the figures from the Cancer Registry of Norway in 2014 (187, 264).

5.3.4 Missing data

Despite cautious planning and execution, missing data remains a challenge in most epidemiological studies. In the NOWAC study, careful consideration to the phrasing and formatting of the questionnaires was done to minimize missingness. Nonetheless, in a population-based study like NOWAC that relies on self-reported data, encountering missing data is unavoidable. Missing data can cause biased estimates depending on its magnitude, the methods used to handle it, and the assumed mechanisms for missingness (294). Three primary mechanisms describe missing data: missing completely at random (MCAR), missing at random (MAR), and missing not at random (MNAR) (295). MCAR refers to missingness that is independent of observed and unobserved data. MAR occurs when the missing data is independent of the unobserved data, i.e. the missing value itself, but not independent of other observed variables. Finally, MNAR is when the missingness is related to the observed and the unobserved data. It is impossible to determine the true mechanism of missingness, because this would require us to know the missing values (296).

There are several ways of handling missing data. A common approach is complete-case analysis, which excludes observations with missing values for any variable in the statistical model. This method is suitable under MCAR conditions, but when missingness is non-MCAR and the magnitude of missingness is high, this method can cause biased estimates (297). Yet, there are certain exceptions where complete-case analysis can be valid under non-MCAR conditions. Specifically, if the reason for missing data in predictor variables are unrelated to the outcome, then results will remain unbiased (203, 298). Another method to handle missing data is single imputation, where missing values are replaced with the mean, median, or mode. These methods can underestimate the standard errors and produce biased effect estimates (203).

In contrast to single imputation, multiple imputations accounts for the statistical uncertainty inherent in the imputations (203). Multiple imputation is an increasingly used approach and is valid when missingness is either MCAR or MAR (203). Briefly, multiple imputation is the use of the distribution of observed data to estimate a set of plausible values for the missing data using multiple datasets (205). A series of regression models estimate the underlying distribution of the variable with missing values based on other variables in the study sample.

MICE is a flexible approach and can handle different variable types (continuous, binary, unordered categorical and ordered categorical). Each variable is modeled according to its distribution, where binary variables are modeled using logistic regression, continuous variables are modeled using linear regression or predictive mean matching, and categorical variables are modeled with ordered or multinomial logistic regression (205, 299). 10 iterations are usually performed to create one imputed dataset in order to stabilize the results.

Our project was constrained by missing data across exposure variables, covariates, and outcome variables. For exposure and covariate variables, missing values were a consequence of both item and unit non-response. Item non-response refers to missing information due to response to some, but not all, survey questions. Unit non-response is a result of non-response of entire surveys, in our scenario follow-up questionnaires. In the NOWAC cohort, some missing values have been replaced under certain assumptions. Examples relevant to this thesis involve family history of breast cancer and parity, where missing values have been replaced by “none” and “0”, respectfully. Missing outcome data on receptor status among breast cancer cases and thus breast cancer subtypes was a major challenge throughout Papers I-III. One reason for this was because receptor status testing practices became standard procedure not before 2005, and our cohort of women were diagnosed from 1991. Thus, a high amount of breast cancer cases diagnosed prior to 2005 had missing subtype status. Nonetheless, missing receptor status data was apparent through all diagnosis years.

As described in section 3.6.2, we performed MICE in Papers I and III to handle missing data. In both papers, effect estimates were similar in complete-case and MICE analyses. However, there remains a possibility that some of the data were not MAR, which could cause biased estimates in calculated results from multiply imputed datasets. In Paper II, the multivariable-adjusted regression models were complete-case analyses. Body fatness exposure variables were based on growth curves, which involves that BMI data were imputed at missing time points for each participant. Nevertheless, missing covariate data amounted to 4% of the study sample. Across Papers I-III, we did not perform multiple imputation on missing subtype data. Given our assumptions that missing receptor status data were likely MAR, multiple imputation could be a good option to increase the sample size. However, reports

indicate that employing complete-case analyses for missing tumor marker data in breast cancer patients yields robust estimates with little differences in model coefficient estimates compared to the multiple imputation approach (300). Moreover, for missing outcome data that are MAR, complete-case analyses with covariate adjustment have been demonstrated to produce unbiased estimates comparable to those from multiple imputation, and advantages of complete-case analyses over multiple imputation in such scenario has been emphasized due to transparency (301). Consequently, we concluded that the benefits of multiple imputation perhaps would not outweigh the simplicity and transparency of complete-case analyses on missing outcome data.

5.3.5 Multiple comparisons

As previously mentioned, several results in this thesis are based on small numbers and, consequently, effect estimates are reported with wide confidence intervals. Universal for Papers I-III, multiple statistical comparisons were made and consequently one cannot exclude chance as an explanation for some of the observed findings. One limitation to this thesis is the absence of correction for multiple testing. As described by Bender er al., proper correction for multiple testing is challenging and is necessary in confirmatory studies, but not in exploratory studies (302). In this thesis, we considered some evaluations that had not previously been published as exploratory. For example, associations between POC use and hormone-receptor subtypes; duration, intensity, timing, and trajectories of body fatness and intrinsic-like subtypes; cumulative hormonal dose and intrinsic-like subtypes, as well as MHT use and mortality and survival of intrinsic-like subtypes. Nevertheless, we have made sure not to overinterpret our findings.

6 Conclusion

This thesis covers three scientific research papers that present a combination of confirmatory findings, which build on well-established relations, and novel exploratory findings that necessitate further validation. The findings in this thesis suggests that the use of OCs, body fatness, and MHT use differed in their associations with breast cancer subtypes. Several conclusions can be drawn:

1) In premenopausal women, COC use was associated with an increased risk of hormone receptor-negative breast cancer, whereas prolonged POC use (five years or more) was associated with hormone receptor-positive breast cancer.
2) In postmenopausal women, the duration, intensity, and timing of body fatness, along with trajectories indicating increasing body fatness, were associated with luminal A-like breast cancer. Compared to those who maintained a stable weight in adulthood, women who reduced their weight from obesity experienced a lower risk of overall and luminal A-like breast cancer. The association between body fatness and hormone receptor-positive breast cancer was confined to women who had never used MHT.
3) In postmenopausal women, MHT use increased risk of both luminal A-like and luminal B-like breast cancer, and was associated with breast cancer-specific and luminal A-like mortality. Among patients with TNBC, pre-diagnostic MHT use was associated with enhanced survival.

Our research makes a novel contribution to the literature on hormonal risk factors and subtypes of breast cancer, highlighting its nuanced effects on etiology and progression.

7 Future perspectives and implications

Despite the expanding body of epidemiological studies evaluating risk factors for breast cancer subtypes, significant gaps persist in our understanding of the underlying biological mechanisms and causal relationships, particularly concerning the less common and more aggressive hormone receptor-negative cancers. Large-scale epidemiological studies incorporating substantial numbers of these subtypes are warranted. Given the adverse effect of progestins demonstrated in this thesis and other studies on exogenous hormones and breast cancer, further investigation into specific progestins and their relation to tumor subtypes could yield deeper insights. Moreover, future studies should aim to evaluate the relationship between MHT use and survival outcome across different breast cancer subtypes to either confirm or refute our finding on improved survival among patients with TNBC. Finally, to mitigate the risk of confounding in future research assessing the relationship between female sex hormones and breast cancer subtypes, obtaining data with frequent and regular repeated measurements or employing Mendelian randomization studies could be viable approaches.

Our results indicate that public health interventions should prioritize sustained healthy weight throughout life as a primary preventive measure against breast cancer. Given the ongoing obesity epidemic and considerable fractions (10-19%) of breast cancers attributed to high BMI (303, 304) and weight gain (305, 306), maintaining a healthy weight could contribute to halting an undue increase in breast cancer incidence. Although our findings suggest a slight increase in breast cancer risk associated with OC and MHT use, these exogenous hormones also exert significant health benefits. For instance, OCs reduce the risk of endometrial and ovarian cancer (307). Moreover, the benefits of MHT use may outweigh the risks for the majority of young, postmenopausal women. Indeed, among women under 60, who are the most likely users of MHT, a reduction in all-cause mortality has been demonstrated (138). The potential for improved quality of life through controlled reproductive health and alleviation of climacteric symptoms should not be neglected. Focus should instead be directed towards pursuing specific regimens of exogenous hormones that cause the least harm. For example, the use of exogenous hormones with natural micronized progesterone should be considered in patients with elevated breast cancer risk (308). As a

final remark, our findings contribute to an enhanced understanding of breast cancer subtype etiology. These insights can be integrated into promising directions of future research as well as navigating in the delicate balance between risks and benefits.

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Paper I

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Progestin-only and combined oral contraceptives and receptor-defined premenopausal breast cancer risk: The Norwegian Women and Cancer Study

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Receptor-defined subtypes of breast cancer represent distinct cancer types and have differences in risk factors. Whether the two main hormonal forms of oral contraceptives (OCs); i.e. progestin-only (POC) and combined oral contraceptives (COC), are differentially associated with these subtypes are not well known. The aim of our study was to assess the effect of POC and COC use on hormone receptor-defined breast cancer risk in premenopausal women in a prospective population-based cohort – The Norwegian Women and Cancer Study (NOWAC). Information on OC use was collected from 74,862 premenopausal women at baseline. Updated information was applied when follow-up information became available. Multiple imputation was performed to handle missing data, and multivariable Cox regression models were used to calculate hazard ratios (HR) for breast cancer. 1,245 incident invasive breast cancer cases occurred. POC use ≥5 years was associated with ER+ (HR = 1.59, 95% CI 1.09– 2.32, $p_{trend} = 0.03$) and ER+/PR+ cancer (HR = 1.63, 95% CI 1.07–2.48, $p_{trend} = 0.05$), and was not associated with ER− ($p_{heterogeneity} = 0.36$) or ER−/PR− ($p_{heterogeneity} = 0.49$) cancer. COC use was associated with ER− and ER−/PR− cancer, but did not increase risk of ER+ and ER+/PR+ cancer. Current COC use gave different estimates for ER/PR-defined subtypes ($p_{heterogeneity} = 0.04$). This is the first study to show significant associations between POC use and hormone receptor-positive breast cancer. The lack of power to distinguish effects of POC use on subtype development calls for the need of larger studies to confirm our finding.

Cancer Epidemiology

Introduction

Breast cancer is the most common cancer in women, and the leading cause of cancer death among females worldwide.[1] Reproductive factors, such as early menarche, late menopause, nulliparity and high age at first birth, are known risk factors for breast cancer.[2–4] The role of these reproductive factors in breast cancer etiology points toward an essential contributive effect of endogenous female sex hormones in the carcinogenesis of breast tissue. Exogenous female hormones are also associated with breast cancer. In addition to hormone therapy (HT), estrogen–progestin contraceptives (combined oral contraceptives; COCs), are classified as carcinogenic to humans with regards to cervical, breast and liver cancer by the International Agency for Research on Cancer.[5]

The association between oral contraceptives (OCs) and breast cancer has been extensively studied for decades. In 1996, a comprehensive pooled analysis of 54 epidemiologic studies found a slightly increased risk of breast cancer associated with current and recent use of COC, and a cessation of risk after 10 years since last use.[6] This has later been confirmed by other studies.[7–10] Some reports suggest a stronger association between OC use and breast cancer in younger women compared to older women,[11–13] reflecting the increase in risk associated with recent OC use. Due to a small proportion of women using OCs <10 years before onset of menopause in the NOWAC cohort (<10%), and to the overwhelming evidence of a time-dependent relationship as a function of time since last OC use,[6,10,14] i.e. no effect after 10 years since last use, the current article concerns premenopausal women only.

Receptor-defined subtypes of breast cancer represent distinct entities of disease and have differences in risk factors.[15–17] These subtypes are defined based on the expression of the hormone receptors estrogen receptor (ER) and progesterone receptor

Key words: breast cancer subtypes, oral contraceptives, tumor heterogeneity, prospective cohort study, multiple imputation

Abbreviations: BMI: body mass index; CI: confidence interval; COC: combined oral contraceptive; ER: estrogen receptor; HR: hazard ratio; NOWAC: The Norwegian Women and Cancer Study; OC: oral contraceptive; POC: progestin-only contraceptive; PR: progesterone receptor

Additional Supporting Information may be found in the online version of this article.

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What's new?

Use of combined oral contraceptives (COC) is associated with an increased risk of breast cancer and, predominantly, its hormone receptor-negative subtypes. Whether progestin-only contraceptives (POC) are also associated with elevated risk of receptor-defined subtypes of breast cancer is unknown. In this prospective, population-based study in Norway, POC use for five or more years was associated with estrogen receptor-positive and estrogen receptor-positive/progesterone receptor-positive but not estrogen receptor-negative or estrogen receptor-negative/progesterone receptor-negative breast cancer subtypes in premenopausal women. The associations contrast with those found for COC use, suggesting that estrogen and progestin serve dissimilar roles in subtype carcinogenesis.

(PR), and human epidermal growth factor receptor 2 (HER2). Prior studies are inconsistent regarding associations between OC use and risk of receptor-defined subtypes of breast cancer. Some propose that OC use is associated with increased risk of hormone receptor-negative breast cancer,[18–22] whereas others suggest a decreased risk of hormone receptor-positive cancers by OC use.[21,23,24] Conversely, positive associations with ER-positive cancer has been reported[25] as well as no association with either subtype,[26–29] or similar associations across subtypes.[19,30]

The pooled analysis found no effect of progestin-only contraceptive (POC) use and breast cancer overall.[31] Previous studies on POC and breast cancer are scarce. To date, no study has addressed associations between POC use and subtypes of breast cancer. Norwegian data is suitable for studying POC use due to a substantial amount of users. Thus, the aim of our study was to assess the effect of POC and COC on hormone receptor-defined breast cancer risk in a representative sample of premenopausal Norwegian women.

Materials and Methods

Study population

The Norwegian Women and Cancer Study (NOWAC) is a prospective national population-based cohort of 172,000 Norwegian women. Initiated in 1991, women aged 30–70 were randomly selected by the Central Population Registry and invited to participate. Out of 327,476 invited women in total during the period 1991 to 2007, 172,478 returned a completed questionnaire, providing an overall participation rate of approximately 53%. Statistics Norway substituted identification numbers with serial numbers on the questionnaires. Questionnaire data on lifestyle and health were collected up to three times at 4- to 6-year intervals to provide updated information on exposures. NOWAC has acceptable external validity[32] and has been described in detail elsewhere.[33]

For the analyses presented here, 88,258 women who were postmenopausal or 53 years of age or older at baseline were excluded.[34] Additional exclusion criteria: HT-users at baseline ($n = 6{,}786$), prevalent cancers at baseline other than non-melanoma skin cancer (ICD-10 C44) ($n = 1{,}018$), women who emigrated or died before baseline ($n = 16$), women who were 10 years or younger at first birth ($n = 2$) and women with missing OC, POC or COC status at baseline ($n = 1{,}540$). This left a total of 74,862 women for the current analyses. Follow-up information from a second questionnaire was collected from 51,850 of these women.

Assessment of OC exposure

Information on exposure to OCs was obtained by self-administered questionnaires. General questions on OC use were asked, such as ever use, age at first use, duration of use and current use. Furthermore, the women were asked to denote specific periods with OC use, which was defined as any continuous use of one specified OC brand for at least 1 month. To facilitate recall, the questionnaires contained a photo booklet with pictures and names of the different OC brands available on the Norwegian market up to the time of mailing. Up to date, no more than 42 different OC brands have been sold in Norway. We stratified OC use into POC use and COC use based on OC brands used. The internal validity with regard to OC use assessment in NOWAC has been found to be satisfying.[33]

Repeated measurements

Updated information on OC exposure was obtained from follow-up questionnaires. Women who reported ever use at baseline and had missing status or reported never use at follow-up were categorized as ever users at follow-up. We applied baseline information on OC exposure until follow-up information became available. Women were censored from the study at the time they reached menopause, started using HT, were diagnosed with incident cancer (except non-melanoma skin cancer), died or emigrated, whichever occurred first. All participants were followed-up until December 31, 2015.

Identification of breast cancer cases, death and emigration

The Norwegian 11-digit national identification number, which includes information on date of birth and sex,[35] allowed linkage of the participants to different national registers. Follow-up information on incident breast cancer was collected annually by linkage to the Cancer Registry of Norway, which is estimated to be virtually complete due to compulsory reporting from all pathological laboratories, hospitals and general practitioners in the country.[36] Year of diagnosis ranged from 1991 to 2015. The classification of breast cancer (ICD-10 C50) was performed according to the 10th revision of the International Statistical Classification of Diseases, Injuries and Causes of Death. Information on death and emigration was obtained through linkage

to the Cause of Death Registry and the Central Population Register, respectively.

Breast cancer subtypes

ER, PR and HER2 status is ascertained by immunohistochemical and *in situ* hybridization techniques conducted at pathological departments across the country and submitted to the Cancer Registry. ER-negative status was defined as <10% reactivity until January 2012, and <1% reactivity from February 2012 and onwards due to change in treatment protocols for breast cancer patients in Norway. PR negativity was defined as <10% reactivity. Contemporary epidemiological studies include HER2 in the breast cancer subtype definition. However, due to large amounts of missing values for this variable, we focused on subtypes based on hormone receptor status only. The subgroups used in the current article (i.e., ER+, ER−, ER+/PR+ and ER−/PR−) are not mutually exclusive and do not add up to the total amount of cases.

Statistical analysis

Repeated measurements of OC, POC and COC use were applied in the analysis of total, ER-defined and ER/PR-defined premenopausal breast cancer. Hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated using Cox proportional hazards models with attained age as the time scale. Separate regression models were constructed for subtype outcomes, allowing women who experienced another subtype than the one defined as failure to be censored at the time when this subtype occurred.[37]

Premenopausal breast cancer was defined as incident breast cancer diagnosed prior to or during the same year as the woman's menopause. Age at menopause was set to the given age at which the woman's menstruation stopped. If age at menopause was missing at baseline, we used reported age at menopause from follow-up questionnaires. Women with unknown menopausal status or irregular menses were considered postmenopausal at age 53 or older. This cut-off was based on the definition used in the Million Women Study,[34] and later in the NOWAC study.[38]

The multivariable analyses included established or potential risk factors as covariates, which were obtained from the questionnaires. If a linear trend was observed for any covariate, this covariate was treated as continuous. Covariates that changed the regression coefficient with <10% were removed from the model, except for age at menarche. The final multivariable model included the following covariates: BMI (continuous), history of breast cancer in mother (yes, no), age at menarche (continuous), alcohol consumption (0, 0.1–4.9, 5–9.9, ≥10 g/day) and a combined variable including parity (0, 1, 2, ≥3 children) and age at first birth (age <25, 25–29, ≥30). For the analysis addressing COC exposure, the model was adjusted for POC use (ever, never), and vice versa.

The HRs of breast cancer subtypes were tested for heterogeneity by the Wald test. For duration variables, heterogeneity between linear trends was tested. All *p*-values were two-sided. The proportional hazards assumption was evaluated by tests of Schoenfeld residuals and by graphical inspection of a log–log survival plot. All analyses were performed using the statistical package STATA, version 14.

Multiple imputation

Under the assumption that data was missing at random,[39] multiple imputation was used to handle missing information. To reduce sampling variability from the imputation simulations, the missing values were replaced by imputed values from 20 duplicate datasets.[39] The imputation model included all covariates used in the multivariable analyses, age at baseline and follow-up, and the Nelson–Aalen cumulative hazard estimator as predictors.

Two types of missing values occurred due to both item and wave non-response. First, values were missing due to missing information in the questionnaires (item non-response). These included missing covariates at baseline (e.g., alcohol consumption [$n = 1{,}651$], age at menarche [$n = 1{,}192$] and BMI [$n = 1{,}477$]), missing duration of OC use at baseline ($n = 1{,}180$) or follow-up ($n = 23{,}850$), and time since last use at baseline ($n = 1{,}293$) or follow-up ($n = 23{,}634$). Second, missing values at follow-up were due to non-response of a second questionnaire (wave non-response, $n = 23{,}012$). These comprised OC status, POC status and duration and COC status and duration. To avoid possible inconsistencies in status of use at the two points in time, we imputed possible changes in status of OC use and used this information to assign the status at follow-up as current, former or never use. Similarly, we computed OC duration at follow-up from the imputation of additional use since baseline to avoid lower imputed values at follow-up compared to baseline.[40] The estimates from 20 imputed datasets were combined using Rubin's rules to obtain HRs and corresponding 95% CIs.[41] Sensitivity analysis was conducted to ensure that risk estimates were similar in complete case analysis and multiple imputation analysis.

Ethics

NOWAC has been approved by the Regional Committees for Medical and Health Research Ethics (REC) and the Norwegian Data Inspectorate. The participants received written information about the study and future linkages to national registers, along with invitation to receive a second questionnaire. Return of a completed questionnaire was considered consent to participate. A second questionnaire was only sent to participants who had agreed to receive one.

Results

A total of 1,245 incident premenopausal breast cancer cases occurred during 580017 person-years of follow-up. Mean follow-up time was 7.8 years. Among the 1,245 cancer cases, there were 679 ER+ cases, 191 ER− cases, 375 cases with missing ER status, 578 PR+ cases, 281 PR− cases and 386 cases with missing PR status. When combining the hormone

Table 1. Baseline characteristics of study population and premenopausal breast cancer cases: The NOWAC Study

	Study population ($n = 74,862$)	Premenopausal breast cancer cases ($n = 1,245$)	p^1
Age (years) at baseline, no. (%)			<0.001
30–34	3,953 (5.3)	104 (8.4)	
35–39	20,853 (27.9)	445 (35.7)	
40–44	19,760 (26.4)	331 (26.6)	
45–49	22,410 (29.9)	297 (23.9)	
>50	7,886 (10.5)	68 (5.5)	
Mean age (SD)	42.6 (0.0)	41.1 (0.2)	
Body mass index (kg/m^2), no. (%)			<0.001
<18.5	2,008 (2.7)	37 (3.0)	
18.5–24.9	52,628 (70.3)	935 (75.1)	
25–29.9	14,717 (19.7)	205 (16.5)	
≥30	4,032 (5.4)	46 (3.7)	
Missing	1,477 (2.0)	22 (1.8)	
Mean BMI (SD)	23.4 (0.0)	22.9 (0.1)	
Age (years) at menarche, no. (%)			0.82
≤11	6,624 (8.9)	112 (9.0)	
12	14,822 (19.8)	241 (19.4)	
13	21,505 (28.7)	370 (29.7)	
14	18,136 (24.2)	294 (23.6)	
≥15	12,583 (16.8)	196 (15.7)	
Missing	1,192 (1.6)	32 (2.6)	
Mean age at menarche (SD)	13.3 (0.0)	13.2 (0.0)	
Parity, no. (%)			<0.001
Nulliparous	7,331 (9.8)	151 (12.1)	
1	9,494 (12.7)	184 (14.8)	
2	33,028 (44.1)	557 (44.7)	
≥3	25,009 (33.4)	353 (28.4)	
Mean number of children (SD)	2.1 (0.0)	2.0 (0.0)	
Age (years) at first birth, no. (%)			<0.001
<20	9,038 (13.4)	132 (12.1)	
20–24	30,373 (45.0)	429 (39.2)	
25–29	19,820 (29.4)	359 (32.8)	
≥30	8,300 (12.3)	174 (15.9)	
Mean age at first birth (SD)	24.2 (0.0)	24.9 (0.1)	
Ever breastfed, no. (%)			0.07
Yes	51,613 (68.9)	899 (72.2)	
No	3,017 (4.0)	39 (3.1)	
Missing	20,232 (27.0)	307 (24.7)	
Mean duration (months) of breastfeeding (SD)	13.5 (0.1)	13.7 (0.4)	
History of breast cancer in mother, no. (%)			<0.001
Yes	3,539 (4.7)	109 (8.9)	

Table 1. Baseline characteristics of study population and premenopausal breast cancer cases: The NOWAC Study (Continued)

	Study population ($n = 74,862$)	Premenopausal breast cancer cases ($n = 1,245$)	p^1
Alcohol consumption (g/day), no. (%)			0.08
None	18,431 (24.6)	285 (22.9)	
0.1–4.9	35,754 (47.8)	609 (48.9)	
5.0–9.9	12,704 (17.0)	209 (16.8)	
≥10	6,322 (8.4)	128 (10.3)	
Missing	1,651 (2.2)	14 (1.1)	
Mean alcohol consumption (SD)	3.7 (0.0)	4.0 (0.2)	
Smoking status, no. (%)			0.07
Never smoker	25,540 (34.1)	395 (31.7)	
Current smoker		24,564 (32.8)	
Former smoker		24,720 (33.0)	
Missing	38 (0.1)	1 (0.1)	

Abbreviations: BMI: body mass index; SD: standard deviance.
Percentages do not add up to 100% for all characteristics because of rounding.
[1] χ^2 Pearson, *p*-values for difference between premenopausal breast cancer cases and the whole cohort.

receptor statuses, they comprised 540 ER+/PR+ cases, 130 ER+/PR− cases, 38 ER−/PR+ cases, 151 ER−/PR− cases and 386 cases with missing hormone receptor status.

Distribution of characteristics at baseline among the study population and premenopausal breast cancer cases is presented in Table 1. In addition to a larger proportion with familial breast cancer, women who developed premenopausal breast cancer tended to be younger, have lower BMI, lower parity and higher age at first birth compared to the whole cohort.

Current OC use, >10 years duration of OC use and <10 years since last use were associated with premenopausal breast cancer as well as all receptor-defined subtypes (Table 2), except for current OC use not being associated with ER+/PR+ cancer. >20 years since last use was also associated with ER−/PR− cancer. In addition, ever and former use of OCs was associated with ER− and ER−/PR− breast cancer.

The main findings of our study are presented in Tables 3 and 4, displaying stratified analysis by POC and COC use. POC use for 5 years or more was associated with ER+ (HR = 1.59, 95% CI 1.09–2.32) and ER+/PR+ (HR = 1.63, 95% CI 1.07–2.48) cancer. In women who were POC users and never COC users, the corresponding increase in risk was 1.87 (95% CI 1.21–2.91) for ER+ cancer (Table 4). However, we observed no significant difference in risk estimates between subtypes with regard to POC use ($p_{\text{ER+ vs. ER−}} = 0.36$ and $p_{\text{ER+/PR+ vs. ER−/PR−}} = 0.49$). Ever, current, former and ≥5 years use of COCs increased the risk of ER− and ER−/PR− disease. The risk of ER−/PR− cancer (HR = 2.39, 95% CI 1.14–5.04) was significantly different from the risk of ER+/PR+ cancer in current COC users ($p_{\text{heterogeneity}} = 0.04$).

Supporting Information Table S1 displays the distribution of OC, POC and COC use and missing values among the study population at baseline and follow-up.

Results were similar in the complete case analyses. Stratified analyses on POC and COC use without using multiple imputation (Supporting Information Table S2) also indicated positive associations between POC use ≥5 years and ER+ cancer (HR = 1.60, 95% CI 1.09–2.35) and ER+/PR+ cancer (HR = 1.64, 95% CI 1.07–2.51).

Discussion

The main finding of our study was that POC use was associated with hormone receptor-positive premenopausal breast cancer if used for 5 years or more. Thus, our prospective, population-based study has unraveled more exact associations between the main hormonal constituents of OCs and receptor-defined breast cancer risk in premenopausal women. Dissimilar associations between POC and COC use on hormone receptor-defined breast cancer suggest that the exogenous hormones estrogen and progestin might have differential roles in subtype carcinogenesis.

Some of our findings confirm existing knowledge: we have observed that OC use slightly increases risk of premenopausal breast cancer. The increase in risk associated with duration of use could reflect long-term users being more likely to be current or recent users. Although the total risk elevation is modest with regard to ever use (12%), it is noteworthy due to the frequent use of OCs among premenopausal women, making OCs a contributing cause for a substantial number of cases.

Associations between OC use and ER− or ER−/PR− breast cancer are in agreement with previous studies.[18,19,21,22] Dolle *et al.* found increased risk of ER-negative breast cancer with several aspects of OC use (i.e., ever use, duration, age at first use and years since first and last use), while no significant associations were found with ER+ breast cancer.[18] Beaber *et al.* found significantly increased risks for both ER-

Table 2. Multivariable adjusted HRs (95% CI) for association between oral contraceptive use and risk of total and hormone receptor-defined premenopausal breast cancer: The NOWAC Study

	All cases		ER+ cases		ER− cases		ER+/PR+ cases		ER−/PR− cases	
	No. (n = 1,245)	HR (95% CI)[1]	No. (n = 679)	HR (95% CI)[1]	No. (n = 191)	HR (95% CI)[1]	No. (n = 540)	HR (95% CI)[1]	No. (n = 151)	HR (95% CI)[1]
General OC use										
Never use	379	1.00 (ref.)	216	1.00 (ref.)	49	1.00 (ref.)	167	1.00 (ref.)	37	1.00 (ref.)
Ever use	866	1.12 (0.99–1.26)	463	1.06 (0.90–1.25)	142	1.48 (1.06–2.06)	373	1.10 (0.91–1.32)	114	1.61 (1.10–2.35)
Current use	129	1.36 (1.09–1.71)	76	1.36 (1.00–1.85)	27	1.93 (1.10–3.37)	60	1.25 (0.87–1.80)	22	1.98 (1.04–3.76)
Former use	737	1.09 (0.96–1.24)	387	1.03 (0.87–1.22)	115	1.44 (1.02–2.01)	313	1.08 (0.90–1.31)	92	1.57 (1.07–2.31)
Duration (years) of use										
1–4	451	1.10 (0.96–1.26)	221	0.97 (0.81–1.18)	70	1.35 (0.93–1.95)	181	1.02 (0.83–1.27)	37	1.50 (0.99–2.28)
5–9	216	1.02 (0.86–1.21)	125	1.04 (0.82–1.30)	38	1.43 (0.92–2.22)	100	1.06 (0.82–1.37)	58	1.46 (0.88–2.41)
≥10	178	1.29 (1.09–1.54)	103	1.33 (1.05–1.67)	32	1.93 (1.24–2.99)	81	1.34 (1.04–1.74)	28	2.11 (1.29–3.46)
p_{trend}[2]		0.02		0.03		0.004		0.04		0.01
Time (years) since last use										
<10	371	1.36 (1.15–1.61)	209	1.34 (1.06–1.70)	68	1.71 (1.10–2.66)	170	1.36 (1.04–1.78)	58	1.78 (1.07–2.96)
11–20	371	1.05 (0.91–1.22)	179	0.94 (0.77–1.15)	60	1.28 (0.86–1.90)	142	0.98 (0.78–1.23)	46	1.44 (0.92–2.25)
>20	113	1.04 (0.89–1.23)	66	1.05 (0.85–1.29)	14	1.57 (1.04–2.37)	53	1.10 (0.87–1.38)	10	1.67 (1.05–2.66)
p_{trend}[2]		0.02		0.17		0.97		0.30		0.86

Abbreviations: CI: confidence interval; ER: estrogen receptor; HR: hazard ratio; OC: oral contraceptives; PR: progesterone receptor.
The subgroups (i.e., ER+, ER−, ER+/PR+ and ER−PR−) are not mutually exclusive and do not add up to the total amount of cases.
[1]Multivariable analysis adjusted for BMI (continuous), history of breast cancer in mother (yes, no), age at menarche (continuous), alcohol consumption (0, 0.1–4.9, 5–9.9, ≥10 g/day) and a combined variable including parity (0, 1, 2, ≥3 children) and age at first birth (age <25, 25–29, ≥30).
[2]p-Value, continuous variable.

Table 3. Multivariable adjusted HRs (95% CI) for association between combined and progestin-only oral contraceptive use and risk of hormone receptor-defined premenopausal breast cancer: The NOWAC Study

	All cases		ER+ cases		ER− cases			ER+/PR+ cases		ER−/PR− cases		
	No. ($n = 1245$)	HR (95% CI)[1]	No. ($n = 679$)	HR (95% CI)[1]	No. ($n = 191$)	HR (95% CI)[1]	p^2	No. ($n = 540$)	HR (95% CI)[1]	No. ($n = 151$)	HR (95% CI)[1]	p^3
COC use[4]												
Never OC use	379	1.00 (ref.)	216	1.00 (ref.)	49	1.00 (ref.)	0.07	167	1.00 (ref.)	37	1.00 (ref.)	
Ever COC use	652	1.10 (0.97–1.26)	353	1.04 (0.87–1.24)	111	1.50 (1.06–2.13)	0.07	288	1.08 (0.88–1.32)	87	1.60 (1.07–2.38)	0.08
Current use	77	1.32 (0.99–1.77)	43	1.17 (0.77–1.78)	19	2.38 (1.25–4.54)	0.10	33	0.91 (0.53–1.55)	16	2.39 (1.14–5.04)	0.04
Former use	575	1.09 (0.95–1.24)	310	1.03 (0.86–1.23)	92	1.44 (1.01–2.05)		255	1.09 (0.89–1.33)	71	1.54 (1.03–2.31)	0.13
Duration (years) of use												
<5	346	1.09 (0.94–1.27)	180	0.99 (0.81–1.22)	52	1.31 (0.88–1.96)		152	1.06 (0.84–1.33)	42	1.44 (0.92–2.27)	
≥5	306	1.11 (0.95–1.30)	173	1.09 (0.89–1.34)	59	1.73 (1.17–2.56)	0.36	136	1.10 (0.87–1.39)	45	1.79 (1.14–2.80)	
p_{trend}[5]		0.755		0.35		0.12			0.73		0.28	0.43
POC use[6]												
Never OC use	379		216	1.00 (ref.)	49	1.00 (ref.)	0.49	167	1.00 (ref.)	37	1.00 (ref.)	
Ever POC use	171	1.16 (0.95–1.42)	97	1.16 (0.89–1.52)	29	1.42 (0.85–2.39)	0.96	79	1.18 (0.87–1.60)	23	1.64 (0.92–2.92)	0.32
Current use	28	1.42 (0.90–2.26)	18	1.52 (0.84–2.77)	6	1.58 (0.48–5.20)	0.47	16	1.75 (0.93–3.28)	5	2.30 (0.69–7.68)	0.69
Former use	143	1.13 (0.92–1.39)	79	1.12 (0.85–1.48)	23	1.41 (0.83–2.40)		63	1.13 (0.82–1.54)	18	1.58 (0.87–2.87)	0.33
Duration (years) of use												
<5	120	1.06 (0.85–1.33)	64	1.01 (0.74–1.37)	23	1.41 (0.80–2.47)		52	1.02 (0.72–1.44)	18	1.59 (0.85–2.99)	
≥5	51	1.45 (1.08–1.95)	33	1.59 (1.09–2.32)	6	1.46 (0.64–3.31)	0.36	27	1.63 (1.07–2.48)	5	1.79 (0.74–4.36)	
p_{trend}[5]		0.067		0.03		0.97			0.05		0.86	0.49

Abbreviations: CI: confidence interval; COC: combined oral contraceptives; ER: estrogen receptor; HR: hazard ratio; OC: oral contraceptives; POC: progestin-only contraceptives; PR: progesterone receptor.

[1]Multivariable analysis adjusted for BMI (continuous), history of breast cancer in mother (yes, no), age at menarche (continuous), alcohol consumption (0, 0.1–4.9, 5–9.9, ≥10 g/day) and a combined variable including parity (0, 1, 2, ≥3 children) and age at first birth (age <25, 25–29, ≥30).

[2]χ^2 Wald, p heterogeneity between estimate for ER+ and ER− cancer.

[3]χ^2 Wald, p heterogeneity between estimate for ER+/PR+ and ER−/PR− cancer.

[4]Analyses on COC use are adjusted for POC use in addition to the aforementioned covariates.

[5]p-Value, continuous variable.

[6]Analyses on POC use are adjusted for COC use in addition to the aforementioned covariates.

Table 4. Multivariable adjusted HRs (95% CI) for association between combined oral contraceptive users among never progestin-only users and progestin-only users among never combined oral contraceptive users and risk of ER-defined premenopausal breast cancer: The NOWAC Study

	ER+ cases		ER− cases		
	No. ($n = 679$)	HR (95% CI)[1]	No. ($n = 191$)	HR (95% CI)[1]	p^2
COC use					
Never OC use	216	1.00 (ref.)	49	1.00 (ref.)	
Ever COC use, never POC use	301	1.03 (0.86–1.23)	95	1.50 (1.06–2.14)	0.06
Current COC use only	38	1.26 (0.81–1.96)	17	2.64 (1.36–5.14)	0.07
Former COC use only	263	1.02 (0.85–1.22)	78	1.42 (0.99–2.04)	0.10
Duration (years) of use					
<5 years	150	0.99 (0.80–1.22)	44	1.31 (0.87–1.98)	
≥5 years	151	1.08 (0.87–1.33)	51	1.73 (1.16–2.58)	
p_{trend}[3]		0.462		0.004	0.03
POC use					
Never OC use	216	1.00 (ref.)	49	1.00 (ref.)	
Ever POC use, never COC use	45	1.11 (0.80–1.53)	13	1.44 (0.78–2.66)	0.46
Current POC use only	9	1.50 (0.62–3.64)	3	2.59 (0.63–10.68)	0.52
Former POC use only	36	1.07 (0.76–1.51)	10	1.33 (0.69–2.57)	0.56
Duration (years) of use					
<5 years	24	0.79 (0.51–1.22)	10	1.40 (0.68–2.85)	
≥5 years	21	1.87 (1.21–2.91)	3	1.54 (0.56–4.29)	
p_{trend}[3]		0.08		0.18	0.69

Abbreviations: CI: confidence interval; COC: combined oral contraceptives; ER: estrogen receptor; HR: hazard ratio; OC: oral contraceptives; POC: progestin-only contraceptives; PR: progesterone receptor.
[1]Multivariable analysis adjusted for BMI (continuous), history of breast cancer in mother (yes, no), age at menarche (continuous), alcohol consumption (0, 0.1–4.9, 5–9.9, ≥10 g/day) and a combined variable including parity (0, 1, 2, ≥3 children) and age at first birth (age <25, 25–29, ≥30).
[2]χ^2 Wald, p heterogeneity between estimate for ER+ and ER− cancer.
[3]p-Value, continuous variable.

positive and ER-negative breast cancer with current use and duration of use,[19] as we did in our analysis.

When stratifying by hormonal content, POC and COC use were differently associated with hormone receptor-defined subtypes. However, heterogeneity tests for POC use were insignificant and unequal associations could be due to a small number of POC users in the hormone receptor-negative groups. As limited power is an issue when addressing POC use in relation to hormone receptor-negative cancer, one cannot rule out the possibility that POC use increases risk of this subtype as well. The increased risk of breast cancer provided by POC use is in line with some[9] and in contrast to other studies.[31,42] Although used as HT, one study found increased risk of breast cancer associated with current use of oral progestins for 4.5 years or more before menopause.[43] These studies have not assessed POC associations in relation to receptor-defined breast cancer.

Our findings further imply that general OC use is also associated with hormone receptor-positive cancers, which is in contrast to most studies.[18–22,29] Since POC use has been more common in Scandinavian countries than in the US,[44] a higher portion of POCs in our data could influence OC associations toward hormone receptor-positivity. Previously mentioned studies defined OC use as equivalent to COC use only,[19,25] or they did not specify what type of OCs were encompassed as such.[18,20,24] Moreover, non-Caucasians are scarce in our cohort which explains the relatively smaller portion of triple-negative cases and consequently hormone receptor-negative cases in our study, as this subtype is more common among African-American women.[45]

The biological mechanism linking progestin to breast cancer development is a subject of controversy. It is hypothesized that the proliferative effect of progestins on mammary epithelium increase breast cancer risk.[46] Moreover, it has been postulated that in breast cancer cells, crosstalk between the ER and the PR enables PR activation to provide estrogen-mediated proliferative response,[47] which also could influence disease development.

Several challenges arise when studying subtypes of disease. First, the potential of misclassification is noteworthy. Pathologists from wide-ranging laboratories conduct hormone receptor status assays across the country. There is a certain degree of variety in laboratory techniques, scoring methods and interpretation of data. In sum, these represent a subjective influence that opens for the possibility of misclassification. Despite that, studies show satisfactory concordance of

hormone receptor status across laboratories with regard to ER+, ER−, ER+/PR+ and ER−/PR− status,[48] as has been the main classifications used in our study. Moreover, a mixture of 1% and 10% cutoff for ER negativity could dilute associations, as contemporary clinical knowledge recognizes 1% cutoff as true-negative ER expression.

Another major issue involves limited statistical power as we restricted our cohort to premenopausal women and only 14% were diagnosed before menopause. Furthermore, there was a considerable amount of missing receptor status data as cases were diagnosed as of 1991, at which time receptor status testing practices was not standard procedure.

Missing information at baseline and follow-up was imputed, assuming the information was missing at random. This was done to keep observations in the analysis and thus improve the accuracy of associations. In analyses with smaller subgroups, this method improved the precision of the relative risk estimates substantially, without changing their values noteworthy. However, there is a possibility that some information was not missing at random, which would result in obtained estimates not being completely free from bias.

To our knowledge, this is the first study to address associations between POC use and receptor-defined breast cancer. Strengths of our study include its prospective design, avoiding concerns of selection and recall bias, which is a problem for case–control studies of OCs and breast cancer.[49] This inevitable concern was the main purpose of creating the prospective NOWAC study. Moreover, its nationally representative, population-based design allows findings to be generalizable to the whole country or broader. Furthermore, NOWAC is designed to study impact of hormonal constituents on cancer risk by providing reliable and detailed assessment of hormone use. Due to few available OC brands on the Norwegian market, our study has reduced potential of exposure misclassification. Potential exposure misclassification is likely to be non-differential due to the prospective design of the study, and estimates would be biased toward unity. Finally, due to including only premenopausal women in our analysis, we get valid results because risk factors and breast cancer characteristics are dissimilar in pre- and postmenopausal women.[50]

Despite numerous strengths of our study, our findings with regard to POC use require further confirmation due to our insignificant heterogeneity tests between subtypes and uncertain biological mechanisms.

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Paper II

Breast Cancer Research

RESEARCH

Open Access

Trajectories of body mass index in adulthood and risk of subtypes of postmenopausal breast cancer

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Abstract

Background Body fatness is a dynamic exposure throughout life. To provide more insight into the association between body mass index (BMI) and postmenopausal breast cancer, we aimed to examine the age at onset, duration, intensity, and trajectories of body fatness in adulthood in relation to risk of breast cancer subtypes.

Methods Based on self-reported anthropometry in the prospective Norwegian Women and Cancer Study, we calculated the age at onset, duration, and intensity of overweight and obesity using linear mixed-effects models. BMI trajectories in adulthood were modeled using group-based trajectory modeling. We used Cox proportional hazards models to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) for the associations between BMI exposures and breast cancer subtypes in 148,866 postmenopausal women.

Results A total of 7223 incident invasive postmenopausal breast cancer cases occurred during follow-up. Increased overweight duration and age at the onset of overweight or obesity were associated with luminal A-like breast cancer. Significant heterogeneity was observed in the association between age at overweight and overweight duration and the intrinsic-like subtypes ($p_{heterogeneity}$ 0.03). Compared with women who remained at normal weight throughout adulthood, women with a descending BMI trajectory had a reduced risk of luminal A-like breast cancer (HR 0.54, 95% CI 0.33–0.90), whereas women with ascending BMI trajectories were at increased risk (HR 1.09; 95% CI 1.01–1.17 for "Normal-overweight"; HR 1.20; 95% CI 1.07–1.33 for "Normal-obesity"). Overweight duration and weighted cumulative years of overweight and obesity were inversely associated with luminal B-like breast cancer.

Conclusions In this exploratory analysis, decreasing body fatness from obesity in adulthood was inversely associated with overall, hormone receptor-positive and luminal A-like breast cancer in postmenopausal women. This study highlights the potential health benefits of reducing weight in adulthood and the health risks associated with increasing weight throughout adult life. Moreover, our data provide evidence of intrinsic-like tumor heterogeneity with regard to age at onset and duration of overweight.

Keywords Breast cancer subtypes, Body fatness, Body mass index, Trajectory, Women

Background

Breast cancer is a heterogeneous disease consisting of at least five distinct molecular subtypes with different etiological pathways and prognosis [1–6]. Owing to the large degree of overlap between these intrinsic subtypes and immunohistochemical subtypes defined by the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), the St.

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Gallen International Expert Consensus panel created an intrinsic-like surrogate definition that has been broadly used in epidemiological research [7]. This classification includes four subtypes: luminal A-like and luminal B-like subtypes, which are predominantly hormone receptor-positive (i.e., ER-positive [ER+] and/or PR-positive [PR+]), and hormone receptor-negative HER2-enriched and basal-like (herein referred to as triple-negative [TNBC]) subtypes.

The risk factors for breast cancer include amongst others exposure to endogenous and exogenous female sex hormones. Hormonal risk factors are associated with hormone receptor-positive and luminal A-like subtypes [8–10]. Less is known about the risk factors for the remaining intrinsic-like subtypes. Adult body fatness, hereafter encompassing overweight and/or obesity, reflects endogenous estrogen exposure through increased aromatization of estrogen precursors in adipose tissue [11–13]. High adult body mass index (BMI) and weight gain are primarily associated with hormone receptor-positive subtypes in postmenopausal women [12, 14–22] and predominantly in never-users of menopausal hormone therapy (MHT) [15–18, 20, 21]. Correspondingly, postmenopausal weight loss reduces the risk of breast cancer among women not using MHT [23–25].

Body fatness is not a static measure but varies over a lifetime, and every woman follows her unique exposure trajectory throughout life. These dynamic aspects are likely relevant for disease development, and such a trajectory approach may provide more insight into the relationship between lifetime exposure intensity, duration and onset, and cancer risk than studying only one or a few measures of exposure. Recent studies have suggested a clear dose–response association between the intensity and duration of body fatness and risk of postmenopausal breast cancer [26, 27]. However, only four previous studies have assessed body fatness trajectories in relation to breast cancer risk [28–31], and only one of them provided estimates based on hormone receptor status [29]. To our knowledge, no previous study has assessed BMI trajectories and the risk of intrinsic-like breast cancer subtypes.

Thus, we aimed to explore whether the intensity, timing, duration, and trajectories of body fatness throughout adult life were associated with breast cancer in postmenopausal women, and whether associations varied according to subtypes.

Methods

Study population

The Norwegian Women and Cancer (NOWAC) study is a nationally representative prospective cohort study initiated in 1991 to investigate cancer etiology among women in Norway. Women aged 30–70 years were randomly sampled from the National Population Register and invited to participate in the study. A total of 172,472 women were enrolled between 1991 and 2007 and completed up to three follow-up questionnaires (1998–2017) distributed 5–10 years apart. The unique national identification number assigned to every resident in Norway allows for complete follow-up through linkages to national registries [32]. The NOWAC study is considered to have high external validity, as the distribution of exposures is independent of the response rate, and the cumulative incidence of cancers is similar to national figures from the Cancer Registry of Norway [33]. The details of the NOWAC study have been described previously [34].

In this study, 164,316 women with at least two self-reported height and weight measurements were eligible for inclusion ($n=8156$ excluded). As physical activity and tobacco smoking affect weight change and hence fluctuations in BMI, we excluded 6697 women without information on these covariates in any of the questionnaires. Excluded women with less than two BMI measurements or missing information on physical activity and tobacco smoking on all time points were slightly older, had higher BMI and lower education than included women (Additional file 1: Table 1). We further excluded women with prevalent cancer (other than non-melanoma skin cancer) at start of follow-up and women diagnosed with cancer within 1 year of the first self-reported weight measurement ($n=8150$), women who had died or emigrated before start of follow-up ($n=457$), and women who reported implausible values for age at menarche (<8 or >20; $n=30$), age at menopause (<25 or >60; $n=111$), or age at first birth (<12 or >50; $n=5$). For the complete-case analyses, women with missing covariates were also excluded ($n=6095$). Thus, the final analytical study sample consisted of 148,866 women, of which 142,771 were included in the complete-case analyses.

Exposure and covariates assessment

Self-reported weights at age 18 years and at the first, second, and third questionnaires (wave 1–3) and height at wave 1 were used to calculate BMI at up to four time points. As weight loss can follow a cancer diagnosis, weight measurements were not considered valid in women who were diagnosed with cancer up to 1 year before returning the questionnaire. BMI was calculated as weight in kilograms divided by the square of height in meters. Body fatness was defined according to the World Health Organization's definition [35].

Relevant covariates were extracted from the wave 1 questionnaires. We used a directed acyclic graph to visualize the assumed causal relationships among the exposure, outcome, and covariates, thereby identifying

Table 1 Characteristics of the study sample at wave 1 according to trajectory group

Total study sample	**T1 «Normal-stable»[a]**	**T2 «Normal-overweight»[a]**	**T3 «Normal-obesity»[a]**	**T4 «Overweight-obesity»[a]**	**T5 «Obesity-decrease»[a]**
N **= 148,866**	**Mean ± SD or *n* (%)**	**Mean ± SD or *n* (%)**	**Mean ± SD or *n* (%)**	**Mean ± SD or *n* (%)**	**Mean ± SD or *n* (%)**
Wave 1 characteristics					
Number of participants	65,507 (44.0)	60,440 (40.6)	18,117 (12.2)	3609 (2.4)	1193 (0.8)
Age at enrollment (yrs)	49.1 ± 0.03	49.2 ± 0.03	48.5 ± 0.06	48.2 ± 0.14	53.9 ± 0.26
Age at menarche (yrs)	13.5 ± 0.01	13.2 ± 0.01	12.9 ± 0.01	12.6 ± 0.02	13.1 ± 0.04
Parity	2.1 ± 0.00	2.3 ± 0.01	2.3 ± 0.01	2.2 ± 0.02	2.3 ± 0.04
Age at first birth (yrs)	24.4 ± 0.02	23.8 ± 0.02	23.6 ± 0.04	23.5 ± 0.08	23.9 ± 0.14
Breast cancer in mother	3572 (5.5)	3178 (5.3)	926 (5.1)	182 (5.4)	67 (5.6)
OC use					
Current	1261 (2.0)	1142 (1.9)	318 (1.8)	81 (2.3)	4 (0.3)
Former	36,961 (57.7)	32,369 (54.9)	9445 (53.4)	1705 (48.7)	459 (39.7)
Never	25,894 (40.4)	25,508 (43.2)	7922 (44.8)	1713 (49.0)	692 (59.9)
MHT use					
Current	8949 (13.9)	7349 (12.4)	1722 (9.7)	258 (7.3)	206 (17.5)
Former	7415 (11.5)	7166 (12.1)	2112 (11.9)	390 (11.0)	141 (12.0)
Never	47,946 (74.6)	44,965 (75.6)	13,964 (78.5)	2896 (81.7)	832 (70.6)
Smoking status					
Current	20,984 (32.2)	17,985 (29.9)	5101 (28.3)	993 (27.6)	518 (43.6)
Former	21,243 (32.6)	21,780 (36.2)	6585 (36.5)	1316 (36.6)	322 (27.1)
Never	23,033 (35.3)	20,441 (34.0)	6368 (35.3)	1286 (35.8)	349 (29.4)
Physical activity					
High	13,826 (21.3)	10,031 (16.7)	2381 (13.3)	358 (10.0)	243 (20.8)
Moderate	38,527 (59.3)	34,790 (58.0)	9269 (51.6)	1591 (44.6)	633 (54.1)
Low	12,617 (19.4)	15,141 (25.3)	6315 (35.2)	1621 (45.4)	295 (25.2)
Education (yrs)					
≤ 9	11,591 (18.5)	13,333 (23.1)	4598 (26.5)	978 (28.3)	423 (39.1)
10–12	20,531 (32.8)	20,422 (35.4)	6288 (36.3)	1267 (36.7)	381 (35.3)
13–16	18,950 (30.3)	15,970 (27.7)	4481 (25.9)	831 (24.1)	199 (18.4)
≥ 17	11,472 (18.3)	7948 (13.8)	1959 (11.3)	379 (11.0)	78 (7.2)
BMI variables					
BMI at age 18 (kg/m^2)	19.7 ± 0.01	21.1 ± 0.01	22.3 ± 0.02	24.9 ± 0.06	34.1 ± 0.10
BMI at wave 1 (kg/m^2)	21.4 ± 0.01	25.0 ± 0.01	29.6 ± 0.02	36.0 ± 0.07	25.4 ± 0.11
BMI at wave 2 (kg/m^2)	21.8 ± 0.01	25.7 ± 0.01	30.4 ± 0.02	36.5 ± 0.08	25.6 ± 0.13
BMI at wave 3 (kg/m^2)	22.1 ± 0.01	26.1 ± 0.02	30.9 ± 0.03	37.1 ± 0.11	26.2 ± 0.23
Predicted BMI variables[b]					
Age at overweight onset (yrs)	N/A	46.5 ± 0.03	32.5 ± 0.04	21.9 ± 0.07	18.0 ± 0.01
Age at obesity onset (yrs)	N/A	N/A	50.5 ± 0.06	35.9 ± 0.10	19.5 ± 0.24
Overweight duration (yrs)	N/A	7.8 ± 0.03	23.1 ± 0.06	32.9 ± 0.13	35.4 ± 0.35
Obesity duration (yrs)	N/A	N/A	4.6 ± 0.04	18.9 ± 0.14	8.4 ± 0.37
Overweight intensity (OWY)	N/A	10.1 ± 0.01	72.5 ± 0.34	206.9 ± 1.61	123.6 ± 2.65
Obesity intensity (OBY)	N/A	N/A	6.7 ± 0.10	76.4 ± 1.04	14.2 ± 0.92

BMI body mass index, *MHT* menopausal hormone therapy, *N/A* not applicable, *OBY* weighted cumulative overweight years, *OC* oral contraceptives, weighted cumulative obesity years, *SD* standard deviation

Number of missing values: 1972 (1.30%) for age at menarche, 9 (0.01%) for parity and age at first birth, 3410 (2.25%) for OC use, 2679 (1.77%) for MHT use, 573 (0.38%) for smoking status, 1232 (0.81%) for physical activity, 6845 (4.52%) for education

[a] Normal weight: 18.5–24.9 kg/m^2; overweight: 25–29.9 kg/m^2; obesity: ≥ 30 kg/m^2

[b] Derived from linear mixed-effects modeling and based on predicted BMI values

confounding factors to be included in the multivariable regression analysis (Additional file 1: Fig. 1). Identified confounders included age (used as time metric), age at menarche (continuous), parity (0, 1, 2, ≥3), age at first birth (<25, 25–29, ≥30 years), history of breast cancer in mother (yes, no), physical activity (low, moderate, high), smoking status (current, former, never), and MHT use (current, former, never). Number of missing values for the covariates are presented in footnotes in Table 1.

Outcome ascertainment

Incident invasive breast cancer cases were identified through linkage to the Cancer Registry of Norway based on the personal identification number assigned to all Norwegians at birth or immigration, and were classified according to the International Classification of Diseases 10th Revision (ICD-10, C50). Information on death and emigration was obtained through linkage to the Cause of Death Registry and the Central Population Register, respectively.

Tumor receptor status

The Cancer Registry of Norway provides information on ER, PR, and HER2 status, assessed using immunohistochemistry (IHC) techniques by pathological departments nationwide. ER negativity was defined as <10% reactivity before January 2012. From February 2012 onward, the threshold for ER-negative tumors was changed to <1% reactivity due to changes in the national treatment guidelines. These official thresholds were used in this study. PR negativity was defined as <10% reactivity. The HER2 expression status was determined using IHC and/or in situ hybridization (ISH). Tumors with no or weak immunostaining were defined as HER2−, while moderate or strong immunostaining was considered HER2+. ISH was generally used to confirm moderate staining. Breast cancer subtypes were defined by IHC surrogates for molecular subtypes according to the St. Gallen 2013 criteria without using the proliferation marker Ki67 in the subtype definition: luminal A-like (ER+PR+HER2−), luminal B-like (ER+PR− HER2− or ER+PR− HER2+ or ER+PR+HER2+), HER2-enriched (ER− PR− HER2+), and TNBC (ER− PR− HER2−) [7].

Menopausal status

Menopausal status was determined based on reported menstrual history. A woman was considered postmenopausal if her menstrual period had ceased naturally or by bilateral oophorectomy. Age at menopause was defined as the age when menstruation stopped. Women with unknown menopausal status or irregular menstrual cycles were considered postmenopausal at 53 years or older. This cutoff has been used previously in the NOWAC study and is based on the Million Women's Study convention [36, 37], as 92% and 96% of the study sample aged ≥53 years who had not had a hysterectomy or used MHT were postmenopausal, respectively. For women who were current smokers, the age of 53 years was substituted with 51 years, as smoking can reduce the age of menopause onset by approximately 2 years [38]. Menopausal status can be masked by a simple hysterectomy or by using MHT before natural menopause; therefore, women in this category were also considered postmenopausal at age 53 years or older. Women were included in the analysis if they were postmenopausal at the start of follow-up or from the age they reached menopause during the follow-up period.

Statistical analyses

BMI variable constructions

To construct variables for age at onset, duration, and intensity of overweight/obesity, we modeled individual BMI trajectories for each study participant as a function of age, physical activity (time-varying), and tobacco smoking (time-varying) [27, 39] using a linear mixed-effects model with a cubic effect of age and with random intercepts and slopes. As the number of samples was considerably larger than the number of measurement occasions, no assumptions were made regarding the covariance pattern of the random effect; therefore, we fitted an unstructured covariance matrix [40]. For each participant, we interpolated the BMI for each year starting from age 18 years until the last valid BMI measurement. From the predicted values, we calculated the years spent with a BMI ≥25 or ≥30, hereafter referred to as overweight and obesity durations, respectively. The duration variables did not necessarily reflect consecutive years of overweight/obesity. Furthermore, we calculated

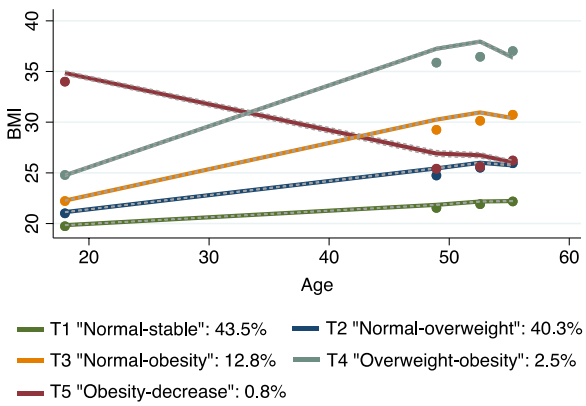

Fig. 1 BMI trajectories (T1 "Normal-stable"; T2 "Normal-overweight"; T3 "Normal-obesity"; T4 "Overweight-obesity"; T5 "Obesity-decrease") with 95% CI

the age at first onset of overweight or obesity from age 18 years. Finally, the weighted cumulative overweight years (OWY) and obesity years (OBY) were computed as measures of intensity by multiplying the duration of overweight/obesity in years by the difference (in BMI units) above the normal BMI (≥ 25 kg/m^2) for overweight and above overweight (≥ 30 kg/m^2) for obesity for each increment of age. Overweight and obesity duration were assessed per 10-year increments and intensity per 100 units, as previously described [26, 27].

Fluctuations in BMI from age 18 years to the age at the last valid BMI measurement were characterized using Nagin's approach to group-based trajectory modeling (GBTM) [41, 42]. GBTM is a semiparametric finite mixture model that allows the definition of relatively homogeneous clusters of BMI evolution over age. Trajectories were constructed using a censored normal model in the Traj package in STATA, and the optimal number of groups and shapes of trajectories were evaluated by the Bayesian information criterion using a two-stage approach [43]. First, the number of groups was determined using a quadratic form for all the trajectory groups. Second, the shape of each trajectory was determined. Using this method, the BMI development among the participants was best described by five-group trajectories based on a cubic function of age and adjusted for time-varying physical activity and tobacco smoking covariates. Finally, the average posterior probability and odds of correct classification were calculated, yielding satisfactory results that demonstrated high assignment accuracy based on Nagin's criteria [43].

Survival analysis

Follow-up began on the date of the last questionnaire used in the BMI modeling if the woman was postmenopausal or at the date of menopause if it occurred later. Women were followed until cancer diagnosis, death, emigration, or the end of the study (December 31, 2020), whichever occurred first. Cox proportional hazards models with attained age as the underlying time metric were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the estimated BMI variables (overweight/obesity duration, intensity and age at onset, and trajectories of BMI) in relation to the overall, ER/PR-, and ER/PR/HER2-defined subtypes of postmenopausal breast cancer. For the intrinsic-like subtypes, we additionally modeled the BMI at wave 1. Separate regression models were constructed for each subtype outcome, censoring women who developed a subtype other than the one defined as failure at the time of diagnosis [44]. We fitted two models per outcome: age-adjusted and multivariable-adjusted. Participants with missing information on the included covariates were excluded from the multivariable-adjusted analysis. The included women were of different ages at their first enrollment into the NOWAC study. Thus, their total follow-up time and their possible time spent with overweight or obesity varied according to age at enrollment. To account for these differences, the regression models for overweight/obesity duration, intensity, and age at onset included age at enrollment in 10-year age groups as stratum variables. This allowed the baseline hazard to vary across age strata while keeping the coefficients equal across groups. The HRs for breast cancer subtypes were tested for heterogeneity by competing risk analyses using the data duplication method and likelihood ratio tests as described by Lunn and McNeil [45, 46]. All p-values were two-sided. The proportional hazards assumption was evaluated by testing Schoenfeld residuals and by graphically inspecting a log–log survival plot. All analyses were performed using the statistical package STATA version 17.0 (StataCorp, College Station, TX, USA).

Results

During 2,221,544 person-years of follow-up, 7223 cases of incident invasive postmenopausal breast cancer occurred. Average follow-up time was 14.9 years (standard deviance [SD] 0.02). Changes in BMI were modeled over a range of 3–58 years, with a mean modeling duration of 36 years (SD 8.74).

Five distinct BMI trajectories were identified (Fig. 1): 43.5% of women had a consistent normal BMI (T1 "Normal-stable"); 40.3% started with normal weight and developed overweight in late adult life (T2 "Normal-overweight"); 12.8% evolved from normal to overweight in early adult life and had obesity in late adulthood (T3 "Normal-obesity"); 2.5% progressed from overweight to obesity (T4 "Overweight-obesity"); and 0.8% had a descending curve from obesity to overweight (T5 "Obesity-decrease").

The individual trajectories for each group are depicted in Additional file 1: Fig. 2.

Study sample characteristics

Compared with the "Normal-stable" (T1) group, the groups with increasing BMI (T2–T4) were less likely to have used exogenous hormones and to be physically active at wave 1 (Table 1).

The age at onset of overweight and obesity decreased, and the overweight duration increased from group T2 to T5. Apart from these differences, the characteristics of T1–T4 were relatively similar. Women in the "Obesity-decrease" (T5) group were more likely to be postmenopausal, never users of oral contraceptives, current users of MHT, current

smokers, and less educated compared with the other trajectory groups. They also had higher physical activity levels than the trajectory groups who experienced weight gain (T2–T4).

Characteristics of cancer cases

Of the 7223 incident invasive breast cancer cases, 5674 ER + (86.8%), 866 ER− (13.2%), 4379 PR + (67.4%), 2114 PR− (32.6%), 719 HER2 + (12.5%), and 5032 HER2− (87.5%) cases were identified (Table 2).

The number of missing cases were 683 (9.5%) for ER status, 730 (10.1%) for PR status, and 1472 (20.4%) for HER2 status. Missing values comprised a higher proportion in the T5 trajectory group but otherwise did not differ considerably across trajectory groups.

Postmenopausal breast cancer overall

Increasing age at overweight was associated with increased risk of postmenopausal breast cancer (p trend < 0.01) and belonging to the "Obesity-decrease" trajectory group decreased risk of breast cancer (HR 0.71; 95% CI 0.52–0.96; Table 3).

Postmenopausal breast cancer by ER/PR/HER2 status

Compared with normal-weight women, women with overweight or obesity at wave 1 had an increased risk of luminal A-like cancer with HRs of 1.11 (95% CI 1.02–1.20) and 1.13 (95% CI 1.00–1.28), respectively (p_{trend} 0.01; Table 4).

Increased age at overweight and obesity onset was associated with an increased risk of luminal A-like cancer (p linear trend < 0.01). Increasing overweight duration increased the risk of luminal A-like cancer (HR per 10-year increment 1.04; 95% CI 1.00–1.07) and decreased the risk of luminal B-like cancer (HR per 10-year increment 0.93; 95% CI 0.88–0.99). Significant heterogeneity was observed across the subtypes with regard to overweight duration and age at overweight ($p_{\text{heterogeneity}}$ 0.03). The HRs were similar to those of overweight/obesity duration when modeling weighted cumulative years of overweight/obesity for luminal A-like cancer. However, for luminal B-like cancer, HRs of 0.85 (95% CI 0.91–0.99) and 0.61 (95% CI 0.38–0.99) were observed for weighted cumulative years of overweight and obesity, respectively. Compared with the "Normal-stable" trajectory, women with constantly increasing BMI during

Table 2 Characteristics of postmenopausal breast cancer cases according to trajectory group

Postmenopausal breast cancer cases	T1 «Normal- stable»[a]	T2 «Normal-overweight»[a]	T3 «Normal- obesity»[a]	T4 «Overweight-obesity»[a]	T5 «Obesity- decrease»[a]
(*n* = 7223)	Mean ± SD or *n* (%)	Mean ± SD or *n* (%)	Mean ± SD or *n* (%)	Mean ± SD or *n* (%)	Mean ± SD or *n* (%)
Cancer characteristics					
Number of cases	3166 (43.8)	2987 (41.4)	885 (12.3)	145 (2.0)	40 (0.6)
Age at diagnosis	63.0 ± 0.13	63.3 ± 0.13	63.7 ± 0.23	62.2 ± 0.52	67.0 ± 1.31
ER/PR status					
ER+PR+	1805 (57.0)	1820 (60.9)	591 (66.8)	94 (64.8)	19 (47.5)
ER−PR−	376 (11.9)	327 (11.0)	91 (10.3)	16 (11.0)	4 (10.0)
ER+PR−	614 (19.4)	517 (17.3)	145 (16.4)	16 (11.0)	7 (17.5)
ER−PR+	26 (0.8)	18 (0.6)	1 (0.1)	2 (1.4)	1 (2.5)
Missing	345 (10.9)	305 (10.2)	57 (6.4)	17 (11.7)	9 (22.5)
ER/PR/HER2 status[b]					
Luminal A-like	1479 (46.7)	1496 (50.1)	481 (54.4)	78 (53.8)	15 (37.5)
Luminal B-like	634 (20.0)	557 (18.7)	167 (18.9)	21 (14.5)	8 (20.0)
HER2+	112 (3.5)	104 (3.5)	25 (2.8)	6 (4.1)	1 (2.5)
TNBC	212 (6.7)	184 (6.2)	58 (6.6)	9 (6.2)	3 (7.5)
Missing	729 (23.0)	646 (21.6)	154 (17.4)	31 (21.4)	13 (32.5)

BMI body mass index, *ER* estrogen receptor, *HER2* human epidermal growth factor receptor 2, *MHT* menopausal hormone therapy, *OC* oral contraceptives, *PR* progesterone receptor, *SD* standard deviation

[a] Normal weight: 18.5–24.9 kg/m^2; overweight: 25–29.9 kg/m^2; obesity: ≥ 30 kg/m^2

[b] Luminal A-like: ER+PR + HER2−; luminal B-like: ER+PR− HER2− or ER+PR− HER2+or ER+PR+HER2+; HER2-enriched: ER- PR- HER2+; TNBC: ER- PR- HER2-

Table 3 Age-adjusted and multivariable-adjusted hazard ratios for the association between body fatness and postmenopausal breast cancer overall

	Study sample ($n=148,866$)	Cases ($n=7223$)	Age-adjusted HR (95% CI)	Complete-case study sample ($n=142,771$)	Cases ($n=6933$)	MV-adjusted HR (95% CI)[a]
Age at onset (yrs)[b]						
BMI ≥ 25						
Never OW	83,606	3939	Ref	79,966	3766	Ref
< 40	26,375	1220	1.01 (0.95–1.08)	25,112	1173	1.00 (0.93–1.07)
40–49	25,208	1356	1.16 (1.09–1.23)	24,422	1311	1.14 (1.07–1.21)
≥ 50	13,677	708	1.18 (1.09–1.28)	13,271	683	1.16 (1.07–1.26)
p_{trend}[c]			< 0.01			< 0.01
BMI ≥ 30						
Never OB	133,840	6504	Ref	128,327	6232	Ref
< 40	3242	120	0.80 (0.66–0.95)	3082	119	0.81 (0.68–0.97)
40–49	5858	286	1.02 (0.91–1.15)	5640	282	1.02 (0.91–1.15)
≥ 50	5926	313	1.17 (1.05–1.32)	5722	300	1.14 (1.02–1.29)
p_{trend}[c]			0.03			0.06
Duration (per 10 yrs)[b]						
BMI ≥ 25	65,260	3284	1.02 (1.00–1.04)	62,805	3167	1.02 (0.99–1.04)
BMI ≥ 30	15,026	719	0.99 (0.94–1.05)	14,444	701	0.99 (0.93–1.05)
Intensity (per 100 units)[b]						
OWY	65,260	3284	1.00 (0.95–1.06)	62,805	3167	1.00 (0.94–1.05)
OBY	15,026	719	0.92 (0.78–1.08)	14,444	701	0.92 (0.78–1.08)
Trajectories[d,e]						
Normal-stable	62,729	3031	Ref	65,507	3166	Ref
Normal-overweight	58,079	2864	1.03 (0.98–1.08)	60,440	2987	1.02 (0.96–1.07)
Normal-obesity	17,374	854	1.03 (0.95–1.11)	18,117	885	1.01 (0.94–1.09)
Overweight-obesity	3454	144	0.88 (0.74–1.04)	3609	145	0.88 (0.75–1.05)
Obesity-decrease	1135	40	0.70 (0.51–0.96)	1193	40	0.71 (0.52–0.96)
p_{trend}[c]			0.45			0.28

BMI body mass index, *CI* confidence interval, *HR* hazard ratio, *MV* multivariable, *OBY* weighted cumulative obesity years, *OWY* weighted cumulative overweight years, *p p*-value

[a] Adjusted for age, age at menarche, parity, age at first birth, breast cancer in mother, smoking, MHT use

[b] Based on linear mixed-effects models

[c] *p* trend, continuous variable

[d] Based on group-based trajectory modeling

[e] Normal weight: 18.5–24.9 kg/m^2; overweight: 25–29.9 kg/m^2; obesity: ≥ 30 kg/m^2

adult life experienced an increased risk for luminal A-like cancer (HR 1.09; 95% CI 1.01–1.17 for "Normal-overweight"; HR 1.20; 95% CI 1.07–1.33 for "Normal-obesity"), whereas those with decreasing weight experienced a nearly 50% reduced risk (HR 0.54; 95% CI 0.33–0.90 for "Obesity-decrease"). With borderline-significance, the "Overweight-obesity" trajectory was associated with decreased risk of luminal B-like breast cancer (HR 0.64; 95% CI 0.41–1.00). No significant associations were observed for HER2-enriched or TNBC subtypes. Results of the age-adjusted analyses are provided in Additional file 1: Table 2.

Postmenopausal breast cancer by ER/PR status and MHT use

The ER/PR-positive breast cancer results were similar to those for the luminal A-like subtype. Body fatness was positively associated with ER/PR-positive breast cancer, whereas we observed no significant association with ER/PR-negative cancer. Specifically, increased age at overweight and obesity onset was associated with ER/PR-positive breast cancer ($p_{trend} < 0.01$; Additional file 1: Table 3). We also observed an increased risk of ER/PR-positive breast cancer by overweight duration (HR per 10-year increment 1.05; 95% CI 1.02–1.08). The

Table 4 Multivariable-adjusted hazard ratios for the association between body fatness and ER/PR/HER2-defined subtypes of postmenopausal breast cancer

	Luminal A-like ($n=3400$)		Luminal B-like ($n=1324$)		HER2-enriched ($n=235$)		TNBC ($n=450$)		p_{het}[b]
	Cases	MV-adjusted HR (95% CI)[a]	Cases	MV-adjusted HR (95% CI)[a]	Cases	MV-adjusted HR (95% CI)[a]	Cases	MV-adjusted HR (95% CI)[a]	
BMI at wave 1[c, d]									
Underweight	72	1.14 (0.90–1.44)	348	0.94 (0.63–1.40)	1	0.20 (0.03–1.40)	12	1.39 (0.78–2.48)	
Normal weight	2038	Ref	852	Ref	154	Ref	271	Ref	
Overweight	966	1.11 (1.02–1.20)	93	0.95 (0.84–1.08)	62	0.99 (0.73–1.33)	125	1.09 (0.88–1.35)	
Obesity	303	1.13 (1.00–1.28)	6	0.81 (0.66–1.01)	18	0.91 (0.55–1.50)	41	1.14 (0.81–1.59)	
p_{trend}[e]		0.01		0.09		0.82		0.54	0.06
Age at onset (yrs)[f]									
BMI ≥ 25									
Never OW	1780	Ref	736	Ref	136	Ref	247	Ref	
< 40	609	1.10 (1.00–1.21)	212	0.90 (0.77–1.05)	39	0.90 (0.63–1.30)	83	1.03 (0.80–1.33)	
40–49	698	1.27 (1.16–1.39)	230	1.00 (0.86–1.16)	46	1.11 (0.79–1.56)	74	0.95 (0.73–1.24)	
≥ 50	313	1.04 (0.92–1.18)	146	1.18 (0.99–1.42)	14	0.68 (0.39–1.19)	46	1.12 (0.81–1.55)	
p_{trend}[e]		< 0.01		0.14		0.41		0.66	0.03
BMI ≥ 30									
Never OB	3024	Ref	1199	Ref	216	Ref	403	Ref	
< 40	63	0.90 (0.70–1.16)	18	0.63 (0.39–1.00)	5	0.96 (0.39–2.34)	7	0.70 (0.33–1.47)	
40–49	145	1.10 (0.93–1.30)	51	0.95 (0.72–1.26)	11	1.11 (0.61–2.06)	22	1.20 (0.78–1.86)	
≥ 50	168	1.23 (1.05–1.44)	56	1.03 (0.79–1.35)	3	0.34 (0.11–1.05)	18	0.98 (0.61–1.58)	
p_{trend}[e]		< 0.01		0.87		0.12		0.87	0.29
Duration (per 10 yrs)[f]									
BMI ≥ 25	1620	1.04 (1.00–1.07)	588	0.93 (0.88–0.99)	99	0.98 (0.86–1.12)	203	0.98 (0.89–1.08)	0.03
BMI ≥ 30	376	1.01 (0.93–1.10)	125	0.88 (0.76–1.03)	19	0.95 (0.67–1.34)	47	0.96 (0.76–1.22)	0.56
Intensity (per 100 units)[f]									
OWY	1620	1.03 (0.96–1.11)	588	0.85 (0.74–0.97)	99	0.96 (0.70–1.30)	203	0.97 (0.78–1.20)	0.16
OBY	376	0.94 (0.75–1.18)	125	0.61 (0.38–0.99)	19	0.82 (0.31–2.15)	47	1.08 (0.63–1.86)	0.35
Trajectories[g]									
Normal-stable	1413	Ref	601	Ref	107	Ref	205	Ref	
Normal-overweight	1432	1.09 (1.01–1.18)	535	0.95 (0.84–1.06)	96	0.96 (0.73–1.27)	176	0.91 (0.74–1.11)	0.16
Normal-obesity	463	1.20 (1.08–1.33)	159	0.94 (0.78–1.12)	25	0.82 (0.53–1.28)	57	0.96 (0.72–1.30)	0.09
Overweight-obesity	77	1.05 (0.84–1.33)	21	0.64 (0.41–1.00)	6	1.00 (0.44–2.30)	9	0.78 (0.40–1.53)	0.3
Obesity-decrease	15	0.54 (0.33–0.90)	8	0.67 (0.33–1.35)	1	0.54 (0.08–3.88)	3	0.76 (0.24–2.37)	0.94
p_{trend}[e]		0.07		0.05		0.42		0.37	

BMI body mass index, *CI* confidence interval, *HR* hazard ratio, *HER2* human epidermal growth factor receptor 2, *MV* multivariable, *OBY* weighted cumulative obesity years, *OWY* weighted cumulative overweight years, *p p*-value, *TNBC* triple-negative breast cancer

[a] Adjusted for age, age at menarche, parity, age at first birth, breast cancer in mother, smoking, MHT use

[b] *p* heterogeneity between ER/PR/HER2-defined subtypes; likelihood ratio test by competing risks analysis

[c] Number of missing values: 21 luminal A-like (0.6%); 6 luminal B-like (0.5%); 0 HER2-enriched; 1 TNBC (0.2%)

[d] Underweight: < 18.5 kg/m^2; normal weight: 18.5–24.9 kg/m^2; overweight: 25–29.9 kg/m^2; obesity: ≥ 30 kg/m^2

[e] *p* trend, continuous variable

[f] Based on linear mixed effects models. Never overweight/obesity as reference group

[g] Based on group-based trajectory modeling

weighted cumulative years of overweight and obesity over time did not significantly change the risk of ER/PR-defined breast cancer. Compared with women belonging to the "Normal-stable" trajectory, women in the "Normal-overweight" and "Normal-obesity" trajectories had increased risk of ER/PR-positive breast cancer, with respective HRs of 1.09 (95% CI 1.01–1.16) and 1.19 (95% CI 1.08–1.31). Significant heterogeneity between ER/

Table 5 Multivariable-adjusted hazard ratios for the association between body fatness and ER/PR-defined subtypes of postmenopausal breast cancer by MHT use

	Ever MHT use[a]					Never MHT use[a]				
	ER+/PR+ ($n=1956$)		ER-/PR- ($n=342$)		p_{het}[c]	ER+/PR+ ($n=2230$)		ER-/PR- ($n=447$)		p_{het}[c]
	Cases	MV-adjusted HR (95% CI)[b]	Cases	MV-adjusted HR (95% CI)[b]		Cases	MV-adjusted HR (95% CI)[b]	Cases	MV-adjusted HR (95% CI)[b]	
Age at onset (yrs)[d]										
BMI ≥ 25										
Never OW	1046	Ref	182	Ref		1151	Ref	261	Ref	
<40	283	0.95 (0.83–1.08)	47	0.89 (0.64–1.23)		469	1.22 (1.09–1.36)	89	1.03 (0.80–1.31)	
40–49	392	1.13 (1.00–1.27)	68	1.11 (0.84–1.47)		455	1.37 (1.22–1.52)	67	0.90 (0.69–1.19)	
≥50	235	1.04 (0.90–1.20)	45	1.18 (0.85–1.64)		155	1.10 (0.93–1.31)	30	1.00 (0.68–1.48)	
p_{trend}[e]		0.21		0.26	0.85		<0.01		0.74	0.04
BMI ≥ 30										
Never OB	1775	Ref	312	Ref		1946	Ref	403	Ref	
<40	22	0.73 (0.48–1.11)	3	0.56 (0.18–1.74)		56	1.00 (0.77–1.31)	9	0.77 (0.40–1.50)	
40–49	66	0.92 (0.72–1.17)	15	1.13 (0.67–1.91)		115	1.26 (1.04–1.52)	21	1.12 (0.72–1.75)	
≥50	93	1.02 (0.83–1.26)	12	0.76 (0.43–1.36)		113	1.56 (1.29–1.89)	14	0.99 (0.58–1.70)	
p_{trend}[e]		0.81		0.45	0.64		<0.01		0.92	0.35
Duration (per 10 yrs)[d]										
BMI ≥ 25	910	1.00 (0.96–1.04)	160	0.98 (0.88–1.09)	0.92	1,079	1.09 (1.05–1.13)	186	0.98 (0.89–1.08)	0.04
BMI ≥ 30	181	0.96 (0.85–1.08)	30	0.91 (0.67–1.23)	0.83	284	1.06 (0.97–1.17)	44	0.97 (0.77–1.24)	0.47
Intensity (per 100 units)[d]										
OWY	910	0.95 (0.85–1.06)	160	0.93 (0.71–1.22)	0.99	1,079	1.10 (1.01–1.20)	186	0.95 (0.76–1.19)	0.21
OBY	181	0.88 (0.62–1.24)	30	1.05 (0.50–2.17)	0.63	284	0.98 (0.76–1.26)	44	0.89 (0.48–1.68)	0.77
Trajectories[f,g]										
Normal-stable	874	Ref	157	Ref		864	Ref	207	Ref	
Normal-overweight	822	1.04 (0.94–1.14)	146	1.02 (0.81–1.28)	0.92	943	1.14 (1.04–1.26)	169	0.86 (0.70–1.05)	0.01
Normal-obesity	222	1.02 (0.88–1.18)	33	0.82 (0.56–1.20)	0.33	349	1.34 (1.18–1.52)	57	0.91 (0.67–1.22)	0.01
Overweight-obesity	26	0.77 (0.52–1.14)	5	0.79 (0.32–1.93)	0.93	67	1.20 (0.93–1.54)	11	0.81 (0.44–1.49)	0.23
Obesity-decrease	12	0.78 (0.44–1.37)	1	0.39 (0.05–2.76)	0.44	7	0.41 (0.20–0.87)	3	0.79 (0.25–2.47)	0.39
p_{trend}[e]		0.64		0.26			<0.01		0.23	

CI confidence interval, *HR* hazard ratio, *ER* estrogen receptor, *MHT* menopausal hormone therapy, *MV* multivariable, *OBY* weighted cumulative obesity years, *OWY* weighted cumulative overweight years, *p p*-value

[a] Based on last reported MHT status prior to censoring

[b] Adjusted for age, age at menarche, parity, age at first birth, breast cancer in mother, smoking, physical activity

[c] *p* heterogeneity between ER+/PR+ and ER−/PR− subtype; likelihood ratio test by competing risks analysis

[d] Based on linear mixed effects models. Never overweight/obesity as reference group

[e] *p* trend, continuous variable

[f] Based on group-based trajectory modeling

[g] Normal weight: 18.5–24.9 kg/m^2; overweight: 25–29–9 kg/m^2; obesity: ≥ 30 kg/m^2

PR-positive and -negative breast cancer was observed for the HRs of the "Normal-obesity" trajectory ($p_{heterogeneity}$ 0.03). Women in the "Obesity-decrease" trajectory had a 43% reduced risk of ER/PR-positive breast cancer (HR 0.57; 95% CI 0.36–0.90). Age-adjusted analyses yielded similar results.

Stratified analyses of MHT use suggested some extent of effect modification by MHT on the association between body fatness and ER/PR-positive breast cancer (Table 5). Significant associations were seen for ER/PR-positive breast cancer in never MHT users, and not in ever users. Specifically, in women who never used MHT, increased age at overweight and obesity onset increased the risk of ER/PR-positive breast cancer ($p_{trend} < 0.01$). Overweight duration per 10 years and weighted cumulative overweight years per 100 unit increase were associated with ER/PR-positive breast cancer (HR 1.09; 95% CI 1.04–1.13 and HR 1.10; 95% CI 1.01–1.19, respectively). Ascending trajectories from normal BMI were associated with ER/PR-positive breast cancer, where the "Normal-obesity" trajectory increased risk by 34% (95% CI 1.18–1.52). Women belonging to the descending trajectory appeared to be at 59% decreased risk (HR 0.41, 95% CI 0.20–0.87). Age at overweight onset ($p_{heterogeneity}$ 0.04), overweight duration ($p_{heterogeneity}$ 0.04) and the "Normal-overweight" ($p_{heterogeneity}$ 0.01) and "Normal-obesity" ($p_{heterogeneity}$ 0.01) trajectory were differentially associated with ER/PR-positive and ER/PR-negative breast cancer among never MHT users.

Discussion

In this exploratory study, we assessed the relationship between BMI trajectories in adult life, duration, intensity, and onset of body fatness and subtypes of postmenopausal breast cancer in a large national cohort of Norwegian women. We observed that obese women who decreased their weight had a reduced risk of hormone receptor-positive or luminal A-like breast cancer compared with women who remained at normal weight throughout their adult life. Adult overweight duration, increased age at onset of overweight or obesity, and ascending BMI trajectories throughout adulthood were associated with an increased risk of hormone receptor-positive and luminal A-like breast cancer. Similar findings were observed in postmenopausal breast cancer overall, likely because the luminal A-like subtype constitutes the largest proportion of breast cancer cases in postmenopausal women. Significant associations between body fatness and hormone receptor-positive breast cancer were predominantly evident in never users of MHT. The findings regarding BMI trajectories are novel and highlight the potential health benefits of weight reduction among adult women with obesity and the health risks associated with consistent weight gain.

Our study aligns with the existing literature revealing that body fatness in adulthood is associated with hormone receptor-positive or luminal A-like tumors [12, 14–22]. A recent prospective study revealed significant associations between postmenopausal obesity and luminal A-like breast cancer, whereas no significant association was observed with either luminal B, HER2-enriched, or TNBC [19]. Furthermore, a German arm of the European Prospective Investigation into Cancer study demonstrated that a higher BMI was associated with luminal A-like breast cancer in postmenopausal women but not with aggressive tumor subtypes [21]. However, controversies exist; while some prospective studies have observed no associations between body fatness and hormone-receptor negative cancer in postmenopausal women [14, 15, 17, 19–21], other studies have reported positive associations [47–50]. For TNBC, case–control studies have observed both positive [48, 49] and inverse [51] associations with body fatness in postmenopausal women. Thus, results are inconsistent as to whether there is an association of BMI and estrogen receptor-negative breast cancer. We did not find previous reports suggesting inverse associations between body fatness and luminal B-like breast cancer as we did for overweight duration, intensity, and weight gain from overweight. To our knowledge, most studies have reported non-significant results [8, 19, 52, 53], and one study reported an increased risk of the luminal B-like subtype among women with obesity compared with normal-weight women [14]. Variations in the study design, age, measure of exposure, sample size, and subtype definition may explain these discrepancies. Of note, the findings for luminal B-like breast cancer need to be interpreted with caution due to low statistical power and thus the possibility that they were made by chance.

While many studies have addressed weight change in relation to the risk of subtypes of breast cancer [8, 16, 17, 20, 54–57], to our knowledge, this is the first study to assess associations between BMI trajectories and breast cancer subtypes. Previous studies on life-course fluctuations of body fatness in relation to breast cancer risk used trajectories of perceived body silhouettes [28–31]. This measure of body fatness may be more prone to misclassification, especially among heavier, shorter, younger, and less educated women [58]. Furthermore, these previous studies started the trajectory modeling in childhood and focused on the mechanisms of pre-pubertal body fatness and breast cancer. As we did not obtain BMI measurements in childhood, we could not extend our modeling to a complete life-course perspective of BMI development.

The mechanisms underlying the association between body fatness and hormone receptor-positive breast cancer in postmenopausal women involve hormonal pathways. Increased circulating levels of bioavailable estrogen are observed with increasing body fatness in postmenopausal women because adipose tissue remains the major site of aromatase activity after menopause, together with reduced production of sex hormone-binding globulin and alterations in androgen metabolism [59–61]. Indeed, women with normal BMI and high body fat percentages have a higher risk of postmenopausal breast cancer [62]. Other studies have revealed that increased estrogen levels largely explain the association between BMI and postmenopausal breast cancer [13]. While the association with luminal A-like breast cancer is evident, similar associations were not observed for luminal B-like breast cancer in the present work. Luminal B-like cancers have a lower expression of the PR protein than luminal A-like cancers [63], which may reflect the importance of the interaction between ER and PR [64]. Hormone receptor-negative subtypes are less prone to estrogen influence, which may explain why we did not observe a significant association with these subtypes. Other potential contributing mechanisms include altered insulin and insulin-like growth factor-I levels and chronic low-grade inflammation [65]. It is not unlikely that body fatness duration and timing influence these key mechanisms.

Our study is consistent with previous reports illustrating that the risk of postmenopausal breast cancer related to body fatness is modified by MHT use [18, 20, 26, 36, 47, 66–69]. We observed that associations between body fatness and hormone receptor-positive breast cancer were largely eliminated in ever-users of MHT. Moreover, overweight intensity significantly increased the risk of hormone receptor-positive subtypes in never-users. A proposed mechanism underlying this phenomenon is the obscuring effect of high exogenous estrogen intake from MHT, leaving relatively negligible endogenous estrogen levels at high BMI.

This study had several limitations. Despite the large sample size, we were restricted by lack of power in the subgroup analyses of the less common subtypes. Under the assumption that receptor status data were missing at random, we chose not to perform multiple imputation in order to maintain transparency and simplicity [70]. Moreover, self-reported weight tends to be underestimated with increasing age and BMI [71], as revealed by the validity assessment in the NOWAC study [72]. However, a substantial agreement was observed between the self-reported values and those measured by medical staff (weighted kappa = 0.73). The 8156 participants who were excluded due to having less than two BMI measurements and the 6697 participants excluded due to missing physical activity or smoking status at all time points differed slightly from the total study sample with regard to age, BMI and education (Additional file 1: Table 1). Hence, the exclusions may have resulted in a slightly slimmer study sample compared to all NOWAC participants since women with high BMI seem to be less likely to repeatedly report their BMI. Reporting bias or misclassification of included covariates may have resulted in residual confounding. Of note, breast density is a potential effect modifier on the association between BMI and breast cancer [50, 73, 74]. Unfortunately, we did not have information about the study participants´ breast density and hence, could not take that into account. Finally, due to the exploratory nature of this study, we did not adjust for multiple testing and results must be interpreted as such [75].

Conclusion

Our exploratory study suggests that decreasing body fatness from obesity in adulthood is inversely associated with overall, hormone receptor-positive and luminal A-like breast cancer in postmenopausal women. Positive associations were observed with increasing body fatness from normal BMI during adulthood. Furthermore, we demonstrated a dose–response relationship between overweight duration and these subtypes, with significant heterogeneity between the intrinsic-like subtypes. As breast cancer is the most frequently diagnosed malignancy in women, and the prevalence of body fatness is increasing, preventive measures, such as weight loss, could contribute to halting an undue increase in breast cancer incidence following the obesity epidemic.

Abbreviations

BMI	Body mass index
CI	Confidence interval
ER	Estrogen receptor
HR	Hazard ratio
MHT	Menopausal hormone therapy
NOWAC	The Norwegian Women and Cancer Study
PR	Progesterone receptor

Supplementary Information

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Additional file 1. Table 1. Comparison of selected characteristics at wave 1 of excluded participants vs. study sample. **Table 2.** Age-adjusted hazard ratios for the association between body fatness and ER/PR/HER2-defined subtypes of postmenopausal breast cancer. **Table 3.** Age-adjusted and multivariable-adjusted hazard ratios for the association between body fatness and ER/PR-defined subtypes of postmenopausal breast cancer. **Figure 1.** Directed acyclic graph on the assumed relations between BMI development in adulthood, postmenopausal breast cancer and covariates. **Figure 2.** Twoway scatterplots of individual BMI trajectories by trajectory group.

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Author contributions
MB performed statistical analyses and drafted the manuscript. GU and EL interpreted the results and revised the manuscript. TW contributed to the interpretation of the data and advised on the statistical methods. CR supervised the study design, statistical analyses, and manuscript preparation.

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Availability of data and materials
The datasets used and/or analyzed in this study are available from the corresponding author upon reasonable request and if legal permissions are in place.

Declarations

Ethics approval and consent to participate
The NOWAC study was approved by the Regional Committees for Medical and Health Research Ethics (REC) and the Norwegian Data Inspectorate. The participants received written information about the study, future linkages to national registers, and invitations to complete a second questionnaire. The return of a completed questionnaire was considered as consent to participate. A second questionnaire was sent to the participants who had agreed to receive one.

Consent for publication
Not applicable.

Competing interests
GU is a journal editor at *Breast Cancer Research*. The remaining authors have no conflicts of interest to declare.

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Publisher's Note

Paper III

Breast Cancer Research

RESEARCH

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Menopausal hormone therapy and incidence, mortality, and survival of breast cancer subtypes: a prospective cohort study

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Abstract

Background Menopausal hormone therapy (MHT) is associated with an increased risk of postmenopausal breast cancer, predominantly the luminal A-like subtype. The impact of MHT on deaths from breast cancer subtypes is less understood. This study aimed to explore associations between MHT use and the incidence, mortality, and survival of intrinsic-like breast cancer subtypes.

Methods Data from 160,881 participants with self-reported MHT use from the prospective Norwegian Women and Cancer Study were analyzed. Among them, 7,844 incident breast cancer cases, and 721 breast cancer-specific deaths occurred. Cox proportional hazard regression was performed to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) for the association between MHT use and the incidence, mortality, and survival of breast cancer subtypes.

Results MHT use was associated with increased risk of overall, luminal A-like, and luminal B-like breast cancer, with respective HRs of 1.44 (95% CI 1.36–1.52), 1.41 (95% CI 1.31–1.52), and 1.23 (95% CI 1.09–1.40) among current estrogen-progestin therapy (EPT) users compared with never users. The risk increased by 4%, 4%, and 2% per year of EPT use for overall, luminal A-like, and luminal B-like breast cancers, respectively. MHT use was also associated with increased risk of overall and luminal A-like breast cancer mortality, with HRs 1.61% (95% CI 1.36–1.91) and 2.15% (95% CI 1.51–3.05) increased risk among current EPT users compared with non-users. Among patients with breast cancer, pre-diagnostic MHT use was not associated with worse survival from overall breast cancer but was inversely associated with survival from triple-negative breast cancer (TNBC; HR death 0.41; 95% CI 0.24–0.73 among current users). Results varied significantly according to tumor subtype ($p_{heterogeneity} = 0.02$).

Conclusions Our study suggests that MHT use increases the risk of incident and fatal overall and luminal A-like, and incident luminal B-like breast cancer but does not decrease overall survival among patients with breast cancer. Further research is needed to elucidate the mechanisms underlying MHT use and breast cancer lethality, and to explore whether MHT use among patients with TNBC is indeed free from harm.

Keywords Menopausal hormone therapy, Breast cancer subtypes, Incidence, Mortality, Survival

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Background

Breast cancer is a heterogeneous disease with intrinsic molecular tumor subtypes that have different risk factors, tumor characteristics, responses to treatment, and survival outcomes [1–5]. These molecular subtypes are commonly cross-classified into a surrogate definition referred to as intrinsic-like subtypes using standard immunohistochemical (IHC) analyses of tumor receptor status [6].

Over the last three decades, numerous studies have identified combined menopausal hormone therapy (MHT) as an important risk factor for postmenopausal breast cancer [7–14]. The latest analyses by the Collaborative Group on Hormonal Factors in Breast Cancer found that all types and regimens of MHT, except vaginal estrogens, were associated with increased risk [13]. The risk escalated with longer use, with estrogen-progestin therapy (EPT) posing a higher risk than unopposed estrogen therapy (ET) compared with non-use [13]. Many studies have investigated the associations between MHT use and intrinsic-like subtypes of breast cancer. A uniform consensus that MHT use is associated with luminal A-like (estrogen receptor (ER)-positive/progesterone receptor (PR)-positive/human epidermal growth factor 2 (HER2)-negative) breast cancer is apparent [15–21], while some studies have indicated a similar association with luminal B-like (ER + /any PR/HER2 + or ER + /PR-/HER2-) subtypes [16, 19–21]. Indications of increased risks of hormone receptor-negative [22] and triple-negative breast cancer (ER-/PR-/HER2-; TNBC) [21] associated with MHT have also been reported, although findings regarding MHT use and hormone receptor-negative subtypes, including TNBC and HER2-enriched (ER-/PR-/HER2 +), are inconsistent.

Contrary to breast cancer incidence, evidence on the impact of MHT use on breast cancer-specific mortality and survival is conflicting. Numerous studies have been published [23–36]; however, the results have been ambiguous and, possibly, afflicted by collider stratification bias. Studies examining breast cancer-specific mortality among the entire study population have reported an increased risk associated with MHT use [23, 26]. Conversely, studies of patients with breast cancer have generally indicated improved survival among pre-diagnostic MHT users [24, 29–33]. A pooled analysis from the Breast Cancer Association Consortium (BCAC) with 121,435 breast cancer cases and 8,554 breast cancer-specific deaths also demonstrated improved survival among MHT users [29]. Studies evaluating the association between pre-diagnostic MHT use and breast cancer subtype-specific mortality and survival are sparse. However, the pooled BCAC analysis found increased survival across all subtypes with EPT and ET formulations [29].

While breast cancer mortality refers to the incidence of breast cancer deaths among initially healthy women, breast cancer survival measures the case-fatality among women diagnosed. Hence, mortality reflects the effects of both incidence and lethality, whereas survival specifically measures lethality and, consequently, more accurately assesses the impact of pre-diagnostic MHT on the developmental pathways of carcinogenesis that may influence tumor aggressiveness. However, survival can be influenced by several biases arising from early detection, typically through cancer screening or high awareness linked to socioeconomic status [37, 38]. These biases can obscure the understanding of cancer lethality. Thus, the importance of interpreting survival in the context of incidence and mortality has been emphasized [38, 39]. Increased knowledge of the relationship between MHT use and mortality and survival in breast cancer subtypes could be valuable for mitigating risks and prognostication for patients with breast cancer. This study aimed to investigate the associations between MHT use and the incidence, mortality, and survival of intrinsic-like breast cancer subtypes.

Methods

Study population

The Norwegian Women and Cancer (NOWAC) study [40], initiated in 1991, is a comprehensive, national prospective cohort study designed to explore cancer etiology in Norwegian women. Participants aged 30–70 years were randomly selected from the National Population Register between 1991 and 2008. A total of 172,472 women participated, completing up to three follow-up questionnaires approximately every 6 years. The unique national identification number for all Norwegian residents allows for complete follow-up through linkages to national registries [41]. The NOWAC study has demonstrated considerable external validity; the distribution of risk factors is independent of response rates, and cancer incidence rates align with national data from the Cancer Registry of Norway [42].

From the total cohort of 172,472, we excluded those with missing MHT status at the start of follow-up (n = 2,063), prevalent cancers (other than non-melanoma skin cancer; n = 7,862), premenopausal breast cancers (n = 1,004), participants who had died or emigrated before follow-up (n = 501), and those with extreme values for age at menarche (< 8 or > 20 years; n = 30), age at menopause (< 25 or > 60 years; n = 125), and age at first birth (< 12 or > 50 years; n = 6). Our final study sample comprised 160,881 participants who completed a baseline

questionnaire between 1991 and 2008. A flowchart of the study sample is presented in Supplementary Fig. 1.

For breast cancer survival analyses, we included 7,832 women diagnosed with incident postmenopausal breast cancer between 1991 and 2020, excluding those without breast cancer and 12 who were diagnosed post-mortem or after emigration.

Exposure and covariates

Information on MHT use, including ever use, current use, age at first use, and duration of use, was obtained from questionnaires. Furthermore, MHT was categorized into specific MHT regimens, with participants providing this information via timeline tables and a photo booklet of all available Norwegian MHT brands. We then categorized MHT use into EPT and ET and calculated cumulative estradiol (E2)- and norethisterone (NETA)-equivalent doses. Patients who previously used EPT were excluded from the ET users' group, leaving a category of patients who had only used unopposed estrogen. MHT status (ever/never, current/former/never) and duration were updated from the follow-up questionnaires to the last non-missing values at start of follow-up.

Covariates of interest were extracted from the questionnaires, and the last non-missing value before inclusion was used. We selected covariates of interest a priori and used directed acyclic graphs (DAGs) to depict their assumed causal relationship with exposure and outcome, thereby identifying potential confounding factors adjusted for in the multivariable models [43]. These covariates included age (used as time metric), body mass index (BMI; continuous), parity (0, 1, 2, ≥ 3) and age at first birth (< 25, 25–29, ≥ 30 years; combined into one variable), age at menarche (continuous), family history of breast cancer (none, mother and sister, only mother, only sister), physical activity (low, moderate, high), smoking status (current, former, never), and education (< 9, 10–12, 13–16, ≥ 17 years of schooling). Separate DAGs were performed for three outcome variables: overall breast cancer incidence, mortality, and survival (Supplementary Figs.2, 3, 4, respectively). To facilitate comparisons with previous literature, supplementary analyses on breast cancer survival were carried out, whereby models were adjusted for tumor stage (I, II, III, IV), surgical status (lumpectomy, mastectomy, other), and age at diagnosis (Model 1), as well as adding these variables to the main multivariable-adjusted analyses (Model 2; Supplementary Table7).

Outcome

Incident breast cancer cases were identified through passive linkage to the Cancer Registry of Norway and classified according to the International Classification of Diseases 10th revision (ICD-10, C50). Breast cancer-specific deaths were identified through the Cause of Death Registry, and emigration status was supplemented by the Central Population Register. These registries provide annual endpoint information, including the date of cancer diagnosis, death, emigration, and cause of death.

Information on tumor markers, characteristics, and mammography screening was obtained from the Cancer Registry of Norway. The registry routinely extracts information on ER and PR status from pathology reports. Receptor status was assessed using IHC by nationwide pathological departments. Before January 2012, ER-negative tumors were defined using a threshold of < 10% reactivity. Owing to alterations in the national treatment guidelines since February 2012, the threshold shifted to < 1% reactivity. This study employed these cutoff points. HER2 status was ascertained using IHC and/or in situ hybridization (ISH) techniques. Tumors exhibiting no or weak immunostaining were classified as HER2-negative, while those exhibiting moderate or strong immunostaining were classified as HER2-positive. ISH was used to verify cases of moderate immunostaining. Finally, molecular subtypes were approximated using the IHC surrogate definition from the St. Gallen 2013 Expert Panel: luminal A-like (ER + PR + HER2-), luminal B-like (ER + PR + HER2- or ER + PR- HER2 + or ER + PR + HER2 +), HER2-enriched (ER- PR- HER2 +), and triple-negative (ER- PR- HER2-) [6]. The Cancer Registry of Norway is estimated to be 98.8% complete [44].

Menopausal status

Participants were considered postmenopausal if their menstrual period had stopped naturally or surgically by bilateral oopherectomy. Those with unknown menopausal age, who reported irregular menses, hysterectomy, or MHT use, were considered postmenopausal at age 53. This cutoff was used to maintain consistency with the Million Women Study convention [7], and previous NOWAC publications [45, 46]. For current smokers, this age was adjusted to 51 years, as smoking can reduce the menopausal age by approximately 2 years [47].

Follow-up

For incidence and mortality analyses, follow-up began at the date of the baseline questionnaire for postmenopausal participants. If menopause occurred later, follow-up began at the age of menopause, age at MHT initiation, or age 53 [51 for smokers]. MHT use at study entry refers to the last questionnaire completed before inclusion in the regression analysis. Exit time was defined as the

date of cancer diagnosis, death, emigration, or end of follow-up, whichever occurred first. For breast cancer survival analyses, follow-up was from diagnosis until death, emigration, or end of follow-up. Participants were censored at 10 years post-diagnosis to retrieve the 10-year risk of death among patients with breast cancer as a measure of survival. The cause and date of death were updated until April 30, 2022, and breast cancer incidence updated until December 31, 2020.

Statistical analyses

Cox proportional hazard regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for associations between MHT use and the incidence, mortality, and survival of overall and intrinsic-like breast cancer subtypes, using age as the underlying time scale. Distinct regression models were fitted for each subtype outcome, censoring patients diagnosed with or dying from a different subtype [48]. The Cox proportional hazard's assumption was evaluated by graphical inspection of Schoenfeld residuals and survival time [49]. To account for variations in cumulative estrogen and progestin doses due to age differences, regression models included age at enrollment as a stratum variable.

A total of 22,434 (14%) participants had missing information on at least one covariate. The percentages of missing covariates are listed in Table 1. Assuming these variables were missing at random, we performed multiple imputation by chained equations (MICE) to handle the missing data. A MICE model was executed for each subtype outcome (overall breast cancer and intrinsic-like subtypes) within the incidence, mortality, and survival analytical samples. MICE models included all covariates, a MHT variable (never, current, or former use of ETP, ET, or an unknown type), age at study entry, a binary outcome variable, and the Nelson–Aalen cumulative hazard estimator. MICE models were constructed using predictive mean matching for continuous variables (BMI, age at menarche, and age at first birth), ordered logistic regression for ordinal categorical variables (physical activity and education), and multinomial logistic regression for non-ordinal categorical variables (smoking status). Family history of breast cancer and parity were used as auxillary variables. To reduce sampling variability during the imputation process, 20 duplicate datasets were created [50]. The estimates and standard errors in the imputed datasets were combined using Rubin´s rule to account for within- and between-imputation variances [51]. Age-adjusted and complete-case analyses were performed as sensitivity analyses.

All *p*-values were two-sided with a type I error rate of 5%. Heterogeneity across breast cancer subtypes was tested using the Wald test after a duplication method for competing risk analysis [52, 53]. All statistical analyses were performed using STATA version 17.0 (StataCorp, College Station, TX, USA).

Results

A total of 160,881 participants were followed for a median of 15.8 years for breast cancer incidence and 18.0 years for breast cancer-specific mortality. At study entry (in median year 2004), these participants were free from breast cancer and were postmenopausal. Among them, 40,974 (26%) were current MHT users (29,522 EPT and 4,370 ET), 17,849 (11%) were former users (11,256 EPT and 1,260 ET), and 102,058 (63%) had never used MHT at study entry. For the 10-year breast cancer-specific survival estimates, 7,832 patients with incident breast cancer (diagnosed in median year 2012) were followed for a median of 8.5 years. Descriptive statistics for the study sample are presented in Table 1, with case characteristics in Supplementary Tables 1 and 2. Notably, MHT users had higher alcohol consumption, higher education, were less likely to smoke, and were more likely to have used oral contraceptives than non-users.

Breast *cancer* incidence

Ever and current use of MHT and EPT at study entry were associated with increased risk of overall, luminal A-like, and luminal B-like breast cancer compared with never use (Table 2), with associations varying by subtype ($p_{\text{heterogeneity}} = 0.02$ and 0.04 for current MHT and EPT use, respectively). The highest HR was for the luminal A-like subtype (HR 1.41; 95% CI 1.31–1.52 for current EPT use). A significant trend for duration of use was observed for the overall, luminal A-like, and luminal B-like subtypes, with HRs increasing by 4%, 4%, and 2% per year of EPT use, respectively. Former EPT and ET use was associated with decreased risk of luminal A-like (HR 0.86; 95% CI 0.75–0.99) and overall breast cancer (HR 0.68; 95% CI 0.49–0.94) compared with never use. Increasing associations with overall, luminal A-like, and luminal B-like breast cancer were observed with increasing cumulative estrogen doses. Cumulative progestin dose was associated with overall (HR 1.66; 95% CI 1.52–1.82), luminal A-like (HR 1.87; 95% CI 1.65–2.12), luminal B-like (HR 1.60; 95% CI 1.30–1.97), and HER2-enriched (HR 1.79; 95% CI 1.08–2.98 for > 2 g NETA equivalence) breast cancer. High estrogen dose (≥ 5 g) combined with low progestin dose (< 1 g) was associated with a twofold increased risk of TNBC (HR 2.23; 95% CI 1.22–4.09). Supplementary Tables 3 and 4 provide corresponding results for non-imputed, age-adjusted and multivariable-adjusted complete-case analyses.

Table 1 Descriptives of study sample according to MHT use at study entry

	MHT use at study entry			
	Never MHT	**Ever EPT use**	**Ever ET use only[1]**	**Ever unknown type**
	Mean ± SD or n (%)			
Number of women, n (%)	102,058 (63.4)	40,778 (25.4)	5,630 (3.5)	12,415 (7.7)
Invasive breast cancer cases	4,297 (4.1)	2,599 (6.4)	262 (4.7)	686 (5.5)
Age at study entry (yrs)	53.9 ± 0.01	53.2 ± 0.03	53.4 ± 0.07	52.6 ± 0.06
Age at menarche (yrs)	13.3 ± 0.00	13.3 ± 0.01	13.2 ± 0.02	13.3 ± 0.01
Missing, n (%)	1,797 (1.8)	524 (1.3)	86 (1.5)	259 (2.1)
Age at menopause (yrs)	49.5 ± 0.02	49.7 ± 0.03	46.2 ± 0.08	48.3 ± 0.06
Missing, n (%)	47,676 (46.7)	10,731 (26.3)	1,193 (21.2)	4,075 (32.8)
Age at first birth (yrs)[2]	24.2 ± 0.02	23.8 ± 0.02	23.3 ± 0.06	23.4 ± 0.04
Missing, n (%)	49 (0.1)	2 (0.0)	0 (0.0)	0 (0.0)
Parity	2.3 ± 0.00	2.2 ± 0.01	2.1 ± 0.01	2.3 ± 0.01
Missing, n (%)	0 (0)	0 (0)	0 (0)	0 (0)
BMI (kg/m^2)	24.7 ± 0.01	24.3 ± 0.02	24.7 ± 0.05	24.6 ± 0.04
Missing, n (%)	2,167 (2.1)	706 (1.7)	119 (2.1)	365 (2.9)
Alcohol consumption (g/day)	3.49 ± 0.02	4.23 ± 0.03	4.03 ± 0.07	3.46 ± 0.05
Missing, n (%)	4,088 (4.0)	2,133 (5.2)	282 (5.0)	845 (6.8)
Education, n (%)				
≤ 9 yrs	22,535 (22.1)	7,793 (19.1)	1,108 (19.7)	3,584 (28.9)
10–12 yrs	32,513 (31.9)	13,593 (33.3)	1,946 (34.6)	3,967 (32.0)
13–16 yrs	26,796 (26.3)	11,070 (27.2)	1,472 (26.2)	2,586 (20.8)
≥ 17 yrs	14,407 (14.1)	6,148 (15.1)	774 (13.8)	1,264 (10.2)
Missing	5,807 (5.7)	2,174 (5.3)	330 (5.9)	1,014 (8.2)
Family history of breast cancer, n (%)				
None	94,481 (92.6)	37,774 (92.6)	5,198 (92.3)	11,491 (92.6)
Mother and sister	301 (0.3)	108 (0.3)	10 (0.2)	40 (0.3)
Mother	5,176 (5.1)	1,996 (4.9)	301 (5.4)	573 (4.6)
Sister	2,100 (2.1)	900 (2.2)	121 (2.2)	311 (2.5)
Missing	0 (0)	0 (0)	0 (0)	0 (0)
Smoking status, n (%)				
Never	37,843 (37.1)	12,842 (31.5)	1,870 (33.2)	3,964 (31.9)
Former	33,783 (33.1)	15,368 (37.7)	2,118 (37.6)	4,099 (33.0)
Current	29,502 (28.9)	12,358 (30.3)	1,602 (28.5)	4,109 (33.1)
Missing	930 (0.9)	210 (0.5)	40 (0.7)	243 (2.0)
Physical activity, n (%)				
Low	22,411 (22.0)	9,386 (23.0)	1,382 (24.6)	3,026 (24.4)
Moderate	54,820 (53.7)	22,735 (55.8)	3,053 (54.2)	6,001 (48.3)
High	17,013 (16.7)	6,507 (16.0)	885 (15.7)	1,782 (14.4)
Missing	7,814 (7.7)	2,150 (5.3)	310 (5.5)	1,606 (12.9)
Oral contraceptive use, n (%)				
Never	43,708 (42.8)	16,551 (40.6)	2,496 (44.3)	5,466 (44.0)
Ever	54,967 (53.9)	23,584 (57.8)	3,015 (53.6)	6,406 (51.6)
Missing	3,383 (3.3)	643 (1.6)	119 (2.1)	543 (4.4)

[1] Never EPT users

[2] Among parous women

EPT estrogen-progestin therapy, *ET* estrogen therapy, *MHT* menopausal hormone therapy, *BMI* body mass index

Table 2 MHT use at study entry and breast cancer incidence by intrinsic-like subtypes

	Breast cancer overall (n = 7,844)		Luminal A-like (n = 3,784)		Luminal B-like (n = 1,480)		HER2 + (n = 264)		TNBC (n = 500)		p_{het} [2]
	n cases	HR (95% CI)[1]	n cases	HR (95% CI)[1]	n cases	HR (95% CI)[1]	n cases	HR (95% CI)[1]	n cases	HR (95% CI)[1]	
MHT use overall											
Never use	4,297	Ref	2,113	Ref	845	Ref	155	Ref	297	Ref	
Ever use	3,547	1.24 (1.18–1.29)	1,671	1.16 (1.10–1.25)	635	1.13 (1.02–1.26)	109	1.07 (0.83–1.37)	203	1.03 (0.86–1.23)	0.51
Current	2,782	1.35 (1.29–1.42)	1,310	1.32 (1.23–1.41)	464	1.17 (1.04–1.31)	77	1.05 (0.80–1.39)	139	0.99 (0.80–1.21)	0.02
Former	765	0.95 (0.88–1.02)	361	0.83 (0.75–0.93)	171	1.04 (0.88–1.23)	32	1.12 (0.76–1.64)	64	1.12 (0.85–1.48)	0.08
Duration											
< 5 yrs	2,250	1.16 (0.10–1.22)	984	1.06 (0.98–1.14)	416	1.13 (1.00–1.27)	78	1.14 (0.86–1.50)	128	0.97 (0.79–1.20)	
≥ 5 yrs	1,243	1.40 (1.31–1.49)	656	1.37 (1.26–1.50)	212	1.15 (0.99–1.34)	30	0.94 (0.63–1.40)	71	1.13 (0.87–1.46)	
Per 1 yr	7,790	1.03 (1.03–1.04)	3,753	1.03 (1.02–1.04)	1,473	1.02 (1.00–1.03)	263	1.00 (0.96–1.04)	496	1.01 (0.98–1.03)	0.04
EPT use											
Never use	4,297	Ref	2,113	Ref	845	Ref	155	Ref	297	Ref	
Ever use	2,599	1.32 (1.25–1.38)	1,248	1.26 (1.17–1.35)	464	1.19 (1.06–1.34)	82	1.16 (0.88–1.52)	147	1.08 (0.88–1.32)	0.45
Current	2,120	1.44 (1.36–1.52)	1,012	1.41 (1.31–1.52)	352	1.23 (1.09–1.40)	58	1.09 (0.81–1.49)	107	1.06 (0.85–1.32)	0.04
Former	479	0.96 (0.87–1.05)	236	0.86 (0.75–0.99)	112	1.09 (0.89–1.33)	24	1.34 (0.86–2.06)	40	1.13 (0.81–1.57)	0.11
Duration											
< 5 yrs	1,559	1.22 (1.15–1.29)	688	1.11 (1.02–1.22)	288	1.18 (1.03–1.35)	56	1.22 (0.89–1.66)	91	1.04 (0.82–1.32)	
≥ 5 yrs	1,028	1.49 (1.39–1.60)	553	1.48 (1.35–1.63)	175	1.22 (1.03–1.44)	25	1.01 (0.66–1.54)	55	1.12 (0.84–1.50)	
Per 1 yr	6,884	1.04 (1.03–1.05)	3,354	1.04 (1.03–1.05)	1,308	1.02 (1.01–1.04)	236	1.01 (0.97–1.06)	443	1.01 (0.98–1.04)	0.05
ET use only											
Never use	4,297	Ref	2,113	Ref	845	Ref	155	Ref	297	Ref	
Ever use	262	0.96 (0.85–1.09)	122	0.89 (0.74–1.06)	52	0.97 (0.73–1.29)	12	1.25 (0.69–2.26)	15	0.80 (0.48–1.35)	0.68
Current	224	1.04 (0.91–1.19)	102	0.95 (0.78–1.16)	47	1.12 (0.83–1.50)	9	1.18 (0.60–2.32)	12	0.81 (0.45–1.45)	0.68
Former	38	0.68 (0.49–0.94)	20	0.65 (0.42–1.01)	5	0.43 (0.18–1.04)	3	1.51 (0.48–4.74)	3	0.76 (0.24–2.37)	0.39
Duration											
< 5 yrs	164	0.97 (0.83–1.13)	78	0.95 (0.76–1.20)	29	0.90 (0.62–1.30)	8	1.34 (0.66–2.72)	8	0.70 (0.35–1.41)	
≥ 5 yrs	96	0.95 (0.78–1.17)	43	0.78 (0.58–1.06)	22	1.05 (0.69–1.61)	4	1.13 (0.42–3.05)	7	0.98 (0.46–2.08)	
Per 1 yr	4,557	0.99 (0.97–1.01)	2,234	0.98 (0.95–1.01)	896	1.00 (0.96–1.04)	167	0.99 (0.89–1.10)	312	0.98 (0.90–1.06)	0.85
Cumulative dose											
Never use	4,297	Ref	2,113	Ref	845	Ref	155	Ref	297	Ref	
Estrogen (E2-equivalence)											
< 5 g	1,999	1.21 (1.15–1.28)	948	1.23 (1.14–1.33)	347	1.13 (1.00–1.29)	69	1.29 (0.97–1.74)	112	1.01 (0.81–1.27)	0.49
5–10 g	827	1.36 (1.26–1.47)	399	1.45 (1.29–1.62)	154	1.39 (1.16–1.66)	22	1.24 (0.78–1.97)	48	1.21 (0.88–1.66)	0.66
> 10 g	192	1.51 (1.30–1.75)	103	1.79 (1.46–2.18)	34	1.46 (1.03–2.07)	5	1.39 (0.57–3.43)	6	0.74 (0.33–1.66)	0.19

Table 2 (continued)

	Breast cancer overall (n = 7,844)		Luminal A-like (n = 3,784)		Luminal B-like (n = 1,480)		HER2 + (n = 264)		TNBC (n = 500)		p_{het}[2]
	n cases	HR (95% CI)[1]	n cases	HR (95% CI)[1]	n cases	HR (95% CI)[1]	n cases	HR (95% CI)[1]	n cases	HR (95% CI)[1]	
Progestin (NETA-equivalence)											
< 1 g	1,411	1.20 (1.13–1.28)	634	1.16 (1.06–1.27)	257	1.18 (1.02–1.36)	46	1.19 (0.85–1.66)	92	1.18 (0.93–1.50)	0.97
1–2 g	695	1.36 (1.25–1.47)	361	1.55 (1.38–1.74)	112	1.19 (0.97–1.45)	18	1.18 (0.72–1.95)	33	0.99 (0.68–1.43)	0.04
> 2 g	608	1.66 (1.52–1.82)	304	1.87 (1.65–2.12)	107	1.60 (1.30–1.97)	18	1.79 (1.08–2.98)	24	1.02 (0.66–1.56)	0.09
E2 dose < 5 g											
NETA dose < 1 g	1,306	1.20 (1.14–1.28)	589	1.16 (1.06–1.27)	237	1.17 (1.01–1.35)	43	1.20 (0.85–1.69)	79	1.09 (0.85–1.41)	0.98
NETA dose ≥ 1 g	439	1.47 (1.33–1.63)	233	1.73 (1.51–1.98)	66	1.20 (0.93–1.55)	12	1.36 (0.75–2.48)	18	0.93 (0.57–1.50)	0.03
E2 dose ≥ 5 g											
NETA dose < 1 g	93	1.20 (0.98–1.48)	40	1.14 (0.84–1.57)	18	1.29 (0.80–2.05)	3	1.30 (0.41–4.09)	11	2.23 (1.22–4.09)	0.27
NETA dose ≥ 1 g	862	1.49 (1.38–1.61)	431	1.66 (1.48–1.84)	153	1.44 (1.20–1.72)	23	1.39 (0.88–2.19)	39	1.04 (0.74–1.48)	0.09

[1] Adjusted for age (underlying time scale), BMI, parity, age at first birth, age at menarche, family history, smoking, physical activity, education

[2] *p* heterogeneity between intrinsic-like subtypes; Wald test by competing risks analysis

CI confidence interval, *ET*, estrogen therapy, *EPT* estrogen-progestin therapy, *E2* estradiol, *HER2* human epidermal growth factor receptor 2, *HR* hazard ratio *MHT* menopausal hormone therapy *NETA* norethisterone acetate, *TNBC* triple-negative breast cancer

Table 3 MHT use at study entry and breast cancer-specific mortality by intrinsic-like subtypes

	Breast cancer deaths overall (n = 721)		Luminal A-like (n = 163)		Luminal B-like (n = 113)		HER2-enriched (n = 33)		TNBC (n = 81)		p_{het} [2]
	n	HR (95% CI)[1]	n	HR (95% CI)[1]	n	HR (95% CI)[1]	n	HR (95% CI)[1]	n	HR (95% CI)[1]	
MHT use overall											
Never use	392	Ref	82	Ref	64	Ref	20	Ref	54	Ref	
Ever use	329	1.27 (1.09–1.47)	81	1.52 (1.11–2.07)	49	1.11 (0.76–1.61)	13	1.00 (0.49–2.04)	27	0.72 (0.45–1.15)	0.10
Current	268	1.48 (1.26–1.73)	65	1.82 (1.31–2.54)	39	1.29 (0.86–1.94)	13	1.43 (0.70–2.94)	16	0.60 (0.34–1.06)	0.03
Former	61	0.78 (0.60–1.03)	16	0.91 (0.53–1.56)	10	0.71 (0.36–1.39)	0	-	11	1.01 (0.52–1.94)	0.80
Duration											
< 5 yrs	220	1.29 (1.09–1.53)	43	1.28 (0.88–1.86)	31	1.10 (0.71–1.69)	10	1.15 (0.53–2.50)	16	0.65 (0.37–1.15)	
≥ 5 yrs	104	1.22 (0.98–1.52)	35	1.86 (1.24–2.78)	17	1.08 (0.63–1.86)	3	0.73 (0.11–1.49)	11	0.89 (0.46–1.72)	
Per 1 yr	716	1.02 (1.00–1.04)	160	1.06 (1.02–1.09)	112	1.01 (0.96–1.06)	33	0.95 (0.83–1.09)	81	0.98 (0.92–1.06)	0.13
ETP use											
Never use	392	Ref	82	Ref	64	Ref	20	Ref	54	Ref	
Ever use	237	1.35 (1.14–1.59)	62	1.74 (1.24–2.44)	37	1.23 (0.81–1.86)	11	1.25 (0.59–2.64)	20	0.78 (0.46–1.31)	0.13
Current	208	1.61 (1.36–1.91)	54	2.15 (1.51–3.05)	31	1.44 (0.93–2.22)	11	1.70 (0.80–3.62)	14	0.74 (0.41–1.33)	0.05
Former	29	0.62 (0.43–0.91)	8	0.77 (0.37–1.60)	6	0.71 (0.30–1.64)	0	-	6	0.90 (0.38–2.11)	0.94
Duration											
< 5 yrs	152	1.38 (1.14–1.67)	30	1.42 (0.93–2.18)	23	1.27 (0.78–2.05)	9	1.60 (0.72–3.59)	11	0.69 (0.36–1.32)	
≥ 5 yrs	84	1.28 (1.01–1.62)	31	2.16 (1.42–3.29)	14	1.15 (0.64–2.08)	2	0.63 (0.15–2.73)	9	0.94 (0.46–1.92)	
Per 1 yr	628	1.02 (1.00–1.05)	143	1.07 (1.04–1.11)	101	1.02 (0.97–1.08)	31	0.95 (0.82–1.10)	74	0.99 (0.92–1.07)	0.10

[1] Adjusted for age (underlying time scale), BMI, parity, age at first birth, age at menarche, family history, smoking, physical activity, education

[2] *p* heterogeneity between intrinsic-like subtypes; Wald test by competing risks analysis

CI confidence interval, *ETP* estrogen-progestin therapy, *HER2* human epidermal growth factor receptor 2, *HR* hazard ratio, *MHT* menopausal hormone therapy, *TNBC* triple-negative breast cancer

Breast *cancer* mortality

Among the entire study sample, MHT use at study entry increased risk of overall breast cancer-specific mortality compared to never use (Table 3; HR 1.61; 95% CI 1.36–1.91 among current EPT users). Ever (HR 1.74; 95% CI 1.24–2.44) and current use (HR 2.15; 95% CI 1.51–3.05) of EPT at study entry were associated with increased risk of dying from luminal A-like breast cancer.

The association with breast cancer mortality increased by 2% per year of EPT use, and ≥ 5 years of EPT use was associated with a twofold risk of dying from luminal A-like breast cancer (HR 2.16; 95% CI 1.42–3.29). No association was observed between MHT use and luminal B-like, HER2-enriched, or TNBC mortality. Associations between current MHT use and breast cancer mortality varied across intrinsic-like subtypes ($p_{\text{heterogeneity}} = 0.03$). Complete-case analysis results are presented in Supplementary Table 5.

Breast *cancer* survival

Among patients with breast cancer, MHT use was associated with increased risk of death from luminal A-like cancer, albeit statistically non-significantly, thus lower 10-year survival compared with non-users (Table 4; HR death 1.36; 95% CI 0.94–1.99 for current EPT use at study entry).

Similarly, the duration of EPT use at study entry was associated with an increased risk of death from luminal A-like breast cancer (HR death 1.04; 95% CI 1.00–1.09 per year increment). Ever (HR death 0.57; 95% CI 0.34–0.96) and current use (HR death 0.48; 95% CI 0.26–0.87) of EPT at study entry was associated with decreased risk of death from TNBC compared with never users. Moreover, current MHT use was differentially associated with survival across intrinsic-like subtypes ($p_{\text{heterogeneity}} = 0.02$). Complete-case analysis findings are presented in Supplementary Table 6. Adjustment for tumor stage, surgical status and age at diagnosis did not substantially alter risk estimates (Supplementary Table 7).

Discussion

In this prospective cohort study with 160,881 participants, 7,844 incident breast cancer cases, and 721 breast cancer-specific deaths, MHT use was associated with increased risks of incident and fatal overall and luminal

Table 4 MHT use at study entry and 10-year survival by intrinsic-like subtypes

	Breast cancer deaths overall (n = 634)		Luminal A-like (n = 148)		Luminal B-like (n = 104)		HER2 + (n = 32)		TNBC (n = 81)		p_{het} [3]
	n	HR (95% CI)[1,2]	n	HR (95% CI)[1,2]	n	HR (95% CI)[1,2]	n	HR (95% CI)[1,2]	n	HR (95% CI)[1,2]	
MHT use overall											
Never use	356	Ref	76	Ref	62	Ref	19	Ref	54	Ref	
Ever use	278	0.95 (0.81–1.11)	72	1.20 (0.86–1.67)	42	0.78 (0.52–1.17)	13	0.90 (0.44–1.86)	27	0.56 (0.35–0.90)	0.10
Current	226	0.97 (0.82–1.15)	58	1.28 (0.90–1.82)	32	0.77 (0.50–1.19)	13	1.14 (0.55–2.35)	16	0.41 (0.24–0.73)	0.02
Former	52	0.85 (0.63–1.13)	14	0.95 (0.53–1.68)	10	0.82 (0.42–1.62)	0	-	11	1.13 (0.59–2.20)	0.78
Duration											
< 5 yrs	181	0.95 (0.79–1.14)	38	1.04 (0.70–1.54)	26	0.78 (0.49–1.25)	10	1.05 (0.48–2.29)	16	0.52 (0.30–0.92)	
≥ 5 yrs	93	0.94 (0.74–1.18)	32	1.43 (0.93–2.19)	15	0.74 (0.42–1.32)	3	0.65 (0.19–2.24)	11	0.66 (0.34–1.28)	
Per 1 yr	630	0.99 (0.97–1.02)	146	1.03 (0.99–1.07)	103	0.98 (0.92–1.04)	32	0.93 (0.81–1.07)	81	0.95 (0.88–1.03)	0.12
ETP use											
Never use	356	Ref	76	Ref	62	Ref	19	Ref	54	Ref	
Ever use	201	0.94 (0.79–1.12)	54	1.24 (0.86–1.77)	31	0.78 (0.50–1.22)	11	1.06 (0.49–2.26)	20	0.57 (0.34–0.96)	0.14
Current	175	0.99 (0.82–1.19)	47	1.36 (0.94–1.99)	25	0.78 (0.49–1.26)	11	1.27 (0.59–2.72)	14	0.48 (0.26–0.87)	0.05
Former	26	0.70 (0.47–1.05)	7	0.77 (0.35–1.67)	6	0.79 (0.34–1.85)	0	-	6	1.03 (0.44–2.44)	0.92
Duration											
< 5 yrs	126	0.97 (0.79–1.19)	25	1.00 (0.63–1.58)	19	0.83 (0.49–1.40)	9	1.38 (0.61–3.09)	11	0.52 (0.27–1.01)	
≥ 5 yrs	74	0.90 (0.70–1.16)	28	1.53 (0.98–2.39)	12	0.71 (0.38–1.33)	2	0.52 (0.12–2.27)	9	0.65 (0.32–1.34)	
Per 1 yr	556	0.99 (0.96–1.02)	129	1.04 (1.00–1.09)	93	0.98 (0.92–1.04)	30	0.93 (0.79–1.08)	74	0.95 (0.88–1.03)	0.08

[1] HRs of breast-cancer specific death

[2] Adjusted for age (underlying time scale), BMI, parity, age at first birth, age at menarche, family history, smoking, physical activity, education

[3] *p* heterogeneity between intrinsic-like subtypes; Wald test by competing risks analysis

CI confidence interval, *ETP* estrogen-progestin therapy, *HER2* human epidermal growth factor receptor 2, *HR* hazard ratio, *MHT* menopausal hormone therapy, *TNBC* triple-negative breast cancer

A-like breast cancers. Longer duration of use and higher cumulative doses of estrogen and progestin at study entry were associated with higher risks of overall, luminal A-like, and luminal B-like breast cancers, indicating a dose–response relationship. We observed differences in risk based on recency, where the strongest HRs were observed with current use at study entry. Despite positive associations between MHT use and breast cancer incidence and mortality, we did not observe worse survival among patients with breast cancer who were pre-diagnostic MHT users. Although based on small numbers, there were indications that MHT use at study entry was associated with a decreased risk of breast cancer-specific death among patients with TNBC. This study provides insights into the nuanced effects of MHT on etiology and progression of breast cancer subtypes.

Our findings on breast cancer incidence align with the empirically grounded consensus that MHT use increases breast cancer risk [13, 21], with effect estimates among current users similar to those of large, prospective studies [9, 12, 21]. Consistent with previous reports, past use was not associated with increased risk of incident or fatal disease [7]. Moreover, the association with an increased risk of luminal subtypes is also reflected in previous studies [16, 17, 19, 21]. We did not observe any association between general MHT use and HER2-enriched or TNBC subtypes, consistent with several studies [16, 17, 19]. However, we observed an association between high cumulative estrogen combined with low cumulative progestin dose and incident TNBC, and increasing cumulative progestin dose and incident HER2-enriched breast cancer. These results are based on small numbers and should be interpreted cautiously. Our results predominantly did not suggest any associations with ET use.

The findings on overall breast cancer mortality and survival partly reflect those reported in existing literature. Our results align with reports that MHT is associated with an increased risk of death from breast cancer among the entire study population [23, 25, 26]. In contrast, and in agreement with previous publications, pre-diagnostic MHT use at study entry was not associated with an increased risk of breast cancer-specific death among patients with breast cancer. There were some indication of inverse associations, as previous studies have disclosed

[24, 29–33, 35], but the results were statistically non-significant. Contrary to these publications, the absence of statistically significant inverse associations with overall breast cancer survival in the present study may be attributed to different recruitment periods. Due to a shift toward increased use of low-dose EPT formulations and non-oral MHT regimens in the early 2000s [54, 55], one could expect studies with recruitment after the millennium shift to report risk estimates of different magnitude than those of older age. In our study with start of follow-up in median year 2004, we anticipate a mixture of user patterns seen prior to and following the millennium shift. A recent publication with contemporary MHT formulations have reported increased risk of comparable magnitude to those of older studies [21]. However, studies evaluating MHT use and breast cancer-specific mortality and survival are generally from earlier recruitment periods and the associations between newer MHT formulations and these outcomes are not well known.

Controlling for mammography screening in analyses of breast cancer survival and mortality has been advocated [25, 26], as MHT users undergo mammography more frequently than non-users [56, 57] and screen-detected cancers tend to be of more favorable grade, early stage, and hormone receptor-positive [56, 58, 59]. The increased survival observed in previous studies could be attributed to mammography screening, producing lead-time bias due to early detection and length bias owing to the identification of slow-growing tumors. However, increased survival has been reported in studies both controlling for mammography [31–33] and those that did not [24, 29]. Furthermore, it has been argued that increased survival associated with MHT use is not explained by mammographic surveillance but by biological mechanisms [33]. We chose not to adjust for mammographic screening in our analysis, as we do not consider it a confounder, but rather a possible intermediate variable in the causal pathway between MHT use and breast cancer subtypes. However, differences in health-seeking behaviors and screening attendance could be related to socioeconomic status [60], affecting MHT use [61] and survival rates. Therefore, we adjusted for educational level. Unfortunately, education level was the only available indicator to capture socioeconomic status and its impact on MHT use and breast cancer death. Thus, residual confounding cannot be excluded. Moreover, unmeasured confounding arising from non-exchangeability between MHT users and non-users, i.e. differences in MHT users and non-users that affect the outcome, cannot be definitively ruled out.

In accordance with mammographic screening, we did not adjust for clinical characteristics such as stage or treatment in our main analyses, as these factors are intermediates between MHT use and breast cancer survival. Evidence supporting a biological chronology in which the molecular subtype precedes tumor characteristics is found in studies where intrinsic-like subtypes have been assessed in pre-cancerous lesions [62, 63]. Upon adjusting for stage, surgical status and age at diagnosis in a supplementary analysis, effect estimates were substantially unaltered, underscoring that the observed associations were not explained by such clinical characteristics.

Another explanation for the opposing risk estimates on overall breast cancer mortality and survival could be the presence of collider stratification bias, also referred to as index event bias, which is introduced when conditioning on an intermediate variable between the exposure and outcome, coupled with unmeasured confounding factors affecting the mediator's impact on the outcome [64–67]. In our scenario, a cancer or subtype-specific cancer diagnosis is an intermediate variable between MHT use and breast cancer survival, and genetic susceptibility to breast cancer represents unmeasured confounding for the effect of a subtype diagnosis on death from breast cancer [68, 69]. We considered this by adjusting for family history of breast cancer, a surrogate variable for genetic susceptibility. However, we cannot completely rule out residual confounding and selection bias. Hence, these results must be interpreted without drawing causal conclusions.

Our findings indicated a reduced HR of death among patients with TNBC who were MHT users pre-diagnosis. The BCAC pooled analysis also demonstrated increased survival among patients with TNBC, with a HR of 0.64 (95% CI 0.48–0.85) of death from TNBC among current EPT users [29]. However, in contrast to our study, they revealed similar effect estimates for all subtypes and did not detect heterogeneity by intrinsic-like subtypes. One study demonstrated an increased risk of incident TNBC with current MHT use [21], aligning with our finding of an association between high cumulative estrogen combined with low cumulative progestin intake and incident TNBC. Potential biological mechanisms linking estrogen and progestin to TNBC as alternatives to the classical ER/PR pathway include receptor conversion, alternative estrogen-binding receptors, androgen receptor stimulation, and paracrine pathways [70]. Although several possible mechanisms exist whereby MHT use could exert associations in triple-negative tumor initiation and progression, the direction of these effects remain unclear.

Our study has some limitations. First, we were limited by small subsamples, particularly in the analyses of mortality and survival of the less common receptor-negative

subtypes. This was partly due to missing data on receptor status and the small number of breast cancer-specific deaths. We chose not to perform multiple imputations on receptor status because imputing outcome data is a subject of controversy [71]. The limited statistical power in these analyses precludes causal interpretations. Second, we used self-reported information on MHT use and covariates. Although a potential for misclassification exists, a validation study on MHT use in the NOWAC cohort demonstrated valid information on current MHT use at baseline and menopausal status among women aged 48–62 [46]. Third, multiple imputations were performed on missing covariate data under the assumption that these variables were missing at random. Similar effect estimates in sensitivity analyses on complete-case data support the robustness of our assumptions; however, we cannot rule out the possibility that some information was missing not at random; thus, our estimates may not be free from bias. Fourth, a multi-state survival model could be a viable approach in understanding the biology behind pre-diagnostic MHT use and breast cancer progression [72]. However, due to the multiple outcomes among breast cancer subtypes, employing this model was outside the scope of our study. Lastly, as we only had information on the first incident breast cancer subtype, some deaths could have resulted from converted or recurrent subtypes that differed from those identified at the initial diagnosis.

Conclusions

We have demonstrated that MHT use was associated with a small increased risk of incident and fatal overall and luminal breast cancers. However, the relationship between MHT use and breast cancer survival is complex. While pre-diagnostic MHT use was not associated with overall breast cancer survival, it was associated with increased survival among patients with TNBC. These findings underscore the intricate relationship between MHT and breast cancer outcomes across subtypes. Further research is needed to elucidate the mechanisms behind differential effects on breast cancer mortality and survival associated with MHT use.

Abbreviations

CI	Confidence interval
ER	Estrogen receptor
ET	Estrogen therapy
EPT	Estrogen-progestin therapy
HR	Hazard ratio
HER2	Human epidermal growth factor receptor 2
IHC	Immunohistochemistry
ISH	In situ Hybridization
MHT	Menopausal hormone therapy
MICE	Multiple imputation by chained equations
NETA	Norethisterone acetate
NOWAC	The Norwegian Women and Cancer Study
PR	Progesterone receptor
TNBC	Triple-negative breast cancer

Supplementary Information

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Additional file 1

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Author contributions

MB performed statistical analyses and drafted the manuscript. GU, EL and SC interpreted the results and revised the manuscript. CR supervised the study design, statistical analyses and manuscript preparation.

Author information

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The NOWAC study was approved by the Regional Committees for Medical and Health Research Ethics (REC) and the Norwegian Data Inspectorate. The participants received written information about the study, future linkages to national registers, and invitations to complete a second questionnaire. The return of a completed questionnaire was considered as consent to participate. A second questionnaire was sent to the participants who had agreed to receive one.

Consent for publication

Not applicable.

Competing interests

GU is journal editor at Breast Cancer Research. The remaining authors have no conflicts of interest to declare.

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Publisher's Note

Appendix I

Supplementary materials Paper I

Appendix Table 1. Distribution of general, combined, and progestin-only oral contraceptive use and missing values according to study population at baseline and follow-up – The NOWAC Study

	Study population at baseline (n = 74,862)	Study population at follow-up (n = 51,850)
General OC use, no. (%)		
Never use	26,251 (35.1)	18,073 (24.1)
Ever use	48,611 (64.9)	33,777 (45.1)
Current use	5,361 (7.2)	1,956 (2.6)
Former use	43,250 (57.8)	31,821 (42.5)
Missing	0 (0)	23,012 (30.7)
Duration (years) of OC use[1]		
1-4	26,656 (35.6)	18,598 (24.8)
5-9	12,246 (16.4)	8,490 (11.3)
≥ 10	8,529 (11.4)	5,851 (7.8)
Missing	1,180 (1.6)	23,850 (31.9)
Time (years) since last OC use[1]		
< 10	16,430 (22.0)	5,569 (7.4)
11-20	22,077 (29.5)	12,949 (17.3)
> 20	8,811 (11.8)	14,637 (19.6)
Missing	1,293 (1.7)	23,634 (31.6)
COC use, no. (%)		
Never use	38,896 (52.0)	26,738 (35.7)
Ever use	35,966 (48.0)	25,112 (33.5)
Current use	3,239 (4.3)	950 (1.3)
Former use	32,727 (43.7)	24,162 (32.3)
Missing	0 (0)	23,012 (30.7)
Duration (years) of COC use[1]		
< 5	19,765 (26.4)	13,992 (18.7)
≥ 5	16,201 (21.6)	11,120 (14.9)
Missing	0 (0)	23,012 (30.7)
POC use, no. (%)		
Never use	65,771 (87.9)	44,737 (59.8)
Ever use	9,091 (12.1)	7,113 (9.5)
Current use	968 (1.3)	379 (0.5)
Former use	8,123 (10.9)	6,734 (9.0)
Missing	0 (0)	23,012 (30.7)
Duration (years) of POC use[1]		
< 5	6,862 (9.2)	5,423 (7.2)
≥ 5	2,229 (3.0)	1,690 (2.3)
Missing	0 (0)	23,012 (30.7)

COC = Combined oral contraceptives; OC = Oral contraceptives; POC = Progestin-only contraceptives.

[1]Among ever-users.

Appendix Table 2. Multivariable adjusted HRs (95% CI) for association between COC use and POC use and risk of hormone receptor-defined premenopausal breast cancer: The NOWAC Study – complete case analyses

	ER+ cases		ER- cases		ER+/PR+ cases		ER-/PR- cases	
	No. (n = 643)	**HR (95% CI)[1]**	**No. (n = 184)**	**HR (95% CI)[1]**	**No. (n = 513)**	**HR (95% CI)[1]**	**No. (n = 145)**	**HR (95% CI)[1]**
COC use[2]								
Never OC use	203	1.00 (ref.)	45	1.00 (ref.)	157	1.00 (ref.)	34	1.00 (ref.)
Ever COC use	338	1.00 (0.89–1.14)	108	1.23 (0.98–1.56)	276	1.01 (0.88–1.16)	84	1.33 (1.03–1.72)
Current use	41	1.12 (0.72–1.73)	19	2.53 (1.32–4.86)	31	0.82 (0.47–1.46)	16	2.52 (1.19–5.34)
Former use	297	1.03 (0.86–1.24)	89	1.48 (1.02–2.13)	245	1.10 (0.90–1.35)	68	1.56 (1.03–2.37)
Duration (years) of use								
< 5	176	1.01 (0.82–1.24)	50	1.33 (0.88–2.01)	149	1.09 (0.86–1.37)	40	1.44 (0.90–2.30)
≥ 5	162	1.07 (0.87–1.32)	58	1.80 (1.20–2.70)	127	1.08 (0.85–1.38)	44	1.84 (1.16–2.92)
p_{trend}[3]		0.80		0.002		0.43		0.01
POC use[4]								
Never OC use	203	1.00 (ref.)	45	1.00 (ref.)	157	1.00 (ref.)	34	1.00 (ref.)
Ever POC use	93	0.99 (0.87–1.13)	29	1.17 (0.92–1.49)	76	0.98 (0.85–1.13)	23	1.26 (0.97–1.65)
Current use	16	1.31 (0.68–2.51)	6	1.68 (0.51–5.55)	14	1.49 (0.75–2.96)	5	2.44 (0.73–8.19)
Former use	77	1.12 (0.85–1.49)	23	1.48 (0.87–2.54)	62	1.14 (0.83–1.57)	18	1.66 (0.91–3.05)
Duration (years) of use								
< 5	61	0.98 (0.71–1.34)	23	1.48 (0.84–2.62)	50	1.01 (0.71–1.43)	18	1.67 (0.88–3.17)
≥ 5	32	1.60 (1.09–2.35)	6	1.55 (0.68–3.54)	26	1.64 (1.07–2.51)	5	1.91 (0.78–4.67)
p_{trend}[3]	203	0.07	45	0.19	157	0.07	34	0.05

CI = Confidence interval; COC = Combined oral contraceptives; ER = Estrogen receptor; HR = Hazard ratio; OC = Oral contraceptives; POC = Progestin-only contraceptives; PR = Progesterone receptor.

[1]Multivariable analysis adjusted for BMI (continuous), history of breast cancer in mother (yes, no), age at menarche (continuous), alcohol consumption (0, 0.1-4.9, 5-9.9, ≥ 10 g/day), and a combined variable including parity (0, 1, 2, ≥ 3 children) and age at first birth (age < 25, 25-29, ≥ 30).

[2]Analyses on COC use are adjusted for POC use (ever, never) in addition to the above-mentioned covariates.

[3]*p* value, continuous variable.

[4]Analyses on POC use are adjusted for COC use (ever, never) in addition to the above-mentioned covariates.

Appendix II

Supplementary materials Paper II

Supplementary file

Supplementary Table 1. Comparison of selected characteristics at wave 1 of excluded participants vs. study sample

	Study sample (n = 148,866)	Excluded due to less than 2 BMI measurements (n = 8,156)	Excluded due to missing physical activity on all time points (n = 6,206)	Excluded due to missing smoking status on all time points (n = 820)
Age, mean ± SD	49.1 (0.02)	53.7 (0.09)	53.7 (0.11)	51.2 (0.31)
BMI, mean ± SD				
Age 18	20.8 (0.01)	21.1 (0.11)	21.3 (0.05)	20.9 (0.11)
Wave 1	24.2 (0.01)	25.1 (0.06)	25.1 (0.05)	24.8 (0.14)
Wave 2	24.9 (0.01)	26.4 (0.19)	26.0 (0.10)	24.7 (0.50)
Wave 3	25.3 (0.02)	26.7 (0.73)	26.8 (0.42)	23.8 (0.58)
Parity, mean ± SD	2.2 (0.00)	2.2 (0.02)	2.4 (0.02)	2.3 (0.04)
Smoking status, %				
Current	34.7	37.4	38.8	N/A
Former	34.6	32.8	28.0	N/A
Never	30.7	29.8	33.2	N/A
Physical activity, %				
High	18.2	20.9	N/A	19.1
Moderate	57.4	54.9	N/A	55.5
Low	24.4	24.2	N/A	25.5
Education, %				
≤ 9	21.8	37.0	53.1	35.7
10-12	34.4	33.3	29.2	35.3
13-16	28.5	19.4	12.7	19.6
≥ 17	15.4	10.2	5.1	9.5

Participants who were excluded due to less than 2 BMI measurements were older, had somewhat higher BMI and had lower education than the study sample. Participants who were excluded due to missing physical activity or smoking status on all time points were older, had somewhat higher BMI and had lower education than the study sample.

Supplementary Table 2. Age-adjusted hazard ratios for the association between body fatness and ER/PR/HER2-defined subtypes of postmenopausal breast cancer

	Luminal A-like (n = 3,549)		Luminal B-like (n = 1,387)		HER2-enriched (n = 248)		TNBC (n = 466)		
	Cases	Age-adjusted HR (95% CI)[a]	Cases	Age-adjusted HR (95% CI)[a]	Cases	Age-adjusted HR (95% CI)[a]	Cases	Age-adjusted HR (95% CI)[a]	p_{het}[a]
BMI at wave 1[b, c]									0.13
Normal weight	2,140	Ref.	893	Ref.	163	Ref.	281	Ref.	
Underweight	74	1.10 (0.87-1.38)	26	0.92 (0.62-1.36)	2	0.37 (0.09-1.48)	12	1.32 (0.74-2.36)	
Overweight	1,001	1.13 (1.05-1.22)	366	0.99 (0.88-1.12)	64	1.00 (0.75-1.34)	130	1.14 (0.92-1.40)	
Obesity	311	1.16 (1.03-1.31)	96	0.86 (0.70-1.07)	18	0.93 (0.57-1.51)	42	1.22 (0.88-1.69)	
Age at onset (yrs)[d]									
BMI ≥ 25									0.03
Never OW	1,869	Ref.	772	Ref.	143	Ref.	255	Ref.	
< 40	634	1.10 (1.01-1.20)	224	0.94 (0.81-1.09)	40	0.92 (0.65-1.30)	85	1.08 (0.84-1.38)	
40-49	724	1.28 (1.17-1.39)	236	1.01 (0.87-1.17)	49	1.17 (0.84-1.62)	78	1.02 (0.79-1.31)	
≥ 50	322	1.04 (0.93-1.18)	155	1.23 (1.03-1.47)	16	0.76 (0.45-1.29)	48	1.17 (0.86-1.61)	
p_{trend}[e]		< 0.01		0.06		0.68		0.37	
BMI ≥ 30									
Never OB	3,164	Ref.	1,256	Ref.	228	Ref.	419	Ref.	0.42
< 40	63	0.87 (0.68-1.12)	19	0.65 (0.42-1.03)	5	0.95 (0.39-2.31)	7	0.71 (0.34-1.50)	
40-49	147	1.09 (0.92-1.28)	51	0.95 (0.72-1.26)	11	1.12 (0.61-2.05)	22	1.24 (0.80-1.90)	
≥ 50	175	1.25 (1.07-1.46)	61	1.11 (0.86-1.44)	4	0.44 (0.16-1.19)	18	0.99 (0.62-1.59)	
p_{trend}[e]		< 0.01		0.75		0.20		0.80	
Duration (per 10 yrs)[d]									
BMI ≥ 25	1,680	1.04 (1.01-1.07)	615	0.95 (0.90-1.00)	105	1.00 (0.89-1.14)	211	1.00 (0.92-1.09)	0.03
BMI ≥ 30	385	1.01 (0.93-1.09)	131	0.89 (0.77-1.03)	20	0.95 (0.68-1.33)	47	0.98 (0.78-1.23)	0.55
Intensity (per 100 units)[d]									
OWY	1,680	1.03 (0.96-1.11)	615	0.87 (0.76-0.99)	105	0.98 (0.73-1.31)	211	0.99 (0.81-1.22)	0.16
OBY	385	0.93 (0.75-1.16)	131	0.62 (0.39-1.00)	20	0.82 (0.32-2.15)	47	1.13 (0.66-1.91)	0.34
Trajectories[f]									
Normal-stable	1,479	Ref.	634	Ref.	112	Ref.	212	Ref.	
Normal-overweight	1,496	1.10 (1.02-1.18)	557	0.95 (0.85-1.07)	104	1.01 (0.78-1.32)	184	0.94 (0.77-1.15)	0.14
Normal-obesity	481	1.21 (1.09-1.34)	167	0.98 (0.82-1.16)	25	0.82 (0.53-1.27)	58	1.01 (0.75-1.35)	0.07
Overweight-obesity	78	1.04 (0.83-1.30)	21	0.65 (0.42-1.00)	6	1.02 (0.45-2.32)	9	0.82 (0.42-1.60)	0.26
Obesity-decrease	15	0.54 (0.32-0.89)	8	0.66 (0.33-1.32)	1	0.51 (0.07-3.67)	3	0.74 (0.24-2.33)	0.95
p_{trend}[e]		0.04		0.09		0.47		0.57	

Abbreviations: BMI: body mass index; CI: confidence interval; HR: hazard ratio; HER2: human epidermal growth factor receptor 2; TNBC: triple-negative breast cancer; OBY: weighted cumulative obesity years; OWY: weighted cumulative overweight years; p: p-value.

[a] *p* heterogeneity between ER/PR/HER2-defined subtypes; likelihood ratio test by competing risks analysis.

[b] Number of missing values: 23 luminal A-like (0.7%); 6 luminal B-like (0.4%); 1 HER2-enriched (0.4%); 1 TNBC (0.2%).

[c] Underweight: < 18.5 kg/m^2; normal weight: 18.5–24.9 kg/m^2; overweight: 25–29.9 kg/m^2; obesity: ≥ 30 kg/m^2.

[d] Based on linear mixed effects models. Never overweight/obesity as reference group.

[e] *p* trend, continuous variable.

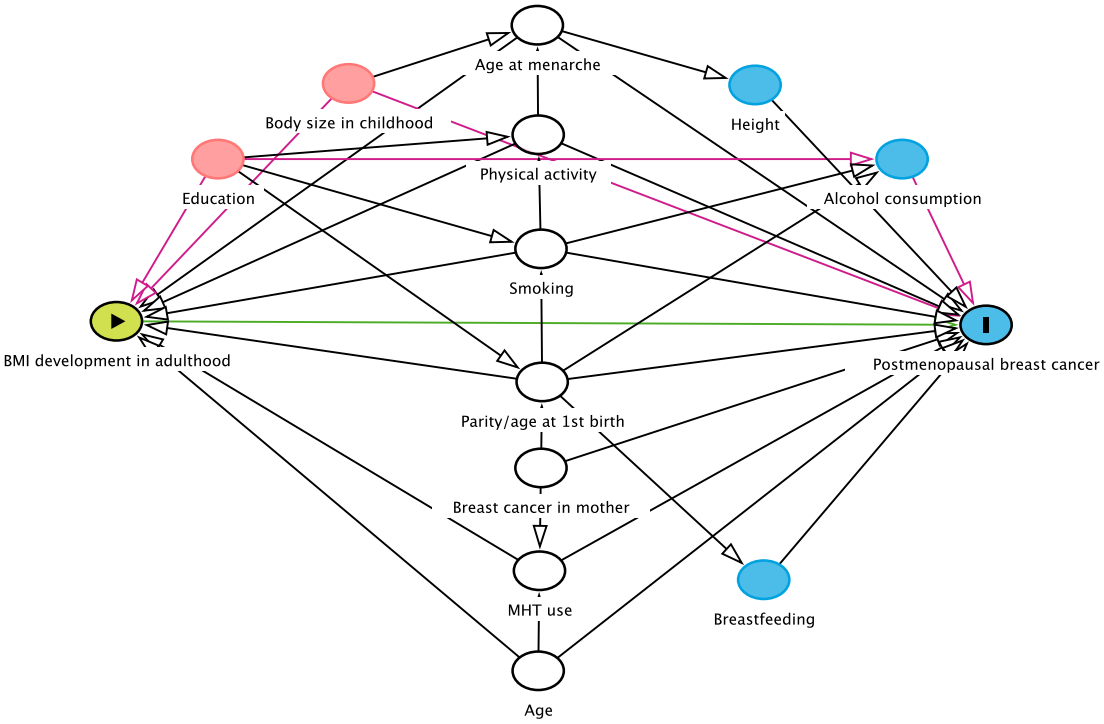
[f] Based on group-based trajectory modeling.

Supplementary Table 3. Age-adjusted and multivariable-adjusted hazard ratios for the association between body fatness and ER/PR-defined subtypes of postmenopausal breast cancer

	ER+/PR+ (n = 4,329)		ER-/PR- (n = 814)			ER+/PR+ (n = 4,150)		ER-/PR- (n = 782)		
	Cases	Age-adjusted HR (95% CI)	Cases	Age-adjusted HR (95% CI)	p_{het}[a]	Cases	MV-adjusted HR (95% CI)[b]	Cases	MV-adjusted HR (95% CI)[b]	p_{het}[a]
Age at onset (yrs)[c]										
BMI ≥ 25					0.18					0.18
Never OW	2,285	Ref.	456	Ref.		2,183	Ref.	439	Ref.	
< 40	779	1.11 (1.02-1.20)	139	0.99 (0.82-1.20)		745	1.10 (1.01-1.20)	136	0.98 (0.80-1.19)	
40-49	869	1.26 (1.17-1.37)	140	1.04 (0.86-1.26)		838	1.25 (1.16-1.36)	133	0.99 (0.82-1.21)	
≥ 50	396	1.09 (0.98-1.22)	79	1.18 (0.92-1.50)		384	1.08 (0.97-1.21)	74	1.11 (0.86-1.43)	
p_{trend}[d]		< 0.01		0.22			< 0.01		0.54	
BMI ≥ 30										
Never OB	3,855	Ref.	740	Ref.	0.23	3,687	Ref.	709	Ref.	0.25
< 40	78	0.88 (0.70-1.10)	12	0.69 (0.39-1.22)		78	0.91 (0.73-1.14)	12	0.70 (0.39-1.23)	
40-49	183	1.11 (0.95-1.28)	36	1.13 (0.81-1.59)		180	1.12 (0.96-1.30)	36	1.13 (0.81-1.58)	
≥ 50	213	1.30 (1.13-1.49)	26	0.88 (0.59-1.30)		205	1.28 (1.11-1.48)	25	0.85 (0.57-1.27)	
p_{trend}[d]		< 0.01		0.67			< 0.01		0.58	
Duration (per 10 yrs)[c]										
BMI ≥ 25	2,044	1.05 (1.02-1.08)	358	0.99 (0.93-1.07)	0.15	1,967	1.05 (1.02-1.08)	343	0.98 (0.91-1.05)	0.14
BMI ≥ 30	474	1.02 (0.95-1.10)	74	0.95 (0.79-1.14)	0.45	463	1.03 (0.95-1.11)	73	0.95 (0.79-1.14)	0.53
Intensity (per 100 units)[c]										
OWY	2,044	1.05 (0.98-1.12)	358	0.96 (0.81-1.13)	0.33	1,967	1.05 (0.98-1.12)	343	0.95 (0.80-1.12)	0.38
OBY	474	0.94 (0.77-1.15)	74	0.95 (0.60-1.53)	0.97	463	0.95 (0.78-1.17)	73	0.95 (0.59-1.52)	0.92
Trajectories[e, f]										
Normal-stable	1,805	Ref.	376	Ref.		1,730	Ref.	361	Ref.	
Normal-overweight	1,820	1.10 (1.03-1.17)	327	0.95 (0.82-1.10)	0.08	1,743	1.09 (1.01-1.16)	311	0.92 (0.79-1.07)	0.06
Normal-obesity	591	1.21 (1.10-1.33)	91	0.89 (0.71-1.11)	0.01	565	1.19 (1.08-1.31)	90	0.88 (0.70-1.11)	0.03
Overweight-obesity	94	1.01 (0.82-1.25)	16	0.81 (0.49-1.33)	0.41	93	1.03 (0.83-1.27)	16	0.80 (0.48-1.32)	0.42
Obesity-decrease	19	0.57 (0.36-0.89)	4	0.60 (0.22-1.60)	0.94	19	0.57 (0.36-0.90)	4	0.62 (0.23-1.66)	0.93
p_{trend}[d]		0.02		0.12			0.06		0.10	

Abbreviations: BMI: body mass index; CI: confidence interval; ER: estrogen receptor; HR: hazard ratio; MV: multivariable; OBY: weighted cumulative obesity years; OWY: weighted cumulative overweight years; p: p-value. [a] *p* heterogeneity between ER+/PR+ and ER-/PR- subtype; likelihood ratio test by competing risks analysis. [b] Adjusted for age, age at menarche, parity, age at first birth, breast cancer in mother, smoking, MHT use. [c] Based on linear mixed effects models. Never overweight/obesity as reference group. [d] *p* trend, continuous variable. [e] Based on group-based trajectory modeling. [f] Normal weight: 18.5–24.9 kg/m^2; overweight: 25–29.9 kg/m^2; obesity: ≥ 30 kg/m^2.

Supplementary Figure 1. Directed acyclic graph on the assumed relations between BMI development in adulthood, postmenopausal breast cancer and covariates



Created from https://dagitty.net. Based on the acyclic graph, we adjusted for a minimal sufficient adjustment set of variables to control for confounding, except for body size in childhood and alcohol consumption due to missing values. Adjustments were made for confounding factors depicted in white. The following assumptions were made when considering covariates for the multivariable model: 1) Age is related to body fatness as older women tend to be leaner than younger women, and various birth cohorts have different BMI development. Age is also a risk factor for breast cancer. We further assumed that age of women affected OC and MHT status; 2) Age at menarche could be related to weight in adulthood as well as breast cancer incidence. One study demonstrated that age at menarche is inversely associated with subsequent obesity[1]; 3) Parity/age at first birth could be related to body fatness in addition to breast cancer incidence, as weight gained during pregnancy is

[1] Yang L, Li L, Millwood IY, Lewington S, Guo Y, Sherliker P, et al. Adiposity in relation to age at menarche and other reproductive factors among 300 000 Chinese women: findings from China Kadoorie Biobank study. Int J Epidemiol. 2017;46(2):502-12

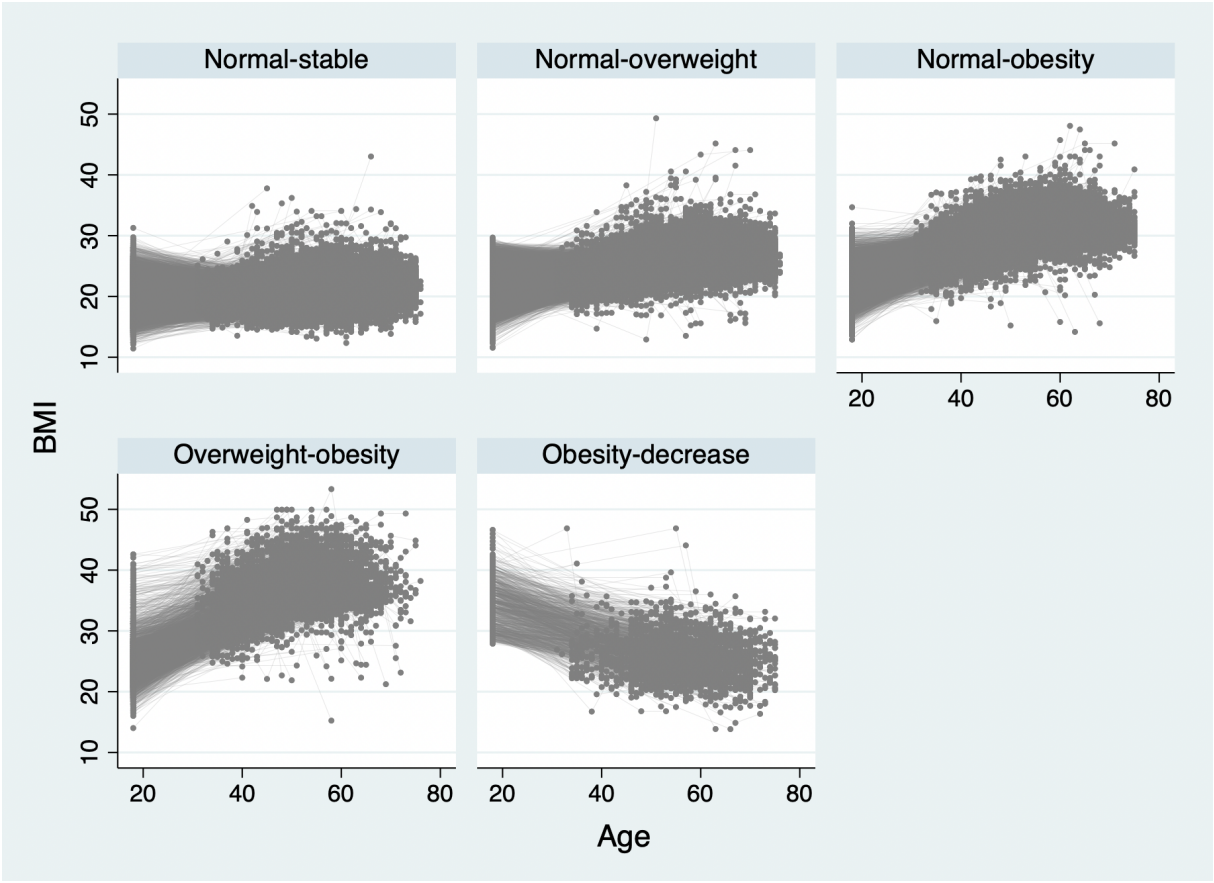
not completely lost following delivery, leading to progressive weight gain over multiple pregnancies and, for some, development of obesity[2]. Parity is also related to breastfeeding duration, alcohol consumption and smoking; 4) MHT use is a risk factor for breast cancer and could potentially cause weight change although no current evidence exists; 5) Breast cancer in mother is a proxy for genetic susceptibility for breast cancer (BRCA1/2 mutations) and could affect subsequent choices such as exogenous hormone use, age at first birth and parity; 6) Physical activity has a protective effect on breast cancer risk, and reductions in habitual levels of physical activity and increased sedentary behaviors is associated with increase risk of obesity[3]. Vigorous physical activity could postpone pubertal onset; 7) Smoking cessation is associated with weight gain[4] as well as being a risk factor for breast cancer. Smoking behavior is related to physical activity and alcohol consumption.

[2] Mannan M, Doi SA, Mamun AA. Association between weight gain during pregnancy and postpartum weight retention and obesity: a bias-adjusted meta-analysis. Nutr Rev. 2013;71(6):343-52.

[3] Piercy KL, Troiano RP, Ballard RM, Carlson SA, Fulton JE, Galuska DA, et al. The Physical Activity Guidelines for Americans. JAMA. 2018;320(19):2020-8.

[4] Bush T, Lovejoy JC, Deprey M, Carpenter KM. The effect of tobacco cessation on weight gain, obesity, and diabetes risk. Obesity (Silver Spring). 2016;24(9):1834-41.

Supplementary Figure 2 – Twoway scatterplots of individual BMI trajectories by trajectory group



Appendix III

Supplementary materials Paper III

Supplementary tables

Supplementary Table 1. Clinical descriptives of cases

	Overall breast cancer cases	**Luminal A-like cases**	**Luminal B-like cases**	**HER2+ cases**	**TNBC cases**	**Unknown subtype**
Number of women, n	7,844	3,784	1,480	264	500	1,816
Number of deaths, n	1,508	440	249	50	126	643
Number of breast cancer-specific deaths, n	721	163	113	33	81	331
Age at diagnosis, mean ± SD	63.2 ± 0.08	64.8 ± 0.11	64.6 ± 0.18	63.1 ± 0.45	64.0 ± 0.32	58.8 ± 0.17
Age at death from breast cancer, mean ± SD	66.7 ± 0.29	69.1 ± 0.60	68.3 ± 0.73	66.5 ± 1.56	66.8 ± 0.87	65.0 ± 0.41
Tumor stage, n (%)						
I	4,218 (53.8)	2,273 (60.1)	781 (52.8)	93 (35.2)	219 (43.8)	852 (46.9)
II	2,277 (29.0)	1,037 (27.4)	446 (30.1)	89 (33.7)	157 (31.4)	548 (30.2)
III	495 (6.3)	208 (5.5)	109 (7.4)	45 (17.1)	48 (9.6)	85 (4.7)
IV	206 (2.6)	51 (1.4)	33 (2.2)	10 (3.8)	20 (4.0)	92 (5.1)
Unknown	648 (8.3)	215 (5.7)	111 (7.5)	27 (10.2)	56 (11.2)	239 (13.2)
Tumor grade, n (%)						
I	1,504 (19.2)	937 (24.8)	204 (13.8)	3 (1.1)	6 (1.2)	354 (19.5)
II	2,730 (34.8)	1,443 (38.1)	559 (37.8)	72 (27.3)	86 (17.2)	570 (31.4)
III	1,560 (19.9)	436 (11.5)	339 (22.9)	155 (58.7)	303 (60.6)	327 (18.0)
Unknown	2,050 (26.1)	968 (25.6)	378 (25.5)	34 (12.9)	105 (21.0)	565 (31.1)
Recent mammography screening (< 2 yrs) prior to diagnosis	5,213 (66.5)	2,716 (71.8)	1,035 (69.9)	181 (68.6)	318 (63.6)	963 (53.0)
Surgical status						
Lumpectomy	5,094 (64.9)	2,775 (73.3)	964 (65.1)	117 (44.3)	315 (63.0)	923 (50.8)
Mastectomy	2,697 (34.4)	1,005 (26.6)	515 (34.8)	146 (55.3)	183 (36.6)	848 (46.7)
Other	53 (0.68)	4 (0.1)	1 (0.1)	1 (0.4)	2 (0.4	45 (2.5)

Abbreviations: HER2: human epidermal growth factor receptor 2; TNBC: triple-negative breast cancer

Supplementary Table 2. Descriptives of cases according to MHT use at study entry

	MHT use at study entry			
	Never MHT	**Ever EPT use**	**Ever ET use only[1]**	**Ever unknown type**
	Mean ± SD or n (%)			
Number of women, n (%)	4,297 (54.8)	2,599 (33.1)	262 (3.3)	686 (8.8)
Age at study entry (yrs)	53.7 ± 0.08	53.1 ± 0.10	52.9 ± 0.35	51.6 ± 0.25
Age at menarche (yrs)	13.3 ± 0.02	13.2 ± 0.03	13.2 ± 0.09	13.3 ± 0.06
Age at menopause (yrs)	49.7 ± 0.09	49.8 ± 0.10	46.7 ± 0.38	48.5 ± 0.25
Age at first birth (yrs)[2]	24.5 ± 0.08	24.1 ± 0.09	24.3 ± 0.31	23.7 ± 0.18
Parity	2.2 ± 0.02	2.1 ± 0.02	1.9 ± 0.07	2.1 ± 0.05
BMI (kg/m^2)	24.9 ± 0.06	24.3 ± 0.07	24.4 ± 0.21	24.3 ± 0.14
Alcohol consumption (g/day)	1.12 ± 0.13	1.31 ± 0.02	1.23 ± 0.05	1.16 ± 0.03
Education				
≤ 9 yrs	894 (22.2)	463 (18.9)	44 (17.5)	186 (29.5)
10-12 yrs	1,373 (34.0)	910 (37.2)	84 (33.5)	211 (33.4)
13-16 yrs	1.165 (28.9)	697 (28.5)	89 (35.5)	156 (24.7)
≥ 17 yrs	605 (15.0)	379 (15.5)	34 (13.6)	78 (12.4)
Family history of breast cancer				
None	3,819 (88.9)	2,338 (90.0)	237 (90.5)	619 (90.2)
Mother and sister	23 (0.5)	16 (0.6)	0 (0.0)	3 (0.4)
Mother	319 (7.4)	173 (6.7)	15 (5.7)	48 (7.0)
Sister	136 (3.2)	72 (2.8)	10 (3.8)	16 (2.3)
Smoking status, n (%)				
Never	1,586 (37.2)	760 (29.4)	80 (30.8)	210 (31.3)
Former	1,447 (34.0)	976 (37.7)	106 (40.8)	228 (33.9)
Current	1,226 (28.8)	853 (33.0)	74 (28.5)	234 (34.8)
Physical activity, n (%)				
Low	1,052 (26.3)	648 (26.4)	64 (26.3)	171 (28.4)
Moderate	2,333 (58.4)	1,452 (59.2)	127 (52.3)	344 (57.1)
High	610 (15.3)	352 (14.4)	52 (21.4)	88 (14.6)
Oral contraceptive use, n (%)				
Never	1,860 (44.7)	1,025 (40.1)	112 (43.8)	285 (42.9)
Ever	2,299 (55.3)	1,533 (59.9)	144 (56.3)	380 (57.1)

[1] Never EPT users

[2] Among parous women

Abbreviations: EPT: estrogen-progestin therapy; ET: estrogen therapy; MHT: menopausal hormone therapy

Supplementary Table 3. MHT use at study entry and incidence by intrinsic-like subtypes – age-adjusted analyses

	Breast cancer overall (n = 7,844)		Luminal A-like (n = 3,784)		Luminal B-like (n = 1,480)		HER2+ (n = 264)		TNBC (n = 500)	
	n	HR (95% CI)	n	HR (95% CI)	n	HR (95% CI)	n	HR (95% CI)	n	HR (95% CI)
MHT use overall										
Never use	4,297	Ref.	2,113	Ref.	845	Ref.	155	Ref.	297	Ref.
Ever use	3,547	1.25 (1.19-1.31)	1,671	1.19 (1.12-1.27)	635	1.16 (1.04-1.28)	109	1.08 (0.85-1.39)	203	1.04 (0.87-1.25)
Current	2,782	1.36 (1.29-1.42)	1,310	1.32 (1.24-1.42)	464	1.19 (1.07-1.34)	77	1.06 (0.80-1.39)	139	1.00 (0.81-1.22)
Former	765	0.97 (0.90-1.05)	361	0.87 (0.78-0.98)	171	1.06 (0.90-1.25)	32	1.15 (0.78-1.68)	64	1.15 (0.88-1.51)
Duration										
< 5 yrs	2,250	1.16 (1.11-1.23)	984	1.06 (0.99-1.15)	416	1.14 (1.02-1.29)	78	1.14 (0.86-1.50)	128	0.98 (0.79-1.20)
≥ 5 yrs	1,243	1.43 (1.34-1.53)	656	1.44 (1.32-1.58)	212	1.20 (1.03-1.39)	30	0.97 (0.66-1.44)	71	1.16 (0.89-1.50)
Per 1 yr	7,790	1.04 (1.03-1.04)	3,753	1.04 (1.03-1.05)	1,473	1.02 (1.00-1.03)	263	1.00 (0.96-1.04)	496	1.01 (0.98-1.04)
EPT use										
Never use	4,297	Ref.	2,113	Ref.	845	Ref.	155	Ref.	297	Ref.
Ever use	2,599	1.33 (1.27-1.40)	1,248	1.29 (1.20-1.39)	464	1.23 (1.10-1.38)	82	1.19 (0.91-1.55)	147	1.10 (0.90-1.34)
Current	2,120	1.44 (1.37-1.52)	1,012	1.43 (1.32-1.54)	352	1.26 (1.11-1.43)	58	1.11 (0.82-1.51)	107	1.07 (0.86-1.34)
Former	479	0.99 (0.90-1.09)	236	0.92 (0.81-1.06)	112	1.13 (0.93-1.38)	24	1.41 (0.92-2.18)	40	1.17 (0.84-1.63)
Duration										
< 5 yrs	1,559	1.22 (1.15-1.30)	688	1.13 (1.04-1.23)	288	1.20 (1.05-1.37)	56	1.24 (0.91-1.69)	91	1.06 (0.83-1.34)
≥ 5 yrs	1,028	1.53 (1.43-1.64)	553	1.57 (1.43-1.72)	175	1.28 (1.08-1.50)	25	1.05 (0.69-1.61)	55	1.16 (0.87-1.55)
Per 1 yr	6,884	1.04 (1.04-1.05)	3,354	1.05 (1.04-1.05)	1,308	1.03 (1.01-1.05)	236	1.02 (0.97-1.06)	443	1.01 (0.98-1.04)
ET use only										
Never use	4,297	Ref.	2,113	Ref.	845	Ref.	155	Ref.	297	Ref.
Ever use	262	0.98 (0.87-1.11)	122	0.92 (0.77-1.11)	52	1.00 (0.76-1.33)	12	1.27 (0.71-2.29)	15	0.82 (0.49-1.37)
Current	224	1.05 (0.92-1.21)	102	0.99 (0.81-1.20)	47	1.16 (0.86-1.55)	9	1.19 (0.61-2.34)	12	0.83 (0.46-1.47)
Former	38	0.70 (0.51-0.97)	20	0.69 (0.45-1.08)	5	0.45 (0.19-1.07)	3	1.57 (0.50-4.94)	3	0.78 (0.25-2.44)
Duration										
< 5 yrs	164	0.98 (0.84-1.15)	78	0.98 (0.78-1.23)	29	0.92 (0.64-1.34)	8	1.35 (0.66-2.75)	8	0.71 (0.35-1.43)
≥ 5 yrs	96	0.98 (0.80-1.20)	43	0.83 (0.62-1.13)	22	1.09 (0.72-1.67)	4	1.16 (0.43-3.14)	7	1.01 (0.48-2.14)
Per 1 yr	4,557	1.00 (0.98-1.02)	2,234	0.98 (0.96-1.01)	896	1.01 (0.97-1.05)	167	0.99 (0.89-1.11)	312	0.98 (0.91-1.06)
Cumulative dose										
Never use	4,297	Ref.	2,113	Ref.	845	Ref.	155	Ref.	297	Ref.
Estrogen (E2-equivalence)										
< 5 g	1,999	1.23 (1.17-1.30)	948	1.20 (1.11-1.29)	347	1.12 (0.98-1.26)	69	1.20 (0.91-1.60)	112	1.01 (0.81-1.26)
5 - 10 g	827	1.39 (1.29-1.49)	399	1.33 (1.19-1.48)	154	1.32 (1.11-1.57)	22	1.05 (0.67-1.65)	48	1.16 (0.85-1.58)
> 10 g	192	1.54 (1.33-1.78)	103	1.62 (1.33-1.97)	34	1.37 (0.97-1.93)	5	1.13 (0.46-2.77)	6	0.68 (0.30-1.53)
Progestin (NETA-equivalence)										
< 1 g	1,411	1.22 (1.15-1.29)	634	1.14 (1.04-1.24)	257	1.17 (1.02-1.35)	46	1.12 (0.80-1.56)	92	1.18 (0.93-1.49)
1 - 2 g	695	1.37 (1.26-1.48)	361	1.42 (1.27-1.59)	112	1.13 (0.93-1.38)	18	1.00 (0.61-1.64)	33	0.94 (0.66-1.35)
> 2 g	608	1.66 (1.52-1.80)	304	1.60 (1.42-1.81)	107	1.44 (1.18-1.77)	18	1.39 (0.85-2.27)	24	0.92 (0.61-1.40)
E2 dose < 5 g										
NETA dose < 1 g	1,306	1.22 (1.15-1.30)	589	1.14 (1.04-1.25)	237	1.17 (1.01-1.35)	43	1.13 (0.81-1.59)	79	1.10 (0.85-1.41)
NETA dose ≥ 1 g	439	1.47 (1.33-1.62)	233	1.54 (1.34-1.76)	66	1.11 (0.87-1.43)	12	1.14 (0.63-2.05)	18	0.87 (0.54-1.39)
E2 dose ≥ 5 g										
NETA dose < 1 g	93	1.25 (1.01-1.53)	40	1.10 (0.80-1.50)	18	1.27 (0.80-2.03)	3	1.14 (0.36-3.57)	11	2.17 (1.19-3.97)
NETA dose ≥ 1 g	862	1.50 (1.39-1.61)	431	1.48 (1.33-1.64)	153	1.35 (1.13-1.60)	23	1.14 (0.74-1.78)	39	0.97 (0.70-1.36)

Abbreviations: CI: confidence interval; EPT: estrogen-progestin therapy; ET: estrogen therapy; E2: estradiol; HER2: human epidermal growth factor receptor 2; HR: hazard ratio; MHT: menopausal hormone therapy; NETA: norethisterone acetate; TNBC: triple-negative breast cancer.

Supplementary Table 4. MHT use at study entry and incidence by intrinsic-like subtypes – complete-case, MV-adjusted analyses

	Breast cancer overall (n = 6,680)		Luminal A-like (n = 3,259)		Luminal B-like (n = 1,275)		HER2-enriched (n = 227)		TNBC (n = 422)	
	n	HR (95% CI)[1]	n	HR (95% CI)[1]	n	HR (95% CI)[1]	n	HR (95% CI)[1]	n	HR (95% CI)[1]
MHT use overall										
Never use	3,660	Ref.	1,827	Ref.	733	Ref.	135	Ref.	253	Ref.
Ever use	3,020	1.22 (1.16-1.28)	1,432	1.14 (1.07-1.23)	542	1.09 (0.98-1.23)	92	1.02 (0.78-1.34)	169	0.99 (0.82-1.21)
Current	2,397	1.34 (1.28-1.42)	1,128	1.29 (1.19-1.39)	414	1.18 (1.04-1.33)	66	1.01 (0.75-1.36)	116	0.96 (0.77-1.20)
Former	623	0.90 (0.83-0.98)	304	0.81 (0.72-0.92)	128	0.90 (0.74-1.08)	26	1.05 (0.69-1.60)	53	1.08 (0.80-1.46)
Duration										
< 5 yrs	1,930	1.15 (1.09-1.22)	850	1.04 (0.96-1.13)	356	1.09 (0.96-1.24)	67	1.09 (0.81-1.47)	106	0.93 (0.74-1.17)
≥ 5 yrs	1,056	1.37 (1.28-1.47)	563	1.34 (1.22-1.47)	183	1.12 (0.95-1.32)	24	0.86 (0.55-1.33)	59	1.08 (0.81-1.44)
Per 1 yr	6,646	1.03 (1.02-1.04)	3,240	1.03 (1.02-1.04)	1,272	1.01 (1.00-1.03)	226	0.99 (0.95-1.04)	418	1.00 (0.97-1.03)
ETP use										
Never use	3,660	Ref.	1,827	Ref.	733	Ref.	135	Ref.	253	Ref.
Ever use	2,255	1.29 (1.23-1.36)	1,090	1.23 (1.14-1.32)	406	1.16 (1.02-1.31)	69	1.07 (0.80-1.44)	125	1.04 (0.84-1.30)
Current	1,850	1.42 (1.35-1.51)	881	1.37 (1.26-1.49)	317	1.23 (1.07-1.40)	50	1.04 (0.75-1.44)	91	1.03 (0.81-1.31)
Former	405	0.91 (0.82-1.01)	209	0.85 (0.73-0.98)	89	0.96 (0.76-1.20)	19	1.17 (0.72-1.91)	34	1.08 (0.75-1.55)
Duration										
< 5 yrs	1,364	1.21 (1.13-1.29)	603	1.09 (1.00-1.20)	255	1.16 (1.00-1.34)	48	1.14 (0.82-1.60)	77	1.01 (0.78-1.31)
≥ 5 yrs	881	1.44 (1.34-1.56)	482	1.44 (1.30-1.59)	150	1.16 (0.97-1.38)	20	0.89 (0.55-1.43)	47	1.08 (0.79-1.48)
Per 1 yr	5,905	1.04 (1.03-1.04)	2,912	1.04 (1.03-1.05)	1,138	1.02 (1.00-1.04)	203	1.01 (0.96-1.05)	377	1.00 (0.97-1.04)
ET use only										
Never use	3,660	Ref.	1,827	Ref.	733	Ref.	135	Ref.	253	Ref.
Ever use	226	0.95 (0.83-1.09)	108	0.89 (0.73-1.08)	45	0.94 (0.69-1.27)	11	1.28 (0.69-2.38)	10	0.61 (0.33-1.15)
Current	197	1.04 (0.90-1.20)	91	0.96 (0.78-1.19)	42	1.10 (0.81-1.52)	9	1.32 (0.67-2.59)	8	0.62 (0.31-1.26)
Former	29	0.60 (0.42-0.86)	17	0.63 (0.39-1.01)	3	0.29 (0.09-0.91)	2	1.15 (0.28-4.67)	2	0.58 (0.14-2.33)
Duration										
< 5 yrs	140	0.94 (0.79-1.11)	69	0.94 (0.74-1.20)	25	0.86 (0.57-1.28)	7	1.29 (0.60-2.76)	6	0.60 (0.27-1.34)
≥ 5 yrs	84	0.97 (0.78-1.21)	38	0.80 (0.58-1.10)	19	1.04 (0.66-1.64)	4	1.29 (0.48-3.50)	4	0.65 (0.24-1.75)
Per 1 yr	3,884	0.99 (0.97-1.02)	1,934	0.98 (0.95-1.01)	777	1.00 (0.95-1.04)	146	1.00 (0.90-1.12)	263	0.93 (0.82-1.04)
Cumulative dose										
Never use	3,660	Ref.	1,827	Ref.	733	Ref.	135	Ref.	253	Ref.
Estrogen (E2-equivalence)										
< 5 g	1,745	1.20 (1.14-1.28)	838	1.23 (1.13-1.33)	307	1.12 (0.98-1.29)	57	1.20 (0.87-1.65)	93	0.96 (0.75-1.23)
5 - 10 g	721	1.34 (1.24-1.46)	352	1.43 (1.27-1.62)	132	1.33 (1.10-1.62)	19	1.20 (0.73-1.98)	41	1.16 (0.82-1.64)
> 10 g	152	1.37 (1.17-1.62)	80	1.59 (1.27-2.00)	29	1.41 (0.97-2.05)	5	1.60 (0.65-3.95)	5	0.70 (0.29-1.70)
Progestin (NETA-equivalence)										
< 1 g	1,232	1.19 (1.11-1.27)	567	1.17 (1.06-1.28)	226	1.16 (0.99-1.34)	38	1.08 (0.75-1.57)	77	1.13 (0.87-1.46)
1 - 2 g	605	1.34 (1.23-1.46)	317	1.54 (1.36-1.74)	99	1.18 (0.95-1.46)	14	1.03 (0.58-1.81)	29	0.98 (0.66-1.46)
> 2 g	519	1.62 (1.48-1.79)	256	1.79 (1.56-2.05)	93	1.57 (1.25-1.96)	16	1.82 (1.05-3.13)	21	1.01 (0.64-1.60)
E2 dose < 5 g										
NETA dose < 1 g	1,143	1.19 (1.12-1.28)	526	1.17 (1.06-1.29)	209	1.15 (0.99-1.35)	35	1.08 (0.74-1.58)	68	1.07 (0.82-1.41)
NETA dose ≥ 1 g	382	1.47 (1.32-1.64)	202	1.71 (1.47-1.98)	59	1.22 (0.93-1.59)	9	1.16 (0.59-2.31)	15	0.88 (0.52-1.49)
E2 dose ≥ 5 g										
NETA dose < 1 g	79	1.14 (0.91-1.42)	36	1.14 (0.82-1.59)	15	1.17 (0.70-1.96)	3	1.42 (0.45-4.50)	8	1.80 (0.88-3.65)
NETA dose ≥ 1 g	740	1.45 (1.33-1.57)	370	1.60 (1.42-1.80)	133	1.40 (1.15-1.69)	20	1.35 (0.83-2.21)	35	1.06 (0.73-1.52)

[1] Adjusted for age (underlying time scale), BMI, parity, age at first birth, age at menarche, family history, smoking, physical activity, education. Abbreviations: CI: confidence interval; ETP: estrogen-progestin therapy; ET: estrogen therapy; E2: estradiol; HER2: human epidermal growth factor receptor 2; HR: hazard ratio; MHT: menopausal hormone therapy; MV: multivariable; NETA: norethisterone acetate; TNBC: triple-negative breast cancer

Supplementary Table 5. MHT use at study entry and breast cancer-specific mortality by intrinsic-like subtypes – complete-case dataset

	Breast cancer deaths overall		Luminal A-like		Luminal B-like		HER2-enriched		TNBC	
	n	HR (95% CI)	n	HR (95% CI)	n	HR (95% CI)	n	HR (95% CI)	n	HR (95% CI)
Age-adjusted	721		163		113		33		81	
MHT use overall										
Never use	392	Ref.	82	Ref.	64	Ref.	20	Ref.	54	Ref.
Ever use	329	1.26 (1.09-1.47)	81	1.48 (1.09-2.02)	49	1.13 (0.78-1.64)	13	0.95 (0.47-1.93)	27	0.74 (0.47-1.18)
Current	268	1.46 (1.25-1.71)	65	1.73 (1.24-2.40)	39	1.29 (0.87-1.93)	13	1.35 (0.66-2.74)	16	0.63 (0.36-1.10)
Former	61	0.79 (0.61-1.04)	16	0.94 (0.55-1.61)	10	0.76 (0.39-1.47)	0	-	11	1.02 (0.53-1.96)
Duration										
< 5 yrs	220	1.29 (1.09-1.52)	43	1.24 (0.85-1.79)	31	1.10 (0.72-1.70)	10	1.11 (0.51-2.39)	16	0.68 (0.39-1.18)
≥ 5 yrs	104	1.22 (0.98-1.51)	35	1.87 (1.26-2.79)	17	1.15 (0.67-1.98)	3	0.69 (0.20-2.33)	11	0.91 (0.48-1.76)
Per 1 yr	716	1.02 (1.00-1.04)	160	1.06 (1.02-1.10)	112	1.02 (0.97-1.07)	33	0.95 (0.83-1.08)	81	0.99 (0.92-1.06)
EPT use										
Never use	392	Ref.	82	Ref.	64	Ref.	20	Ref.	54	Ref.
Ever use	237	1.33 (1.13-1.57)	62	1.68 (1.21-2.35)	37	1.25 (0.83-1.89)	11	1.20 (0.57-2.53)	20	0.81 (0.48-1.35)
Current	208	1.58 (1.34-1-88)	54	2.01 (1.43-2.85)	31	1.44 (0.93-2.22)	11	1.62 (0.77-3.40)	14	0.76 (0.42-1.38)
Former	29	0.62 (0.43-0.91)	8	0.79 (0.38-1.64)	6	0.75 (0.32-1.74)	0	-	6	0.92 (0.39-2.14)
Duration										
< 5 yrs	152	1.37 (1.13-1.65)	30	1.35 (0.89-2.06)	23	1.27 (0.78-2.05)	9	1.57 (0.71-3.48)	11	0.71 (0.37-1.37)
≥ 5 yrs	84	1.27 (1.00-1.61)	31	2.16 (1.42-3.27)	14	1.23 (0.69-2.20)	2	0.60 (0.14-2.57)	9	0.97 (0.48-1.97)
Per 1 yr	628	1.02 (1.00-1.05)	143	1.08 (1.04-1.12)	101	1.03 (0.98-1.09)	31	0.94 (0.81-1.10)	74	0.99 (0.92-1.07)
MV-adjusted[1]	585		128		92		28		67	
MHT use overall										
Never use	309	Ref.	65	Ref.	50	Ref.	19	Ref.	44	Ref.
Ever use	276	1.30 (1.10-1.53)	63	1.44 (1.01-2.05)	42	1.16 (0.76-1.75)	10	0.83 (0.37-1.82)	23	0.74 (0.45-1.24)
Current	226	1.51 (1.27-1.80)	49	1.66 (1.14-2.42)	35	1.41 (0.91-2.19)	10	1.18 (0.53-2.61)	13	0.59 (0.32-1.11)
Former	50	0.80 (0.60-1.09)	14	0.99 (0.55-1.77)	7	0.61 (0.28-1.35)	0	-	10	1.10 (0.55-2.21)
Duration										
< 5 yrs	195	1.40 (1.16-1.67)	36	1.30 (0.86-1.97)	29	1.27 (0.80-2.02)	9	1.10 (0.49-2.51)	14	0.69 (0.38-1.28)
≥ 5 yrs	79	1.13 (0.88-1.45)	25	1.62 (1.01-2.58)	13	0.99 (0.53-1.84)	1	0.27 (0.04-2.01)	9	0.87 (0.42-1.80)
Per 1 yr	583	1.01 (0.99-1.04)	126	1.05 (1.01-1.09)	92	1.00 (0.95-1.06)	29	0.89 (0.73-1.07)	67	0.98 (0.91-1.06)
EPT use										
Never use	309	Ref.	65	Ref.	50	Ref.	19	Ref.	44	Ref.
Ever use	200	1.35 (1.13-1.61)	47	1.56 (1.06-2.28)	33	1.29 (0.82-2.01)	9	1.06 (0.47-2.40)	18	0.83 (0.48-1.45)
Current	174	1.60 (1.32-1.93)	39	1.83 (1.22-2.74)	28	1.54 (0.96-2.46)	9	1.45 (0.64-3.30)	12	0.75 (0.39-1.44)
Former	26	0.66 (0.44-0.99)	8	0.91 (0.43-1.90)	5	0.67 (0.27-1.69)	0	-	6	1.03 (0.44-2.45)
Duration										
< 5 yrs	137	1.48 (1.21-1.81)	25	1.40 (0.88-2.23)	23	1.51 (0.92-2.49)	8	1.46 (0.62-3.44)	10	0.75 (0.37-1.49)
≥ 5 yrs	63	1.14 (0.86-1.50)	22	1.81 (1.11-2.96)	10	0.96 (0.48-1.90)	1	0.33 (0.04-2.53)	8	0.98 (0.45-2.09)
Per 1 yr	509	1.01 (0.99-1.04)	112	1.07 (1.02-1.11)	83	1.01 (0.95-1.07)	28	0.91 (0.75-1.10)	62	1.00 (0.92-1.08)

[1] Adjusted for age (underlying time scale), BMI, parity, age at first birth, age at menarche, family history, smoking, physical activity, education

Abbreviations: CI: confidence interval; EPT: estrogen-progestin therapy; HER2: human epidermal growth factor receptor 2; HR: hazard ratio; MHT: menopausal hormone therapy; TNBC: triple-negative breast cancer

Supplementary Table 6. MHT use at study entry and 10-year survival by intrinsic-like subtypes – complete-case analyses

	Breast cancer deaths overall		Luminal A-like		Luminal B-like		HER2+		TNBC	
	n	HR (95% CI)[1]	n	HR (95% CI)[1]	n	HR (95% CI)[1]	n	HR (95% CI)[1]	n	HR (95% CI)[1]
Age-adjusted	634		148		104		32		81	
MHT use overall										
Never use	356	Ref.	76	Ref.	62	Ref.	19	Ref.	54	Ref.
Ever use	278	0.92 (0.79-1.08)	72	1.11 (0.80-1.54)	42	0.74 (0.50-1.10)	13	0.85 (0.42-1.72)	27	0.57 (0.36-0.91)
Current	226	0.94 (0.79-1.11)	58	1.15 (0.82-1.63)	32	0.75 (0.49-1.16)	13	1.06 (0.52-2.16)	16	0.43 (0.24-0.75)
Former	52	0.84 (0.63-1.13)	14	0.96 (0.54-1.71)	10	0.84 (0.43-1.65)	0	-	11	1.12 (0.58-2.16)
Duration										
< 5 yrs	181	0.93 (0.77-1.11)	38	0.96 (0.65-1.43)	26	0.76 (0.48-1.21)	10	1.00 (0.46-2.18)	16	0.53 (0.30-0.93)
≥ 5 yrs	93	0.91 (0.72-1.15)	32	1.33 (0.87-2.03)	15	0.76 (0.43-1.35)	3	0.58 (0.17-1.97)	11	0.66 (0.34-1.27)
Per 1 yr	630	0.99 (0.97-1.01)	146	1.03 (0.99-1.07)	103	0.98 (0.93-1.04)	32	0.92 (0.81-1.06)	81	0.95 (0.88-1.03)
EPT use										
Never use	356	Ref.	76	Ref.	62	Ref.	19	Ref.	54	Ref.
Ever use	201	0.91 (0.77-1.09)	54	1.14 (0.80-1.63)	31	0.77 (0.50-1.20)	11	1.00 (0.47-2.12)	20	0.57 (0.34-0.96)
Current	175	0.96 (0.80-1.15)	47	1.22 (0.85-1.77)	25	0.76 (0.48-1.22)	11	1.20 (0.57-2.53)	14	0.49 (0.27-0.88)
Former	26	0.69 (0.46-1.03)	7	0.79 (0.36-1.72)	6	0.81 (0.35-1.88)	0	-	6	0.99 (0.42-2.33)
Duration										
< 5 yrs	126	0.93 (0.76-1.15)	25	0.92 (0.58-1.45)	19	0.80 (0.48-1.34)	9	1.35 (0.61-3.00)	11	0.53 (0.28-1.02)
≥ 5 yrs	74	0.87 (0.68-1.13)	28	1.42 (0.92-2.21)	12	0.73 (0.39-1.37)	2	0.47 (0.11-2.03)	9	0.65 (0.32-1.33)
Per 1 yr	556	0.99 (0.96-1.01)	129	1.04 (1.00-1.08)	93	0.99 (0.93-1.05)	30	0.92 (0.79-1.07)	74	0.95 (0.88-1.03)
MV-adjusted[2]	515		116		85		27		67	
MHT use overall										
Never use	280	Ref.	60	Ref.	48	Ref.	17	Ref.	44	Ref.
Ever use	235	1.00 (0.84-1.19)	56	1.16 (0.80-1.69)	37	0.87 (0.56-1.36)	10	0.78 (0.35-1.73)	23	0.59 (0.35-0.99)
Current	193	1.02 (0.85-1.23)	44	1.19 (0.80-1.77)	30	0.91 (0.57-1.46)	10	0.97 (0.44-2.18)	13	0.41 (0.22-0.78)
Former	42	0.90 (0.65-1.25)	12	1.08 (0.58-2.03)	7	0.74 (0.33-1.66)	0	-	10	1.30 (0.64-2.61)
Duration										
< 5 yrs	162	1.05 (0.86-1.28)	32	1.08 (0.70-1.68)	25	0.97 (0.59-1.58)	9	1.00 (0.44-2.30)	14	0.56 (0.31-1.04)
≥ 5 yrs	72	0.91 (0.70-1.19)	23	1.28 (0.78-2.11)	12	0.75 (0.39-1.43)	1	0.26 (0.03-2.01)	9	0.66 (0.32-1.38)
Per 1 yr	514	0.99 (0.96-1.02)	115	1.03 (0.98-1.08)	85	0.98 (0.92-1.04)	27	0.88 (0.72-1.07)	67	0.95 (0.88-1.04)
EPT use										
Never use	280	Ref.	60	Ref.	48	Ref.	17	Ref.	44	Ref.
Ever use	172	0.99 (0.81-1.20)	41	1.14 (0.76-1.72)	28	0.87 (0.54-1.41)	9	0.96 (0.42-2.20)	18	0.62 (0.36-1.09)
Current	148	1.02 (0.83-1.25)	34	1.19 (0.77-1.83)	23	0.89 (0.54-1.48)	9	1.15 (0.50-2.65)	12	0.50 (0.26-0.95)
Former	24	0.82 (0.54-1.25)	7	0.97 (0.44-2.13)	5	0.80 (0.31-2.02)	0	-	6	1.24 (0.52-2.95)
Duration										
< 5 yrs	115	1.07 (0.86-1.33)	21	1.01 (0.61-1.67)	19	1.04 (0.60-1.78)	8	1.29 (0.54-3.04)	10	0.58 (0.29-1.15)
≥ 5 yrs	57	0.86 (0.65-1.15)	20	1.34 (0.80-2.26)	9	0.66 (0.32-1.37)	1	0.32 (0.04-2.42)	8	0.70 (0.33-1.52)
Per 1 yr	452	0.99 (0.96-1.02)	101	1.04 (0.99-1.09)	76	0.98 (0.91-1.05)	26	0.89 (0.73-1.09)	62	0.97 (0.89-1.06)

[1] HRs of breast cancer-specific deaths

[2] Adjusted for age (underlying time scale), BMI, parity, age at first birth, age at menarche, family history, smoking, physical activity, education

Abbreviations: CI: confidence interval; EPT: estrogen-progestin therapy; HER2: human epidermal growth factor receptor 2; HR: hazard ratio; MHT: menopausal hormone therapy; TNBC: triple-negative breast cancer

Supplementary Table 7. MHT use at study entry and 10-year survival by intrinsic-like subtypes – MI dataset, adjusted for clinical characteristics

		Breast cancer deaths overall (n = 634)		Luminal A-like (n = 148)		Luminal B-like (n = 104)		HER2+ (n = 32)		TNBC (n = 65)	
		n	HR (95% CI)[1]	n	HR (95% CI)[1,2]	n	HR (95% CI)[1,2]	n	HR (95% CI)[1,2]	n	HR (95% CI)[1]
Model 1[2]											
MHT use overall											
Never use		356	Ref.	76	Ref.	62	Ref.	19	Ref.	54	Ref.
Ever use		278	0.98 (0.84-1.15)	72	1.15 (0.83-1.60)	42	0.81 (0.54-1.20)	13	0.86 (0.42-1.75)	27	0.61 (0.38-0.97)
	Current	226	0.98 (0.83-1.16)	58	1.18 (0.83-1.67)	32	0.77 (0.50-1.19)	13	1.06 (0.52-2.16)	16	0.45 (0.26-0.79)
	Former	52	0.98 (0.73-1.32)	14	1.06 (0.59-1.88)	10	0.95 (0.48-1.87)	0	-	11	1.29 (0.66-2.51)
Duration											
	< 5 yrs	181	1.03 (0.86-1.24)	38	1.00 (0.67-1.49)	26	0.82 (0.51-1.30)	10	1.11 (0.51-2.40)	16	0.60 (0.34-1.05)
	≥ 5 yrs	93	0.90 (0.72-1.14)	32	1.39 (0.91-2.12)	15	0.77 (0.43-1.37)	3	0.51 (0.15-1.75)	11	0.65 (0.34-1.25)
	Per 1 yr	630	0.99 (0.96-1.01)	146	1.03 (0.99-1.07)	103	0.98 (0.93-1.04)	32	0.92 (0.80-1.05)	81	0.95 (0.88-1.02)
EPT use											
Never use		356	Ref.	76	Ref.	62	Ref.	19	Ref.	54	Ref.
Ever use		201	0.98 (0.82-1.16)	54	1.17 (0.82-1.67)	31	0.80 (0.52-1.24)	11	1.03 (0.48-2.19)	20	0.62 (0.37-1.04)
	Current	175	1.01 (0.84-1.21)	47	1.24 (0.85-1.79)	25	0.79 (0.49-1.26)	11	1.21 (0.57-2.57)	14	0.52 (0.29-0.94)
	Former	26	0.80 (0.53-1.19)	7	0.85 (0.39-1.87)	6	0.88 (0.38-2.07)	0	-	6	1.16 (0.49-2.74)
Duration											
	< 5 yrs	126	1.04 (0.85-1.27)	25	0.94 (0.59-1.48)	19	0.84 (0.50-1.41)	9	1.54 (0.69-3.47)	11	0.59 (0.31-1.14)
	≥ 5 yrs	74	0.88 (0.69-1.14)	28	1.49 (0.95-2.32)	12	0.75 (0.40-1.41)	2	0.42 (0.10-1.83)	9	0.66 (0.33-1.36)
	Per 1 yr	556	0.99 (0.96-1.01)	129	1.04 (1.00-1.08)	93	0.99 (0.93-1.05)	30	0.91 (0.78-1.06)	74	0.95 (0.88-1.03)
Model 2[3]											
MHT use overall											
Never use		356	Ref.	76	Ref.	62	Ref.	19	Ref.	54	Ref.
Ever use		278	1.00 (0.85-1.18)	72	1.24 (0.89-1.74)	42	0.82 (0.55-1.23)	13	0.95 (0.45-1.99)	27	0.59 (0.37-0.95)
	Current	226	1.01 (0.85-1.19)	58	1.31 (0.92-1.87)	32	0.79 (0.51-1.23)	13	1.17 (0.55-2.45)	16	0.43 (0.25-0.76)
	Former	52	0.98 (0.73-1.32)	14	1.03 (0.57-1.84)	10	0.93 (0.47-1.84)	0	-	11	1.32 (0.68-2.57)
Duration											
	< 5 yrs	181	1.05 (0.88-1.27)	38	1.08 (0.72-1.61)	26	0.86 (0.53-1.37)	10	1.16 (0.52-2.57)	16	0.59 (0.33-1.03)
	≥ 5 yrs	93	0.91 (0.72-1.15)	32	1.49 (0.97-2.29)	15	0.73 (0.41-1.32)	3	0.60 (0.17-2.13)	11	0.63 (0.33-1.23)
	Per 1 yr	630	0.99 (0.97-1.01)	146	1.03 (0.99-1.07)	103	0.98 (0.92-1.03)	32	0.93 (0.80-1.06)	81	0.95 (0.88-1.02)
EPT use											
Never use		356	Ref.	76	Ref.	62	Ref.	19	Ref.	54	Ref.
Ever use		201	1.00 (0.84-1.20)	54	1.29 (0.90-1.85)	31	0.81 (0.52-1.27)	11	1.13 (0.52-2.47)	20	0.60 (0.35-1.01)
	Current	175	1.04 (0.86-1.25)	47	1.40 (0.96-2.04)	25	0.80 (0.49-1.29)	11	1.32 (0.60-2.89)	14	0.50 (0.27-0.90)
	Former	26	0.82 (0.54-1.22)	7	0.86 (0.39-1.88)	6	0.86 (0.37-2.04)	0	-	6	1.18 (0.50-2.80)
Duration											
	< 5 yrs	126	1.08 (0.88-1.33)	25	1.04 (0.66-1.66)	19	0.89 (0.52-1.51)	9	1.60 (0.70-3.66)	11	0.58 (0.30-1.12)
	≥ 5 yrs	74	0.89 (0.69-1.14)	28	1.59 (1.02-2.50)	12	0.70 (0.37-1.33)	2	0.48 (0.11-2.15)	9	0.64 (0.31-1.31)
	Per 1 yr	556	0.99 (0.96-1.01)	129	1.04 (1.00-1.08)	93	0.98 (0.92-1.04)	30	0.92 (0.79-1.07)	74	0.95 (0.88-1.03)

[1] HRs of breast cancer-specific deaths

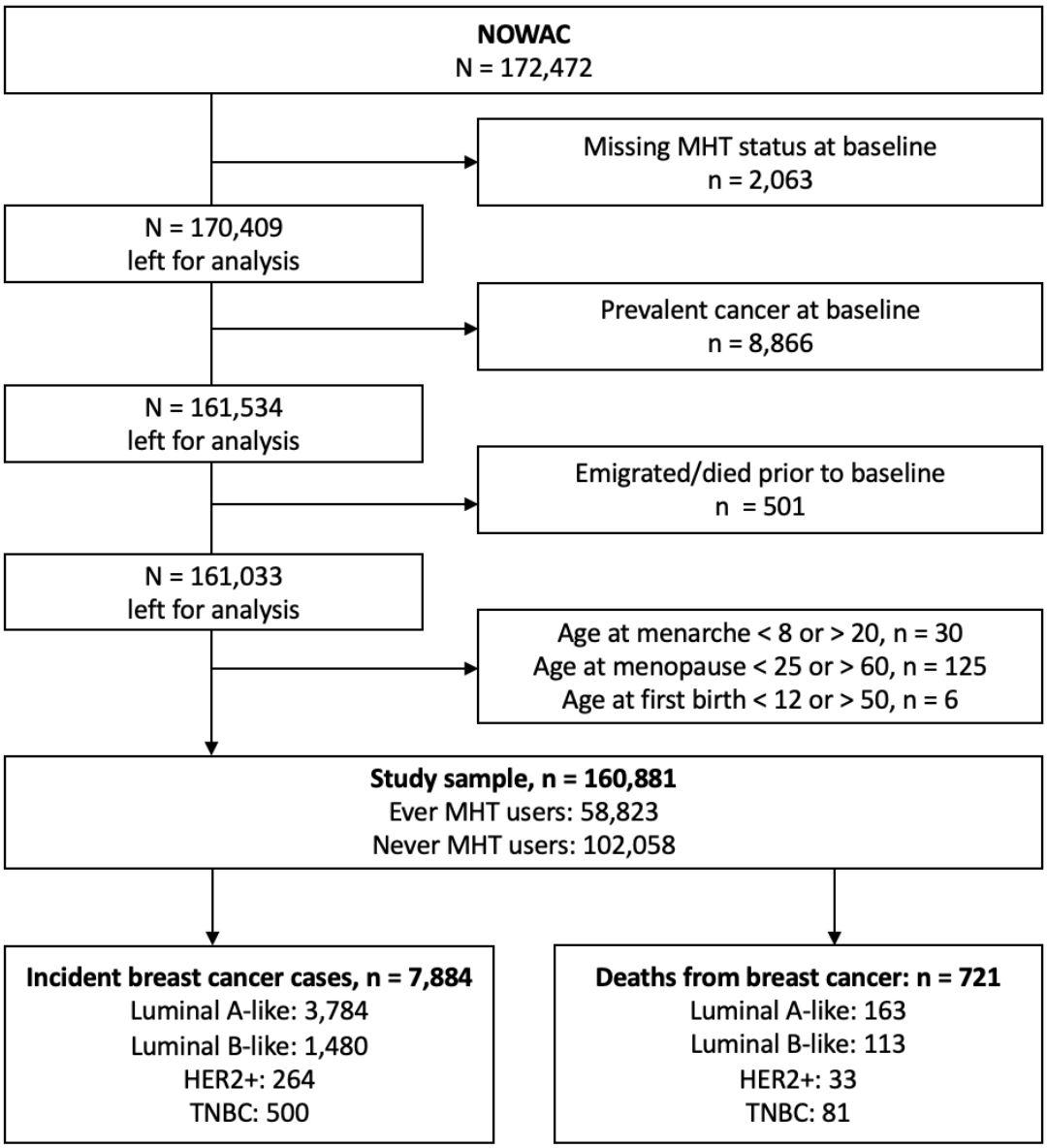
[2] Adjusted for stage, surgery status, age at diagnosis

[3] Adjusted for age (underlying time scale), BMI, parity, age at first birth, age at menarche, family history, smoking, physical activity, education, stage, surgery status, age at diagnosis

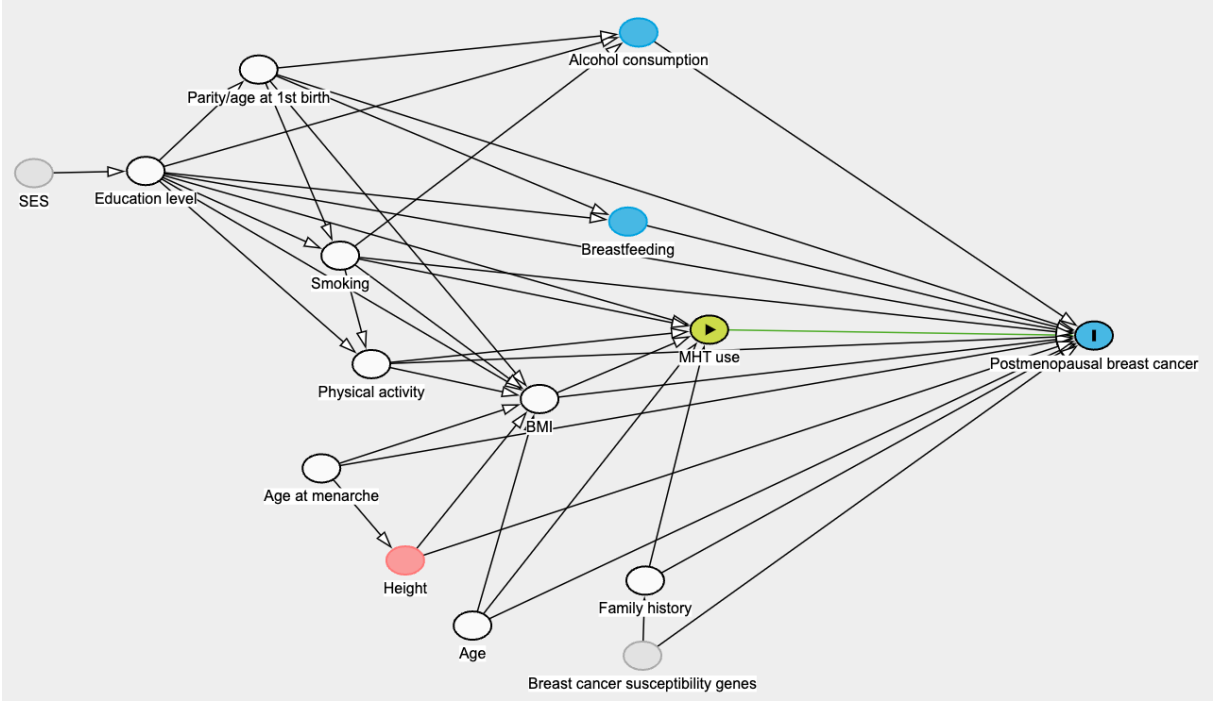
Abbreviations: CI: confidence interval; EPT: estrogen-progestin therapy; HER2: human epidermal growth factor receptor 2; HR: hazard ratio; MHT: menopausal hormone therapy; TNBC: triple-negative breast cancer

Supplementary figures

Supplementary Figure 1. Flow chart of study sample



Supplementary Figure 2. Directed acyclic graph on the assumed relations between MHT use and incidence of postmenopausal breast cancer



Created from https://dagitty.net. Color coding: blue – ancestor of outcome; red – ancestor of exposure and outcome; white – adjusted variable; grey – unmeasured variable. Minimal sufficient adjustment set, depicted in white, included age, age at menarche, BMI, education level, family history of breast cancer, parity/age at first birth, physical activity and smoking. The DAG is based on the following implications of common causes of exposure and outcome: 1) Age affects likelihood of MHT use as well as breast cancer incidence; 2) Education level is associated with both MHT use and postmenopausal breast cancer[1]; 3) Smoking affects age at menopause and thus MHT use, and is also a risk factor for breast cancer[2]; 4) Physical activity, directly or mediated by BMI, is assumed associated with MHT use, and also affect breast cancer risk[3]; 5) BMI is related to both MHT use and postmenopausal breast cancer[4]; 6) Reproductive history such as parity, age at first birth, and age at menarche are assumed indirect ancestors of MHT use, and comprise risk factors for breast cancer[5]; 7) Family history of breast cancer likely affects subsequent use of MHT, and increases risk of breast cancer[6].

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[1] Dong JY, Qin LQ. Education level and breast cancer incidence: a meta-analysis of cohort studies. Menopause. 2020;27(1):113-8.

[2] Gaudet MM, Carter BD, Brinton LA, Falk RT, Gram IT, Luo J, et al. Pooled analysis of active cigarette smoking and invasive breast cancer risk in 14 cohort studies. Int J Epidemiol. 2017;46(3):881-93.

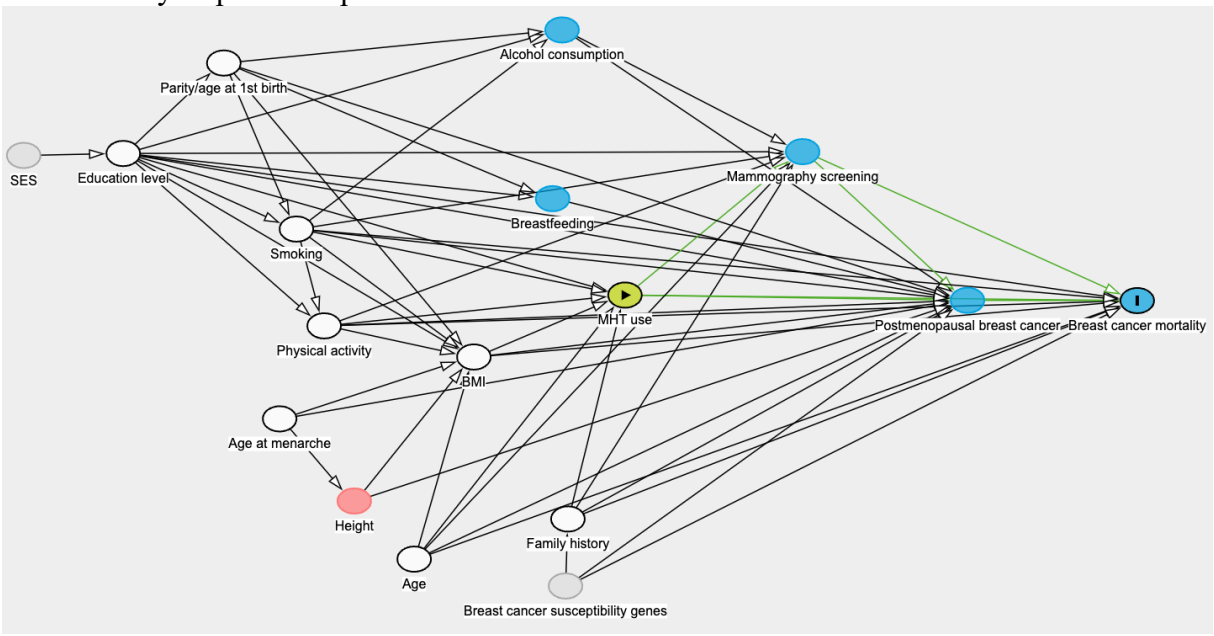
[3] Pizot C, Boniol M, Mullie P, Koechlin A, Boniol M, Boyle P, et al. Physical activity, hormone replacement therapy and breast cancer risk: A meta-analysis of prospective studies. Eur J Cancer. 2016;52:138-54.

[4] Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F, Straif K, et al. Body Fatness and Cancer--Viewpoint of the IARC Working Group. N Engl J Med. 2016;375(8):794-8.

[5] Collaborative Group on Hormonal Factors in Breast C. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. Lancet Oncol. 2012;13(11):1141-51.

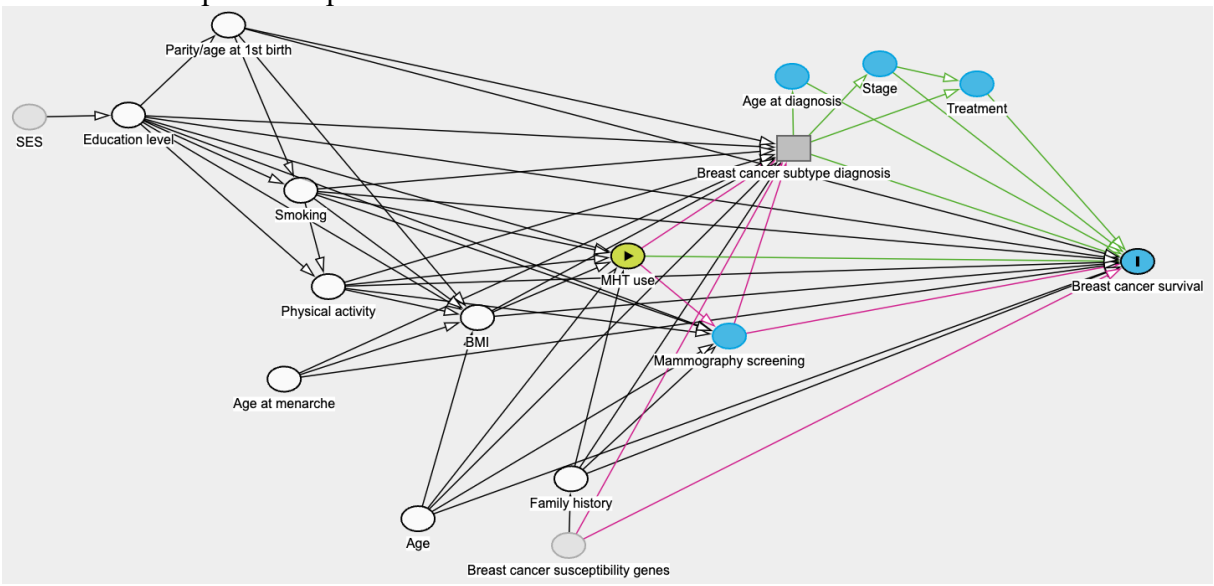
[6] Collaborative Group on Hormonal Factors in Breast C. Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. Lancet. 2001;358(9291):1389-99.

Supplementary Figure 3. Directed acyclic graph on the assumed relations between MHT use and mortality of postmenopausal breast cancer



Created from https://dagitty.net. Color coding: blue – ancestor of outcome; red – ancestor of exposure and outcome; white – adjusted variable; grey – unmeasured variable. Minimal sufficient adjustment set, depicted in white, included age, age at menarche, BMI, education level, family history of breast cancer, parity/age at first birth, physical activity and smoking. The DAG is based on the same assumptions as those of MHT use and breast cancer incidence, as a breast cancer diagnosis will also increase risk of breast cancer-specific mortality on a population level.

Supplementary Figure 4. Directed acyclic graph on the assumed relations between MHT use and survival of postmenopausal breast cancer



Created from https://dagitty.net. Color coding: blue – ancestor of outcome; white – adjusted variable; grey – unmeasured variable. Adjustments were made for age, age at menarche, BMI, education level, family history of breast cancer, parity/age at first birth, physical activity and smoking. The DAG is based on the following implications of common causes of exposure and outcome: 1) Age affects likelihood of MHT use as well as survival from breast cancer; 2) Education level is associated with MHT use as well as breast cancer survival[7]; 3) Smoking is related to MHT use by affecting age at menopause and is associated with decreased breast cancer survival[8]; 4) Physical activity is assumed related to both MHT use and breast cancer survival[9]; 5) BMI could influence the use of MHT, and is associated with breast cancer survival[10]; 6) Reproductive history, including age at menarche, age at first birth, and parity, are assumed indirectly related to MHT use, and could be related to breast cancer survival[11][12][13]; 7) Family history of breast cancer affects MHT use as well as breast cancer survival[14]. In contrast to the mentioned covariates which are assumed to cause confounding if not properly adjusted for, the variables mammography screening, stage, and treatment are intermediates between MHT exposure and breast cancer survival.

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[8] Passarelli MN, Newcomb PA, Hampton JM, Trentham-Dietz A, Titus LJ, Egan KM, et al. Cigarette Smoking Before and After Breast Cancer Diagnosis: Mortality From Breast Cancer and Smoking-Related Diseases. J Clin Oncol. 2016;34(12):1315-22.

[9] Cannioto RA, Hutson A, Dighe S, McCann W, McCann SE, Zirpoli GR, et al. Physical Activity Before, During, and After Chemotherapy for High-Risk Breast Cancer: Relationships With Survival. J Natl Cancer Inst. 2021;113(1):54-63

[10] Protani M, Coory M, Martin JH. Effect of obesity on survival of women with breast cancer: systematic review and meta-analysis. Breast Cancer Res Treat. 2010;123(3):627-35.

[11] Warren Andersen S, Newcomb PA, Hampton JM, Titus-Ernstoff L, Egan KM, Trentham-Dietz A. Reproductive factors and histologic subtype in relation to mortality after a breast cancer diagnosis. Breast Cancer Res Treat. 2011;130(3):975-80.

[12] Kroman N, Wohlfahrt J, Andersen KW, Mouridsen HT, Westergaard T, Melbye M. Parity, age at first childbirth and the prognosis of primary breast cancer. Br J Cancer. 1998;78(11):1529-33.

[13] Orgeas CC, Hall P, Rosenberg LU, Czene K. The influence of menstrual risk factors on tumor characteristics and survival in postmenopausal breast cancer. Breast Cancer Res. 2008;10(6):R107.

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Appendix IV

Information Letter, Norwegian Women and Cancer Study

INSTITUTT FOR SAMFUNNSMEDISIN
UNIVERSITETET I TROMSØ
9037 TROMSØ
Telefon 77 64 48 16

KVINNER OG KREFT

Orientering om undersøkelsen

Institutt for samfunnsmedisin ved Universitetet i Tromsø gjennomfører en spørreundersøkelse om levesett og kreft blant norske kvinner. En slik undersøkelse gir et verdifullt grunnlag for å studere mulige sammenhenger mellom f. eks. barnefødsler, p-piller, solvaner og utviklingen av kreftsykdommer som særlig rammer kvinner. Resultatene vil bli publisert i dagspressen og i internasjonale fagtidsskrifter. Ansvarlig for undersøkelsen er professor Eiliv Lund.

Du forespørres hermed om å delta i undersøkelsen. Alle som blir forespurt er trukket ut tilfeldig. Statistisk Sentralbyrå har trukket utvalget og står for utsending av spørreskjemaene.
Med noen års mellomrom fram til 2017 vil vi sammenholde opplysningene som er gitt i undersøkelsen med opplysninger fra Kreftregisteret og Dødsårsaksregisteret. Alle opplysninger fra undersøkelsen og fra registrene vil bli behandlet konfidensielt og etter de regler Datatilsynet har gitt i sin tillatelse. På spørreskjemaet er navn og fødselsnummer erstattet med et løpenummer slik at ingen av de som mottar og tar hånd om skjemaene vil kjenne din identitet. Undersøkelsen er tilrådd av den regionale etiske komite for Nord-Norge.

Vi vil be deg om å besvare det vedlagte spørreskjemaet så riktig som mulig. Dersom ingen av oppgitte svaralternativ dekker din situasjon, sett kryss for det alternativet som ligger nærmest. Gi eventuelt tilleggsopplysninger i skjemaet. Du behøver ikke å svare på alle spørsmål.

Vi spør også alle som deltar om tillatelse til fornyet skriftlig kontakt om noen år i form av et liknende spørreskjema. For et mindre, tilfeldig utvalg ønsker vi i tillegg mer detaljerte opplysninger om siste døgns kosthold.

Det er frivillig om du vil være med i undersøkelsen. Det er også adgang til å trekke seg senere, hvis du skulle ønske det. Du kan få slettet dine opplysninger hvis du krever det.

Ditt bidrag til undersøkelsen vil være å svare på spørsmålene i det spørreskjemaet som følger med. For spørsmål om hormoner og p-pille bruk finner du bilder i denne brosjyren som skal være et hjelpemiddel til å svare riktig (brosjyren skal ikke returneres). Spørreskjemaet returneres i vedlagte konvolutt med betalt svarporto.

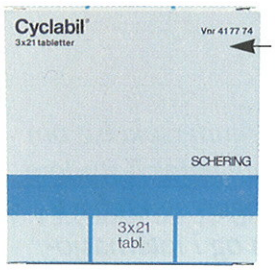
Med hilsen

Eiliv Lund
Professor dr. med.

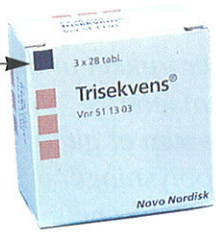
Bruk av hormoner i og etter overgangsalderen

Denne brosjyren er et hjelpemiddel for å huske riktig navn på de hormontabletter/plaster/salver/stikkpiller du har brukt. Under bildene er det oppgitt hvilke år disse var i salg. For noen hormontabletter/plaster finnes det esker med samme utseende, men med ulik styrke av hormonene. Vi ber deg tenke nøye gjennom navnet på de hormon-tabletter/plaster/salver/stikkpiller du har brukt. Eldre avregistrerte preparater er ikke gjengitt med bilder, det gjelder:

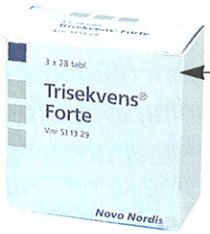
Nr. 201 **Dietylstilbøstrol** 1mg stikkpiller til skjeden (1976-92)
Nr. 202 **Dietylstilbøstrol** 0,1 mg tabletter (1980-85)
Nr. 203 **Dietylstilbøstrol** 0,5 mg stikkpiller (1976-81)
Nr. 204 **Primodos** tabletter (1961-74)
Nr. 205 **Østriol** 1 mg tabletter (1975-95)
Nr. 206 **Østriol** 0,25 mg tabletter (1961-83)



Nr. 101. Solgt fra 1978



Nr. 102. Solgt fra 1978

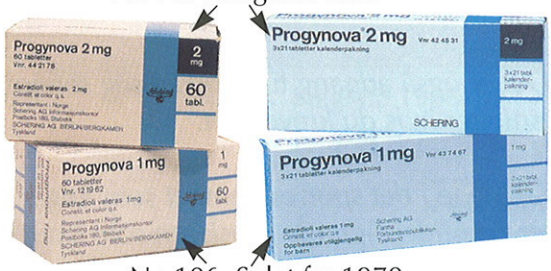


Nr. 103. Solgt fra 1978

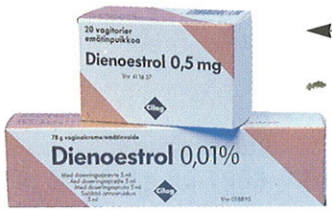
Nr. 104. Solgt fra 1953

Nr. 105. Solgt fra 1988

Nr. 107. Solgt fra 1967

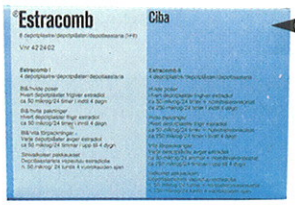


Nr. 106. Solgt fra 1970



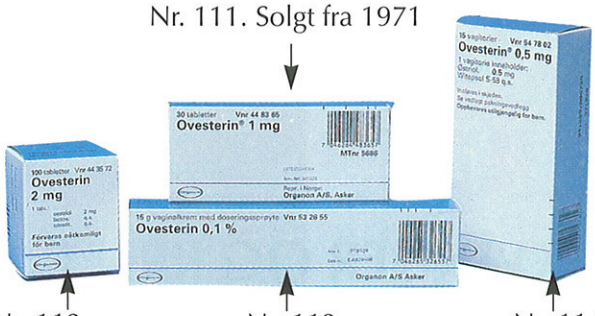
Nr. 108. Solgt fra 1976

Nr. 109. Solgt fra 1954



Nr. 110. Solgt fra 1994

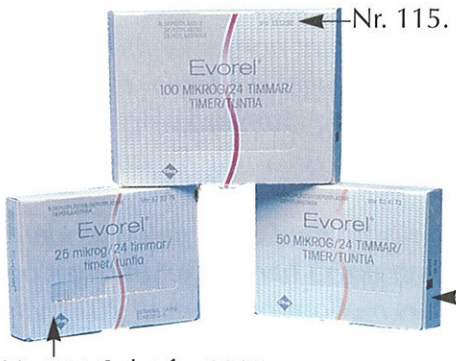
Nr. 111. Solgt fra 1971



Nr. 112. Solgt fra 1989

Nr. 113. Solgt fra 1983

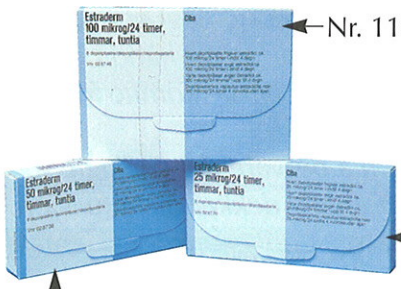
Nr. 114. Solgt fra 1984



Nr. 115. Solgt fra 1995

Nr. 117. Solgt fra 1994

Nr. 116.Solgt fra 1995



Nr. 118. Solgt fra 1989

Nr. 120. Solgt fra 1989

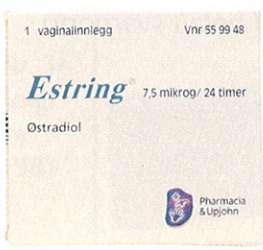
Nr. 119. Solgt fra 1989

Nr. 121 Solgt fra 1996.

Nr. 123 Solgt fra 1996.

Nr. 122 Solgt fra 1996.

Nr. 124 Solgt fra 1996.



Nr. 125 Solgt fra 1996.

P-PILLE MERKER

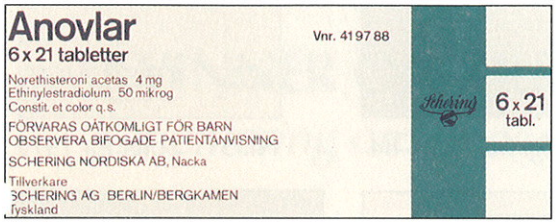
Denne brosjyren er et hjelpemiddel for å huske riktig navn på de p-piller du har brukt. Bildene er ordnet alfabetisk. Under bildene er det oppgitt hvilke år p-pillene var i salg.

For noen p-piller finnes det esker med samme utseende, men med ulik størrelse, avhengig av om de inneholder p-piller for en eller flere måneder.

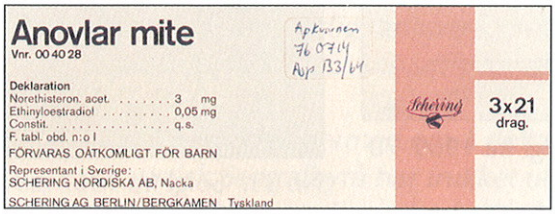
Vi ber deg tenke nøye gjennom navnet på de p-pillene du har brukt.

Av tre p-pillemerker har vi ikke bilder, det gjelder:

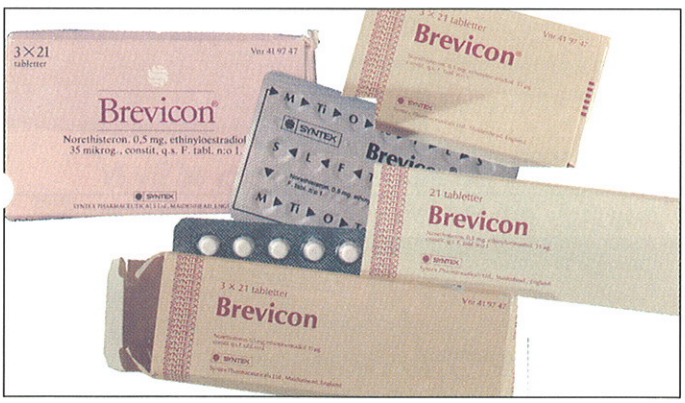
Nr. 1. **Follistrel,** solgt fra 1973–76
Nr. 2. **Menokvens,** solgt fra 1971–72
Nr. 3. **Novokvens,** Solgt fra 1969–70



Nr. 4. Solgt fra 1965–68



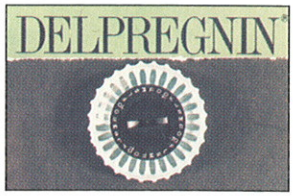
Nr. 5. Solgt fra 1967–69



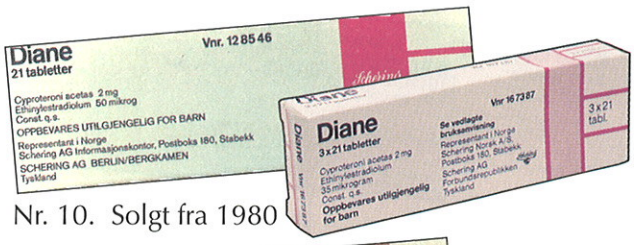
Nr. 6. Solgt fra 1980

Nr. 7. Solgt fra 1971

Nr. 8. Solgt fra 1968–70

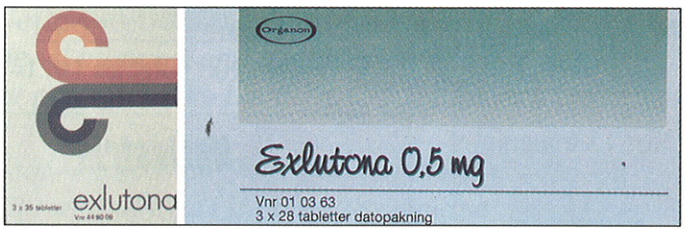


Nr. 9. Solgt fra 1968–71

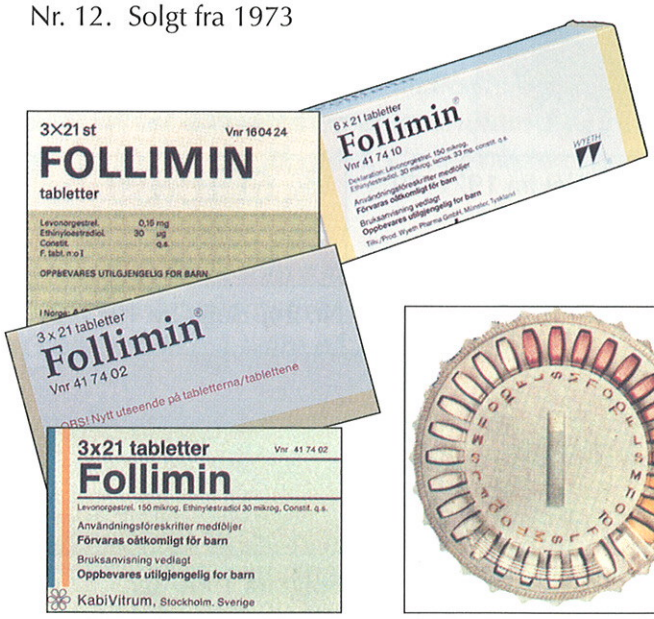


Nr. 10. Solgt fra 1980

Nr. 11. Solgt fra 1969

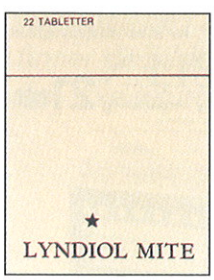


Nr. 12. Solgt fra 1973

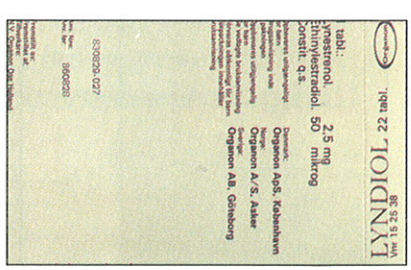


Nr. 13. Solgt fra 1978

Nr. 14. Solgt fra 1971–75

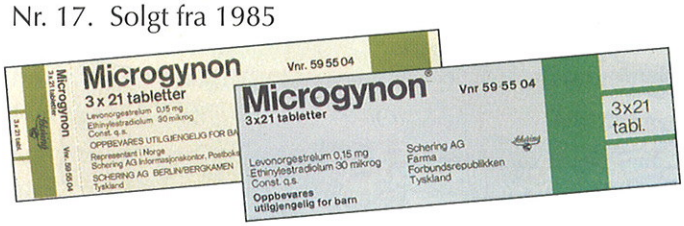


Nr. 15. Solgt fra 1966–72



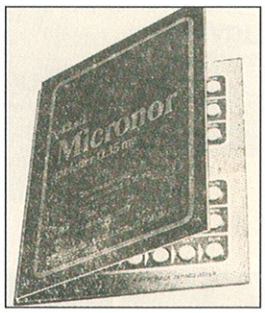
Nr. 16. Solgt fra 1965

Nr. 17. Solgt fra 1985

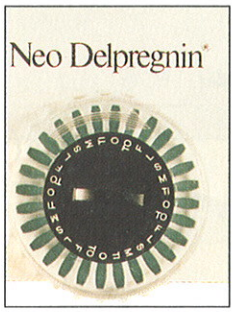


Nr. 18. Solgt fra 1975

Nr. 19. Solgt fra 1973

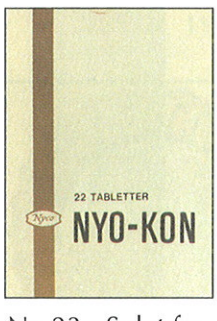


Nr. 20. Solgt fra 1971–79



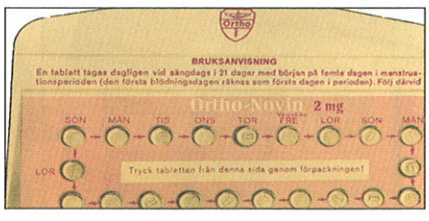
Nr. 21. Solgt fra 1971–79

Nr. 22. Solgt fra 1965–80

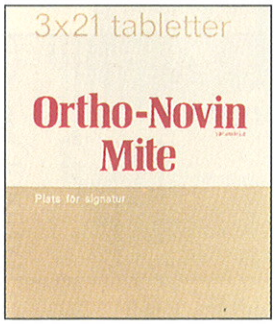


Nr. 23. Solgt fra 1968–70

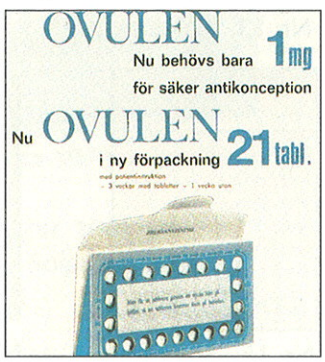
Nr. 24. Solgt fra 1971–81



Nr. 25. Solgt fra 1966–69



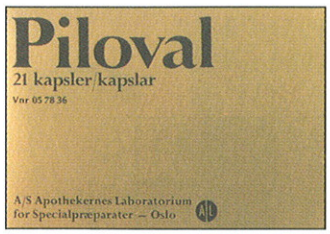
Nr. 26. Solgt fra 1968–72



Nr. 27. Solgt fra 1965–71

Nr. 28. Solgt fra 1970

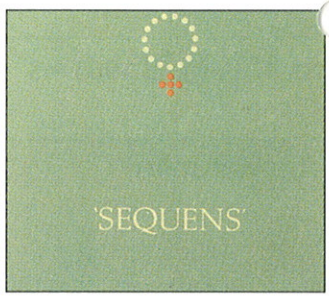
Nr. 29. Solgt fra 1973–82



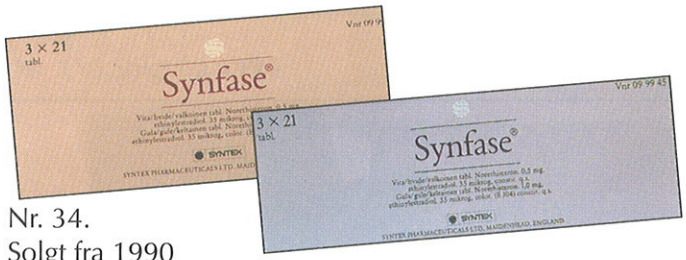
Nr. 30. Solgt fra 1968–84

Nr. 31. Solgt fra 1977

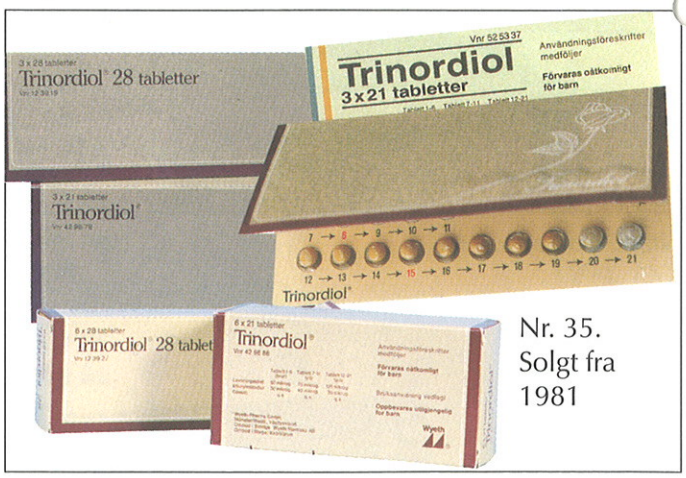
Nr. 32. Solgt fra 1969–70



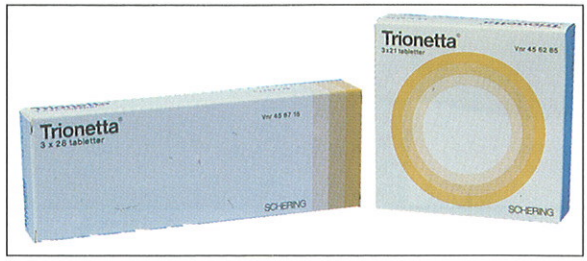
Nr. 33. Solgt fra 1967–69



Nr. 34. Solgt fra 1990



Nr. 35. Solgt fra 1981

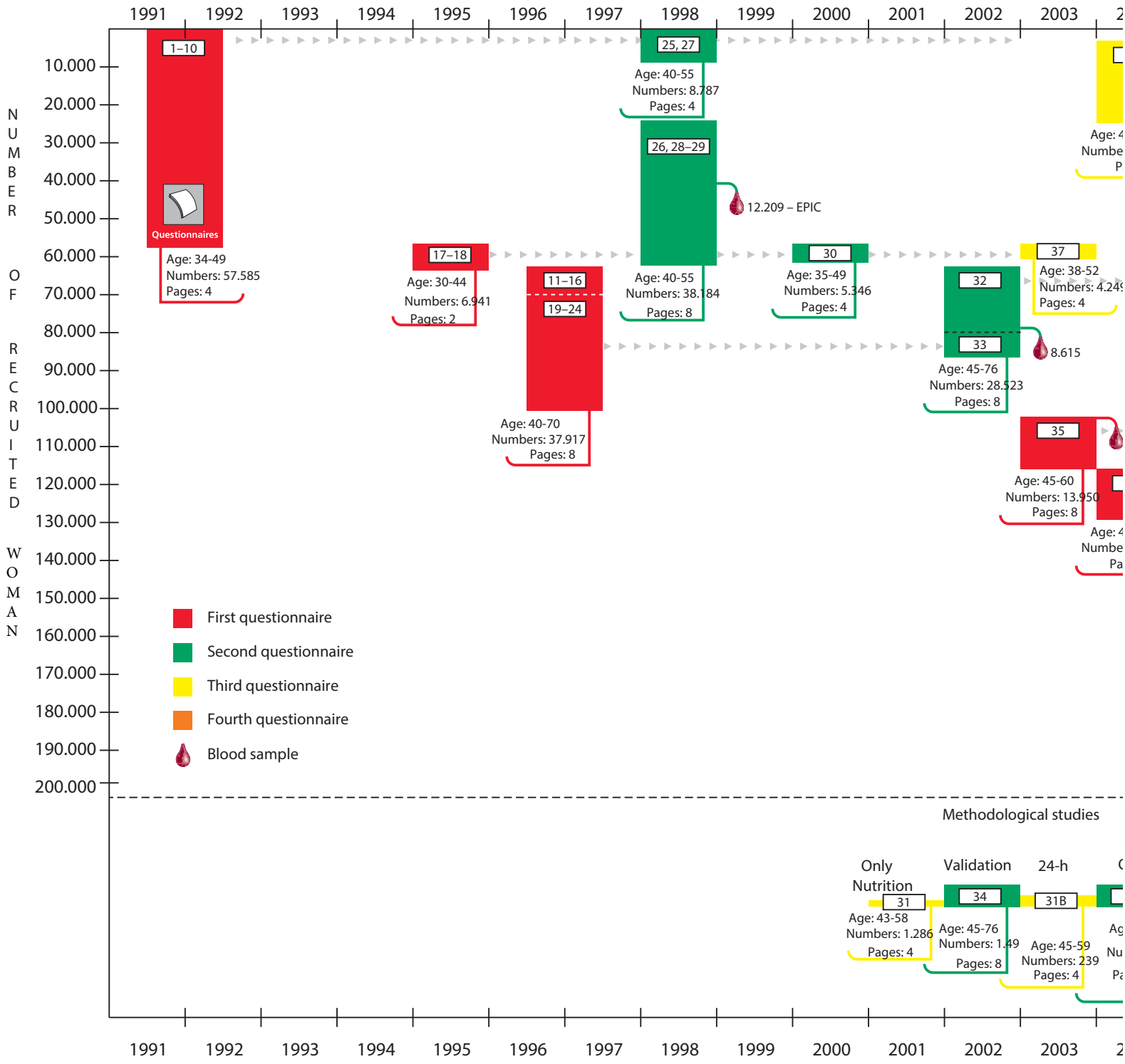


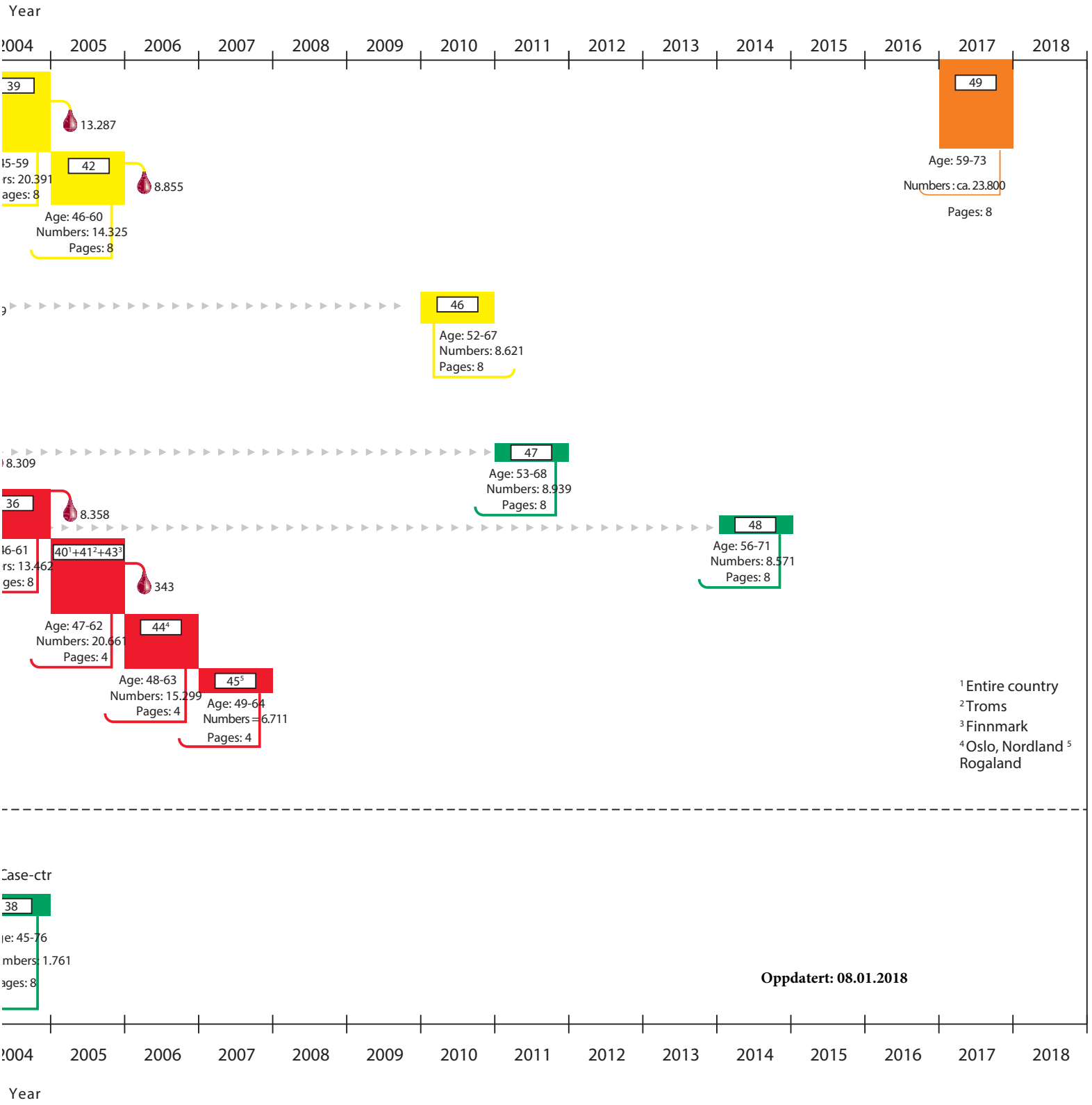
Nr. 36. Solgt fra 1981

LUNDBLAD GRAFISK AS, TROMSØ - TLF 77 67 51 01

Appendix V

Norwegian Women and Cancer study timeline


1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
NUMBER OF RECRUITED WOMAN
10.000
20.000
30.000
40.000
50.000
60.000
70.000
80.000
90.000
100.000
110.000
120.000
130.000
140.000
150.000
160.000
170.000
180.000
190.000
200.000
1–10
Questionnaires
Age: 34-49
Numbers: 57.585
Pages: 4
17–18
Age: 30-44
Numbers: 6.941
Pages: 2
11–16
19–24
Age: 40-70
Numbers: 37.917
Pages: 8
25, 27
Age: 40-55
Numbers: 8.787
Pages: 4
26, 28–29
12.209 – EPIC
Age: 40-55
Numbers: 38.184
Pages: 8
30
Age: 35-49
Numbers: 5.346
Pages: 4
32
33
8.615
Age: 45-76
Numbers: 28.523
Pages: 8
37
Age: 38-52
Numbers: 4.249
Pages: 4
35
Age: 45-60
Numbers: 13.950
Pages: 8
First questionnaire
Second questionnaire
Third questionnaire
Fourth questionnaire
Blood sample
Methodological studies
Only Nutrition
31
Age: 43-58
Numbers: 1.286
Pages: 4
Validation
34
Age: 45-76
Numbers: 1.49
Pages: 8
24-h
31B
Age: 45-59
Numbers: 239
Pages: 4


Year
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
39
13.287
42
8.855
Age: 46-60
Numbers: 14.325
Pages: 8
49
Age: 59-73
Numbers : ca. 23.800
Pages: 8
46
Age: 52-67
Numbers: 8.621
Pages: 8
47
Age: 53-68
Numbers: 8.939
Pages: 8
48
Age: 56-71
Numbers: 8.571
Pages: 8
36
8.358
40[1]+41[2]+43[3]
343
Age: 47-62
Numbers: 20.661
Pages: 4
44[4]
Age: 48-63
Numbers: 15.299
Pages: 4
45[5]
Age: 49-64
Numbers = 6.711
Pages: 4
[1] Entire country
[2] Troms
[3] Finnmark
[4] Oslo, Nordland [5] Rogaland
Case-ctr
38
Oppdatert: 08.01.2018
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
Year